8th ICUP
ZURICH 2014

Proceedings of the 8th International Conference on Urban Pests

Editors

Gabi Müller,
Reiner Pospischil,
and
William H Robinson

Published 2014

ISBN xxx-xxx-xx-xxxx-x

Printed by OOK-Press Kft., H-8200 Veszprém, Pápai u.37/A, Hungary, in cooperation with the Executive Committee of the International Conference on Urban Pests

The 8th International Conference on Urban Pests

University of Zurich

July 20 – 23, 2014

8th ICUP ZURICH 2014

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Publicity was provided by: Beckmann Verlag (DpS), International Pest Control Magazine and Pest (Independent UK pest management magazine).

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INTRODUCTION

It has been six years since the International Conference on Urban Pests was held in Europe. After the Conference in Budapest (2008) we traveled to Ouro Preto, Brazil (2011).
The return to Europe has provided many academic and industry scientists the opportunity to contribute papers and attend a meeting where they can exchange information and ideas with their colleagues. Some of the presenters from the earlier conferences have returned, and we welcome them back.

Ants and termites remain at the top of the list of important household and structural pests around the world. However, research on the increasing threat of mosquito- and tick-borne diseases is well represented during this Conference. The global importance of bed bugs is apparent at the 8th ICUP. There are eight presented papers, covering biology and habits and control from Australia, Germany, United Kingdom, and the United States. The distribution, control problems, and involvement of the professional pest control industry with bed bugs are reminiscent of infestations of the German cockroach in the 1970s and 1980s.

The success of every ICUP is linked to the dedication and commitment of a small number of people. These individuals give the Conference their time and skills for the satisfaction that of knowing they have contributed to urban pest management. They have provided a place and a forum for scientists from around the world, and we sincerely thank the Organising Committee for this. The dedication and commitment of Gabi Muller to the Conference success, and the work of Reiner Pospischil in getting the manuscripts and the Proceedings prepared is greatly appreciated.

William H Robinson
Chair, Executive Committee, International Conference on Urban Pests

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CONTROL OF FLOODWATER MOSQUITOES IN WETLANDS

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Abstract Flood water mosquitoes have been successfully controlled in Switzerland for 25 years with products based on *Bacillus thuringiensis israelensis* (Bti). These mosquitoes have a flying range of up to 10 km in search of a blood meal. Before the interventions the quality of life of residents in villages near the mosquito breeding areas was greatly reduced, and tourism suffered financial losses. The logistic to block the mass development of the flood water mosquitoes, dominated by *Aedes vexans* and *Ochlerotatus sticticus* is demanding. Accurate forecast of precipitation in the breeding areas is an important for the planning stage. Flooded breeding sites require monitoring for the presence of mosquito larvae. Only a few days are available for Bti-interventions. Time slots for aerial treatments by helicopter have to be obtained. Ground application equipment has to be ready. Mosquito larvae have to be eliminated in their third instar, once in the fourth instar the efficacy of Bti drops. Post-treatment monitoring has to show 95% to 99% elimination of mosquito larvae. It is the goal to limit the Bti-treatments to one or two interventions per year. Bti-treatments to control flood water mosquitoes requires governmental approval.
Key words Mosquito control in wetlands, *Bacillus thuringiensis israelensis*.

INTRODUCTION

In temperate climatic zones, especially in Europe, mosquitoes were during the second half of the past century no major issue. Malaria had been eradicated, and other mosquito borne infectious diseases were mainly limited to the tropical belts. At present this situation is changing drastically, as will be highlighted later-on during this conference. Urbanization, human mobility, world-wide passive transport of mosquitoes as well as re-naturation and extension of wetlands is leading to an increased exposure of the European population to mosquitoes. This development is accelerated by the climate change, especially warming and extreme weather situations.

The impact by flood water mosquitoes which is emphasized here, on humans but also on farm animals seems to be on a steady increase due some of the above mentioned factors. In many instances control of these mosquitoes has become indispensable. The main breeding sites are located in wetlands which are periodically flooded. Wetlands are fragile ecosystems and need protection. Therefore, interventions with biocides is very delicate. The only approved biocides are based on *Bacillus thuringiensis israelensis*. The simultaneous work with two organisms, on the one hand side a bacterium on the other hand an insect, is highly demanding. The control of mosquitoes has to be effective, and at the same time the biosafety of the microbial control agent has to be assured. Thus the risk-benefit factor becomes a key issue along with the cost-benefit ratio.

The goal of our work was and is the control the mass development of flood water mosquitoes to limit the disturbance for residents in the vicinity of breeding sites. The flood water mosquitoes belong to the two genera *Aedes* and *Ochlerotatus*. *Aedes vexans* is the dominating flood water species in many European

countries including Switzerland. Their mass development is directly correlated with the severity of flooding (Rettich et al., 2012). The dynamic of these mosquitoes differs greatly from other mosquitoes. The eggs are deposited in moist soil, temporarily flooded usually during spring and early summer. Hatching occurs synchronously as soon as the eggs are inundated. Depending on the water temperature the development of the aquatic stages may take one to two weeks. The striking characteristic is the extreme high population densities with often more than 1'000 larvae per liter. Masses of adult females may appear after sunset in the search for blood meals, covering distances of up to 10 km. The longevity averages four to six weeks. A single blood meal is sufficient for the development of at least 100 eggs which are deposited again in temporarily flooded soil.
The only measure to curb the mass invasion of flood water mosquitoes into residential areas is the control of the larvae in their breeding places. The check the widely dispersed adults is not feasible. The use of adulticides in residential areas should be avoided. Insecticides such as pyrethroids provide only short-term relieve, and they are known for their potential side effects towards other insects such as bees.

MATERIALS AND METHODS

***Bacillus thuringiensis israelensis* (Bti).** Over the past twenty-five years the commercial product Vectobac-G® (Valent BioSciences, Libertyville, IL, USA) has been used exclusively for the control of the flood water mosquitoes. It is a granular formulation with potency of 200 International Toxic Units/mg. The product is based on a Bti strain designated as AM65-52. The toxic effect against the mosquito larvae is caused by the Cry-proteins Cry4, Cry10, Cry 11 supported by the Cyt-proteins, Cyt1 and Cyt2. All these proteins are coded by genes located on a mega plasmid designated as pBtoxis (Schnepf et al., 1998).
Application technique and equipment. The mosquito breeding areas in the Plain of Magadino bordering Lago Maggiore, and the upper end of the Lac de la Gruyére are treated from the air. Fertiliser spreaders with a loading capacity of 200 kg of Vectobac-G are hooked on to a helicopter. The Bti-granules are distributed by a rotating disk covering a swath of 20 m. The delivery rate of Vectobac-G is adjusted to 14 kg/ha with a flying speed of 60 km/h at an altitude of 80 m. In the smaller flooded sites of the natural reserve of the Thurauen where the river Thur joins the Rhine, treatments are carried out by ground equipment with a motorized knap sack sprayer adjusted for the delivery of Vectobac-G granules. The same application rates are used.
Pre-and post-treatment logistics. During spring and early summer months preparation for the interventions against the mosquitoes have to start on time. Weather reports and predictions of flooding, issued by the Federal Office for the Environment (FOEN) are used as early warning systems. The increase of the water levels in the project areas is followed via the internet. The field work starts with the monitoring of the mosquito larvae. If an intervention is necessary a slot for the helicopter treatment has to be requested. At the time of the treatment enough Vectobac-G, packaged in bags of 18.1 kg, has to be on the site. Including reloading, a helicopter treatment takes two to four hours depending on the extension of the flooding. Post-treatment controls for surviving larvae are carried out 24 h following the intervention. If required, the presence and density of adults is monitored with CDC traps.
Administrative issues. Each mosquito control project is supervised by a working group composed of representatives of the local population, of the Cantons and the Federal Government. Detailed reports on the Bti-interventions have to be submitted annually. Permission for the following year has to be granted by the various government offices.

RESULTS AND DISCUSSION

Efficacy of the Bti Treatments

The long-term interventions against the flood water mosquitoes carried out since a quarter of a century in the Plain of Magadino, and since 18 years at the Lac de la Gruyère were highly satisfactory. In 2013 a first Bti-treatment was performed in breeding sites near the village of Ellikon neighboring the Thurauen. The residents could be protected from major disturbances by mosquitoes which prior to the Bti-treatments reduced severely the quality of life during the summer months.

Decision Making Parameters for Interventions

The essential reference point is the degree of nuisance caused by the flood water mosquitoes to man. Thus, recurrent complaints by residents are the key factors. Complaints have to be verified and carefully recorded and inter-linked with the locations and size of the temporarily flooded zones and the concentration of the mosquito larvae. This information forms the basis for decisions to carry out Bti-treatments. Despite regular interventions the density of the mosquito larvae remained rather constant in a given breeding zone with numbers ranging between 100 and 1'000 per liter. The minimum number of larvae requiring an intervention is linked to the level of complaints and falls into a range between 3 and 30 larvae/liter. For example in the German mosquito control program along the upper Rhine valley the threshold is reached with 3 to 5 larvae/liter (n. Becker, personal communication) and for example with 30 larvae/liter in a mosquito abatement program in the Fraser Valley Regional District, Canada (2014).

Timing of Bti Treatments

The timing of Bti-operations is crucial and highly demanding. Synchronous hatching takes place as soon as the eggs are inundated. The minimum temperature is 10^0C. But 20^0C to 25^0C during spring and summer flooding are reached rapidly, shortening the development of the aquatic mosquito stages to 7 – 10 days. The efficacy of Bti is highest when applied to larvae in their 2^{nd} and 3^{rd} stages. The time window for the intervention is 2 to 3 days. The treatments should be carried at the last moment anticipating a possible decrease of the water level and the size of the flooded area. Once mosquito larvae have entered the 4^{th} and final stage, treatments cannot be postponed even if the weather forecast calls for more rain with an extension of flooding. In such situations another treatment is required, but covering only sites additionally covered by water with newly hatched larvae. The number of treatments per season has to be kept to a minimum if ever possible limited to one or two interventions.

Bti Application Rates

Long-term experience shows that under the conditions encountered in Switzerland a minimum dosage of 14 kg/ha of Vectobac-G is required to achieve a satisfactory reduction of the mosquitoes by 95% to 99%. This corresponds to about $3x10^9$ International Toxic Units/ha. The flooded wetlands to be treated are covered often with more than one canopy of dense vegetation consisting of trees, shrubberies, reed and grass. Hence a uniform distribution of about140 Vectobac-G granules/m^2 is required to reach the mosquito larvae in the water. Post-treatment controls at 24 h and 48 h will reveal the success of a Bti-treatment. After one day 90% of the larvae should be eliminated, and at day 2 surviving larvae should be encountered rarely. A re-treatment of the same flooded area due to the application of an insufficient Bti-concentration has to be avoided. The costs are too high and the addition of another dose of the biocide is not desirable.

The Cost-Benefit Relationship

Long-term control of flood water mosquitoes is only feasible if the benefit for the society is substantially higher than the costs incurred. Maintaining an acceptable quality of life for the residents within the perimeter of flood water mosquitoes cannot be measured in money. The key economic factors refer to losses incurred by restaurants and tourisms as well as costs for repellents providing at least partial protection from mosquito bites. The cost-benefit factor is the highest in the densely populated plain of Magadino with tourism being an essential source of income for the region. Invaded by mosquitoes, restaurants are not able to serve their guests in the open especially after sunset. In the plain of Magadino the cost-benefit factor was calculated at 1:50. The cost-benefit factor in the mosquito control project at the Lac de la Gruyère is about 1:5. Here the population affected amounts to 5‘000 persons being about ten times lower than in the Plain of Magadino, and the number of tourists spending their holiday is less. Within the third and smallest mosquito control project, initiated 2013 in the region of the nature reserve of the Thurauen the cost-benefit factor is at least 1:2, still in favor of the benefit. Ten ha were treated by ground application to protect the small village of Ellikon with 90 inhabitants and two restaurants visited by day tourists.

Biosafety of Mosquito Control with *Bacillus thuringiensis israelenis*

Bacillus thuringiensis in general, and in this context the subspecies *israelensis* has an outstanding biosafety record. Bti-products are used world-wide since decades in various formulations, adapted to the type mosquito breeding site. The safety is based on the unique properties of the Cry-proteins which differ among Bt-subspecies (Schnepf et al., 1998). The high specificity is due to a multistep cascade starting with the dissolution of the Bti-crystals via an enzymatic activation process leading to receptor mediated binding with lethal pore formation in the gut epithelium membrane of the target larvae.

The insecticidal activity of the Bti Cry-proteins supported by the Cyt-proteins is limited to a few genera of *Nematocera* (Diptera). Mosquito (*Culicidae*) species are the most susceptible. Many studies have demonstrated the safety of the Bti insecticidal proteins (Lacey and Siegel, 2000; Tetreau, 2012). The World Health Organization (WHO, 1999) approved the global use of Bti, recommending it as an addition to stored drinking water to prevent development of container breeding mosquitoes.

Bti-spores are applied along with the Cry-proteins. The long-term fate of the spores in the environment has been investigated by Guidi et al., (2011). Proliferation was not found. The spore concentration decreased continuously after each treatment.

Indirect impacts on the food web in areas where Bti is regularly applied has become a controversial issue. Poulin et al. (2010) found a reduction of the progeny of house martins caused by a decrease of Nematoceran prey species. Becker et al. (2010) did not find any evidence that mosquitoes served as a major nutrient source for birds. Very likely both authorships were correct. A temporary mass development of flood water mosquitoes could have led via the food web to an increased availability of nutrients for the house martins. Non-flood water mosquitoes are only marginally or not at all affected by the Bti-treatments since their life cycles vary, and they occupy different breeding sites.

Legal Aspects

The majority of wetlands as the main breeding sites of flood water mosquitoes are protected areas where intervention is generally banned. A control of flood water mosquitoes is only admissible if the disturbance caused in urban areas outweighs nature conservation. Products based on Bti are the only permitted biocides. Authorities approve the use of Bti in protected wetlands with great diligence and reserve. Maintenance of a balance between the different interests of the stakeholders remains a complex problem.

At present it is uncertain whether Vectobac-G may be used beyond 2014 since the approval granted by the Swiss authorities has expired on 30 September 2013 and the manufacturer has to our knowledge not filed a request for re-registration. The Bti-products held on stock may be applied for one more year. Switzerland follows closely the EU directives on biocides (The EU Biocidal Products Regulations No 528/2012).

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Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

A PROACTIVE BED BUG SUPPRESSION PROGRAM FOR MULTI-UNIT HOUSING

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Abstract In 2012, a proactive bed bug suppression program was implemented in a low-income housing facility and evaluated over the course of one year. The mean treatment time and application cost were calculated for each unit. In 2013, both the number of initial infestations and the costs associated with bed bug treatments decreased by 26%.
Key Words *Cimex lectularius*, diatomaceous earth, integrate pest management

INTRODUCTION

In the United States, we have been battling the bed bug (*Cimex lectularius* L.) resurgence for over ten years. Current treatment methods are labor intensive, time consuming, and very expensive. Bed bug remediation costs vary depending on the method(s) used, but an insecticide treatment in a single apartment costs ~$500.00, and it is recommended that three treatments be made at two-week intervals (Potter et al., 2010). The average cost of a whole unit heat treatment in an apartment ranges from $800 to $1200. Low-income, multi-unit housing facilities are being financially devastated by the cost of bed bug remediation.

Low-income, multi-unit apartment facilities are particularly at risk for multiple bed bug infestations because the residents are often elderly, or physically or mentally handicapped. These residents may not be able to recognize the signs of an infestation. Infestation problems are exacerbated in multi-unit facilities because units often share common walls, and ventilation, electrical, or plumbing systems. Wall voids and conduits between adjoining units allow bed bugs to travel from one apartment to another (Potter et al., 2010) thus increasing control costs. Apartment managers are desperate to reduce bed bug control costs and prevent future infestations. Implementation of a proactive bed bug suppression program could potentially decrease remediation costs by limiting bed bug movement and increasing the early detection.

Diatomaceous earth (DE) is a desiccant dust that can be applied by facilities staff as part of a proactive bed bug suppression program. When applied correctly, diatomaceous earth applications may reduce the spread of infestations between units. Because DE has a broad application label, and is inexpensive, it is a practical bed bug management tool. With the proper training and certification, even housing facilities staff can apply the diatomaceous earth to help reduce the number and cost of future infestations (Wang et al., 2012). The purpose of this study was to successfully implement a proactive bed bug suppression program in a low-income apartment community and to determine if the number of bed bug infestations, and subsequent treatment costs, could be reduced one year after the implementation.

MATERIALS AND METHODS

Suppression Program Components

Multiple methods of bed bug suppression were selected for their efficacy, ease of application, low cost, and practicality for use in different types of facilities. Ultimately, the program included seven components that could be used together or separately, but were most effective when employed together. The foundation of the suppression program was the application of a barrier of diatomaceous earth (Mother Earth D Pest Control Dust; BASF Corp. St. Louis, MO) around the entire perimeter of the apartment unit. The DE was applied using a power duster (Cyclone Power Duster™; GL Enterprises Inc. New Braunfels, TX; Exacticide power duster, Technicide ™, San Clemente, CA). Another potential component of the suppression program was the construction of a Do-It-Yourself heat chamber (Pereira et al., 2009). The heat chamber would be used to treat infested belongings that could not be treated with chemical insecticides. Additional components of the suppression program might include monitoring devices, mattress and box spring encasements, vacuuming, and the use of a household clothes dryer. The final component was resident and staff education.

Study Site

Lineweaver Apartments is a government subsidized housing community for elderly and disabled residents (265 North Main St. Harrisonburg, VA 22803). The building consists of 47 efficiency units and 74 one-bedroom apartments. The first three reported bed bug infestations occurred in December 2010. Prior to these reported infestations, there had been no bed bug incidence for the previous nine years. The apartment management hired a local company to provide “whole-unit” heat treatments using the Thermal Remediation System from Temp-Air (Temp-Air, Burnsville, MN). The contract costs were $625 for efficiency units and $825 for 1-bedroom units. Although only three infestations were reported, an additional four units were treated because they were adjacent to infested units (total cost $4,975). In 2011, there were an additional 12 infestations and the management paid $14,585 for both heat treatments and inspections. It was in 2011 when the Lineweaver Apartment manager contacted the Dodson Urban Pest Management Laboratory (DUPML) at Virginia Tech.

Suppression Program Implementation

Beginning in January 2012, multiple components (not all) of the bed bug suppression program were implemented in the Lineweaver facility. Upon entering each unit and prior to application of the DE perimeter barrier, staff members vacuumed the perimeter at the floor-wall junction to remove debris and old bed bug evidence. After vacuuming, DE was applied behind vinyl baseboards along the entire perimeter of the unit, and behind plumbing penetrations, electrical outlets, and switch plates. Because management could not afford to purchase bed bug monitors or mattress encasements for the entire building, these items were made available for residents to purchase at wholesale cost. Initially, the Lineweaver management was very interested building a portable heat chamber on a trailer. But as of December 2013, the proposed heat chamber had not been built. Currently, residents are using the on-site coin operated dryers to kill bed bugs on their belongings. Finally, bed bug education seminars were offered to the Lineweaver residents and staff. Residents were taught how to identify live bed bugs and how to install passive monitors and encasements. Staff members were given hands-on instruction in how to implement all bed bug suppression methods including the application of DE using a power duster.

Protocol Implementation

In each apartment, the amount of time spent on unit preparation and dust application was recorded, in addition to the number of laborers, the unit size, and amount of diatomaceous earth applied (g). Total labor cost for implementing the program at Lineweaver was calculated at a rate of $1 per minute (break-even cost for the pest management industry) per laborer. The total cost of the diatomaceous earth applied was based on the market price of $69.95/10 lb. container of Mother Earth D Pest Control Dust.

Program Efficacy Assessment

Bed bug treatment records (Jan. 2011- Dec. 2013) were used to calculate the number and cost of whole unit heat treatments (only remediation method used) for each year. In 2012, the bed bug suppression program was implemented. The costs of the suppression program were added to the cost of the heat treatments. The number of new infestations, and the total cost of all bed bug management measures were recorded for each year. Cost data were used to calculate percent change in bed bug remediation expenses from year to year.

Statistical Analysis

Mean application time (2 laborers), and amount of DE applied per unit were calculated for units of different sizes, treated with different dusters. These means were compared using Tukey's HSD (JMP Pro 10, 2011, SAS Institute, Cary, NC) to detect significant differences in time or product used.

RESULTS AND DISCUSSION

The use of reduced toxicity bed bug management is not new. Several evaluations of different integrated management programs for bed bugs have been conducted (Wang et al., 2012). The study presented here is the first to include perimeter barriers (of desiccant dust) as part of a program to suppress bed bug spread in multi-unit facilities.

Cost of Suppression Program Application

The total application time spent in the Lineweaver community from Jan-June 2012 was 94 hours and 37 minutes, or 47.3 minutes per unit with two staff working in each unit (Table 1). If the labor cost is calculated at $1/min (break even cost for a pest management professional), the cost per unit would be $47.3 min x 2 (staff) or $94.6/unit. Total labor cost for treating the entire community was estimated to be $11,355. This $1/min takes into account a pest control technician's wage, mileage, pest control insurance, marketing, and product. This $1/min cost does not take into account the pest management company's profit. Therefore, if a pest management company were paid to make the perimeter DE applications described in this study, we would expect the price to increase by at least 100%, if not more. The apartment facilities staff is not in the pest control business. Therefore, the costs associated with having these individuals apply the DE in-house would be considerably less than a $1/min.

The total amount of DE applied in the Lineweaver community was 7140 g. The amount applied in each unit varied with unit size, and the duster used. The mean amount of DE applied per unit 60 g. The cost of the diatomaceous earth was $110.10 ($0.92 per unit) to treat all units. The total cost of the proactive DE perimeter barrier application was $11,465.10. Consider that applying a DE barrier in the building is a one-time expense. DE and other dusts do not degrade over time and there is currently there is not data to suggest that the DE barrier ever needs to be reapplied during the life of the building.

Preliminary suppression program evaluation

While it will take multiple years of data collection to truly assess the financial impacts (if any) of the bed bug suppression program, preliminary data collected from 2011-2013 is presented here. Between January 2011 and December 2013, the Lineweaver community paid for 105 whole-unit heat treatments. Forty of these treatments were initial treatments (new infestations or units surrounding infested units). Sixty-five of these treatments were re-treatments, where bed bugs were found again at some time after the initial treatment was completed. In 2011, Lineweaver Apartments paid for a total of 13 treatments, 7 initial and 6 retreatments. However, in 2012, the year that the DE portion of suppression program was being applied, there was 171.4% increase in initial treatments (19 units), and a 383.3% increase in re-treatments (29 units).

Interestingly, this large increase in treatments can be attributed to the discovery of additional infestations by another researcher working within the community. In 2013, the number of initial treatments and re-treatments was actually reduced (26.3% and 8.3% respectively) from those of 2012. This was the first year (2013) since 2010 that the number of treatments had not increased (and the number of new infestations actually decreased). However, the total number of treatments in 2013 was still much greater than they had been in 2011.

Total annual expenses for bed bug management in Lineweaver Apartments were assessed and compared for years 2011, 2012 and 2013. In 2011, the number of known bed bug infestations was relatively low and the treatment costs for the entire year were $8,500. In 2012, the bed bug treatment costs increased over the previous year by 442.4% (due to additional bed bug infestations discovered, and the DE application costs). Total cost of bed bug management in 2012 was $46,240. In 2013, the number of new bed bug infestations was reduced, and the number of re-treatments only increased by one unit, resulting in an overall decrease in treatments costs of 26% from 2012. Lineweaver Apartments total expenditures for bed bug remediation during the three year study period were $88,840.00.

One year after the implementation of a bed bug suppression program, there was a reduction (albeit small) of both the number of new bed bug infestations and the subsequent costs associated with bed bug treatments. Perhaps the most compelling result of this study so far, was that in 2013 the number of treatments did not increase as they had every previous year. However, it will take several years to fully determine if this decrease was anomalous, or the beginning of a trend. This suggestion of a downward trend in infestations is reinforced by several noteworthy observations. The proactive bed bug suppression program was cost efficient and therefore, much more sustainable than reactive bed bug treatment alone. Labor was the most expensive cost factor and yet there is no doubt that if these applications are implemented by facilities staff, the labor costs would be far less than those quoted here (based on pest management company costs).

Table 1. Mean application time (min) for two laborers and mean amount of diatomaceous earth applied per unit (g) based on unit size and duster were used to determine the potential financial investment for a multi-unit facility to apply the proactive perimeter diatomaceous earth barrier.

	Efficiency Unit		**1-Bedroom Unit**	
	Exacticide	Cyclone™	Exacticide	Cyclone™
Mean Treatment Time with Two Laborers (min)	60.5 ± 6.45 AB*	33.37 ± 2.46 C	73.44 ± 9.32 A	45.28 ± 2.97 BC
Cost per laborer	*$60.50*	*$33.37*	*$73.44*	*$45.28*
Mean Diatomaceous Earth Applied (g)	54.33 ± 8.07 a	48.54 ± 1.84 b	74.13 ± 6.79 b	67.83 ± 2.29 a
Cost	*$0.84*	*$0.75*	*$1.14*	*$1.05*
Mean Cost per Unit	*$121.84*	*$67.49*	*$148.02*	*$91.61*

* Means not followed by the same letter are significantly different.

While the efficacy of the proactive bed bug suppression program will only be known over the next few years, we consider the installation of the program to be a success. Our preliminary data indicates a flat to downward trend in new bed bug infestations since the beginning of 2013, and a subsequent reduction in bed bug treatment costs. We anticipate that the number of new bed bug infestations and the cost of bed bug remediation will continue to decline over the next several years. We are optimistic that the success of the DE application will result in additional components of the suppression program being adopted in the future, particularly, the construction and utilization of a heat chamber. As of 2013, additional multi-unit facilities have been trained to implement this proactive bed bug suppression program. In each case, the program was easily modified to fit the specific needs of each community. These communities will be observed over the next three years to determine the true potential of the proactive bed bug suppression program to reduce the number of infestations and bed bug remediation costs.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

GONADAL DEVELOPMENT IN WINGED FORMS OF ANTS (HYMENOPTERA: FORMICIDAE) WITH APPLICATION TO MANAGEMENT

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Abstract Development of the reproductive stages in the life cycle of ants varies among species. Formicine ants usually overwinter or spend development time in the nest between eclosion and the mating flight. Many of the Dolichodorinae and Myrmecinae mate in the nest and colonies reproduce by budding. A formicine example, *Camponotus modoc*, emerges as winged forms in August and remains in the colony until swarming the following spring. The emergence of reproductives in structural infestations throughout the winter months creates management concerns as winged forms, particularly males, make sporadic weak fights to windows or lighted areas. Reproductive systems of males and winged females were examined following eclosion and at three-month intervals until the spring nuptial flight to determine development of sexual organs. Spermatogenesis was complete by emergence, with no further development of the male gonads and apparent shrinkage of the testes in the first week following eclosion. Ovariole number varies among species of *Camponotus*. Each ovary of *C. modoc*, composed of 48-50 ovarioles, contained oocytes with varying degrees of development through the winter until by spring the terminal ovum in many ovarioles had completed vitellogenesis. Oogenesis and vietllogenesis occur throughout the life of the queen. Winged females and males occur in structures particularly in winter months before the nuptial flight and challenge management strategies in inspections for location and treatment.
Key words Carpenter ants, ovaries, testes, management

INTRODUCTION

Identification

Urban pest ants fall into four subfamilies: Formicinae, Dolichodorinae, Myrmicinae, and Ponerinae. These ants are found worldwide and have been introduced throughout the world. Oi and Vail (2011) cited 30 pest ants in 21 genera with a generous approximation of 13% of the North American ant species. Klotz et al. (2008) named 25 genera with seven in subfamily Formicinae, six in Dolichodorinae, ten in Myrmicinae, and two in Ponerinae. In comparison of exotic ants introduced into North America, 27 species in 14 genera have been identified. Ants introduced into Europe include 16 species in 11 genera (Klotz et al., 2008). These introduced, exotic, or tramp species can become serious pests. Nine genera have been recorded as introduced into both North America and Europe. Ant identification has taken on a worldwide perspective. Management of pest ants relies first on the proper identification and second on the knowledge of the biology of specific ants. Variations in life histories within a species differ with latitude and with specific locations. Keys in books, publications, the Internet, and research institutions are available for correct identification to species. Following proper identification, biological features of the species provide clues to management. Knowledge of reproductive activity is critical to understanding the biology.

Reproduction

Polygynous colonies reproduce by fission or budding when one or more of the many queens leave the nest along with workers and brood to establish a new colony. Pest species belonging to the

Dolichodorinae and Myrmicinae commonly establish new colonies by budding (Klotz et al., 2008). Because these colonies share the same gene pool, these open societies lack intraspecific aggression. Many exotic or tramp ants fall into this category and include: *Linepithema humile* (Mayr), *Tapinoma melanocephalum* (Fabricius), *T. sessile* (Say)*, and Monomorium pharaonis* (L.). In some colonies flights occur at irregular times or mating occurs within the nests. Queens in polygynous colonies are short-lived, usually less than one year (Passera, 1994).

Colonies of formicine species generally reproduce through mating flights where winged males and females take flight in a synchronized fashion following specific environmental conditions. Winged reproductives are an energy expense to the colony and are more common in northern latitudes. In three pest genera of formicine ants (*Camponotus, Prenolepis* and some species of *Lasius*), reproductives overwinter in the nest and the nuptial flights occur the following spring (Klotz et al., 2008). Many formicine queens in monogynous colonies live for many years.

Life History

Carpenter ants remain the most common structurally damaging insect in northern United States and Europe (Akre et al., 1995). Although some species are nuisance pests in urban environments, a number of species are classified as structurally damaging: *Camponotus modoc* Wheeler and *C. vicinus* Mayr in western half of North America, *C. pennsylvanicus* (DeGeer) and *C. chromaiodes* Bolton in the eastern half of North America, *C. herculeanus* (L.) in both northern Europe and northern North America, and *C. ligniperda* Latreille in northern Europe are most commonly identified (Butovitsch, 1976). Altogether 24 species of *Camponotus* spp. have been recorded as either nuisance or structurally damaging in North American and Canada (Hansen and Klotz, 2005). Depending on latitude, carpenter ant colonies enter diapause in September or October and break diapause from March through June (Klotz et al., 1999). Although differences occur in life history of each species, homeowners are most aware of infestations in structures when the mating flights occur between April and June. If nests occur within structures winged males and females are attracted to windows and lights as they begin their nuptial flight. Males emerge before females often as early as December when temperatures in structures are higher than temperatures in the natural environment. These ants generally return to nests and remain in overwintering sites until conditions warrant the spring flight.

Carpenter ant colonies are monogynous and nests producing winged forms may be found in either parent or satellite nests. A parent or main nest contains the queen, workers, brood, and winged forms. A satellite contains all but the queen. Usually parent nests are located in a natural area, outside a structure, while satellite nests may occur within structures (Hansen and Akre, 1985). Winged forms are produced when colonies are mature, usually over eight to ten years old, in the late summer and overwinter in either the parent nest or satellite nest until the following spring. These overwintering winged adults are perceived by homeowners to be the damaging stages and management of these forms is imperative (Cannon and Fell, 1990) (Fowler, 1985).

Female Reproductive System

The female reproductive system consists of a pair of ovaries, each composed of ovarioles; the oviduct; spermatheca; and accessory glands. Each ovariole consists of a terminal filament that supports the ovary, the egg tube where oogenesis occurs and the pedicel that unites the ovariole with the lateral oviduct. The spermatheca located on the common oviduct serves as the sperm storage organ once the female has been inseminated. The polytrophic ovarioles have nurse cells or trophocytes associated with each developing egg. Oogenesis starts when the germ cells at the terminal end of the ovariole produce the oocyte and trophocytes. Oocytes grow in the vitellarium as nurse cells deposit yolk and the mature eggs are forced down the egg tube by the continuous development of more oocytes and nurse cells. The oocyte completely absorbs the nutriments from the nurse cells to produce the mature egg at the end of the ovariole. An ovariole resembles a string of beads increasing in size with the alternation of egg chambers and nutritive cells as they progress through the egg tube (Wigglesworth, 1972) (Snodgrass, 1956).

Male Reproductive System

The male reproductive system consists of a pair of testes composed of follicles, the vas deferens, seminal vesicles, and accessory glands. Spermatogenesis occurs in the follicles during the larval and pupal stages (Snodgrass, 1956) and the male ant ecloses with all the sperm that he is ever going to produce (Hölldobler and Bartz, 1985). Spermatogonia develop into spermatocysts and are transformed into mature spermatozoa where they are stored in the seminal vesicles. Degeneration of the testes and movement of spermatozoa into seminal vesicles follows eclosion (Wheeler and Krutzsch, 1992). Two large curved accessory glands are attached to the posterior of the seminal vesicles (Snodgrass, 1956) and produce secretions that mix with spermatozoa to form spermatophores (Wigglesworth, 1972).

The objective of this paper is to review reproductive strategies by examining development of reproductive structures and to relate these and life histories to management of urban pest ants, particularly carpenter ants. Over-wintering colonies in the laboratory, inspecting infestations in structures, and examining the development of the winged males and females before the nuptial flights have provided additional information on the development of winged reproductives.

MATERIALS AND METHODS

Overwintering Females and Males

Development of ovaries and testes in the winged females and males of *C. modoc* were studied at three month intervals: fall, winter, and spring (Witherell, 1991). *C. modoc* nests containing workers and brood were collected in May and maintained in the laboratory. Males and winged females emerged from pupae in the nest during the first week in August. In October, January and May males and winged females were randomly selected from these nests for dissection and examined for changes in development (Table 1). Dissections were made with 0.75% NaCl and reproductive organs from both males and winged females were removed and placed in alcoholic Bouin's fixative, dehydrated with ethanol and butanol and embedded in paraffin as described by Gatenby and Beams (1950). Tissues were sections at 12 µ, transferred to slides, and stained with Delafield's hematoxylin and Eosin B.

Table 1. Winged *Camponotus modoc* dissected for examination of reproductive structures.

Sex	No.	Collection site	Gross Dissect	Histology	Season
female	12	Spokane Co	21-Oct	21-Oct	fall
	12	Spokane Co	20-Jan	20-Jan	winter
	3	Spokane Co	26-May	26-May	spring
	2	Clallam Co	26-May	26-May	spring
males	12	Spokane Co	21-Oct	21-Oct	fall
	3	Whitman Co	10-Oct	10-Oct	fall
	5	Clallam Co	20-Jan	20-Jan	winter
	4	Clallam Co	1-Mar	1-Mar	spring

Dissections of Males and Winged Females for Morphology

Males and winged females were selected from nests collected in June and maintained in the laboratory. Ants were dissected from nests in early August before and following eclosion. Winged females of other *Camponotus* spp. also were collected and dissected, with emphasis on recording ovariole numbers. Dissections were made in 10% ethanol with methylene blue. Fat bodies and tracheae were removed exposing respiratory, digestive and reproductive systems for gross examination.

Infestations in structures involving winged forms of *Camponotus* spp.

Notes on 210 inspections of infestations for five years (2006-2011) were reviewed to determine the occurrence and timing of winged males and females observed by homeowners within or on the exterior of the structure. Infestations were selected from referrals made by extension personnel and through personal contacts. On inspections, attempts were made to locate parent and satellite nests or to determine conducive conditions where nests might be located.

RESULTS AND DISCUSSION

Overwintering Females and Males

Gross dissection of males. The adult male reproductive system of *C. modoc* includes a pair of testes that are small globular structures (0.3 mm) with tracheae intertwined. The tracheae appear to add structural support anchoring testes to the body wall. Seminal vesicles are tubes leading from the testes to accessory glands and have a diameter 0.1 mm and 3.5 mm long. The distal third of the seminal vesicles are coiled. The proximal ends are constricted as each enters the ventral surface of the accessory gland. These structures are large sacs (l.3 mm x 0.5 mm) that are milky white. The posterior ends of the accessory glands fuse to form the ejaculatory duct. In comparison of the male reproductive tissue from October to January to May, differences were not observed. In fall dissections the fat body was opaque, pearly white, in spring dissections the fat body was reduced in size and translucent (Witherell, 1991).

Gross dissection of winged females. Organs dissected from *C. modoc* gynes included the ovaries that consist of numerous white thread-like structures, the ovarioles (4 mm in length). The posterior end of each ovariole is wider than the anterior and some ovarioles are bead-like in appearance. Tracheae were highly intertwined with the ovarioles and appear to help support the ovary structure by anchoring the ovary to the body wall. The ovarioles terminate in the lateral oviduct. The pair of lateral oviducts unites at their posterior ends forming the common medial oviduct. In the winter and spring dissections, the size and number of 'beads' in the ovarioles increased. The number of ovarioles containing eggs (beads) also increased in winter and spring dissections. The fat body occupied a major portion of the gaster cavity and surrounded the digestive and reproductive systems. The fat body became more translucent in the spring dissection. The changes in appearance are similar to changes reported by Cannon and Fell (1990). They investigated *C. pennsylvanicus* and found that lipids and proteins are consumed during the over-wintering phase.

Histological examination of males. Corresponding to the gross dissections of the male reproductive system in *C. modoc*, there were no histological changes during these time intervals. Remnants of the testes were all that remained in the adult. The seminal vesicles were filled with spermatozoa producing a spiral pattern. The large accessory glands were filled with secretions of proteins and carbohydrate to mix with spermatozoa in the formation of spermatophores (Wigglesworth, 1972) (Wheeler and Krutzsch, 1992). Folds at the posterior ends of the accessory glands were observed in the spring specimens of *C. modoc*. The number of follicles in each testis was nine for *C. modoc* (Figure 1) but varies with other *Camponotus* spp. *C. pennsylvanicus* has 12-16 follicles; *C. ligniperda* has 17 follicles

(Forbes 1954) *C. festinatus* (L.), and *C. sayi* Emery, both possess 9 follicles and *C. mina* Forel has 10-11 follicles (Wheeler and Krutzsch, 1992). *Lasius niger* (Buckley) possesses 7 follicles (Forbes, 1954). In *C. herculeanus* and *C. ligniperda,* spermatogenesis is not yet completed at the time of ecdysis but continues up to 25 days before migrating into the seminal vesicles (Hölldobler, 1966).

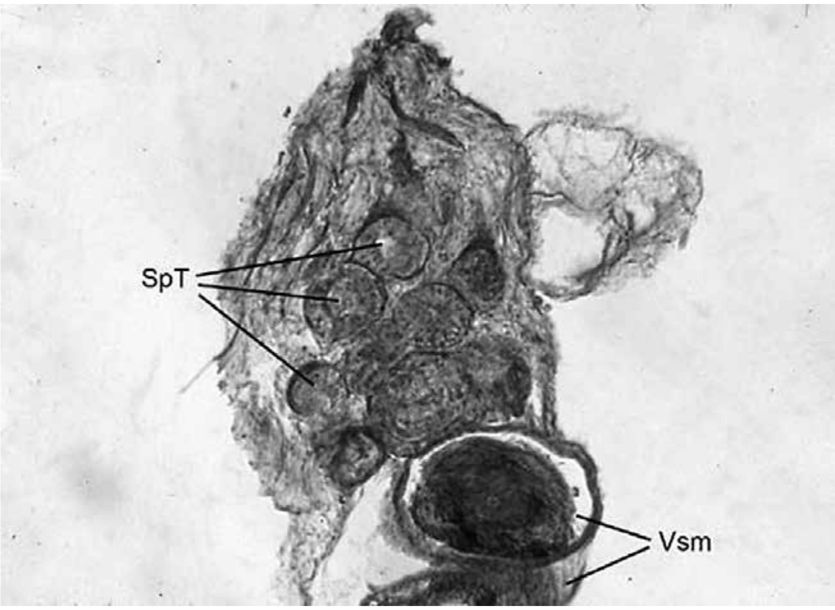

Figure 1. Histological section of testis of *Camponotus modoc* male showing nine spermatic tubes or follicles (SpT) and beginning of the seminal vesicle (Vsm) on a fall dissection.

Histological examination of winged females. Cross-sections of the *C. modoc* ovaries showed that each was composed of 48-50 ovarioles. Individual follicles at the proximal end of a number of ovarioles were observed in sections from each season. In ovarioles of fall gynes, developing oocytes (75 μ) with their accompanying trophocytes were vestigial (Figure 2). In ovarioles of the winter gynes, the ovum had increased in size, due to partial absorption of nurse cells (Figure 3). The terminal ovum (310 μ) in spring dissections was larger in size due to vitellogenesis. Trophocytes of spring eggs had been absorbed leaving only the mature ovum in the follicle at the proximal end of the ovariole (Figure 4).

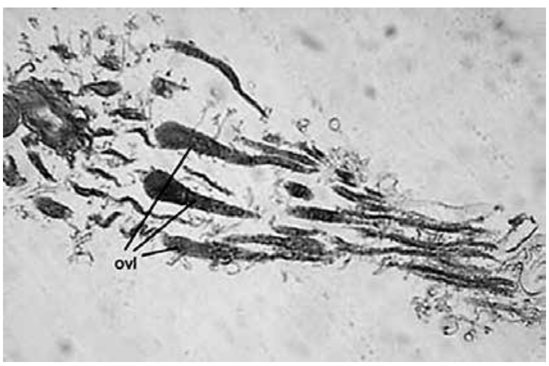

Figure 2. Longitudinal section of *Camponotus modoc* winged female showing ovarioles (Ovl) in a fall dissection.

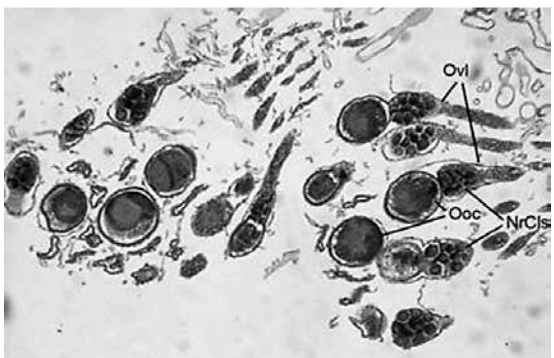

Figure 3. Longitudinal section of *Camponotus modoc* winged female showing ovarioles (Ovl), oocytes (Ooc), and nurse cells (NrCls) in a winter dissection.

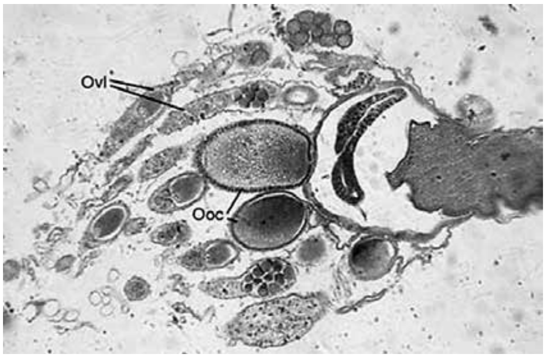

Figure 4. Longitudinal section of *Camponotus modoc* winged female showing terminal oocyte (Ooc) in an ovariole (0vl) in a spring dissection.

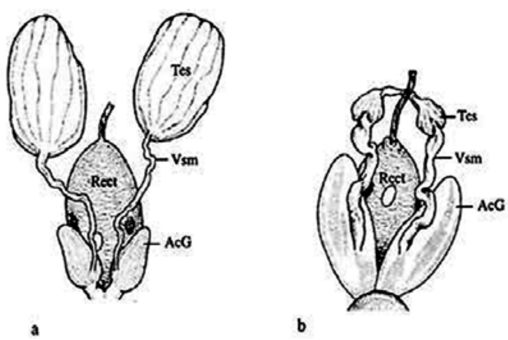

Figure 5. Reproductive systems in male *Camponotus modoc* at (a) 1 day and (b) 1 week following eclosion showing accessory gland (AcG), seminal vesicle (Vsm), testis (Tes). These lie near the rectum (Rect).

Dissections to Study Morphology

Winged females. Numbers of ovarioles per ovary varied among the *Camponotus* species examined: 13 to 48 with the highest numbers occurring in *C. modoc* (Table 2). Number of ovarioles may be linked to size of colonies and polygyny as the largest colonies are *C. modoc* and the only polygynous species is *C. vicinus* (Akre et al., 1994).

Table 2. Ovariole number in winged females of Camponotus spp.

Camponotus spp.	No. dissected	Range	Ave. No.
C. essigi M.R. Smith	4	12-16	13
C. herculeanus (L.)	2	43-45	44
C. modoc Wheeler	14	46-52	48.3
C. noveboracensis (Fitch)	1	44	44
C. pennsylvanicus (DeGeer)	4	30-38	34
C. semitestaceus Snelling	1	22	22
C. vicinus Mayr	2	20-22	20.5

Males. Dissections of males made on day one and day seven following eclosion revealed the testes had become drastically reduced after one week. On day one the testes were enlarged and connected to narrow seminal vesicles and small accessary glands. By day seven the testes were reduced and the seminal vesicles and accessory glands were enlarged (Figure 5).

Structural Infestations

Between 2006 and 2011, 210 structures were inspected for carpenter ant infestations in the Pacific Northwest (Table 3). Workers were observed in all infestations and winged forms were observed in

45%. In the months of January-February and November-December very few workers were observed. Workers were found where water was available, in bathrooms and kitchens. In the months of March through October, the numbers of workers were higher when foraging occurred outside the structures. Highest numbers of winged ants were observed from March through June; 33% of the structures had winged ants during the period from eclosion to swarming (September-February).

Table 3. Number infestation sites in 2006-2011 with *Camponotus modoc*, *C. vicinus*, or *C. essigi* where males, winged females or both winged forms were observed.

Months	***C. modoc***			***C. vicinus***			***C. essigi***		
	males	**females**	**Both**	**males**	**females**	**Both**	**males**	**females**	**Both**
Jan-Feb	4	0	1	3	0	0	5	3	3
Mar-Apr	8	4	1	5	0	0	6	3	3
May-Jun	6	8	3	5	3	2	0	0	0
July-Aug	0	0	0	0	3	3	0	0	0
Sep-Oct	0	0	0	0	0	0	1	2	2
Nov-Dec	3	0	0	1	0	0	2	1	0
Total	21	12	5	14	6	5	14	9	8

Winged forms of carpenter ants often over-winter in satellite nests that commonly occur in structures. Whereas in a natural environment these forms generally do not appear until the nuptial flight, in heated structures, males and often females leave over-wintering sites and appear at windows in an attempt to begin a flight. Males stimulate flight by the pheromone produced in the mandibular gland (Hölldobler and Maschwitz, 1965). This pheromone appears to be released only when the environmental conditions for flight are established. When the pheromone is not released winged forms usually return to over-wintering sites.

CONCLUSIONS

Dissections and histological sections of adult males of *C. modoc* in fall, winter and spring showed no development of gonadal tissues between the time of eclosion and swarming. However, spermatozoa were moved from the testes to the seminal vesicle during the first week following eclosion. C. *modoc* winged females showed considerable development throughout the period from eclosion in summer and mating flights the following spring with the development of mature oocytes. Ovariole numbers varied among *Camponotus* species studied with 48-50 found in *C. modoc.*

In management of structural infestation, eliminating these winged forms can be difficult (Hansen, 2007). Baits are not effective, as the winged forms are not foraging. The ants may be isolated in small satellite nests under insulation, in attics, or other voids. Finding these nests to directly apply dusts, aerosols, or sprays can be challenging. Winged forms produce ‘rustling’ noises when disturbed and drumming on the void or ceiling may elicit this response and the treatment can be confined to the affected void. Drilling into voids may be necessary to locate satellite nests with reproductives.

Understanding this phase in the biology of carpenter ants is helpful to understanding the infestation and explaining the problem to clients. The winged forms are developing and waiting for the proper environmental conditions for nuptial flights before leaving the site to mate and establish new colonies. The winged forms do not cause damage, but workers that are present with the winged forms in satellite nests may cause damage. These ants are attracted to water sources in structures and liquid or gel baits may be effective. Follow-up inspections during the foraging season are prescribed because parent nests are located outside the structure and additional satellite nests may be located during the next overwintering season.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

CONTROL OF INDIGENOUS AND EXOTIC MOSQUITOES IN GERMANY

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Abstract In Germany since more than three decades products based on *Bacillus thuringiensis israelensis* (Bti) and *Lysinibacillus sphaericus* have been successfully used as biological control agents against floodwater mosquitoes, mainly *Aedes vexans* and *Ochlerotatus sticticus* as well as against house mosquito *Culex pipiens*. Over 4.000 km^2 of breeding areas have been treated with various *Bti*-formulations as ice-granules by helicopters, fluid and powder formulations mixed with water by ground application, resulting in a reduction of the mosquito population year by year more than 95% and without evidence of any harmful impact on the environment. Globalization resulting in increased international trade and human mobility is responsible for the quick spread of pathogens and neozoa such as container-breeding *Aedes/Ochlerotatus* mosquitoes. Amongst them the most important ones are *Ae. albopictus* and *Oc. japonicus* in Germany. The control of container breeding mosquitoes is mainly based on environmental management and the use of Bti-tablets in the frame of community participation. Since 2013 copper as a mosquitocidal metal has been tested successfully to avoid mosquito breeding.
Key words Copper, *Bacillus thuringiensis israelensis*, container breeding mosquitoes

INTRODUCTION

The control of mosquitoes in Germany has a long history. In the 1920s and 1930s breeding sites were treated with petroleum oils. During the 1950s and 1960s adulticides were used. However, in the early 1970s, the mosquito population was extremely high because of frequent fluctuations of the water level of the Rhine. The outdoor attack rate on humans was more than 500 female mosquito bites per minute, greatly restricting the time village residents could spend outside. As a reaction to this natural disaster, towns and communities on both sides of the River Rhine merged their interests into the GMCA/KABS, a united mosquito control programme founded in 1976 (Becker, 1997). Now 102 cities and municipalities along a 310 km stretch of the Upper Rhine River, with a total population of 2.7 million people, have joined forces to control the mosquitoes, mainly *Aedes vexans* and *Ochlerotatus sticticus* over a breeding area of some 600 km^2 of the Rhine's flood plain. The budget of the programme is approximately 3.4 million Euros a year, which results in overall costs per person per year of approximately 1.2 Euros/Person/year. The overall goal of the KABS is to control mosquitoes while conserving biodiversity. This goal can be reached effectively only, when biological control methods are used. The control of *Aedes* mosquitoes by GMCA/KABS is based mainly on the use of Bti products. In the last 30 years about 340.000 hectares of floodwater breeding sites have been treated with more than 4000 tons of various Bti formulations mainly based on ice granules resulting in a reduction rate year by year by 95% of the floodwater mosquitoes. Domestic mosquitoes *Culex pipiens* are controlled mainly by the use of Culinex®/Vectobac® DT-Bti-tablets in containers and septic tanks, as well as by the application of *B. sphaericus* to eutrophic ponds and ditches.

Bti-tablets are used e.g. in Italy against *Ae. albopictus* and in Germany against all container breeding mosquitoes by millions. Also in tropical countries they can contribute to the integrated control of dengue (Kroeger et al., 1995). The fizzy tablets are sterilized by Gamma radiation before usage, that only the protein crystals produced by the bacilli as active ingredient and not spores or bacilli are applied (Becker, 2002). Another recent development has been reported with a combined formulation of *Bacillus thuringiensis israelensis* and *Lysinibacillus sphaericus*, (VectoMax®) which has a residual mosquitocidal effect against container breeders for several weeks.

Increased mobility of humans as well as the international trade, facilitate the dispersal and in some cases, the establishment of exotic mosquito species outside their original area of distribution. Within the about 30 species known to have established in new areas throughout the world, 3 species merit special recognition for their dispersal potential and also for their significance as vectors of human diseases such as *Aedes aegypti*, *Ae. albopictus* and *Ochlerotatus japonicus*. In Europe other exotic species occur such as *Oc. koreicus*, *Oc. atropalpus* and *Oc. triseriatus* (Becker et al., 2012). Once established, these species can spread by vehicles, trains and boats to neighboring countries. In Europe, *Ae. albopictus* is the most problematic species. In the early 1990s it was passively introduced into Italy, due to the international trade of used tires. Since 1999, *Ae. albopictus* has been found in various southern and central European countries, including France, Bosnia & Herzegovina, Montenegro, Belgium, Switzerland, Greece, Malta, Monaco, Croatia, San Marino, Slovenia, Spain, the Netherlands, Vatican City, Germany, Austria, Slovakia, Czech Republic, Hungary, and Romania (ECDC, 2012).

In 2000, *Oc. j. japonicus* was first recorded in Europe when larvae were found in a storage yard of imported used tires in France and Belgium. Finally in 2008 the species was detected in northern Switzerland. Since 2009 this species is recorded in several States of Germany as well as in Austria and Slovenia. Due to the threat of these exotic species in a joint cooperative project the WHO and EMCA developed 2012/13 Guidelines for the control of mosquitoes of public health importance in Europe to prevent further spread of invasive species especially *Ae. albopictus.* In Germany the rapid spread of *Ae. albopictus* and *Oc. japonicus* increased the awareness of authorities, scientists and the public and led to an intensive surveillance program for exotic mosquito species, including the development of new control tools to combat exotic mosquitoes. During the surveillance activities related to the spread of *Oc. japonicus* in Germany it has been observed that no mosquito larvae were found in copper vases in cemeteries.

In this study the efficacy of copper to combat container-breeding mosquitoes such as *Oc. japonicus*, *Cx. pipiens/torrentium* and *Ae. aegypti* has been evaluated in order to use copper coins against container-breeding mosquitoes and to limit the distribution of exotic mosquitoes such as *Oc. japonicus* and *Ae. albopictus*.

MATERIALS AND METHODS

In a series of experiments the effect of copper and other metals on the development of container breeding mosquitoes such as *Cx. pipiens* s.l., *Ae. aegypti* and *Oc. japonicus* have been investigated. Especially the effect of 1 Euro Cent (weight: 2.3 grams; surface: 2.8cm^2) and 5 Euro Cent ((weight: 3.9±0.1 grams; surface: 8.3 cm^2) applied in regular grave vases (volume: 750 ml) have been evaluated.

Series 1: Each five copper, steel, Zink and glass cups have been filled with 38 ml of tap water and 20 second/third instars of either *Cx. pipiens* s.l. or *Ae. aegypti* have been added in each 2 ml of water to the test vessels, a small amount of Tetra-Tabimin served as food resource. Mortality reading has been conducted after 1, 2, 3, 5, 7, 9, 10 and 13 days.

Series 2: 15 standard green plastic grave vases were filled each with 750 ml of tap water (pH: 7.2; conductivity: 70 uS), 20 second/third instars of *Ae. aegypti* have been added into each test vessel including some Tetra-Tabimin powder as food resource. In each of five vases one 5 cent coin or one 1 cent coin were added, respectively. 5 vases without coins served as control. The same test set-up was used for tests with *Cx. pipiens* s.l. second/third instars. The mortality reading was conducted at 1, 2, 3, 5, 7, 10, 13 and 15 days.

Series 3: In this series the effect of copper ions on larvae of *Ae. aegypti* and *Oc. japonicus* was tested. All 20 grave vases were filled with 750 ml of tap water (pH: 7.2; conductivity: 70uS). Into ten vases 20 second/third larvae of *Ae. aegypti* or *Oc. japonicus* were added in addition to small amounts of TetraTabimin. Five vases of each set were treated with one 5 cent coin and 5 vases remained untreated as control. The mortality reading was done 1, 2, 3, 5, 7, 10, 12, 13 and 14 days.

RESULTS

In the first experiment the effect of different metals on the development of *Culex pipiens* and *Ae. aegypti* larvae has been evaluated. *Cx pipiens* sl. and *Ae. aegypti* larvae in the copper vessels were all dead after 10 days, In the same time period in the Zinc vessels only 45 % and 50% of *Ae.aegypti* and *Cx, pipiens* s.l. larvae and in the steel vessels 33.3% and 41.6% died, respectively. In the glass vessels 1% of the both species died (Figure 1).

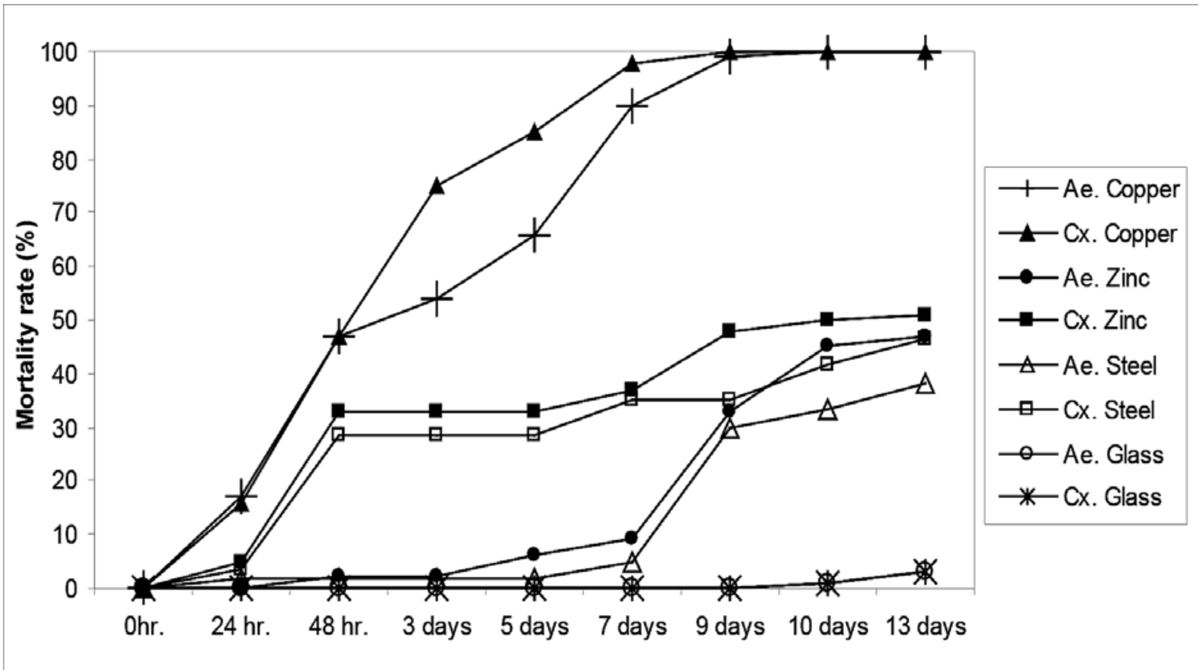

Figure 1. Effect of copper, zinc, steel and glass breeding vessels on development of *Ae. aegypti* and *Cx. pipiens* larvae.

In the second experiment the effect of one cent and 5 cent coins in standard grave vases have been tested. After 15 days all larvae were dead in the 1 cent treated as well as in the 5 cent treated vases. Not only was there no difference recorded between the 1 or 5 cent coin treated vases, but A*edes* and *Culex* larvae were similar sensitive (Figure 2).

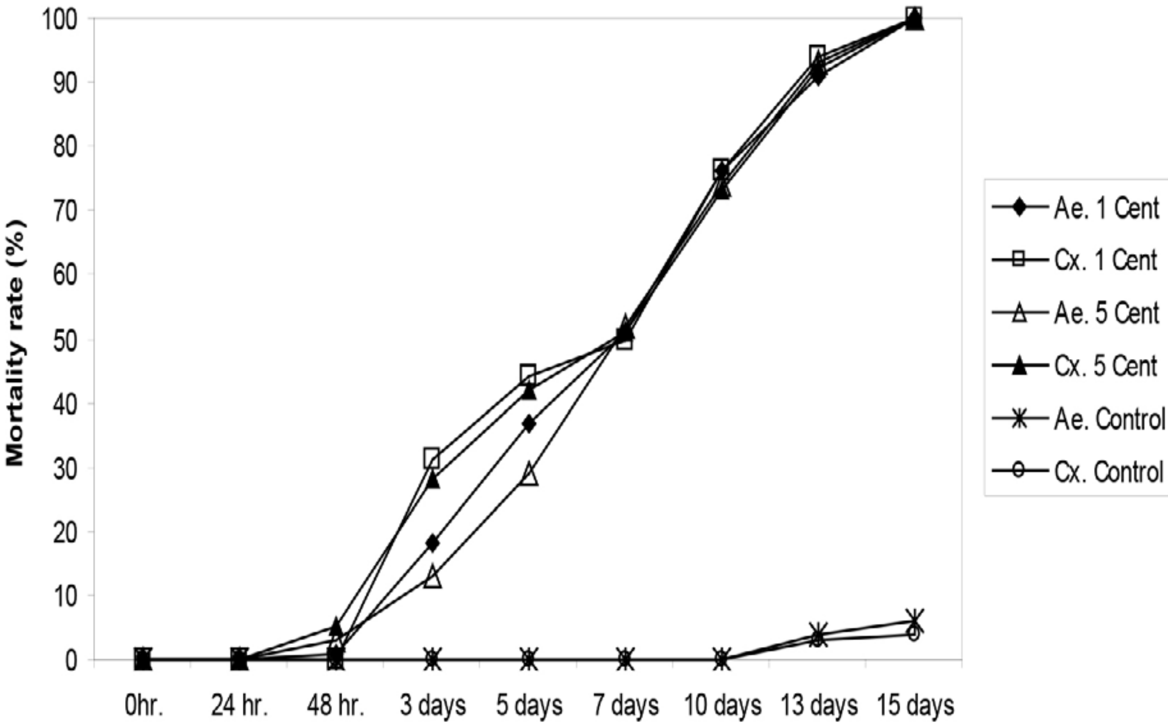

Figure 2. Effect of 5 cent and 1 cent coins on development of *Ae. aegypti* and *Cx. pipiens* larvae.

In the third experiment the effect of 5 cent coins on the development of *Ae. aegypti* and *Oc. japonicus* larvae in standard grave vases have been evaluated. After 14 days all larvae of both species were dead (Figure 3). The larvae of *Oc. japonicus* are slightly more sensitive than the larvae of *Ae. aegypti*. After 5 days 72% of the larvae of *Oc. japonicus* died whereas only 35% of the *Ae. aegypti* were dead.

DISCUSSION

In the course of the German surveillance programme of exotic mosquitoes especially cemeteries have been found infested by *Oc. japonicus*. Cemeteries seem to be an ideal breeding ground for container breeding mosquitoes because the resources are excellent for the development of container breeding mosquitoes: vases provide breeding sites, flowering plants serve for nectar feeding of the adults, humans provide blood meals and bushes are resting places for the adults. Whereas, Bti-tablets can be successfully used in wells or larger water containers, the control of mosquitoes in vases remain mainly on environmental sanitation (e.g. emptying of the vases each week) which is seldom done. In this study it was shown that container breeding mosquitoes can be easily controlled by the use of copper coins.

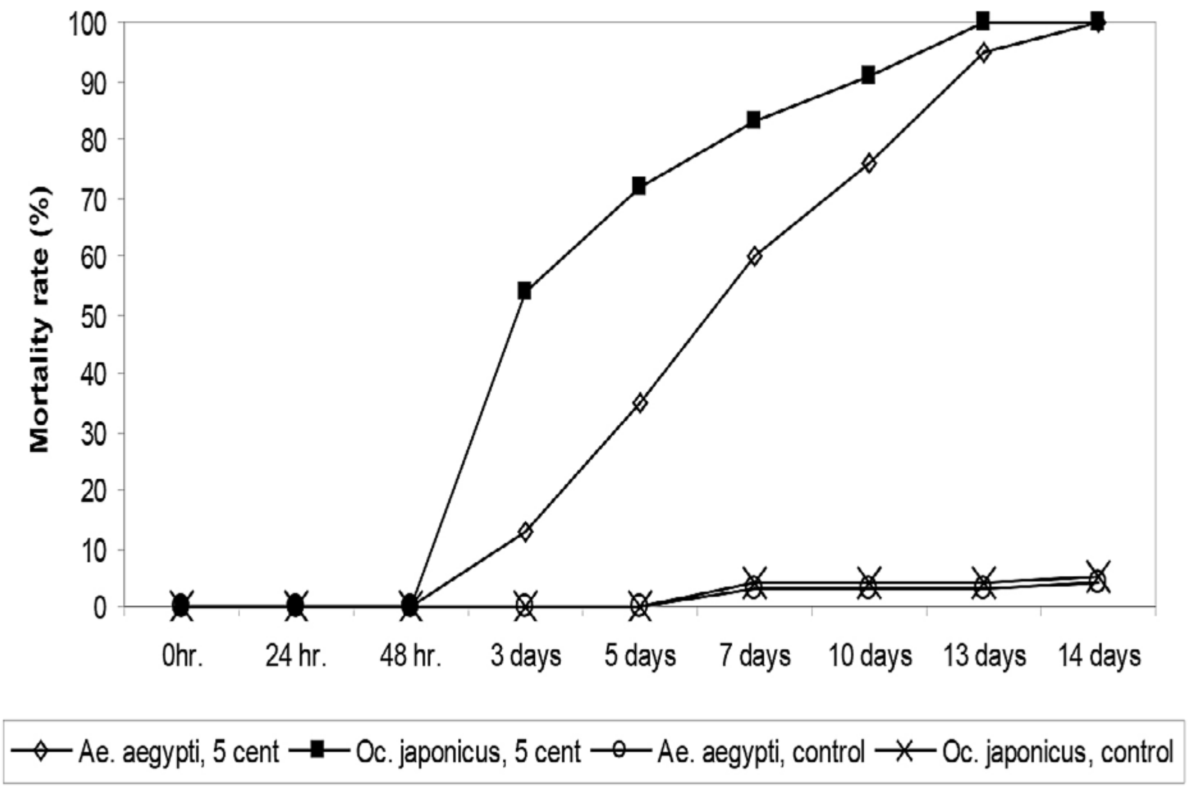

Figure 3. Effect of 5 cent coins on development of *Ae. aegypti* and *Oc. japonicus* larvae.

The results obtained here prove the results of Romi et al. (2007) who found that copper prolonged the development of mosquito larvae and even killed them at a certain copper concentration. In a research program in Florida it was shown that the toxic effect of bronze limits the development of *Ae. aegypti* and *Ae. albopictus*. Copper formulations are known as fungicides for more than 100 years. Copper is a trace element for humans and is not toxic at low dosages. WHO allows up to 2 ppm copper concentrations in drinking water. In further studies different application forms of copper will be tested to provide an easy tool for the control of container breeding mosquitoes for the general public.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

LOCAL AUTHORITY PEST CONTROL SERVICES IN THE UK: PESTS, PERFORMANCE AND PROFIT

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Abstract The National Pest Advisory Panel of the Chartered Institute of Environmental Health (CIEH) in the UK was established in 2001 to advise the CIEH on pest control policy. It developed a pest survey to understand the way in which pest management services within local authorities were operationalised and delivered in the UK. This paper examines some of the changes that have taken place between the 2002 survey and the 2012 survey. Within this interval the economic downturn resulted in significant job cuts in the public sector. There was a significant reduction in the number of local authorities that continued to provide an in-house pest management service to its residents. Significant reductions were found in the proportions of local authorities providing pest management services for specific public health pests.
Key words Urban pest management, local authorities, in-house pest control services.

INTRODUCTION

The challenges facing the continued resourcing of local authority in-house pest management services may seriously impact on public health in the UK. Pest control is a core element of environmental health with specialist knowledge and practices that tackle pest issues and ultimately protect public health within the wider context (Bonnefoy et al., 2008). Focusing on the wider determinants of health makes an important contribution to the improvement of society's quality of life, health and wellbeing (Burke et al., 2002).

The National Pest Advisory Panel of the Chartered Institute of Environmental Health (NPAP) was set up in 2001 to advise the CIEH on pest control policy (NPAP, 2002). The object of the CIEH in its charter is to promote for the public benefit the theory and science of environmental health in all its aspects and the dissemination of knowledge about environmental health. It is a professional and educational body dedicated to the promotion of environmental health and encouraging the highest possible standards in the training and work of environmental professionals.

Pest management has been a neglected area of public health policy in the UK. There has been little consistent, reliable data on activities of the local authorities and municipalities in controlling pest species and this has in turn resulted in a fragmented approach to the control of pests of public health importance. Following the economic downturn in 2008/9, the UK Government's Spending Review (HM Treasury, 2010) laid out the approach to dealing with the deficit. It detailed the likely loss of 490,000 public sector jobs and an average cut of 19% over a 4 year period in government departmental budgets. The pattern of cuts was not uniform across the country.

Planned cuts (which excluded education, fire and police services) between 2009/10 and 2011/12 exceeded 15% in approximately one quarter of local authority areas whilst in another quarter, they were less than 6% (Crawford and Philips, 2012). UNISON, which is one of the largest trade unions, representing staff who provide public services, examined the potential impact of these cuts by surveying their members who worked in environmental health services (n = 4,000) in June 2012; they received 422 responses. These employees reported a diminishing workforce and reduced services. Respondents expressed concern about landlords, highlighting that with less active regulatory staff, rogue landlords could become more confident about acting with impunity. Respondents pointed out the impact of poor housing and exposure to pests, such as bed bugs, on the health of families and particularly the effect on children. A large proportion expected that some services across their authority would be withdrawn altogether in the future and a number reported that they no longer provided a pest control service for their residents. Over half of the respondents reported the introduction or increase in charging for pest control services, with residents, when they could afford to, trying to control pest problems themselves. The need for reliable data about the way in which pest management services across the UK are operationalised and delivered became apparent to the NPAP, as anecdotal evidence indicated wide variations of approach.

MATERIALS AND METHODS

The NPAP questionnaire was sent to all Chief Officers in England, Wales and Northern Ireland in 2002 (n = 406) and 2012 (n = 368), allowing an overview of the changes that occurred in the 10 year window.

RESULTS

In 2002, 268 of the 406 local authorities returned questionnaires and in 2012, 151 of the 368 local authorities (reduction in numbers of local authorities as a result of some reorganisations) returned questionnaires giving response rates of 64% and 41% respectively.

Results confirmed a significant shift in the provision of in house pest management services in this period. In 2002 only 1% (n = 254; n = 3) of those who responded did not provide an in house pest management service. By 2012, this had risen to 10% (n = 151; n = 15) (Fisher's exact test $p<0.005$). Those who no longer offered an in-house pest management service were asked when the service had been withdrawn (Table 1). Between 2004 and 2009, respondents reported the withdrawal of pest control services from four authorities; however between 2010 and 2012, 10 authorities reported the cessation of their in-house pest management services. The survey was first sent out early in 2012 and may not have captured the full extent of the closures during that year. Financial and/or budgetary cuts were cited as the reasons why all of these in house pest control services had been withdrawn.

The proportion of local authorities offering in-house service to treat for pests in 2002 and 2012 are detailed in Table 2. Significant reductions in the numbers of local authorities offering in-house services between 2002 and 2012 were found for all the pests listed, apart from in-house services to control birds. These results suggest that for a number of important public health pests, local authorities had reduced their services.

Table 1. Year local authorities ceased offering in-house or out-sourced pest control.

Year	In house	Out-sourced	Both	Total
2004	1	0	0	1
2005	0	1	1	2
2009	0	1	0	1
2010	2	1	0	3
2011	3	2	0	5
2012	1	1	0	2
Total	7	6	1	14

Table 2. Percentage of respondents offering in-house pest service in 2002 and 2012.

In-house services provided for:	2002	2012	Significance
Rats	87%	76%	P= 0.005
Mice	86%	75%	P = 0.002
Birds	28%	24%	P = 0.44
Wasps	86%	71%	P= 0.001
Flies	60%	47%	P=0.015
Cockroaches	86%	67%	P <0.005
Fleas	88%	66%	P< 0.005
Bed bugs	87%	65%	P<0.005
Dermestid beetles	49%	37%	P=0.023

Table 3. Percentage of respondents only offering advice for pests in 2002 and 2012.

In-house services provided for:	2002	2012	Significance
Rats	6.5%	10.9%	P= 0.149
Mice	7.3%	12.2%	P = 0.118
Birds	51%	54.5%	P = 0.531
Wasps	8.4%	5.4%	P= 0.298
Flies	34.6%	38.8%	P=0.424
Cockroaches	7.9%	20.4%	P <0.005
Fleas	9.%	20.8%	P< 0.005
Bedbugs	9.4%	20.4%	P=0.004
Dermestid beetles	36.3%	35.2%	P=0.829

The proportions of local authorities that provided only advice for pests are presented in Table 3. The proportions of those providing advice only for infestations of cockroaches, fleas and bed bugs had increased significantly between 2002 and 2012.

DISCUSSION AND CONCLUSION

The findings of the two NPAP surveys in 2002 and 2012 have provided a rich source of information about the way in which pest control within LAs is operationalised across England, Wales and N. Ireland and how these services have changed during this 10 year period. Whilst many local authorities continue to provide a comprehensive in-house pest management service, the UK Government's Spend Review has had an impact, with fewer in-house services available and a reduction in the proportions of local authorities providing services to control a number of important public health pests.

Whilst historically pest management has been viewed as a core environmental health function providing key public health protection for its residents, the pace of change in the past 10 years is of concern. The strategies and arrangements in place to control pests is of concern. Local arrangements to deal with pests will reinforce an already fragmented approach and seriously impact on the UK's capacity to deal with a future outbreak of a pest-borne illness. The currency of the points identified in 2002 regarding provision of pest management services remain pertinent: 1) The nature and impact of an apparent uncoupling of pest control services from core EH activities in some Local Authorities; 2) The variation in the provision of a structured training/development programme for staff; 3) The complexities of the charging policies adopted for pest treatments; 4) Assessment of procedures and policies relating to contracted out pest control services; 5) The apparent variations in the nature and extent of the liaison between Sewerage Undertaker and LA to control rats in sewers; 6) The inconsistencies in funding arrangements between Sewerage Undertaker and LA for sewer baiting; 7) The variability in the membership of pest liaison groups; and 8) A review of the mechanisms to facilitate the dissemination of good practice in pest management.
The substantial fall in the response rates in the intervening years may be further evidence that many of the local authorities that did not reply had already removed the provision of a pest management service in the intervening period. This deduction in pest services is of concern to the Chartered Institute of Environmental Health.

ACKNOWLEDGEMENTS

The authors wish to thank the Chartered Institute of Environmental Health for their continued support in the work of NPAP and to all local authorities that completed the survey. Jonathan Peck was a driving force behind the establishment of the National Pest Advisory Panel. We continue to mourn his loss and will always remember him with great fondness.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

20 YEARS HISTORY AND FUTURE PERSPECTIVE OF THE INTERNATIONAL CONFERENCE OF URBAN PESTS

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Abstract The International Conference of Urban Pests (ICUP) is a link between academic and industry researchers, regulatory officials, government agency leaders, manufacturer representatives and students and presents the latest results in research and development in the field of urban pest control for the past 20 years. The earlier conferences took place in different places all over the world and gave an excellent survey on the special topics of urban pest management on the different continents. About 500 full (complete) manuscripts and abstracts of about 320 poster presentations were published since 1993 in the proceedings and are available on the ICUP homepage. The importance of urban pest management will increase strongly in future. Urban areas are growing steadily. The modern architecture of carbon dioxide neutral buildings provides shelter to many organisms. The facades of these houses contain now are now covered with insulating material which offers many species excellent nesting sites, a fact that has become a strong challenge for the pest management. Expanding worldwide trade and tourism will further increase the introduction of alien organisms. The urban pest management must face these challenges and would benefit from the research and development of universities and scientific institutes, to protect our urban environment against pests. Already in the past the ICUP has been an important source for troubleshooting in urban pest management and even more so this is true for the future.
Key words Urban pest management, insulation materials, natural compounds, urban farming, exotic species

HISTORY

The International Conference on Urban Pests (ICUP) started more than 20 years ago with the first conference in Cambridge, UK in 1993. It was followed by Edinburgh, Scotland (1996), Prague, Czech Republic (1999), Charleston, USA (2002), Singapore (2005), Budapest, Hungary (2008) and Ouro Preto, Brazil (2011). With the meeting in Zurich 2014 we now look back on more than 20 years worldwide competence in urban pest control.

The first ICUP started with a lecture on 'Trends in World Urbanization', which is still relevant today (Watson, 1993). This presentation was followed by several future topics like "Urban entomology perspectives" (Robinson, 1993). Three years later, in Edinburgh, it was decided to include rodents and birds into the ICUP topics. The importance of various pests and pest management changed constantly in these two decades. Table 1 shows these changes by the subject matter of the presented papers and poster presentations. The number of contributions on conventional insecticides was on a constant high level over the past 20 years. Lectures on natural compounds and biological control were already present from 1993 to 1999 with 4 to 9 presentations per conference. The importance of these two topics declined in the following conferences but recovers in Zurich with 6 contributions. Heat treatment makes now a high percentage of alternative methods. Cockroaches were the most recognized pests over the first 12 years and declined slightly in the following conferences. Ants started to be more important from 2002 on, and flies remained on a low level over the 20 years. Medical entomology has a large share of the conference from the beginning in 1993. Mosquitoes have a high percentage in this session since 1993 followed by ticks. Fleas were only represented with 1 to 3 contributions in the first 4 conferences. Bed bugs were not a big concern in the ICUP until 2005 but started to be a major topic in Budapest 2008 with eight presentations and a well frequented workshop and with 12 presentations in 2011(Ouro Preto). Bed bugs are again represented in the current conference with 10 contributions. Exotic species are in the focus since 2008 and are now present with 12 contributions on mosquitoes, ants, bugs and beetles. Wood protection is dominated by lectures on termites which have a high proportion on the total number of contributions particularly in Charleston, Singapore and Ouro Preto.

Table 1. Topics of oral and poster presentations at the meeting of the International Conference on Urban Pests from 1993 to 2014 (Contributions which refer to different topics, are mentioned in each one.)

	Cambridge	Edinburgh	Prague	Charleston	Singapore	Budapest	Ouro Preto	Zurich
Year	1993	1996	1999	2002	2005	2008	2011	2014
Oral presentations	67	84	81	50	76	68	73	64
Poster presentations	32	38	69	52	45	27	61	29
Topics								
Urban Entomology – Trends and perspectives	11	7	9	2	0	3	3	1
Conventional Insecticides	22	26	15	28	25	22	24	17
Natural compounds + biological control	7	9	4	0	5	3	2	6
New formulation- + application techniques	5	2	1	1	1	0	4	2
Alternative methods	5	7	3	3	5	1	5	10
Biocidal directives and Legislation	1	3	1	0	7	1	5	2
Pest Management	2	6	8	6	3	11	4	4
Cockroaches	26	27	30	13	21	10	12	11
ants	4	5	8	15	16	11	19	11
flies	5	2	9	8	3	4	2	4
Medical Entomology	25	34	48	17	31	37	40	32
(Mosquitoes)*	(9)	(19)	(17)	(4)	(19)	(18)	(18)	(17)
(Bedbugs)*	(0)	(0)	(0)	(0)	(0)	(8)	(12)	(10)
Exotic pests	0	0	0	1	0	5	8	12
Wood protection	16	10	20	28	31	10	20	6
(Termites)*	(6)	(8)	(19)	(27)	(30)	(10)	(16)	(2)
Vertebrates	0	6	6	5	6	3	12	8
Others	13	14	6	10	4	7	11	9

*Numbers in brackets are also included in “Medical Entomology” and “Wood protection”, resp.

FUTURE URBAN PEST MANAGEMENT

As a main topic for the next twenty years the ICUP has to present a worldwide picture of the future needs and trends in pest management. To achieve this we need leaders in the different scientific fields and governments who are prepared to attend this conference and to present their knowledge. For the last 20 years ago pest management has been seen as a national task by most nations, but now it has become a worldwide concern. Bed bugs and invasive species such as ants have demonstrated that understanding their behavior and control is only possible when thinking globally (Klotz et al., 2008).

INTRODUCTION OF 'ALIENS' – A GROWING CHALLENGE FOR PEST MANAGEMENT

The development of new and effective pest management strategies has become a global challenge. Global trade and tourism allow organisms from tropical areas to travel undetected within a few days around the world. Global warming is an important prerequisite for these species to establish themselves even in temperate zones of the northern or southern hemisphere, where in the past the low temperatures did not allow survival outside of buildings (Rabitsch and Essl, 2010; Nentwig, 2011).

Most alien arthropods have a different biology and behavior than native species and require special control strategies. Descriptions of economically important species are in reference texts in their countries of origin or in the Brazilian, American, Australian, and Japanese literature (Zorzenon and Junior, 2006; Yasutomi and Umeya, 1995; Gerozisis et al., 2008; Robinson, 2005). Pest management requires knowledge of pest control strategies and the study of global pest control literature. Some control advice may also be found in the global net (Pospischil, 2011).

CONSEQUENCES OF MODERN LIFESTYLE FOR PEST MANAGEMENT

The environment is driven by ongoing changes. People's expectation of better housing, health care, vector control, and their tolerance of pest control measures has changed, particularly in urban areas. In many places, people refuse treatment with synthetic insecticides or insist on doing the control themselves (Lüscher et al., 2008; Lüscher et al. 2014). In these situations, alternatives to synthetic insecticides should be provided wherever possible. Some natural compounds are well known and have been in use for a long time, including pyrethrum, an extract from *Tanacetum* spp. (Asteraceae) and different extracts of the neem tree (*Azadirachta indica*), which contains more than 100 active compounds like nimbidin, azadirachtin, salannin and others (Casida and Quistad, 1995; Mehlhorn et al., 2011; Hinson et al., 2014). Institutes and industry has dedicated personnel and resources in the research and development of natural compounds. Natural compounds have to be treated as other active ingredients of insecticides, and be tested for toxicological hazards and allergic reactions.

Typical integrated pest management measures, such as guidance of clients, monitoring and preventive measures against pest infestation are now the main tasks of a pest control professional. These measures require a thorough education including identification of pests. Modern control strategies with baits require a thorough knowledge of the biology and behavior of pests with special regard to new invaders (Zungoli et al., 2014). Recently introduced alien species are often well known in their country of origin and specific information of their control is available. It is an objective of the ICUP to make this information accessible worldwide.

IMPACT OF MODERN BUILDINGS TO PESTS

In temperate zones modern buildings are often provided with an outer insulation to reduce heat loss. Modern insulation with polystyrene and other foam materials with high insulating properties are excellent habitats for pests, such as ants, wasps, woodlice, beetles, mice, rats, and birds (including woodpeckers, starlings and parakeets). The age of the insulation layer plays no role in the attack. Other insulation materials, such as cellulose and mineral wool are less suitable as nesting sites. In individual cases, sheep's wool is used as natural resource for insulation (Zack et al., 2012). However, this natural insulation material is vulnerable to clothing moths (*Tineola* spp.) and carpet beetles (Dermestidae). Effective procedures are urgently needed to protect these insulations. Unfortunately, there is only very little knowledge that is based on sound research. Meanwhile, it is accepted that many animals like to use new, convenient options in their environment to build their nests (Pospischil and Pospischil, 2014). Urban farming started some years ago in several cities like, Singapore, New York, and Berlin (Yuen and Wong, 2005; Wong et al., 2003). It is a strategy to produce food in direct neighborhood of people on roof-tops in larger towns and starts now to enter economic level. It may be an important tool to provide people in mega cities with fresh food. However, monitoring strategies have to be established before pests become a hazard for urban farming (Pospischil, 2012). The pest control business has to consider these changing conditions and establish a training of the pest management professionals to provide them with the right answers to the new situation in pest control.

CONCLUSIONS

For the last twenty years the ICUP has been a unique conference by providing a forum for discussion of the different aspects of urban pest management, including insects, vertebrates, mites and spiders, new control strategies, and regulatory affairs. The Conference has served the need for information and recommendations from worldwide researcher on ways to overcome the challenges of pests arising from our lifestyle and environment. For scientists, industry researchers, regulatory officials, government agency leaders, and pest management professionals not able to attend the Conference there is the bound Proceedings. This book is provided to participants at the start of the Conference and is available to those not able to attend; alternatively there is a compact disk version of the complete Proceedings.

A world without pests is unattainable – therefore pest management will be an even more important task in future. The future preoccupation with urban pests means to learn from invasive pests, from the environment and from nature.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

BED BUG NATION: IS THE UNITED STATES MAKING ANY PROGRESS?

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Abstract Contrary to recent news reports, the United States continues to be inundated with bed bugs (*Cimex lectularius* L.). Based on a nationwide survey of pest professionals, infestations still are increasing. While most pervasive in residences, the bugs are becoming more common in schools, offices, public transportation and other locales where beds are not normally present, paralleling historical trends for this insect. Bed bugs continue to be the most challenging pest encountered by professionals. The cost of extermination is exceeding the budgets of many households and businesses, the majority of whom are averse to spending more on pro-active inspections and other preventative measures. Pest managers in the U.S. are becoming more experienced with bed bugs, and have better tools for providing relief to those who can afford their services. Prospects are less hopeful for the poor, and widespread resistance to insecticides is a global predicament. Absent of a coordinated societal response with affordable science-based pest management tactics, prospects for curtailing the resurgence appear unlikely.
Key words Survey, infestation status, management, monitoring, insecticides

INTRODUCTION

One of the remarkable things about bed bugs, *Cimex lectularius*, has been the reach of their resurgence. When pest management firms from 43 countries were surveyed in 2010, respondents confirmed that infestations were increasing worldwide (Potter et al., 2010). The following year, pest managers in the United States were surveyed again, revealing that infestations were becoming more common in 'non-bed' environments such as schools, office buildings, theaters, libraries, and on public transportation (Potter et al., 2011). Many respondents also reported ineffective, often desperate attempts at self-treatment by the public, including dangerous misuse of insecticides, heaters, open flame, and flammable chemicals.

In 2012, media coverage of bed bugs in the U.S. waned. Health officials in some notably infested cities (New York, Cincinnati) also received fewer calls on their bed bug help lines, prompting speculation that the problem was in decline (Palmer, 2013; Maseru, 2013). This paper provides a different perspective on the status of bed bugs, based on a recent survey of the pest management industry.

Survey Parameters

An online survey consisting of 32 questions was developed, covering topics ranging from frequency of bed bug infestation, to management and business practices. Many of the questions were similar to those in previous surveys in order to permit year-to-year comparisons. Responses were collected from January 19 to February18, 2013. Letters requesting participation were emailed to 6972 contacts from member companies of the National Pest Management Association (NPMA).This resulted in 251 completed surveys for a 3.6% response rate. Respondents worked for companies ranging in size from fewer than 10 individuals to thousands of employees. Participation by region was Midwest (33%), South (26%), Northeast (21%) and West (20%) (Potter et al., 2013).

STATUS OF INFESTATION

Nearly every pest manager (>99%) indicated their company treated or was asked to treat for bed bugs in the past year. When asked about the incidence of bed bugs in their region, 72% of respondents said infestations were increasing while 25% said they were staying about the same. Only 3% felt that incidence of bed bugs was decreasing. Regionally, more pest management firms in the West (79%), Midwest (77%) and South (74%) felt that bed bugs were increasing, than those from states in the Northeast (53%).

About half (49%) of respondents said their company receives more bed bug calls at certain times of the year. Fifty-six percent of those that did report a "busy season" mentioned receiving more bed bug calls during summer, while 24%, 12% and 7% said their busiest season for bed bugs, respectively, was fall, spring and winter. The same seasonal trend was noted in a previous industry survey (Potter et al., 2011). Mabud et al. (2014) also reported a seasonal cycle of infestation (peaking in summer, waning in winter), based on calls received by the Philadelphia Department of Public Health. Travel, relocation, and other activities tend to increase during warmer months of the year, which could boost the chances of encountering bed bugs. During summer, the ambient temperature of some dwellings also tends to be higher, which would result in a faster development time.

Bed bugs continue to be most pervasive in residences, with almost all respondents encountering them in apartments, condominiums and houses (Figure 1). Occurrences also are becoming more frequent in schools, offices, public transportation and other areas where beds are not normally present. When pest managers in the U.S. were surveyed in 2007 (Potter, 2008), only 5-6% mentioned finding bed bugs in schools and hospitals, and fewer than 1% encountered them in office buildings. By 2013, 41%, 36% and 33% of respondents, respectively, reported finding bed bugs in these places. Discovering bed bugs in diverse locations is consistent with the historical infestation pattern of this insect (Potter, 2011). This trend will likely continue now that the bugs are entrenched in housing, and brings into question how such occurrences should be handled. Bed bugs are less prolific when access to stationary hosts is intermittent. Consequently, when problems arise in places such as schools, office buildings and libraries, bed bug numbers tend to be small and the threat to the public minimal disproportionate, at least, to the unease and adverse publicity the incidents sometimes evoke.

MANAGING INFESTATIONS

Pest managers in the U.S. continue to find bed bugs harder to control than other key pests. More than three fourths (76%) of those polled said bed bugs were the most difficult pest to control; ants were deemed most difficult by 17% , cockroaches by 6%, and termites by only 1%. This opinion has not changed from previous industry surveys, despite many more educational seminars, workshops, articles, research, new products and technology. More than 20 U.S. universities are now conducting research on bed bugs, compared to only a few as recent as seven years ago. Significantly there were more papers presented on bed bugs at recent annual meetings of the Entomological Society of America, than on termites, ants or cockroaches (Figure 2).

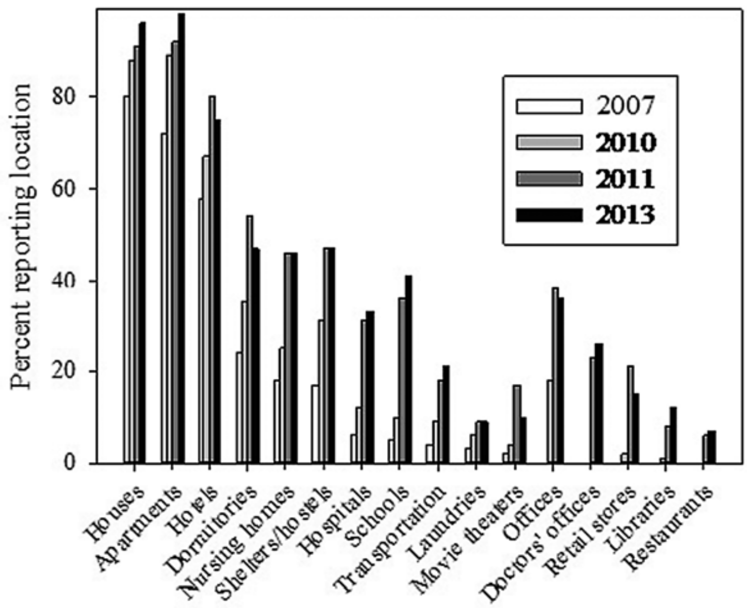

Figure 1. Locations by survey year where respondents reported finding bed bugs.

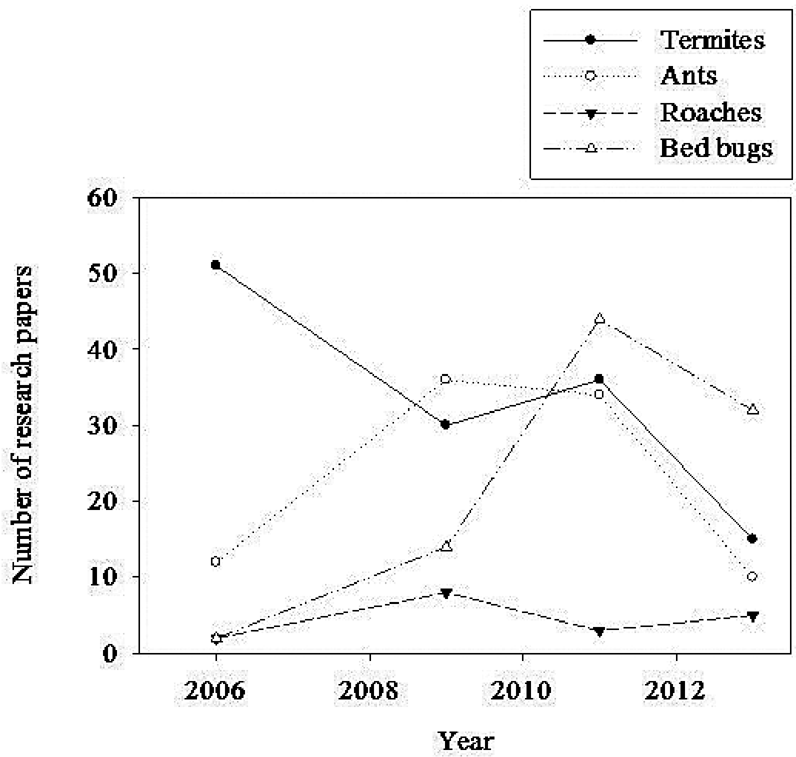

Figure 2. Number of papers on key pests presented at Entomological Society of America annual meetings.

The time initially spent treating an "average size bed bug infestation" in a residential setting, ranged from less than one hour to more than five hours. (Estimated median 3.4 hours, compared to 2.9 and 2.7 hours, respectively in 2011 and 2010 surveys.) Most respondents (94%) said their company spends more than one hour on the initial service. Thirty percent said one to two hours were spent, while 64% said more than two hours were typically spent on the initial service. The majority of respondents (69%)

said two or three services were typically needed to control infestations (16% said one visit was satisfactory, while 12% said more than three visits were typically needed).

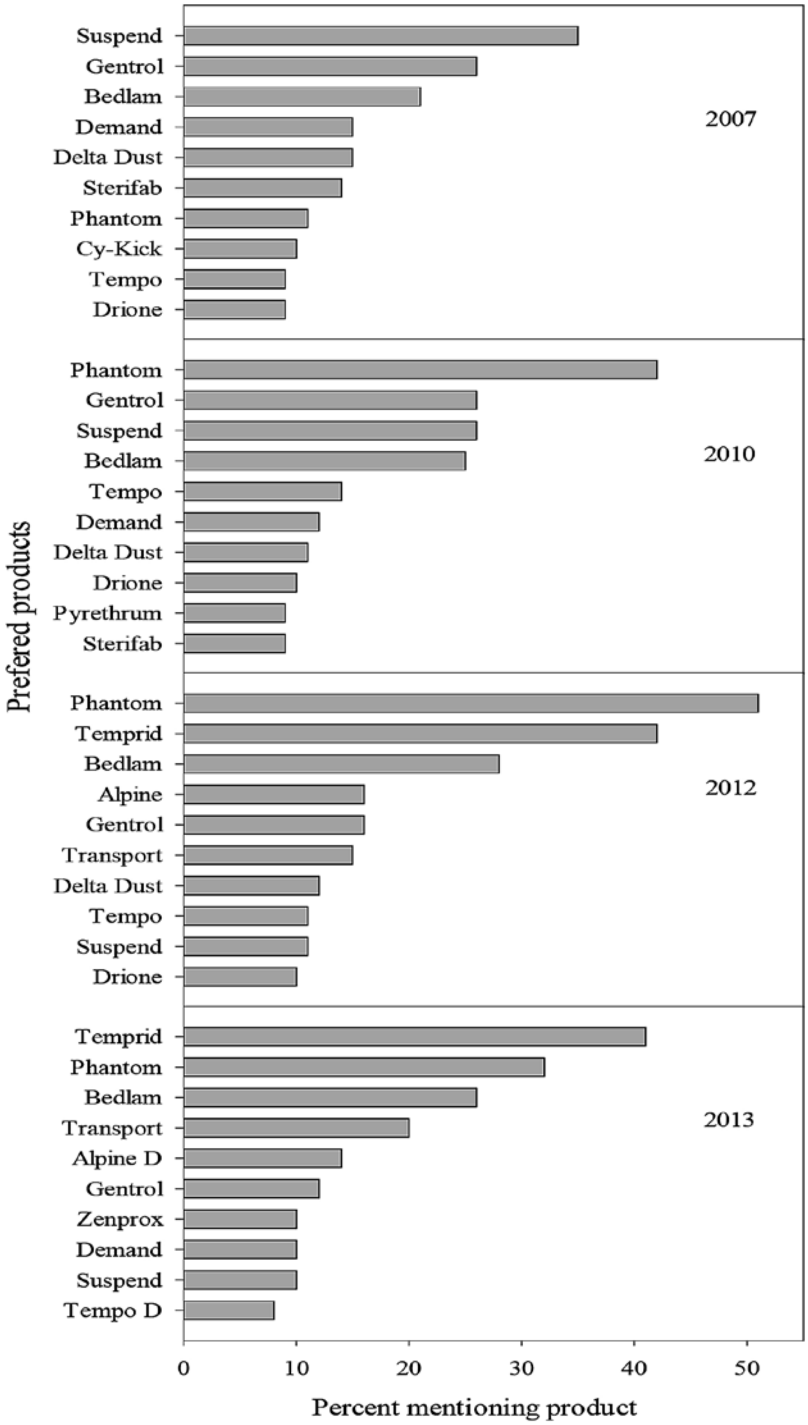

Figure 3. Most commonly used insecticides by survey year.
Alpine (dinotefuran/DE); Bedlam (d-phenothrin); Cy-Kick (cyfluthrin); DeltaDust (deltamethrin); Demand (λ-cyhalothrin); Drione (silica gel/PBO/pyrethrins); Gentrol (hydroprene); Phantom (chlorfenapyr); Sterifab (d-phenothrin); Suspend (deltamethrin); Tempo, Tempo D (cyfluthrin); Temprid (β-cyfluthrin/imidacloprid); Transport (bifenthrin/acetamiprid);

Monitoring, Detection, Prevention

Vigilance and early detection have long been considered keys to managing bed bugs. Detecting infestations early simplifies treatment within individual living units and limits dispersal to other areas of the building. Nearly all companies surveyed (99%) conduct visual inspections, but other approaches are gaining in popularity. Half (50%) of those polled mentioned using pitfall traps under the legs of beds and sofas, compared to 46% and 25% who mentioned using them in 2011 and 2010. More than a third (35%) also mentioned using monitoring devices emitting heat and/or carbon dioxide (versus 27% and 14% in 2011and 2010). Conversely, utilization of glue traps declined (46% in 2013 versus 59% in 2011) — reflecting perhaps a g reater realization by the industry that bed bugs tend to avoid capture by sticky surfaces. Also notable was the continued use of canine scent detection teams, either owned or sub-contracted through another company. In 2013, 45% of respondents said their company had used canines to find bed bugs, up slightly from 43% in 2011. Only 17% used the dogs when the pest management industry was surveyed in 2010. Undoubtedly there will be more bed bug detection devices in the future. Most promising will be those that reliably detect infestations at low levels, and are economical and inconspicuous.

More than two thirds of current respondents (68%) felt that customers are very (16%) or somewhat (52%) interested in some form of preventative service for bed bugs. In respect to the willingness of customers *to pay* for preventative inspections, 54% sensed they were very (7%) or somewhat (47%) willing to pay for bed bug prevention, while the rest felt their clients were not interested (31%) or were unsure (15%). Sixty ercent of respondents said their company currently conducts some type of proactive service for bed bugs. Services performed by those that do include ongoing visual inspection (by 29%), monitoring/trapping (by 18%), canine inspections (18%), and insecticide applications (17%).

Treatment Methods

When pest managers were asked which methods they normally use to control bed bugs, 96% mentioned insecticides. Encasing beds was also widely mentioned by 85% of respondents, which was comparable to their usage in 2011. Utilization of vacuums (by 70%) and steamers (by 43%) was also similar to 2011. Volumetric heating of rooms and buildings was employed by 42% of this year's respondents, compared to 32% of those polled in 2011 and 17% in 2010. Other treatment methods employed included container heat treatment (by 19%), pesticide-impregnated bed liners (by 4%), and spot freezing (3%).

Insecticides

Insecticides continue to be the most universal control method for bed bugs. Ninety-six percent of respondents said they apply insecticide liquids, 90% dispense dusts, 52% use aerosols, and 22% employ resin strips impregnated with a volatile organophosphate insecticide. These were virtually the same use patterns reported in 2011. When asked which products they routinely use, Temprid containing both pyrethroid (β-cyfluthrin) and neonicotinoid (imidacloprid) formulation ingredients was mentioned by 41% of respondents. The next most utilized product Phantom (chlorfenapyr) was cited by 32% (28% mentioning the liquid formulation, 4% the aerosol). Chlorfenapyr was the most mentioned product (by 51% and 42% of respondents) in 2011 and 2010. Rounding out the third and fourth most-mentioned products in 2013 were Bedlam aerosol (d-phenothrin + synergist), and another dual formulation, Transport (bifenthrin + acetamiprid). Usage of pyrethroid-neonicotinoid combinations for bed bugs has increased dramatically in recent years. In 2010, fewer than 5% of those polled routinely used these formulations. Clearly the choice of insecticides has changed rapidly, due in large part to resistance concerns of the industry (Romero et al., 2007) (Figure 3).

When pest management firms were asked if they were satisfied with the performance of current insecticides, 24% said "very satisfied," 61% were "somewhat satisfied" and 15% were either "not very" or "not at all satisfied." Industry satisfaction with available products has increased modestly since we asked this question in 2010.

As in previous surveys, opinions varied on the extent to which bed bugs are insecticide resistant. About a third of 2013 respondents (34%) said they had encountered resistant populations in the field, 42% felt they had not, and 24% were unsure. Bed bug resistance tends to be less obvious in the field than in the laboratory. One reason for this is that pest controllers often employ multiple tactics, which can offset reduced effectiveness of insecticides. Encasement of beds, for example, can purge many bed bugs from a dwelling, especially when augmented by laundering, vacuuming, steam treatment, etc. Insecticides also tend to perform better when bed bugs (even moderately resistant ones) are contacted directly. Apart from the active ingredients, bed bugs can be impacted by solvents, propellants, synergists, and other formulation components. This is especially true with aerosol and oil-based materials. Newer products with different or dual modes of action (e.g., chlorfenapyr, β-cyfluthrin + imidacloprid, bifenthrin + acetamiprid), also generally perform better against populations resistant to pyrethroids (Romero et al., 2010; Potter et al., 2012). Such factors may help to explain why many respondents do not consider resistance to be a widespread problem in the field.

Nonetheless bed bugs are endowed with diverse toxicological defenses to resist insecticides (Yoon et al., 2008; Adelman et al., 2011; Mamidala et al., 2011; Zhu et al., 2013). Although the new pyrethroid-neonicotinoid combo formulations are performing better than pyrethroids alone, resistance to these compounds has recently been documented also (Gordon et al., 2014). Pest managers should be watchful for declining performance in the field, and vary their approach to preserve current materials.

BUSINESS AND OPERATIONAL CONSIDERATIONS

Bed bugs command considerable time and resources of the pest management industry, yet still comprise a rather modest percentage of total revenue. Almost half the respondents in our survey (49%) said bed bug-related services generated five percent or less of their firms' annual income. Nonetheless, 26%, 24%, and 11% of firms from the Northeast, West and Midwest reported revenue from bed bugs exceeding 20%. On the other hand, costs for treatment are outpacing the budgets of many households and businesses. Two-thirds (67%) of respondents said the average amount customers in single-family homes paid for treatment was between \$500 and \$1500, with a median expenditure of about \$1000. The amount that apartment and condominium managers spent treating their buildings for bed bugs ranged from \$1000 to more than \$50,000. Similar costs were incurred by owners and managers of hotels and motels. These expenditures far exceed what consumers are used to paying for relief from household pests. Companies have had limited success to date convincing clients to invest in pro-active inspections or pre-emptive treatments for bed bugs. Targeted preventive applications using non-repellent residual insecticides are currently being evaluated in hotels and apartments by some U.S. companies (Potter et al., 2012).

Several respondents stressed that the bed bug resurgence cannot be curtailed without the public's cooperation and vigilance. When pest managers were asked what the biggest customer oriented challenges were during treatment, most often mentioned were "too much clutter" and "not following their advice." While many U.S. firms provide detailed preparation instructions to clients, the lists are often tedious and difficult to fully comply with, especially for the elderly and infirmed. Consequently, some companies have begun basing the level of requested preparation on extent of the infestation, i.e., low infestation, less preparation; heavy infestation, more preparation. Additional preparatory measures may be required as treatment proceeds.

Other respondents emphasized the need for effective and affordable solutions for the poor, pointing out that neglected infestations can also become massive reservoirs capable of infecting entire communities. Where the funding would come for community-wide bed bug eradication and prevention is unclear. Respondents also voiced concern about the devaluation of services as the bed bug market matures and prices decline. Some lamented that firms should not be doing bed bug work if their service lacks thoroughness, since this ultimately misleads the public. Unease was also expressed regarding the public's misuse of pesticides and flammable materials, which is also posing a risk for service technicians.

More U.S. cities are passing legislation defining the rights and responsibilities of tenants and landlords. Responsibility for treatment is usually incumbent upon property managers, while tenants are expected to promptly inform them of infestations and cooperate fully with treatment. One controversial issue is whether landlords should be required to inform prospective renters of infestations. While such notification may seem reasonable, it raises complex questions about when a formerly infested unit can be considered bed bug free. Another ethical dilemma is whether tenants have a right to know about the presence of bed bugs elsewhere in the building and especially in nearby units— or if this violates the privacy rights of those living in the infested units. Several lawsuits involving bed bugs are presently working their way through the U.S. courts, including the first case approved for class action status involving hundreds of poor, elderly and disabled tenants (Pitt, 2013).

What is clear from the current survey is that bed bugs in the U.S. are not subsiding, as some reports have suggested. Infestations continue to appear wherever people live, work and gather. Bed bugs continue to be the most challenging pest, by far, encountered by the pest management industry. Companies are becoming more experienced and have better tools for treating infestations. Yet there are no 'silver bullets' or easy fixes and the threat of insecticide resistance poses a global predicament. As a nation, the United States is making progress, but the lowly bed bug is proving to be a formidable adversary.

ACKNOWLEDGEMENT

We thank J. Fredericks, M. Henriksen and B. Rosenberg with the National Pest Management Association for their assistance in administering and compiling results of the survey.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

MANAGEMENT OF BED BUGS ON COMMERCIAL AIRCRAFT

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Abstract Bed bug (*Cimex lectularius*) infestations on board aircraft is a growing concern and has substantial financial impact on commercial airlines, in some cases it has resulted in Port Health Authorities grounding aircraft. Airlines that have a proactive approach to bed bug management fare much better than those with a reactive approach, suffering 80% fewer seats infested and 69% fewer insects in the heaviest seats. A range of detection systems were evaluated with detection rates on known infestations ranging from 12.5% to 95% accuracy. The withdrawal of methyl bromide left European carriers without an eradication system, its reputation of achieving 100% eradication in a single treatment was confirmed in this study. With limited insecticides holding Aerospace Materials Standard (AMS) 1450A approval and the ethical issues of pesticide use in passenger cabins new technologies were needed. Chemical treatment strategies achieved an average 15% reduction in infested seat count. Heat treatment in its different forms was seen as a direct replacement to methyl bromide. Open and recirculating heat capsule systems were evaluated and achieved 95% and 92.5% reduction in infested seat count respectively. However the frequency that recirculating heat treatments caused damage to aircraft and the reduced efficacy compared to open systems made this technique unpopular with aircraft operators.
Key words Aircraft fumigation, heat treatment, methyl bromide

INTRODUCTION

Biting insects on aircraft have been a concern since the beginning of commercial and international air travel (Griffitts and Griffitts, 1931). Swain (1952) predicted that aircraft would be a major distributor of insect pests, Sullivan (1958) reported on how insects may survive on aircraft. Public health pests have received the most attention (Evans et al., 1963; Basio et al., 1970; Otaga et al., 1974; Russell et al., 1984; Goh et al., 1985). The World Health Organisation (WHO) published a revised bulletin to cover the increased risk of vector borne disease. Disinsection of cabin environments to control mosquito species is required under the WHO International Health Regulations when flying from certain destinations. An overview of pest control methods, both chemical and non-chemical is provided by Story (1985) and Gratz et al. (2000). The resurgence of bed bugs (*Cimex lectularis*) around the world (Robinson and Boase, 2011) has included their appearance on commercial aircraft. The negative publicity suffered by airlines (Haiken, 2011) will affect a passengers' choice when selecting a carrier.

Over a three-year study comprising over 100 infested aircraft inspections of different carriers, aircraft models and configurations. The range and severity of infestation was monitored. Many infestations had spread through multiple cabins and consisted of multiple insects per seat. Cases ranged from single insects in single seats to infestation of many thousand insects spread through multiple cabins. The complex nature of aircraft interiors and the time constraints of a commercial aviation operation limit the potential detection systems that could be deployed. A range of systems including human

inspection, refuge monitors, lure based monitors, electronic detectors and scent detection dogs were assessed. The ability of detection dogs to locate live bed bug infestations in a controlled environment is well known (Pfiester et al., 2008) however their performance in a highly complex environment with multiple competing scents was unknown.
The withdrawal of methyl bromide in Europe left European carriers without an effective eradication system, the insecticidal properties of fumigants including methyl bromide in upholstered environments is well documented (Sherrard 1942). With limited insecticides holding AMS approval and the ethical issues of wide spread pesticide use in passenger cabins, new and innovative eradication technologies were needed. Different airlines approached this issue in different ways allowing the assessment of multiple technologies. Aircraft were inspected prior to and 28 days after treatment to assess the efficacy of the treatment process. The objective of this study was to assess the current management systems with the view to providing base line data to guide further developments.

MATERIALS AND METHODS

All detection technologies were assessed based on their ability to detect known infestations in a commercial aircraft environment. The accuracy of the systems was assessed along with their acceptance in the aviation industry. Due to legal constraints, refuge and lure based monitors could not be tested on aircraft, as none of these technologies would pass the fire safety tests needed to be installed on a commercial aircraft. As such these monitors were tested on infested seat sets that had been removed from the aircraft. All other systems were assessed on active aircraft.
Detection and infestation levels. A primary hand search was undertaken by experienced bed bug technicians to confirm the presence of bed bugs to test criteria. To prevent cabin disruption biasing test results, only one technology was deployed per aircraft per day. The following detection systems were deployed, refuge monitors, lure monitors, electronic air sampling detector and scent detection dogs. Refuge and lure monitor devices were installed 1 per seat in the test arena. The electronic detector was worked systematically through the test cabin sampling likely refuge sites following the search protocol recommended by the supplier. The search dog handlers were instructed to search the test cabins as a normal search. All systems were assessed based on percentage accuracy of known infestations, number of false positive readings and the acceptance of the system by contributing airlines. Infestations were graded based on the number of seats infested and peak insect count in the seat of heaviest activity. Aircraft presenting bed bug activity were inspected using canine and visual inspection process to identify the spread and severity of the infestation. Insects were removed from infested seats using a vacuum powered aspirator and insects counted post inspection in a counting arena.
Eradication. Eradication systems were assessed based on efficacy and acceptance within the aviation industry. Using a combined approach of canine and visual inspection, known infestations where documented prior to and 28 days after treatment to assess the efficacy of the systems. The following systems were analysed. Methyl Bromide Fumigation, this was carried out by a specialist aircraft fumigation company. The whole aircraft was fumigated not just the infested area as this was their normal process. High concentration of 100% methyl bromide was maintained for the treatment period.
Two available chemical treatments were evaluated, only Ficam W and K-othrine have approval to Aerospace materials specification 1450A. Applications were made using compression sprayers applying the pesticide to label conditions onto non-passenger contact surfaces. Seat covers were removed to facilitate access to key harbourages and replaced after the application had dried.
Two heat treatments were evaluated. The first where the heat capsule is closed with the air recirculating

within, with hot air heaters placed in the capsule either electrically powered or using ethylene glycol transfer systems. The second system used forced hot air ducted into the treatment capsule and vented to prevent the capsule pressurising. Both systems require the treatment to run until the core temperature of all treated material reached 60°C this exceeds the known thermal tolerance of bed bugs (Schrader and Schmolz, 2011) and should result in complete control of the infestation.

RESULTS

Detection

	Replicates	Mean infested seat count	% known infested seats detected	Mean false positive per replicate	Airlines surveyed deploying system
Refuge Monitors	2	8	12.5%	0	0
Lure Monitors	2	8	62.5%	0	0
Electronic detector	10	14.3	59.44%	26.2	2
Scent detection Dogs	10	15.6	95.51%	0.3	2

Infestation Levels

	Sample Size	1-10 infested seats	11-20 infested seats	21-50 infested seats	50+ infested seats	Mean peak seat insect count (range)
Proactive Detection	76	59	12	3	2	22(1-82)
Reactive Detection	32	11	5	7	9	71(10-140)

Eradication

	Sample size	Mean Infested seats before treatment	Mean Peak seat insect count before treatment	Mean Infested seats 28 days post treatment	Mean Peak seat insect count 28 days post treatment
Methyl Bromide	1	114	140	0	0
Ficam W	4	22.5	23	14	7
K-Othrine	4	19.25	21	21.5	9
Recirculating heat treatment	3	62.6	46.3	4.6	6
Forced air heat treatment	5	71.2	38.2	3.2	3.6

DISCUSSION

Detection

Harbourage and lure based monitors were the first to be assessed by the airlines and were quickly discounted as unfeasible. As these systems need an initial visit to deploy the monitors and follow up visits to check for activity. This alone made them unfavourable with airline operators. Harbourage based devices do not seem to be able to compete with the refuge rich environment in an airline seat however as this system was evaluated in the absence of a host it is possible that the absence of stimulus skewed this result. The only real advantage of these systems is that they produce no false positive results. Although in this trial the lure based devices produced reasonable results, none of the airline operators surveyed in this study are using either system due to the fire safety implications.

The air sampling device gave a large number of false positive results, this system is based on an Infrared absorption cell calibrated to the absorption spectrum of gasses resulting from digestion. It is likely that these false positives are due to the localised warm air pockets on aircraft seats caused by in seat electronics such as in flight entertainment units. It also did not detect some of the known infestations. Even with these limitations two airlines are operating this system as reactive survey system following passenger complaints.

Scent detection dogs provided the most reliable data to enable eradication efforts to be focused. A small number of false positive results were noted predominantly on the perimeter of the known infestations. Similarly the small number of infested seats that were not indicated by the scent detection dogs were on the perimeter of an infestation where a higher scent load towards the core of infestation drew the dog away. Throughout this survey accuracy in excess of 95% was achieved however the use of scent detection dogs must still be seen as a presumptive test not an evidentiary result as is the case with narcotic and explosive detection dogs.

Infestation Levels

Infestations studied ranged vastly in terms of spread and severity. Many of the proactively detected infestations consisted of single or small clusters of insects in a limited number of seats. The heaviest infested aircraft in this study contained 126 infested seats with a peak seat insect count of 140, in this case over 25g of live insects were collected during the survey. This level of activity was the exception rather than the rule however it illustrates the extent that infestations can develop to if left unchecked. As would be expected there is clear advantage to proactive detection in terms of infestation size and severity, not to mention the legal and ethical obligations to passengers. Those airlines that were engaged in a process of proactive detection averaged 80% fewer seats infested and 69% fewer insects in the heaviest seat, when compared with reactive airlines. In the absence of details of uninfested aircraft it is not possible to pass comment on the frequency of infestation, however due to the nature of a mass transit system it is likely that commercial aircraft are exposed to bed bugs on a daily basis and that the rate of transference is relatively low.

Eradication

Methyl bromide fumigation was the primary eradication system up until its revocation. The closed nature of aircraft favour a fumigation based approach and the non-corrosive, non-flammable properties made this system the benchmark that all treatment systems are measured against. Due to the timing of this study only one fumigation was assessed, however it was the only treatment strategy that achieved 100% eradication in a single treatment. Chemical treatment produced disappointing results. In some cases a dispersal of the infestation was noted. This was probably due to natural progression of the infestation rather than a repellent effect of the treatment. Deltamethrin is known to increase mobility in bed bug populations (Romero et al., 2009) and in a closed aircraft environment resulted in an increase in passenger complaints. As chemical control is the mainstay of the global bed bug control industry it was hoped that a chemical solution would replace aircraft fumigation. However the complex nature of aircraft seating products and the restrictions on dismantling seats does not allow the technician access to properly apply a pesticide. The aggressive nature of a cabin environment degrades both AMS approved preparations exceptionally quickly resulting in negligible residual value to chemical treatments, coupled with the elevated tolerance profile of field strain bed bugs (Boase et al., 2006; Romero et al., 2007) it is unlikely that a pesticide based approach will achieve the levels of control needed, even with further approval of pesticides for cabin use. Heat treatment being an environmental manipulation technique has many supporters in the aviation industry. The levels of control achieved are the closest to methyl bromide fumigation but still a degree of survival did occur. If carried out correctly it has no deleterious effects on the aircraft, however close attention needs to be paid to temperature monitoring to ensure safe treatment and the desired level of control. In one recirculating system treatment overheating of the environment resulted in warping of plastic components in seating products and cabin side walls. The need to use either fluid transfer or electric filament heaters within the aircraft raised further objections to recirculating heat treatments due to the risk of fire or flood. As only ducted hot air enter the aircraft and the more controllable nature of a forced air systems this has become the favoured eradication system. The increased air turbulence in a forced air treatment capsule results in greater energy transfer to the treatment substrate and consequently a greater reduction in population. With further developments in this area or if coupled with another eradication technology it is possible that the levels of eradication achieved with methyl bromide will be matched without the environmental impact.

CONCLUSION

Bed bugs on commercial aircraft will become more of an issue over the coming years. Most of the aviation industry have a reactive approach to bed bug management, relying on passenger complaints to drive treatment. Massive advances have been made over the past 3 years in detection and eradication systems resulting in workable proactive detection and risk management systems. As the bed bug issue develops globally the swing from reactive bed bug control to proactive management is inevitable.

The complex nature of aircraft seating products and regulatory constraints limit the detection systems that can be used. By far the most accurate and time efficient method in this environment is the use of scent detection dogs. This is followed by hand searching in terms of accuracy but the time input needed makes this system less attractive.

The severity and spread of bed bug activity is obviously directly correlated with duration since the inoculating event. Once established within the aircraft cabin, infestations develop very quickly due to the stability of the environment, extensive harbourage close to the host and the abundance of feeding opportunities. Those airlines that were engaged in a process of proactive detection averaged 80% fewer seats infested and 69% fewer insects in the heaviest seat, when compared with infestations detected by passengers or Port Health authorities. As preventing the inoculation is not possible, the early detection of infestations is vital particularly in view of reduced efficacy of eradication systems. In view of an aircrafts' likely exposure to bed bugs further research is needed to study passenger boarding behaviour with a view of reducing the rate of inoculation. Seating product design could also be vastly improved to reduce rates of establishment following inoculation and subsequent spread throughout the cabin environment.

As yet there is not a direct replacement for methyl bromide fumigation. No system evaluated during this study achieved the levels of control that were possible before its withdrawal. However workable alternatives are available, forced air heat treatment shows great potential and with future development it is likely that this system will become the direct replacement for methyl bromide.
The aviation industry harbours widespread misconceptions regarding the management of bed bugs. Many who responded to our survey questionnaire believe that bed bugs can be eradicated in a single treatment as was possible with methyl bromide. Some believe that only aircraft flying to USA are at risk of infestation. There is a clear need for a central resource centre for the aviation industry to allow best practice to be shared across the industry.

ACKNOWLEDGEMENTS

The pest controllers, airlines and suppliers who participated in the study. Their frankness, openness and willingness to share knowledge and experience played a major part in the project.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8 200 Veszprém, Papái ut 37/a, Hungary

NESTING AND QUESTING ACTIVITY IN BED BUG POPULATIONS: MALE AND FEMALE RESPONSES TO HOST SIGNALS

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Abstract A large-arena bioassay is used to examine gender differences in the spatiotemporal patterns of bed bug, *Cimex lectularius* Latreille 1802, behavioural responses to either a human host or CO_2. After release in the center of the arena, 90% of the newly fed bed bugs move to hiding places in the corners within 24 h. They require 3 days to settle down completely in the arena, with generally low activity levels and the absence of responses to human stimuli for 5 days. After 8-9 days, persistent responses can be recorded. Gender differences are observed, in which females are more active during establishment, respond faster after feeding, expose themselves more than males during the daytime, and respond more strongly to the host signal. The number of bed bugs that rest in harbourages is found to vary significantly according to the light setting and gender. Both genders stay more inside the harbourages in daylight compared with the night, and males hide more than females during the daytime but not during the night. The spatial distribution of the bed bugs is also found to change with the presence of CO_2, and peak aggregation around the odour source is observed after 24 min. Both male and female bed bugs move from the hiding places or the border of the arena toward the centre where the CO_2 is released. Peak responses are always highest during the night. Bed bug behaviour and behaviour-regulating features are discussed according to control methods.
Key words Bed bug, *Cimex lectularius*, *Cimex hemipterus*, host location.

INTRODUCTION

Bed bugs (*Cimex lectularius*) are closely connected to their host. Each nymphal stage must have a blood meal to proceed in its lifecycle, and adults need blood to successfully produce offspring (Reinhardt and Siva-Jothy, 2007; Usinger, 1966). During feeding, the bed bugs elevate their mortality risk by exposing themselves to the host when leaving their concealed and safe harbourages. The time spent on or around the host is therefore minimized, and the bed bugs remain hidden from humans for the majority of their lives. This cryptic way of living is one of the major challenges in bed bug control because it reduces our chances of bed bug detection and makes the efficient application of killing agents difficult. Bed bugs are currently increasing worldwide (Davies et al., 2012). To overcome pesticide resistance (Kilpinen et al., 2011; Romero et al., 2007; Tawatsin et al., 2011; Zhu et al., 2010) and cope with their concealed biology, improved control strategies are needed (Koganemaru and Miller, 2013; Weeks et al., 2010). To find potential bed bug weaknesses that can be exploited to develop efficient integrated pest management solutions, increased knowledge about bed bug behaviour is necessary.

Bed bug behaviour consists of sub-social elements, such as aggregation and alarm signals (Levinson et al., 1974; Olson et al., 2009; Siljander et al., 2008). They also benefit from nesting activity, in which high-density accumulations reduce water loss (Benoit et al., 2007). Odours from blood secretion, feces, and exuviae combine with residues from the alarm signals to produce a volatile cue that in conjunction with tactile stimuli allows bed bugs to aggregate in their nests after feeding on their host (Domingue et al., 2010; Olson et al., 2009; Siljander et al., 2008). Host locations also depend on olfactory cues (Anderson et al., 2009; Harraca et al., 2012; Singh et al., 2012), and the bed bug appears to be governed by an assembly of volatiles that regulate movement and resting.

Awareness of the complexity of olfactory signals (Bruce et al., 2005; Lazzari, 2009; Logan and Birkett, 2007) and increased knowledge of olfactory-mediated bed bug behaviour (Siljander, 2006; Weeks et al., 2010) may be utilized as tools to improve the efficiency of control methods (Benoit et al., 2009). In many other pest situations, semiochemicals are used for early detection, push-pull strategies, mass trapping, luring and killing strategies, luring and infecting strategies, and mating disruption (Agelopoulos et al., 1999; Cook et al., 2007; El-Sayed et al., 2009; El-Sayed et al., 2006; Witzgall et al., 2010). The spatiotemporal patterns of bed bug questing, together with descriptions of life stages and gender-specific responses to different semiochemicals need to be considered to properly time and direct treatment. Some disparities in bed bug activity and movement are known across sexes and between life stages (Domingue et al., 2010; Pfiester et al., 2009; Romero et al., 2010; Weeks et al., 2011), but gender differences in host perception have not been investigated, although this is considered an important applied aspect of semiochemical control (El-Sayed et al., 2006).

Laboratory behavioural studies are integral parts of the development of semiochemical control methods, and bioassays should allow the insects to act in a nearly natural way. This is difficult to achieve with bed bugs, however, because removal from stock cultures, handling, and transfer to the bioassay clearly interfere with the concealed biology of the insect and may consequently influence their responses. Behavioural studies that described bed bug activity have often been performed in Petri dishes or in small-scale experimental arenas where behaviour is measured during a limited time span (Harraca et al., 2012; Olson et al., 2009; Pfiester et al., 2009; Romero et al., 2010; Weeks et al., 2013; Weeks et al., 2011). These experimental setups are valuable tools for identifying basic behavioural elements, but a low spatiotemporal scale may make field application of the results more difficult. Larger-scale arena trials, which more closely mimic a natural indoor bed bug pest situation, provide information about the attraction potential of CO_2 and chemical lures (Anderson et al., 2009; Singh et al., 2013; Singh et al., 2012; Wang et al., 2009; Wang et al., 2013).

To fully describe natural bed bug responses, all regulating factors need to be considered, and a bioassay should allow bed bugs to behave as naturally as possible. In the present study, we seek to describe a large-scale arena bioassay to identify elements that are necessary to produce quantifiable behavioural responses to host signals. Gender and day-night differences in responses are described, and we investigate the effect of the time since feeding on the level of responsiveness. Using the arena bioassay, we provide detailed measures of the bed bugs' response profiles when stimulated by volatiles from a human. We also investigate responses to a pure CO_2 point source to describe its effects on spatial distribution in the arena.

MATERIALS AND METHODS

Insects. The bed bug stock culture was collected in 2009. The initial population consisted of 40 adult specimens that originated from a hotel in Oslo, Norway. Cultures were maintained in 140 ml polyethylene boxes (7 cm height, 5 cm inner diameter) that contained folded paper towels to provide harbourages and allow the bed bugs to move, mate, and lay eggs. All of the cultures were maintained in a 15 h/9 h light/dark cycle at room temperature (20-22ºC) and 50-60% relative humidity. Stock cultures were fed artificially and on rodents until experiments were performed.

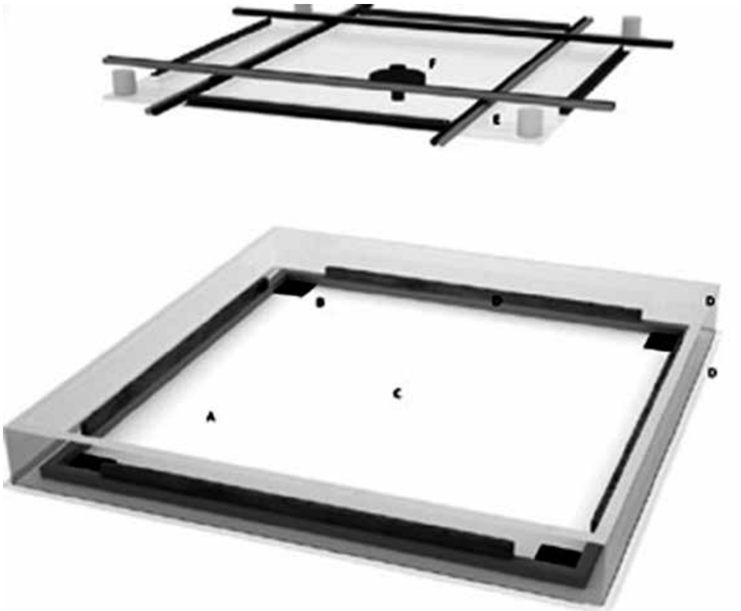

Figure 1. Three-dimensional rendering of the bed bug arena. (A) Area of movement. (B) Harborages. (C) CO2 release point. (D) Safety barriers (polished plastic wall with copper weights, Plexiglas wall, insect glue-coated overhang, and mineral oil-filled duct). (E) White, red, and infrared light sources. (F) Vivotech IP camera.

Bed bug arena. The experimental arena (Figure 1) was placed in a 15 m^2 room (3 m x 5 m) without windows. The room was air-conditioned and maintained at an average (± SE) temperature of 22.3 ± 0.1ºC and relative humidity of 31.7 ± 3.3%. The room was only furnished with a chair and desk, and the walls and ceiling were white or grey to minimize visual cues. All of the light sources were positioned directly above the arena and consisted of a set of eight Plexiglas tubes (1.2 cm diameter, 110 cm length), each with 96 light-emitting diode (LED) lights positioned 1 cm apart (8 W, Northlight LED, Clas Ohlson, Oslo, Norway) and four infrared (IR) lamps (Ecoline IR illuminator, TV6700, ELFA, Kolbotn, Norway). The eight tubes were placed in a grid fashion and the 768 LED lights were directed toward the arena. The four IR lamps were directed toward the ceiling. This lighting setup provided shadow-free and even light conditions in the arena to facilitate night and day observations of the bed bugs. Half of the Plexiglas tubes were coated with red plastic foil. Day was simulated by turning all of the light sources on. Night was simulated by turning on only the red tubes and IR lights. The light cycle was 15 h/9 h (light/dark) to keep the conditions similar to the rearing facilities and what is commonly found in a bedroom. The dark cycle began at 15:00 hours and lasted until 00:00 hours. A day- and night-vision Internet Protocol (IP) camera (Vivotek, FD 8361, Multicom, Åmli, Norway) was used to record a 1200 x 1600 pixel video with five frames per second. Sharp night videos were obtained using the built-in IR-cut filter and iris technology. The recording software was Vivotek web-client, version 1.00.

The bed bug arena was placed 90 cm above the floor. The arena base was constructed from a 2-cm-thick white plastic plate (Polyoksymetylen, copolymer: 30 kg/m^2, Plastkompaniet A/S, Oslo, Norway) that measured 150 cm x 150 cm. A 15 cm high and 2 mm thick Plexiglas wall, with a 2 cm insect glue-coated overhang, surrounded the arena. As an additional safety measure to prevent bed bug escape, the wall was bordered by a duct filled with mineral oil. An inner frame constructed from polished hard plastic defined the borders of the area where the bed bugs could move (130 cm x 130 cm). This frame was held in place by brass rods that kept in place one white piece of paper (134-white seamless background paper, 175 g/m^2) that covered the arena floor and provided grip for the moving bed bugs. In six experimental series, the arena was used in its entirety. In two experimental series, the inner part of the arena was split in two halves (130 cm x 65 cm) by an additional plastic wall. Harbourages, made from 10 cm x 10 cm transparent dark-red Plexiglas (Plexiglas, GS268, Plastkompaniet A/S, Oslo, Norway), were positioned in each of the corners of the arena. The Plexiglas harbourage had a corner that faced the centre of the arena, elevated approximately 3 mm.

Experimental protocol. Fifth instar nymphs were fed on rodents until fully engorged; which provided newly emerged adults after 2 weeks. The emerged adults were sexed by registration of the genitalia and offered a rodent blood meal. Immediately after feeding, an equal number of fully engorged male and female bed bugs were transferred to the experimental room and released in the arena. The room was left empty on the initial day to allow the bed bugs to establish themselves without interference. During the experimental series, human stimulation was performed by one person who entered the room and sat in a chair next to the bed bug arena or by CO_2 that was presented to the bed bugs by placing a small Petri dish (55 mm diameter) that contained an average (± SE) of 17.1 ± 0.2 g dry ice in the middle of the arena. Each stimulation lasted 30 min. As a control for human stimulation, we used the room with no humans in it. As a control for CO_2 stimulation, we used an empty Petri dish. Experimental bed bugs were only used in one 14-day series and were killed by freezing upon completion of the experiment.

To describe the response of the bed bugs to their natural human host, we initially used five males and five females in the arena. From the second day of experimentation, a human entered the room every third hour from 07:00 hours to 22:00 hours to provide six stimulations per day (Table 1). Three stimulations occurred during the day, and three at night. This experiment lasted 14 days and repeated three times. As a control, three additional 14-day experimental series were performed with no stimulation in the room.

To investigate gender differences, the bed bug arena was split into two halves. Ten males were released in one half, and ten females in the other half. From the second day of experimentation until day 4, we did not expect host-initiated activity, and the bed bugs were stimulated only once by a human in the daylight (Table 1). From day 5-9 in the experimental series, a human entered the room every third hour from 10:00 hours to 19:00 hours to provide four stimulations per day, three hours apart. Two stimulations occurred during the day, and two at night. From day 10 to day 14, the four daily human stimulations were replaced by either CO_2 or an empty Petri dish (Table 1). The daily order of CO_2 stimulation and the control were alternated to achieve an overall balance between which treatment being introduced first during both the day and night. The split population experiment was repeated twice.

Video analysis and quantification of behaviour. In all of the experiments, the bed bugs and their activity in the arena were video-recorded. When analysing the video files, we scored behaviour for 1 min every 6 min. This gave a total of 10 min of recording every hour and a total of 5 min of recording during stimulation. During each minute of recording, the total number of bed bugs that occupied the open spaces of the arena, number of bed bugs that rested in their harbourages, and cumulative number of bed bug individuals that moved during the 1 min period of observation were counted. In the split series, the spatial distribution of the bed bugs was investigated when the CO_2 or empty control Petri dish was presented as a point source. Changes in the bed bugs' positions were analysed by dividing the entire arena into an 8 x 8 square grid. The number of individuals in each of the 64 squares (16 cm x 16 cm) was counted in the freeze frame at the beginning of each minute of recording (6 min before stimulation, every sixth minute during stimulation, and 18 min after stimulation).

Table 1. Experimental set-up of the day/night regimen, with methods and periods of stimulation in the bed bug arena in mixed and split populations. White represents day (white, red, and infrared lights on), and grey represents night (only red and infrared lights on). ○ = no stimuli, ●= human, ◎ = empty Petri dish, x = Petri dish with dried ice to release CO_2.

	MIXED POPULATION		SPLIT POPULATION		
Stimuli	*Human* (Day 2-14)	*Control* (Day 2-14)	*Human* (Day 2-4)	*Human* (Day 5-9)	*CO_2/Empty Petri dish** (Day 10-14)
00:00-07:00	○	○	○	○	○
07:00-07:30	●	○	○	○	○
07:30-08:00	○	○	○	○	○
08:00-10:00	○	○	○	○	○
10:00-10:30	●	○	●	●	⦿
10:30-11:00	○	○	○	○	○
11:00-13:00	○	○	○	○	○
13:00-13:30	●	○	○	●	◎
13:30-14:00	○	○	○	○	○
14:00-16:00	○	○	○	○	○
16:00-16:30	●	○	○	●	⦿
16:30-17:00	○	○	○	○	○
17:00-19:00	○	○	○	○	○
19:00-19:30	●	○	○	●	◎
19:30-20:00	○	○	○	○	○
20:00-22:00	○	○	○	○	○
22:00-22:30	●	○	○	○	○
22:30-23:00	○	○	○	○	○
23:00-24:00	○	○	○	○	○
Repetitions:	*14 days n = 3*	*14 days n = 3*	*3 days n = 2*	*5 days n = 2*	*5 days n = 2*

*The order of CO_2/empty Petri dish was alternated daily.

Statistical analysis. Data were analysed using SigmaPlot 12 (Systat Software, San Jose, CA, USA). Data were checked for normality, and multiple comparisons were performed using analysis of variance (ANOVA). Pairwise comparisons were performed using *t*-tests or paired *t*-tests. The level of significance was set to 0.05, and differences between multiple comparisons were identified using the Tukey test. If the tests of normality failed, then we used the nonparametric Mann-Whitney test, Mann-Whitney Rank Sum Test, or Kruskal-Wallis ANOVA.

RESULTS

General activity. No response to human stimulation was observed during the initial period of observation, which was considered establishment in the arena and excluded from further analysis. We observed generally higher activity among females compared with males, and the split populations were two- to three-times more active as the mixed populations during the initial 3 days. Almost all of the bed bugs (90.0 ± 4.9%) moved from the open spaces of the arena where they were released to the corners within 24 h.

Overall activity in the control series with no stimulation was low, with an average (± SE) of 0.02 ± 0.01 individuals that moved during the 10 min observation period per hour in daylight, and 0.62 ± 0.05 individuals moved at night. The bed bugs were active according to the light regimen, and distinct activity peaks could be observed during the night (Figure 2A) when the maximum number of individuals that moved within a 1 min observation period reached five of 10. Overall activity during the human stimulation series was significantly higher than in the control series, in which 0.82 ± 0.07 individuals moved in daylight (Mann-Whitney Rank Sum Test: $T = 85075$, $P < 0.001$) and 1.43 ± 0.13 individuals moved at night (Mann-Whitney Rank Sum Test: $T = 74870$, $P < 0.001$). In the stimulation series, spikes of activity related to human presence could be observed during both the night and day (Figure 2B). Maximum activity was reflected by movement of all 10 individuals in a 1 min period of observation. This occurred at night, either during human stimulation or following the presence of a host.

Responses to host. A change in activity occurred in the mixed populations on day/night 9 in response to human presence (Figure 3A, B). At night, no difference was found between the control and human presence from day 4-8 (paired *t*-test: $t = 0.78$, $P = 0.480$), activity was significantly higher with a human present from day 9 onward (paired *t*-test: $t = 12.84$, $P < 0.001$; Figure 3A). During daylight, activity in the control series was close to zero, and human presence significantly increased activity during both periods (paired *t*-test: day 4-8, $t = 3.53$, $P = 0.012$; day 9-13, $t = 6.69$, $P = 0.003$; Figure 3B).

When males and females were separated, the level of female activity during human stimulation gradually increased until day 8, whereas male activity was low. Females always moved more than males but only significantly more on day 8 (Mann-Whitney Rank Sum Test: $T = 773.5$, $P < 0.001$; Figure 3C) and day 9 (Mann-Whitney Rank Sum Test: $T = 740.5$, $P = 0.002$; Fig. 3C). When humans were replaced with CO_2, this difference in responsiveness persisted, but males increased activity throughout the last 10 days to a similar level as females. A significant gender difference in response to CO_2 was found only on day 10 ($P < 0.020$; Figure 3C).

When the stimulation events were divided into 6 min intervals, we observed a distinct response that began when the host entered the room and when dry ice was placed in the arena. Activity continuously increased until the stimulus was removed and then gradually decreased during the next hour. Peak responses were always the highest during the night. In the mixed population, the response profile appeared to be similar, regardless of night or day, but was less prominent from day 4 to day 8 compared with the interval from day 9 to day 13 (Figure 4A, B). In the split series, only one of five tests exhibited a significant gender difference in response to CO_2, and the data were pooled across sexes to create a response profile (Figure 4C). CO_2 stimulation appeared to be similar to a human odor source with regard to the temporal change in activity, but CO_2 stimulation produced more responders. Night stimulation in the split series occurred during the first half of the night, and a general increase in activity was observed in both the control and stimulation conditions during this period (Figure 4C).

Spatial distribution. Six minutes before stimulation, the number of bed bugs that rested in the four squares that contained a harbourage varied significantly according to the light setting and gender (Kruskal-Wallis ANOVA on Ranks: $H = 36.092$, $df = 3$, $P < 0.001$). The Tukey *post hoc* test revealed that both genders positioned themselves more in the harbourages in the daylight compared with the night, and males hid more than females during the daytime but not during the night (Figure 5). The spatial distribution of the bed bugs also changed with the presence of CO_2, and peak aggregation in the four centre squares that surrounded the odour source was observed after 24 min (Figure 6). Compared with the control series, a significant increase in the number of individuals that resided in the centre squares was observed after 6 min (Mann-Whitney Rank Sum Test: $T = 515.5$, $P = 0.002$, or $P < 0.001$ for all tests after 6 min; Figure 6). Both males and females moved from the harbourages or border of the arena toward the centre, whereas the control treatments maintained the initial distribution. During peak aggregation at 24 min, females comprised 63% of the animals during the daytime and 44% during the night. The maximum number of bed bugs observed around the CO_2 source during a single stimulation was 12 of 20 individuals. After removal of the CO_2 source, the bed bugs again successively dispersed from the centre of the arena.

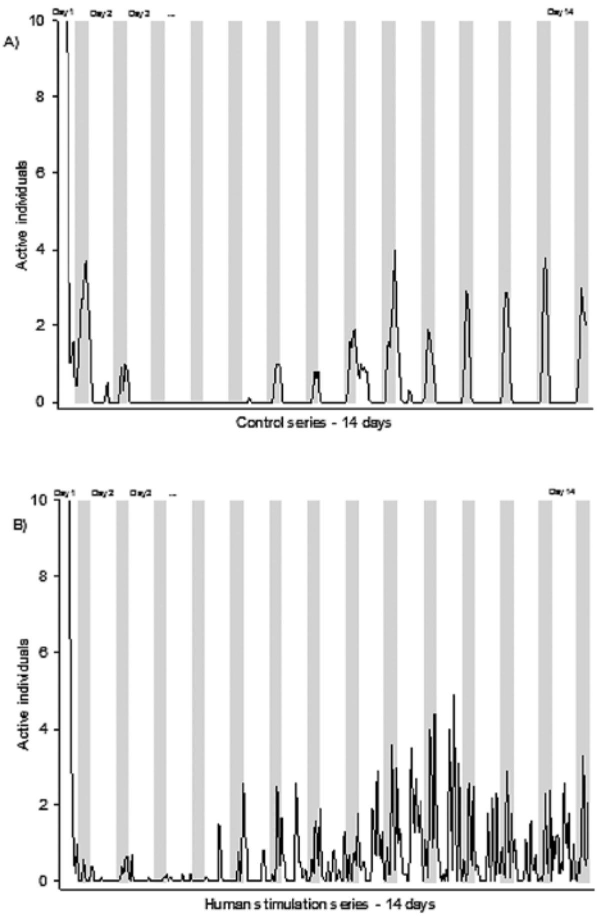

Figure 2. Typical recordings of Cimex lectularius activity in a population of 5 males and 5 females for14 days. (A) Control series without stimuli. (B) Human stimulation series. Gray background = night, white background = lights on.

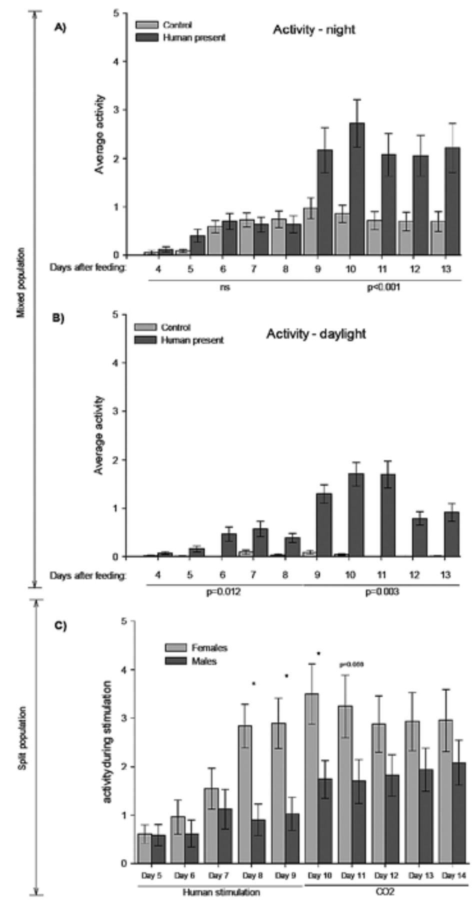

Figure 3. Cimex lectularius activity (average ± SE) during human stimulation of 5 males and 5 females (A) at night and (B) in daylight and (C) during human stimulation from day 5-9 and CO2 stimulation from day 10-14 with 10 males and 10 females in the each half of the arena

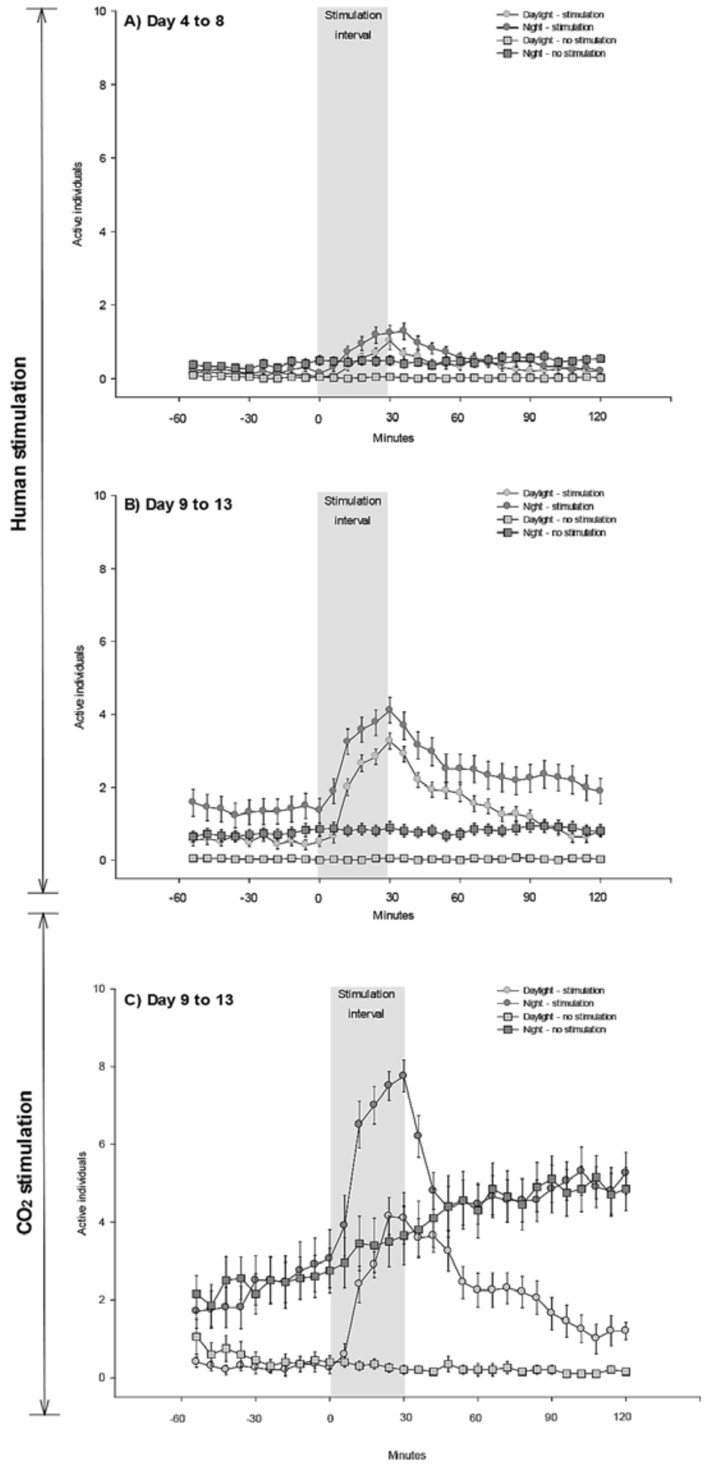

Figure 4. Mean number (± SE) of active Cimex lectularius during stimulation (grey area) with (A) humans on day 4-8 after feeding, (B) humans on day 9-13 after feeding, and (C) CO2 on day 9-13 after feeding.

DISCUSSION

The behaviour observed in the bed bug arena is consistent with bed bug habits that have been previously described in both field and laboratory studies. Activity peaks occur during the night (Romero et al., 2010), with a lack of activity after feeding (Usinger, 1966), modulated movement with the presence of a host signal (Anderson et al., 2009; Harraca et al., 2012), and only a low level of spontaneous movement in daylight (Romero et al., 2010). These behavioural responses are in agreement with bed bug biology in an urban setting. Our large arena and the use of a 14-day behavioural series appear to mimic natural conditions and may represent a tool for studies that seek to improve field applications in pest control.

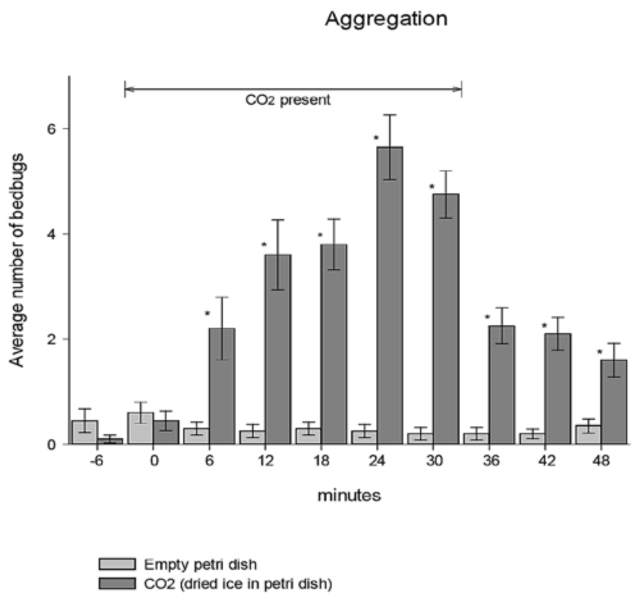

Figure 5. Percentage (± SE) of 10 male and 10 female Cimex lectularius that hid in the corners of the arena during the day and night 6 min prior to stimulation.

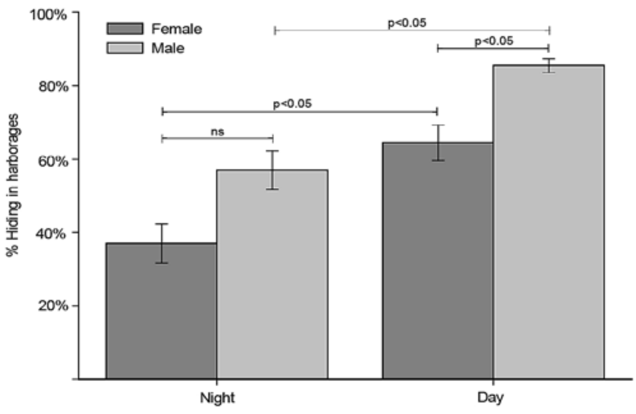

Figure 6. Aggregation of 20 Cimex lectularius (average ± SE) at different times around odour source of dry ice in a Petri dish in middle of arena. Petri dish was removed at 30 min. Empty Petri dish used in the control treatment. *P < 0.05, significant difference in aggregation between stimulation and control.

The present study finds gender differences in host signal responses. Females expose themselves more than males during the day, respond more strongly to the host signal, and respond earlier in terms of the time since feeding. Female egg production will sequester proteins and other elements needed for metabolic processes (Boggs, 2009), and male sperm may be produced and remain viable for a longer time without high metabolic cost. The recorded day-to-day change in the level of responsiveness agrees well with such a nutritional demand scenario because the onset of female activity directed toward a host appears after resource-draining egg production occurs (after 4-8 days (Usinger, 1966)). The males' gradual increase in activity may be linked to a more fixed metabolic rate. Fitness profit connected to dispersal strategies is an additional explanation of the gender differences. Bed bugs are known to walk long distances in urban environments, and this behaviour occurs mostly in adults (Wang et al., 2010). The sex ratio among dispersing individuals is partially described (Domingue et al., 2010; Pfiester et al., 2009), but the efficient colonization of new habitats by single individuals clearly requires a fertile and inseminated female. Together with the potential location of new habitats, the location of new harbourages for offspring around the present host may be an additional benefit. The distribution of eggs at several locations may be a part of a bet-hedging strategy among females to reduce the total risk of exposure and offspring mortality (Hopper, 1999). Males, in contrast, have no opportunity to recolonize new areas alone and must do this indirectly by mating with females. The chances of finding a new host and a female at the same time may be so low that their optimum strategy is to await mating opportunities in the established nest.

The observed response patterns have implications for the field application of killing agents. As females lay eggs, they are the main target for control and should be the focus of population suppression and eradication (El-Sayed et al., 2006). Most females in the present study remain rather inactive for 5-7 days, even with stimuli present. Such a low level of activity is problematic in terms of exposing bed bugs to killing agents outside their resting places. Because of bed bugs' passivity immediately after feeding (Usinger, 1966), a reasonable strategy will be to leave rooms empty for some days before the application of pesticides or desiccant dusts. This may prevent bed bugs from remaining hidden when the killing agents are most potent. It is not expected that natural populations are as synchronized in feeding as our experimental animals, and frequent and almost continuous feeding and egg laying may occur within a population (Pereira et al., 2013; Reinhardt et al., 2010). Therefore, if artificial host signals are to be utilized efficiently in pest management, then the time since the last feeding and overall hunger state of the population need to be considered. Waiting at least 5 days before applying killing agents may increase the blood lust of a larger proportion of the population to spur the activity of as many individuals during as few stimulation events as possible. Shortening the passive time of the bed bugs might also be possible by raising room temperatures to increase metabolism, the need for a host, and questing activity during artificial stimulation (Reinhardt et al., 2010). Such approaches must be weighed against an increase in egg production and potential hunger-mediated dispersal to adjacent rooms or out of apartments (Wang et al., 2010).

Regardless of when treatment is performed or how it is connected to the feeding habits of the resident population, we demonstrat an effect of CO_2 on the activity level of the bed bugs. Relatively inexpensive and simple activators, such as CO_2 released from dry ice, regulated tanks, or sugar-yeast solutions, can thus be utilized to improve control (Singh et al., 2013). CO_2 is also used to activate bed bugs to make them move across treated substrates (Wang et al., 2013). The present study shows that this single component may be sufficient to manipulate their behaviour to lure them out from their harbourages. The similar response profiles to humans and CO_2 also indicate that CO_2 is the major component of the host signal. The addition of other attractive host compounds (Anderson et al., 2009; Harraca et al., 2012) may further improve such effects. Ideally, all behaviourally active host compounds should be included in the odour replica to lure as many bed bugs as possible into questing and exposing themselves. Currently, full activation can only be achieved with a natural host (i.e., humans), but using natural bait is ethically questionable because many killing agents are toxic to humans, and even residual pesticides may have a negative effect on the health of the residents (Mostafalou and Abdollahi, 2013). This means that during treatment with potential harmful substances, an artificial host signal is needed to activate the bed bugs to ensure contact with the killing agents. Host signals also appear to more strongly influence egg-laying females, which are an important focus of control efforts.

To identify the full host signal, more behavioural studies are needed, and bioassays that are capable of quantifying bed bug responses should be further developed. Interestingly, in the present study the relative response in terms of activated animals compared with the control condition is largest with the lights on. In daylight, the stimulation produces a peak response with a more than 10-fold increase in activity, whereas activity is only four-times higher at night. Significant differences between human presence and the control condition are also detected earlier (i.e., on day 4-8) only when the lights are on. In terms of measuring differences in odour-released activity, experiments may be performed in the daylight to distinguish small responses from general night-time movements in the arena. Both genders also hide more when experiencing daylight. This facilitates the detection of activity when they leave their harbourages. This rather surprising result may be influenced by the use of habituated laboratory animals that normally feed in the daylight. However, the clear interactions between sex, light setting, and time since feeding should be considered when designing bed bug bioassays.

Arenas appear to be powerful tools for understanding the dynamics of the questing and nesting activity of bed bugs. Our arena contains hiding places. Because the arena is not cleaned between the experimental series, the harbourages likely contain attractive and arresting odours. The human odours are presented in a natural way by simply entering the room, and this triggers a fairly strong and persistent bed bug response. This allows quantification and comparisons with artificial cues. The bed bugs are also found to locate the point source of CO_2 quite efficiently when positioned in the centre of the arena, showing that this single component is sufficient to allow spatial orientation and host location in both daylight and darkness. In addition to providing knowledge for improving control methods, such studies can be used to test, evaluate, and improve the efficiency of traps and lures that are intended to monitor or suppress bed bug populations. The observed behaviour also indicates responses and orientation mechanisms in bed bugs that differ from the more rapid responses and distinct movement patterns found during optomotor anemotaxis in flying insects (Carde and Willis, 2008). The use of more detailed video tracking systems might provide valuable quantification of the behavioural mechanisms that lead to source location in crawling insects that manoeuvre in darkness. Although only an incremental step in elucidating bed bug chemical ecology and the dynamics of questing and nesting activity, we make observations that may contribute to improvements in control strategies. More experimental approaches, combined with field studies, are clearly needed to fully understand the dynamics of a growing bed bug population. Properly revealing the behaviour-regulating features of bed bugs may allow the development of more efficient control methods.

ACKNOWLEDGEMENTS

Pest control technician Thomas Riise provided us with the initial bed bug stock culture, and Masters of Architecture Lina Grundstrøm and Frederic Bachman made the three-dimensional photo rendering of the bed bug arena. Special thanks are given to senior engineers Magne Føllesdal and Eivind Arntzen for expert advice and help building and designing the bed bug arena. We also extend our gratitude to Professor Tone Birkemoe who provided valuable comments on the manuscript.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

OCCURRENCE OF BED BUGS IN BUDAPEST, HUNGARY

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Abstract The number of bed bug controls in Budapest has been steadily increasing since 2000, an abrupt rise, however, began from 2007. To apply an effective control strategy, it is essential to identify the causes of the increase in the number of bed bugs. To determine the causes, during the past one and a half year, simultaneously with the treatments performed by Bábolna Bio in apartments and institutions in Budapest, a questionnaire was also filled out. The survey was conducted over a 18-month period between March 1, 2012 and August 30, 2013 in 91 flats and in 14 institutions, at a total of 105 locations. We surveyed the level of bed bug infestation in the flats, the type of buildings, the layout and the furniture of the flats. We identified the presumed mode of introduction and inquired about how long the tenants had been aware of the presence of bed bugs. In addition, we also investigated signs indicative of bed bugs and their occurrence and location within the flat or room.
Key words *Cimex lectularius,* bed bug survey.

INTRODUCTION

Based on the data available, we can say that the occurrence of bed bugs in Hungary was quite general in the beginning of the 20th century, but their number steadily increased from the 1940-ies. In The Fauna of Budapest, Pénzes (1942) reported that 75% of the apartments in Budapest were infested with bed bugs. At the end of the 1950s, infestations decreased significantly, due primarily to the treatments carried out with hydrocyanic acid and later with DDVP. Between 1970 and 1990 bed bugs remained at a low level, but they began to reappear in the late1990s both in Europe, the United States and Australia (Boase, 2008; Doggett, 2006; Kilpinen, et al., 2008). The number of bed bug treatments in Budapest performed by Bábolna Bio has been steadily increasing since 2000, with a dramatic increase since 2007 (Figure 1).

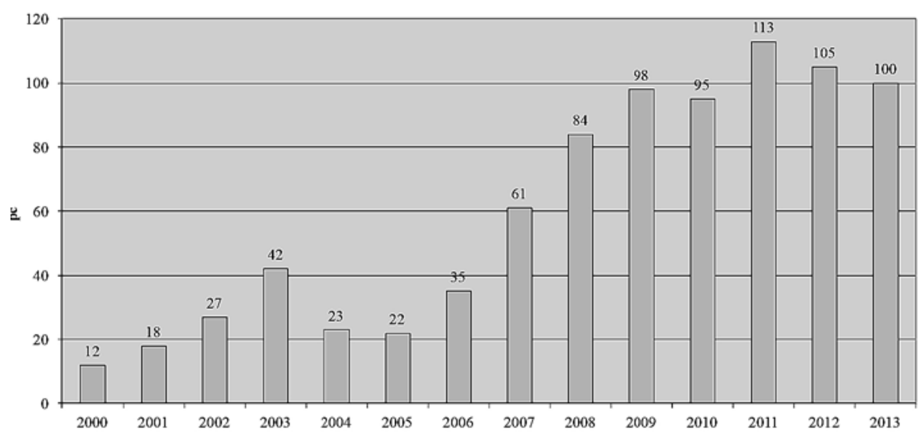

Figure 1. Bed bug treatments per year by Bábolna Bio in Budapest: 2000-2013.

Researchers in pest control give various explanations for the abrupt increase in bed bugs at certain locations. They attribute the increase to general and partly insecticide-related reasons. General reasons: lack of knowledge or experience, increased traveling activity: tourism and especially the traffic of guest workers worldwide, inappropriate chemical treatments, increased sale of second-hand furniture, junk clearance, neighborhood clean-up, lack of controls and inspections by the authorities, improper pest control practices. Insecticide-related reasons: decrease in the number of insect control treatments by spraying as compared to gel treatments, decrease in the types of insecticide actives available for spraying as a result of the adverse effect of the European Biocidal Products Directive, worldwide resistance to pyrethroids and carbamates (Bajomi et al., 2012; Doggett, 2006; Boase, 2008).
Bed bugs are insects in a hidden habitat with high reproduction potential, and their control is difficult. For an effective control strategy, it is of important to know why bed bugs are increasing. Successful control of bed bugs largely depends on thorough and careful inspection (Papp, 2011).

MATERIALS AND METHODS

In this presentation we are reporting on the results of a survey conducted by means of questionnaire. During the past one and a half year, simultaneously with the bed bug treatments carried out by Bábolna Bio in apartments and institutions in Budapest, survey forms were also filled out. The survey was conducted in 105 locations between March 1, 2012 and August 30, 2013. We surveyed the level of infestation of the apartments and institutions to be treated, the type of buildings, the layout and the furniture of the apartments. We identified the probable mode of introduction and inquired about how long the people at the location had been aware of the presence of bed bugs. We also investigated signs indicative of bed bugs as well as their location within the apartment or the room. We used a questionnaire for the purpose of the survey.

RESULTS AND DISCUSSION

During the inspections and controls performed by Bábolna Bio over the 18-month period, bed bug infestations were detected on 91 occasions (86.7%) in apartments and on 14 occasions (13.3%) in public institutions. We are unaware of the number of pest control companies and the bed bug treatments performed by these companies during this period in Budapest, a city with 1.8 million inhabitants.
When surveying in apartments, and before treatment, the level of insect infestation in the areas to be treated. The goal of each survey was to confirm or to exclude an active bed bug infestation or, in case of an active infestation, to determine its extent. According to the number of bed bugs found in an apartment, four categories were determined: low level (1-10 bed bugs per apartment), medium level (11-30 bed bugs per apartment), high level (31-50 bed bugs per apartment), extremely high level (over 50 bed bugs per apartment). Figure 2 shows various infestations levels of the apartments. Before and during treatments in the apartments, we found low levels in 57 apartments which represent 62.6% of the treated apartments. Medium, high and extremely high levels were detected in 25 (27.5%), 8 (8.8%) and 1 apartments (1.1%), respectively.

We examined the building technology of the houses and found that more than half of the 91 treatments (52 occasions, 57.1%) were done in apartment buildings, mostly in old brick buildings in the central districts of Budapest (Figure 3). The number of apartments found infested in the panel-type buildings in housing estates was 37 (40.7%). This building technology favors bed bug infestation and active movement or migration of the insects within the building. Infestations in separate family houses were found on only 2 occasions (2.2%).

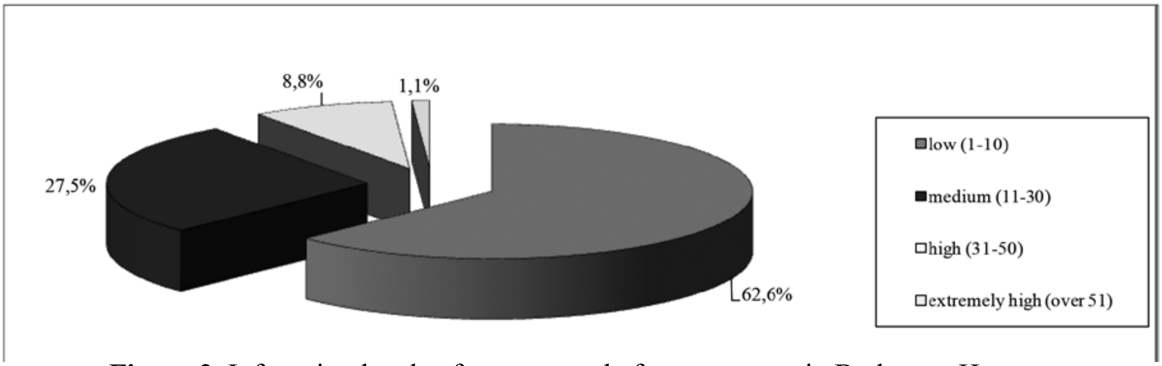

Figure 2. Infestation levels of apartments before treatment in Budapest, Hungary.

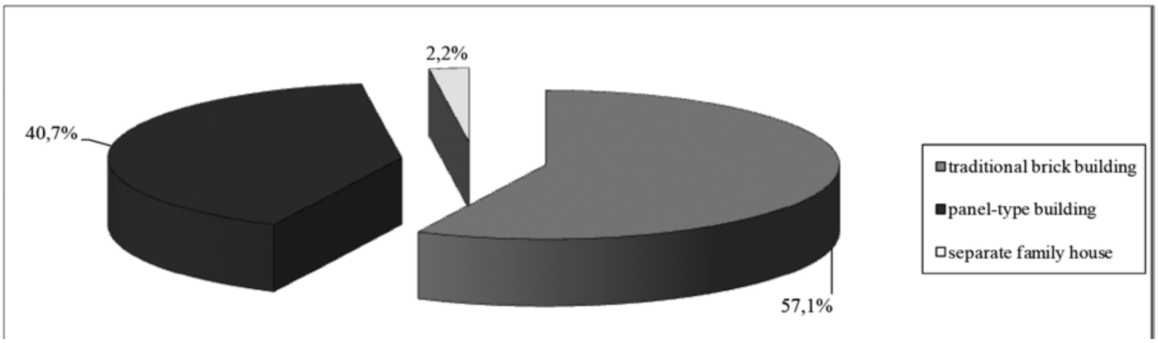

Figure 3. Ratio of infested apartments by building type in Budapest, Hungary.

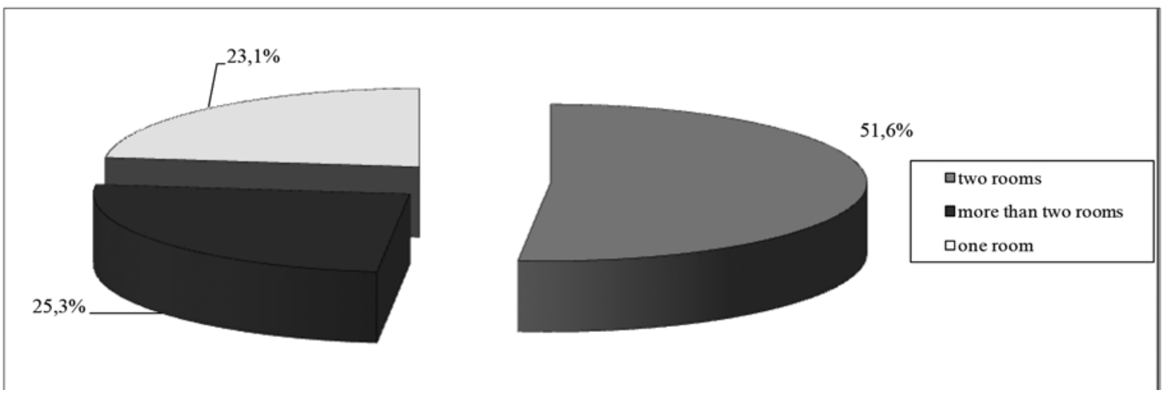

Figure 4. Number of rooms in bed bug infested apartments in Budapest, Hungary.

The ratio of bed bug infested multiple-room apartments represented 23.1%, 51.6% and 25.3%, respectively. 4.4% of them had lofts (Figure 4). This is an important element which requires special attention both during the survey and the control works as the wooden structures of lofts provide numerous harborages for bed bugs. The loft spaces had beds installed, and these places proved to be the most infested. 20%, 60% and 20% of bed bug infested apartments were under-furnished, fairly furnished and over-furnished, respectively.

As bed bugs primarily spread in a passive way, we wondered how people living or working in the apartments thought the infestation might have started. Figure 5 shows the presumed modes of introduction. For the most part (21.9%), people blame visits by relatives or guests for introducing bed bugs to their apartment. 21.9% of the respondents think they might have taken the bed bugs home from their workplace. It is worthy of note that half of them (12.4%) work in the public hygiene sector. 20% of the people inquired believed that infestation started from various articles for personal use and furniture. To obtain food, bed bugs move from one apartment to another mostly along the electric wires, water ducts and gas pipes of the panel-type buildings (Erdős et al., 2002). 16.2% of the tenants think that the infestation originated from their neighbor. Public transport in big cities also provides conditions suitable for bed bugs, and 11.4% of the persons participating in the survey considered this as the possible source of infestation of their apartment. 8.6% of the people associated the infestation with a trip inland or abroad.

Upholstered furniture, wall coverings, panel-works, loft spaces made of wood, wall-papers, crowded and cluttered apartments make their detection more difficult. Bed bugs leave dark, fluent traces of excrement immediately after feeding. These are often regarded as the first signs of an infestation (Doggett, 2006; Papp, 2011). In case of early infestations, excrement can be found near the bed only. It is advisable to check bedding for the stains of bed bug excrement. These traces may be distinctly visible on the wallpaper or light textile surfaces. As the exuviae remain intact for long, they are not suitable to differentiate between active and older infestations (Madge, 2011).

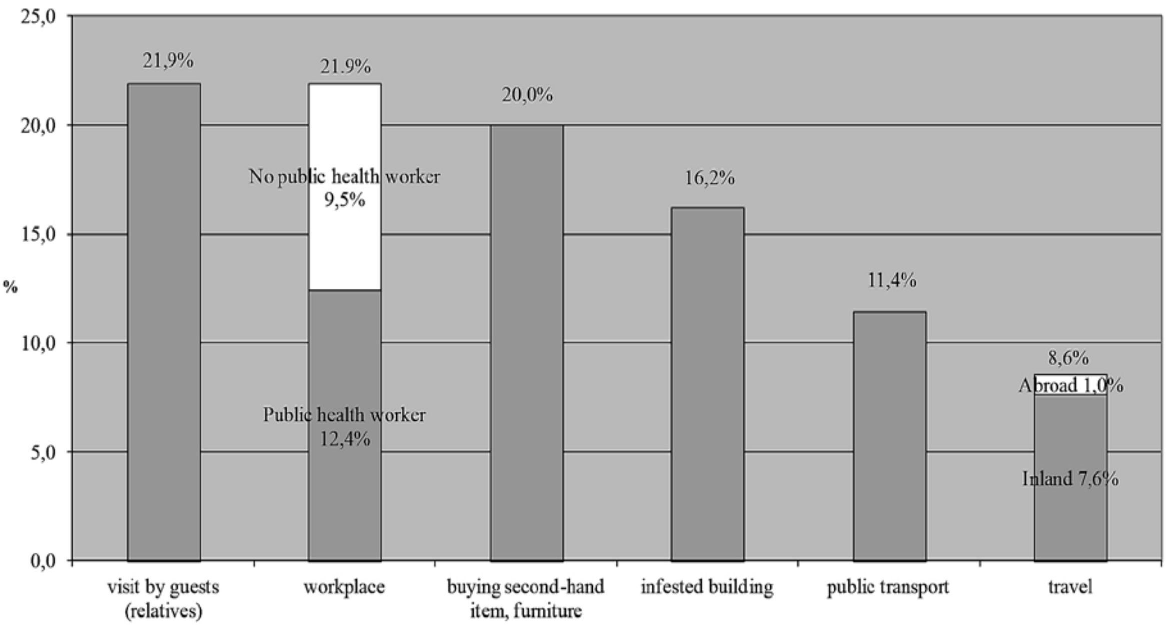

Figure 5. Presumed mode of introduction of bed bugs into apartments surveyed.

Active bed bug infestations are typically indicated by the bites. At 80 out of the 105 surveyed locations, the people living or working there reported bites. During the survey, pest control operators detected live or dead insects on 43 and 16 occasions, respectively, exuviae were found on 3 occasions. The excrement and the distinctive blood-stain denoted the presence of bed bugs in 26 and 23 cases, respectively. In case of minor infestations bed bugs are almost always found in or very close to the bed structure. The next question revealed where the insects were hiding within the apartment (Figure 6). In 67.6% of the cases (125) they were found close to the bed or immediate surroundings. Bed-frames and headboards represented 36.2% (67 cases), mattresses and mattress racks account for 31.4% (58 cases). The remaining 32.5% occurrence (60 cases) was divided as follows: couch, armchair 10.3% (19 cases), wall carpet, wall decoration 8.6% (16 cases), furniture 7.6% (14 cases), parquet floor 3.8% (7 cases), other locations 2.2% (4 cases).

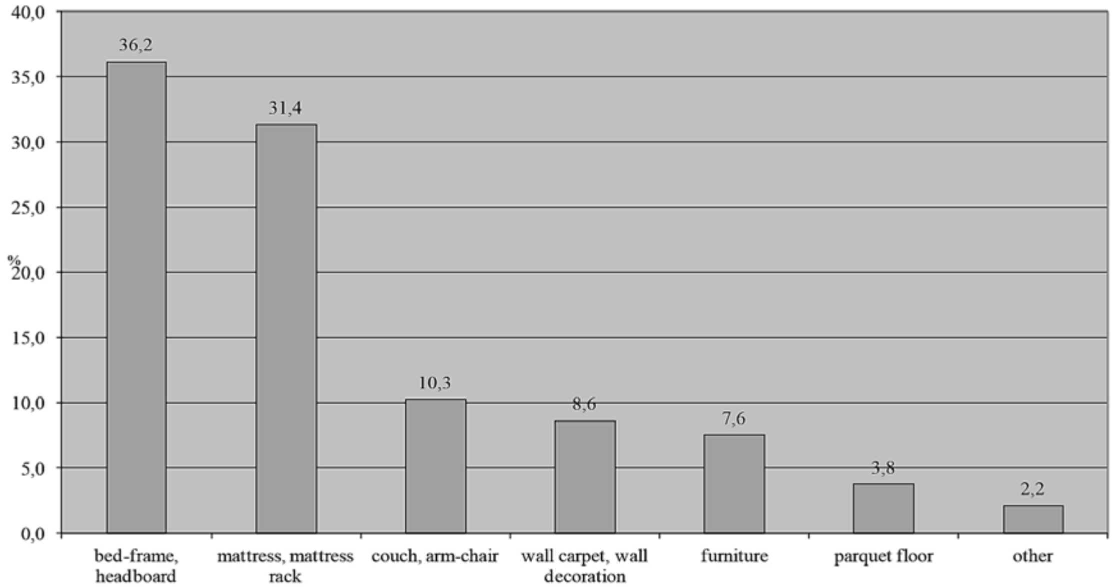

Figure 6. Occurrence of bed bugs in apartments surveyed.

Comparison of these data to the surveys made by the University of Kentucky in apartments in the United States reveals the similarities summarized in Table 1. The data in the table clearly show that the most infested spot in the rooms are the bed and its immediate surroundings. This is confirmed by both surveys: Bábolna Bio: 67.6%, University of Kentucky: 71.8%. About 30% of the bed bugs can be found further off the bed in any part of the rooms. This information is very important during control.

CONTROL

Bed bug control was mainly carried out by using Biopren 6 EC. The product has rapid flushing out and killing effect, low toxicity and can be applied to various textile surfaces including mattresses. The flushing out and the rapid killing action is ensured by the synergised natural pyrethrum, while the S-methoprene is responsible for disruption of the growth of eventually surviving larvae. The insect growth regulator active ingredient of the S-methoprene disrupts the life cycle of the insects by preventing their metamorphosis into adults (Bajomi, et al., 2011; Bajomi, et al., 2012).

In the highly infested places a residual insecticide (containing cifenotrin and d-tetramethrin active substance) was simultaneously used by adding to the working solution prepared with Biopren 6 EC (tank mix). In 28.6%, 35.2% and 16.2% of the total of 105 infested locations the bed bug infestation was eliminated with only one, two and three treatments, respectively. In 14.3% of the cases, an additional treatment (four in total) was necessary. 57.2% of the apartment bed bug infestations could be eliminated with one or two treatments. In locations with extremely high infestation five treatments were necessary but this represents only 5.7% (Figure 7).

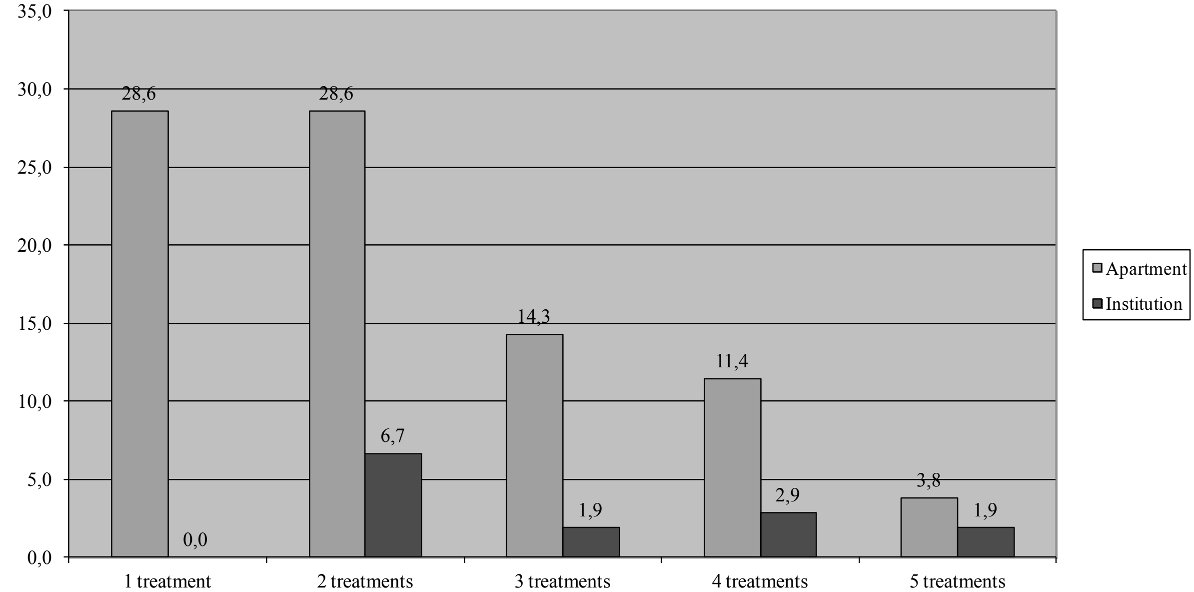

Figure 7. Number of treatments to control of bed bugs in apartments and institutions

It is worth examining the relation between the number of insecticide treatments necessary to achieve bed bug control and the initial insect infestation. We examined the initial level of bed bug infestation in those apartments where four or five treatments were necessary for the total elimination of bed bugs (Table 2). From these data one cannot establish a close relation between the initial infestation level and the number of treatments. There were cases when four treatments had been performed even if the initial infestation was low or medium. When five treatments proved to be necessary, the initial infestation was extremely high in one case only, while on the other three occasions it was medium. It is possible that the crowdedness of the apartments or the re-infestation during the treatments have a bigger role in terms of the number of treatments necessary to achieve full elimination than the initial bed bug infestation. Consequently, it can be declare that appropriate formulations and methods for bed bug control are available.

Table 1. Surveys of places bed bugs occurred during treatments.

Locations	**Bábolna Bio Ltd. %**	**University of Kentucky %**
Bed-frame, headboard	36.2	13.4
Mattress, mattress rack	31.4	57.0
Couch, armchair, easy-chair	10.3	22.6
Wall carpet, wall decoration, walls/ ceiling	8.6	2.3
Furniture	7.6	---
Other (parquet floor, etc.)	6.0	3.0
Wood slats	---	1.4

Table 2. Level of bed bug infestation in apartments and the number of treatments necessary to achieve bed bug free conditions.

Number of treatments	Initial Infestation Per Apartment			
	Low (1-10)	Medium (11-30)	High (31-50)	Extremely high (over 50)
4 treatments	3	6	3	0
5 treatments	0	3	0	1

CONCLUSIONS

The survey conducted in Budapest was the first ever attempt to obtain an objective picture of the existing infestation during bed bug control treatments. It can be definitely stated that the presence of bed bugs in Budapest, in line with international tendencies, has been gradually increasing over the last decade, but an abrupt increase occurred in 2007.

The survey shows that the great majority (90.1%) of the 91 apartments had low or medium bed bug infestation level at the time of the survey. Contrary to what had been expected, bed bug infestations in Budapest were more frequently (57.1%) found in traditional buildings made of brick than in panel-type buildings in housing estates. This confirms that bed bugs spread mainly by way of introduction instead of active migration.

Based on our findings, visits by guests or relatives, take-away from workplace, purchase of second-hand furniture, infestation on means of public transport or during travels play a major role in terms of bed bug introduction. It is especially worthy of note how often persons working in public hygiene take bed bugs home. The occurrence of bed bugs within the apartments is closely associated with beds (67.6%), (mainly upholstered) furniture (17.9%), walls and wall decorations or cracks in the parquet floor (12.4%).

In the majority of cases, the insecticide spray containing natural pyrethrum and S-methoprene IGR, occasionally complemented with residual cifenotrin tank mix, achieved total elimination with two or three treatments (63.9% and 16.2%, respectively). We did not find close relation between four or five treatments performed at the same location and the initial level of infestation.

For a successful control, performance of an initial inspection and a thorough control done with the utmost diligence seem to be more important. Success definitely requires close co-operation with the tenants or owners of the premises. Experience shows that bed bug infestations can be eliminated with the insecticides and methods that we have available.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

STUDIES ON PYRETHROID RESISTANCE IN CIMEX LECTULARIUS (HEMIPTERA: CIMICIDAE), IN BERLIN, GERMANY

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Abstract Pyrethroid-resistance in bed bugs, *Cimex lectularius,* has been described in many countries, but up to now, for Germany no data are available. However, pest control companies increasingly report difficulties in controlling bed bugs with pyrethroids. In the present study four bed bug strains were collected from infested apartments in Berlin and reared in the laboratory without insecticide selection pressure. In this work bed bug colonies are referred to as strains, which denotes their origin but individuals within a strain and not genetically uniform. A filter contact bioassay was developed and susceptibility of the collected bed bug strains against deltamethrin was determined in comparison to a pyrethroid susceptible laboratory strain. Resistance ratios, calculated from LD_{50}-values were between R_r 3.8 and R_r 5.1. Molecular studies regarding two mutations V419L and L925I in the voltage gated sodium channel α-subunit gene, which have been reported to be involved in knockdown resistance (*kdr*) in bed bugs collected from the USA, were also performed. Pyrosequencing of genomic DNA fragments showed the presence of mutation L925I in each of the four studied field populations with allele frequencies between 30% and 59%, while it was not detectable in the laboratory strain. Furthermore, none of the tested strains had the substitution V419L. The results demonstrate that decreased pyrethroid susceptibility of bed bugs is present in Germany but resistance levels are considerably lower than reported from the USA and Australia.
Key words Pest control, contact bioassay, Pyrosequencing, *kdr*-mutation.

INTRODUCTION

Over the last ten years there has been an upturn in reports on bed bug infestations in hotels, public buildings and private houses worldwide. International travel and migration, the trade of second-hand articles, regulatory restriction of chlorinated hydrocarbon-, organophosphate- and carbamate- insecticides and the evolution of insecticide resistance against all of them are considered to be responsible for the expansion of the bed bug *Cimex lectularius* (Davies et al., 2012; Doggett et al., 2012). Due to their low mammalian toxicity and a rapid effectiveness (knock down effect), pyrethroids are mainly used for bed bug control. However, the presence of pyrethroid-resistant bed bugs has frequently been reported in recent years (Moore and Miller, 2006; Romero et al., 2007; Boase, 2008; Yoon et al., 2008; Lilly et al., 2009; Zhu et al., 2010; Suwannayod et al., 2010; Tawatsin et al., 2011). German pest control companies have also observed increasing difficulties in controlling bed bugs with pyrethroids, but for Germany no published data are available yet. Knockdown resistance (*kdr*) to pyrethroids has been shown to be associated with the presence of two point mutations (V419L or L925I) in the voltage gated sodium channel α-subunit gene (Yoon et al., 2008; Zhu et al., 2010; reviewed by Davies et al., 2012). In the present study, susceptibility to deltamethrin in four field strains of *C. lectularius* was determined in a filter contact bioassay. Furthermore, pyrosequencing of genomic DNA fragments of single and pooled bed bug samples was performed in order to analyze the presence of the two polymorphisms (V419L, L925I) and to evaluate the genotypes by which they appear.

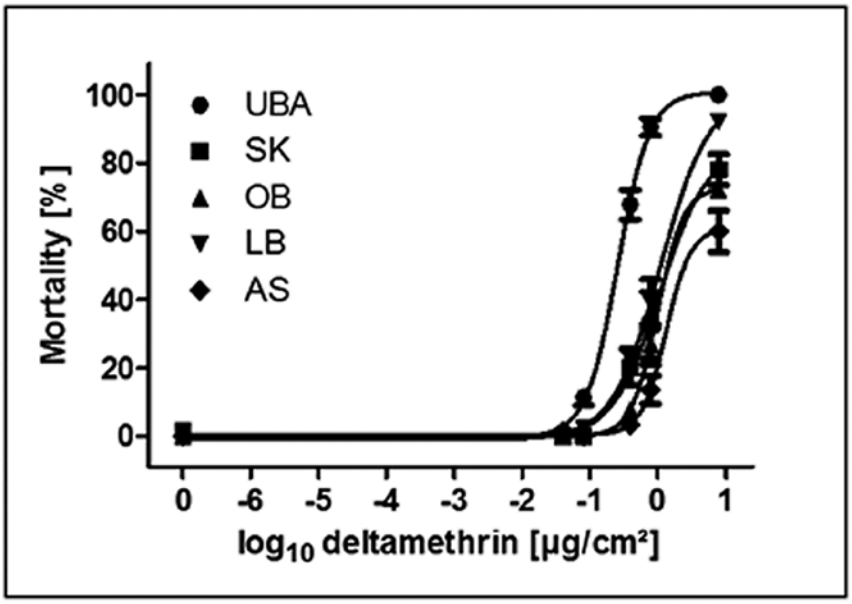

Figure 1. Dose response curves for effects of deltamethrin on mortality (%) of five Cimex lectularis strains in a filter contact bioassay. Male bed bugs were exposed to the active substance for 24 h. UBA is the susceptible reference strain, strains SK, OB, LB and AS were recently collected and isolated in Berlin and successfully adapted to laboratory maintenance.

MATERIALS AND METHODS

Bed bug populations. In this work bed bug colonies are referred to as strains, which denotes their origin but individuals within a strain and not genetically uniform. Bed bug field strains were collected from 20 infested apartments in Berlin. Of these 20, twelve collected samples consisted of less than 30 bed bugs, which was insufficient to establish a laboratory strain. From the eight remaining strains, laboratory rearing of four field strains was successful up to now (SK, OB, LS and AS strain). Three strains failed to breed, and one strain has not been included in our study due to technical reasons. Rearing was performed without insecticide selection pressure. Sufficient numbers of bed bugs were obtained about one year after initial introduction to the laboratory. The insecticide-susceptible laboratory *C. lectularius* strain of the Federal Environment Agency (UBA) has been kept since 1947, with several refreshments of the genetic pool. All strains were held in petri dishes with filter paper (Whatman® round filter, 70 mm Ø, 87 g/m^2, thickness 180 µm) kept in an incubator (24 h darkness; 25 ± 3°C and 45 ± 10% humidity) and were fed weekly on rabbits.

Filter contact bioassay. Male bed bugs of both field and laboratory strains were exposed for 24 h to deltamethrin on filter papers (Whatman® filter, 87 g/m^2, thickness 180 µm) in 24-well cell culture plates (adapted from Romero et al., 2007). The bed bugs had a total of seven feedings and were tested eight days after their last blood meal. Concentrations of technical grade deltamethrin (99% active ingredient, Bayer Environmental Science) were generated by dilution in acetone (for analysis, 99.8%, Merck KGaA) and ranged from 0.0005 µg/µl, 0.001 µg/µl, 0.005 µg/µl, 0.01 µg/µl to 0.1 µg/µl. An amount of 50µl was pipetted on each filter paper disc (0.64 cm^2). Controls consisted of acetone treated filter papers only. All filter papers were allowed to dry for 30 minutes, before being placed in the bottoms of 24-well cell culture plates with forceps. Each concentration was tested on a plate with 18 individual bed bugs which were exposed to deltamethrin and six bed bugs as a control. In order to prevent aggregation, only one bed bug was placed into each well. Plates were closed and stored in darkness at room temperature. Testing of each concentration was repeated five times (total number of bed bugs: n = 90 insecticide-exposed bed bugs and n = 30 as controls for each concentration). The UBA strain has always been tested in parallel to the field strains. Mortality was determined after 24 h exposure by gently touching each male bed bug with a forceps. Individuals were categorized as vital by showing a normal movement behavior or as dead when no or only uncoordinated movement was observed and bed bugs in dorsal position were not able to turn back into ventral position (bed bugs did not recover). LD_{50} values were calculated by using logit analysis and resistance ratios ($R_r = LD_{50}$ resistant strain/ LD_{50} susceptible strain) were determined. Statistical differences between the LD_{50} values for each field strain and the susceptible UBA strain were calculated with a sum of square F-Test in Graphpad Prism 5.0 and p-values were adjusted using the Bonferroni correction.

Pyrosequencing of genomic DNA. Genomic DNA of 100 pooled male *C. lectularius* per strain was isolated with the NucleoSpin® 8 Tissue Kit (Macherey-Nagel) and examined for the presence of the two *kdr*-polymorphisms (V419L and L925I) and their allele frequencies. For the study on single bed bugs, DNA from ten males per population was isolated individually with the same method. DNA fragments with the potential point mutations V419L and L925I were amplified by PCR (Phusion Hot Start II High-Fidelity DNA Polymerase, Thermo Scientific or Biozym) using sequence specific primer pairs (V419Lup: 5´- GTGGCACATGTTGTTCTTCATAGT-3´, V419Llo(biotin) 5´-CGCCTTCTTTGCAGTTCA-3´; L925Ilo: 5´- CCCATCACAGCAAAGATGAAAAT-´3, L925Iup(biotin): 5´-ATTATGGGCAGAACAGTGGGT-3´). For the subsequent quantitative analysis of both genetic DNA modifications, pyrosequencing (PyroMark® Q24 System and Software, Qiagen) was performed as described in the PyroMark® Q24 User Manual (sequencing primer for V419L: 5´-CCTGGGATCATTCTACC-3´and L925I: 5´-ACACAAAAGTTAAATTACCA-3´). For each of the four field strains five technical repeats were analyzed. Differences between the susceptible UBA strain and the field strains were tested for statistical significance with a one way ANOVA and p-values were adjusted with Tukey's multiple comparison post test (Graphpad Prism 5.0).

RESULTS AND DISCUSSION

Susceptibility to Deltamethrin

The differences in deltamethrin LD_{50} values between the UBA strain and the four field strains were found to be statistically highly significant ($p<0.0053$ for all strains; Fig. 1; Table 1). As expected, the LD_{50} value of the insecticide susceptible laboratory strain was low (0.258 µg/cm^2), confirming high susceptibility against deltamethrin. LD_{50} values of the field strain bed bugs were 1.072 µg/cm^2 for the SK strain, 0.989 µg/cm^2 for the OB strain, 1.095 µg/cm^2 for the LB strain and 1.319 µg/cm^2 for the AS strain. However, there was no significant difference in the LD_{50} values between the four field strains. Resistance ratios of the field populations relative to the UBA control strain ranged between 3.8 and 5.1 (Table 1).

Table 1. Comparison of deltamethrin LD50 values (n=90 males) between the susceptible reference strain (UBA) and the four recently to the laboratory adapted strains collected in Berlin (The differences in LD50 values were found to be statistically highly significant with p<0.0053 for all strains). Resistance ratios (Rr =LD50 resistant strain/ LD50 susceptible strain) and coefficients of determination (R2) for all dose response curves are given.

Strain	LD_{50} (µg/cm²)	R^2	Rr
UBA	0.258	0.95	-
SK	1.072	0.8844	4.2
OB	0.989	0.9632	3.8
LB	1.095	0.0637	4.2
AS	1.319	0.9192	5.1

It has to be noted that the resistance ratios observed herein are considerably lower than those reported from the USA, amounting to e.g. 5,200 (Adelman et al., 2011) and 12,800 (Romero et al., 2007) or resistant ratios higher than 432,000 in Australia (Lilly et al., 2009). A possible explanation for low R_rs might be the rearing of the field strains without insecticide selection pressure over a period of more than one year, causing loss of mutations causing resistance (Zhu et al., 2013). The latter explanation is supported by the observation that resistance ratios in the first collected field strain SK considerably decreased between initial filter contact bioassays in 2009 (unpublished data) and the re-examining in 2012 as presented in this paper. Another reason could be that in our studies only R_rs of those field populations were determined, which we were able to reproduce in the laboratory. Rearing of three field strains could not be accomplished, possibly due to fitness disadvantages caused by the presence of insecticide-resistance, which has been observed in other insects (Roush and McKenzie, 1987; Brito et al., 2013). This would implicate, that in our studies sample selection of the field populations was not random and R_rs were selectively determined in populations with lower insecticide susceptibility. Up to now, nothing is known about the genetic structure of German bed bug populations. It is also possible that the field strains in our study descended from local reservoirs where the prevalence of pyrethroid-resistance causing alleles is low. However, the differences in R_rs as seen in our study compared to those reported from other countries may also reflect the actual occurrence of R_rs and may not be influenced by methodological biases. If this would be the case, a possible explanation for lower R_rs in Germany would be a result of differing bed bug control methods. Up to now, no comparative data on practical control routines in different countries are available.

Allele Frequencies of the Two Point Mutations V419L and L925I

Pyrosequencing revealed the absence of the two mutations in the susceptible laboratory strain. Only the L925I (CTT→ATT) substitution was identified in the four collected field strains. Pyrosequencing indicated allele frequencies of 30% mutated fragments in the LB strain. The OB strain showed the highest frequency of the mutated allele with 59%. The allele frequencies of the SK and AS strain were intermediate with 44% and 51%. None of the tested strains had the V419L substitution (GTC→CTC), which generally rarely occurs in bed bug populations (Table 2). These results are supported by several studies from the United States (Zhu et al., 2010; Zhu et al., 2013). Statistically significant differences in allele frequencies were found between the susceptible UBA strain and each of the four field strains ($p<0.0005$ for all strains). In addition, significant differences between SK and OB strain ($P<0.0005$), SK and LB strain ($p<0.005$), OB and LB strain ($p<0.0005$) and LB and AS strain ($p<0.0005$) were observed. These results demonstrate that the point mutation L925I is present in German bed bug populations. However, the finding of Zhu et al. (2010) that even the single mutation L925I could confer significant deltamethrin resistance and thus high resistance ratios could not be confirmed. In contrast, it was found that although the allele frequencies of the four field strains differed significantly between the strains there were no significant differences in their in vitro susceptibility to deltamethrin. None of the published studies provides information about the impact of homo- and heterozygosity of both point mutations on the resistance status of bed bug populations. Results from ongoing tests on single bed bugs showed in each field strain non-mutated as well as heterozygous bed bugs for the allele L925I were present. Additionally, *kdr* homozygous bed bugs were found in the OB, LB and AS strains (Table 2). Therefore, a possible explanation for the low resistance ratios observed in our study could be that although the mutation L925I was detected in each tested field strain, only bed bugs which are homozygous regarding the mutated allele show high tolerance against pyrethroids. This hypothesis is supported by results regarding other *kdr* mutations in insects which indicate that the *kdr* allele is fully recessive resulting in a close positive correlation between high LD_{50} values for pyrethroids and the frequency of *kdr* homozygotes in flies or mosquitoes (Huang et al., 2004; Saavedra-Rodriguez et al., 2007). This may also hold true for the *kdr* alleles in bed bugs and could explain the considerably lower susceptibility to pyrethroids which we found in our study.

Table 2. Frequencies (among n=100 males) of both SNPs (V419L and L925I) and the different genotypes (homozygous susceptible (HS), heterozygous (H) and homozygous mutated (HM)) of the L925I mutation in 10 single male bed bugs of the four field strains and the susceptible UBA strain, respectively. Background signals of approximately 10% for both alleles were often detected in the absence of the mutation.

Strain	Allele frequencies (%)				Genotypes of L925I		
	V419L	SD	L925I	SD	HS	H	HM
UBA	4	2.96	11	2.07	+	-	-
SK	4	2.34	44	4.82	+	+	-
OB	4	2.97	59	6.11	+	+	+
LB	4	3.03	30	4.28	+	+	+
AS	5	3.63	51	6.87	+	+	+

It has been shown that the *kdr* mutations are not the only mechanisms which can cause pyrethroid resistance in bed bugs. An increased expression of genes coding for cuticular proteins and an up-regulation of ABC transporters which are responsible for the translocation of many substrates and xenobiotics causing lower concentration of insecticides at their target site should also be considered to be responsible for pyrethroid resistance in *C. lectularius*. An increased metabolic detoxification by esterases, glutathione S-transferases, and especially by cytochrome P450s have been described as additional mechanisms causing pyrethroid resistance in bed bugs (Bai et al., 2010; Adelman et al., 2011; Mamidala et al., 2011; Mamidala et al., 2012; Zhu et al., 2013). To determine whether multiple resistance mechanisms are interacting in the bed bug strains collected in Berlin, further analyses of enzyme expression patterns are currently in progress.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

ASSESSMENT OF NATURAL-BASED PRODUCTS FOR BED BUG (HEMIPTERA: CIMICIDAE) CONTROL

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Abstract Four essential oil-based products and a liquid-based borate spray were tested for their efficacy against adult, pyrethroid-resistant, field-strain and Harold Harlan susceptible-strain bed bugs using direct spray applications. The most effective product was Green Bug (100% mortality in one hour or less based on strain) followed by Bed Bug Patrol (100% mortality in one-two weeks based on strain). All other products failed to exceed 70% mortality over the two-week trial. Despite promising results of two of the products tested, results of field applications may differ due to: a) the difficulty of locating bed bugs for direct applications, b) the uncertainty of repellency in the presence of hosts and associated host cues, c) a lack of data on mortality as a result of contacting an insecticide-treated surface, and d) the duration of product efficacy (repellency or mortality) when applied to a surface. Considerations for future research are discussed.
Key words *Cimex lectularius*, insecticides, essential oils.

INTRODUCTION

The bed bug (*Cimex lectularius* L.) is a cryptic, nocturnal, hematophagous insect that has been a pest of humans for at least four millennia (Panagiotakopulu and Buckland, 1999). While *C. lectularius* was considered to be fairly common in the USA and other countries until the middle of the 20th century (Usinger, 1966), the 1940's marked the advent of dichlorodiphenyltrichloroethane, an insecticide more commonly known as "DDT". At first considered highly effective, some bed bug populations had begun showing resistance to DDT as early as the 1940's, with Johnson and Hill (1948) reporting a pesticide resistant population from Pearl Harbor in 1947. DDT was banned in the 1970's due mainly to environmental concerns and resistance. Next generation insecticides (carbamates and organophosphates) replaced DDT and were generally effective, but have since been banned due to regulatory concerns (Weeks et al., 2011,; Boase, 2001). Our most effective modern insecticides, particularly pyrethroids, are now showing poor control due to widespread insecticide resistance (Romero et al., 2007).

Today, integrated pest management (IPM) techniques are needed and used to control bed bug infestations. Aspects of IPM programs may involve proper inspection, isolation, and treatment of infested items/areas prior to the arrival of a pest control firm, which may use heat, cold, or pesticide treatments singularly or in conjunction (Potter, 2008, Lewis et al., 2009). Such involved and expensive treatments have opened a market for alternative strategies. As expected, a variety of "do it yourself" bed bug products have emerged on the scene. Claiming to kill or repel bed bugs, these products consist principally of extracts from aromatic plants (thyme, cedar, lemongrass, etc.). Although essential oils have been shown to be environmentally safe, and exhibit repellent and insecticidal properties (Isman, 2000), there is a lack of scientifically credible data on the application of these products for bed bug control. An essential oil may prove to be a valuable tool when attempting to control bed bugs, but an ineffective oil may serve no other purpose than to delay the implementation of effective treatment.

The objective of this study was to examine the efficacy of four commercially available essential oil products and one liquid-based borate product when applied as a direct spray to adult, field-strain, pyrethroid-resistant (referred to as field-strain bugs) and Harold Harlan-strain, susceptible bed bugs (referred to as Harold Harlan strain bugs).

MATERIALS AND METHODS

Four commercially available essential oil products and a borate-based spray were examined for direct-spray efficacy. Four of these products were specifically labeled for bed bugs, including Bed Bug Patrol® (Nature's Innovation: Buford, GA), Green Bug (All Natural Pest Control: Beaufort, SC), Cymex® (Nisus Corporation: Rockford, TN), and Rest Easy® (Green Rest Easy: Memphis, TN). Triple Action Neem Oil® (Southern Agricultural Insecticides, Inc.: Boone, NC) was also examined to compare bed bug products to an essential oil not labeled for bed bugs. Bed Bug Patrol ingredients included 0.03% Clove Oil, 1.0% Peppermint Oil, 1.3% Sodium Lauryl Sulfate, and Citric acid, Glycerin, Oleic acid, and water as 97.67% inert ingredients. Green Bug ingredients consisted of 90% silane fluid and 10% cedar oil. Cymex consisted of 8.5% Disodium Octaborate Tetrahydrate and 91.5% "other ingredients". Triple Action Neem oil included 70% clarified hydrophobic extract of neem oil and 30% inert ingredients. A control treatment consisting of distilled water was included.

A total of 240 adult bed bugs (120 Harold Harlan strain, 120 field strain) maintained at 26°C, ~40% R/H, and 12L/12D photoperiod were removed from colony and fed defribrinated rabbit blood five days prior to treatment. Bed bugs were fed to repletion (~30 minutes) using an artificial feeding system. Bed bugs were housed in altered condiment cups during and after direct sprays. Each container was created by removing the bottom of a 59 ml (~60 mm diameter x ~30 mm height), plastic condiment cup with scissors before using melted wax to fasten one piece of 90 mm Whatman filter paper to the bottom of the cup, forming a new base. This base was intended to absorb excess essential oil and prevent pooling and envelopment of bed bugs. A snap-on lid was modified for each cup by removing a circle ~2.5 cm in diameter from the lid's center. A small circle of mesh was then waxed over the hole. This design prevented escape, and provided bed bugs with a fresh air supply. Five bed bugs were placed in each of 48, 30 ml (~40 mm diameter x ~45 mm height) medicine cups several hours before spray applications. As bed bugs were incapable of climbing medicine cups, but were able to climb the sides of the larger condiment cups, bed bugs were dumped from medicine cups to condiment cups seconds prior to being sprayed. Although the essential oil products examined did not include label rates, bed bugs were sprayed using a Pistol Pro (B&G Equipment Company: Jackson, GA) at a rate similar to commonly available industry products. The mortality of bed bugs was assessed at 1 minute, 1, 3, 6, 12, and 24 hours, and once per day for 14 days by prodding bed bugs with a toothpick. One toothpick was used per condiment cup to avoid transferring product to other bed bugs. Bed bugs that exhibited no movement were marked as dead. Proportion of bed bugs killed per product, per time, were compared within each strain using ANOVA followed by Fisher's LSD test. The model used for the analyses included terms for product (d.f.=5), block (d.f.=3), and error (d.f.=15). Products were considered significantly different when p-values were less than $\alpha=0.05$.

RESULTS AND DISCUSSION

The efficacy of the products differed based on product and time post application (Figures 1 and 2). Green Bug produced 100% mortality among Harold Harlan-and field-strain bugs within one hour, while Bed Bug

Patrol produced 100% mortality at 7 days among field-strain bugs, and 14 days among Harold Harlan-strain bugs. Though comparisons among all products and all time blocks are too detailed to present in full, Fisher's LSD tests revealed that Green Bug differed significantly from all other products until Bed Bug Patrol reached a similar mortality at day two (field strain bugs) or day five (Harold Harlan-strain bugs). Within the exception of Bed Bug Patrol (day 13), Green Bug and Bed Bug Patrol differed from all other products throughout the remainder of the trial. Rest Easy and Cymex were moderately effective. Rest Easy differed from the control from 1 hour until the end of the trial for field-strain bugs, and from 1 hour until the end of the trail for Harold Harlan-strain bugs, with the exception of day 10. Cymex differed from the control for days 12-14 only for both bed bug strains, and only differed from Rest Easy at 1 hour for Harold Harlan-strain, and 1 hour through 13 days for field strain. Triple Action Neem Oil failed to differ significantly from the control at any time for either strain. Curiously, Triple Action Neem Oil, Bed Bug Patrol, and even the control applications produced higher mortality at an earlier period for pyrethroid resistant bed bugs. This may be due to some unknown fitness cost associated with resistance, a phenomenon which has been documented among insect resistance to *Bacillus thuringiensis* (Gassmann et al., 2009).

Of the five products tested, only Green Bug and Bed Bug Patrol produced mortality exceeding 70%. As studies of bed bug population dynamics have shown that infestations often originate from a few bed bugs or even a single mated female (Booth et al., 2012), 70% is an unacceptable mortality level. Considering that bed bugs were directly sprayed, maintained on a sprayed surface, and deprived of nutrition and harborage for 14 days, even these generally poor kill rates likely exceed those that would occur in the field, where bed bugs would have the option of returning to an untreated harborage to aggregate and await additional blood feeding opportunities. Previous research has shown that bed bug aggregations reduce mortality (Benoit et al., 2007), and that some insects can recover from insecticides when provided with meals during or after exposure (Cox and Parish, 1991).

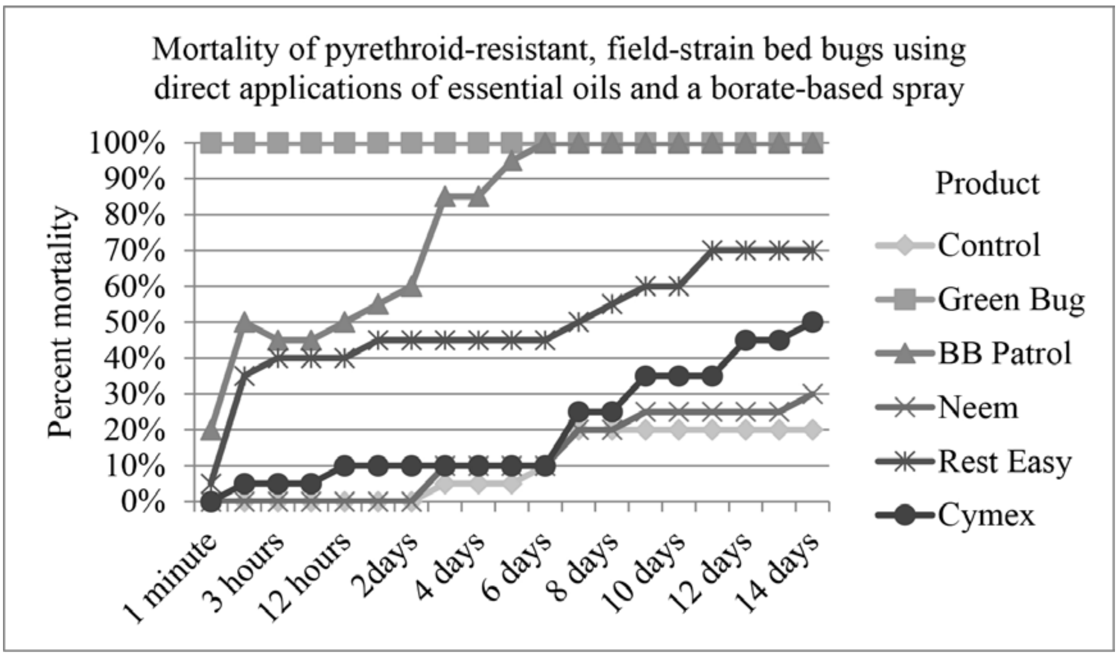

Figure 1. Mortality of pyrethroid-resistant, field-strain bed bugs expressed as percent mortality over a 14 day period.

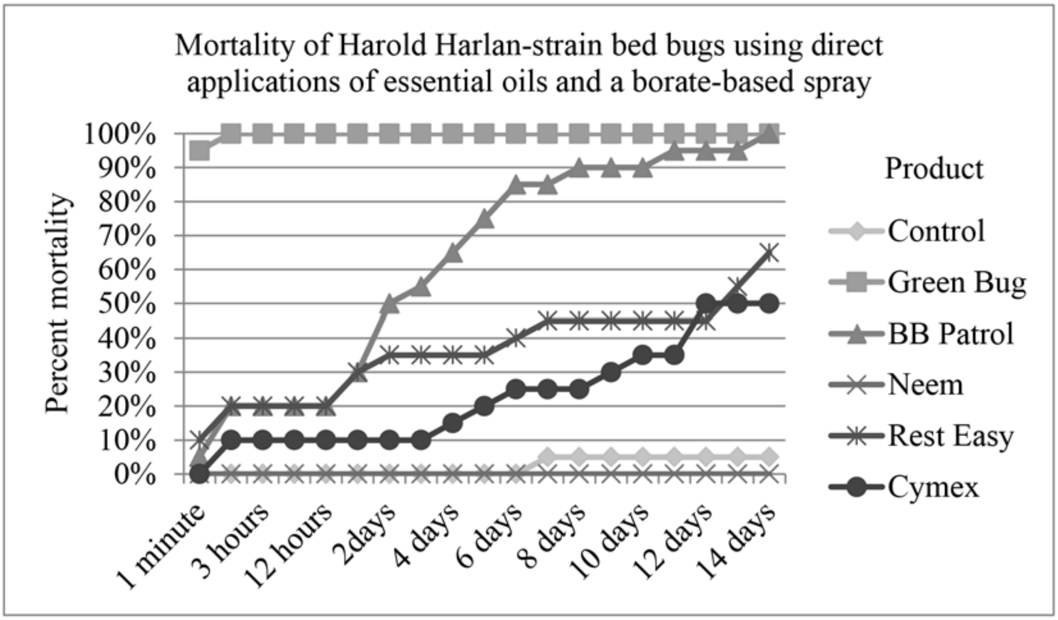

Figure 2. Mortality of Harold Harlan-strain bed bugs expressed as percent mortality over a 14 day period.

CONCLUSION

Although Green Bug produced 100% mortality within 1 hour for both strains, such seemingly effective products should still be viewed with skepticism. Effective bed bug products should have residual efficacy, due to the fact that bed bugs are difficult to locate and eliminate with direct sprays (Romero et al., 2009). Many manufacturers of essential oils claim that their products kill or repel bed bugs, yet fail to define any parameters relating to such claims. Future research will focus on whether these products yield any level of mortality when applied to a surface, as well as the duration of the product's efficacy. Claims of repellency, and duration, should be investigated by other researchers, and should be defined as repellency in the presence or absence of a host. Whether these products leave any type of stain or residue should also be investigated. Additional, replicated and controlled studies should examine silane fluid and cedar oil separately to determine factors that contributed to the high mortality rate produced by Green Bug.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

INSECTICIDE RESISTANCE IN *CIMEX LECTULARIUS* (HEMPTERA: CIMICIDAE) IN AUSTRALIA

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Abstract Insecticide resistance in bed bugs (*Cimex lectularius* L. and *C. hemipterus* F.) is a major factor in the pest's resurgence. Various studies have demonstrated resistance to the pyrethroids, carbamates and the organophosphates. Resistance has been suspected in Australia, with anecdotal reports of poor product performance. Laboratory studies have demonstrated insecticide resistance in a previously suspected-resistant field strain of bed bugs, with substantial differences in LD_{50} values when compared to a susceptible strain. The resistance factors for each compound were: permethrin = 1.4 million, deltamethrin = 430,000, bendiocarb = 240, pirimiphos-methyl = 2.8, imidacloprid = 2.7. Resistance was confirmed in the field-collected strain with the pyrethroids and carbamates, but not the organophosphates or neonicotinoids. Professional pest management operations require the development of new strategies to combat the pest. Regulatory authorities must consider the implications of resistance to multiple insecticide groups when registering new products utilizing either existing or new modes of action.

Key words Bed bugs, pyrethroid, carbamate, organophosphate, neonicotinoids.

INTRODUCTION

Over the last two decades a major bed bug resurgence has occurred internationally. Australia has not been immune, with infestations increasing over the years 2000-2006 by 4,500% compared with pre-2000 (Doggett and Russell, 2008). A Sydney based pest management company alone reported a 700% increase in the number of treatments for the period 2000-04 (Doggett et al., 2004), with this trend continuing to an eventual 3,500% increase for the decade 2001-2011 (unpublished data).The development of resistance amongst populations of bed bugs is one of the commonly mentioned theories for their resurgence internationally (Doggett et al., 2004; Doggett et al., 2012, Myamba et al., 2002; Romero et al., 2007; Wang and Cooper, 2011).

Resistance to insecticides has become an increasingly serious international problem. Since the earliest known resistance to DDT in field populations of house flies (*Musca domestica* L.) was reported (Lindquist and Wilson, 1948; Sacca, 1947), over 540 species of insect have developed resistance to one or more insecticides (Yu, 2008a). A key problem of insecticide resistence is that it limits the available insecticidal control options that could be classed as efficacious whilst simultaneously forcing reliance on alternatives that are 'either expensive, not readily available, or not particularly effective' (Robinson and Boase, 2011). Understanding the basis and function of insecticide resistance is, therefore, critical to detecting when it evolves, in order to promptly implement effective resistance mitigation programs and develop improved control techniques to manage its effects in field situations.

Resistance in field collected *C. lectularius* to several common pyrethroid insecticides has been confirmed in the United States (Adelman et al., 2011; Koganemaru et al., 2013; Moore and Miller, 2006; Romero et al., 2007; Yoon et al., 2008). Similarly, in the United Kingdom resistance has been found to the pyrethroids and also the carbamates (Boase et al., 2006). Resistance to permethrin has also been found to be prevalent amongst infestations in Denmark, although not correspondingly so with chlorpyrifos (an organophosphate) with only low rates of resistance being detected (Kilpinen et al., 2011).

The degree of resistance detected in the above studies has been typically high, although it has since been discovered the magnitude of pyrethroid resistance may vary according to the class of the compound assayed. Type I ('non-cyano' pyrethroids such as permethrin, d-phenothrin, pyrethrins etc.) will cause predominantly lower mortality compared to Type II compounds ('α-cyano' pyrethroids such as deltamethrin, cyfuthrin, cypermethrin and fenvalerate etc.) when applied to a suspected resistant field strain (Anderson and Cowles, 2012).

Pyrethroid resistance exhibited in *C. lectularius* in the United States appears widespread and the result of multiple resistance mechanisms (Zhu et al., 2013; Zhu et al., 2010). Two *kdr*-type target site point mutations (V419L and L925I) have been identified (Yoon et al., 2008) and subsequently found to be highly prevalent, being detected (in various haplotypes) in 88% of 110 bed bug infestations sampled from across the country (Zhu et al., 2010). A *C. lectularius* strain from Richmond, VA has also been found to exhibit over expression of genes consistent with cytochrome P450 monooxygenase and carboxylesterase mediated metabolic resistance, in addition to *kdr*-type (L925I) resistance (Adelman et al., 2011). The same Richmond, VA strain has since been found to exhibit cuticular resistance with a three-fold difference in mortality when insecticide is applied via injection as opposed to topical application (Koganemaru et al., 2013). Zhu et al. (2013) also identified resistance-associated genes expressed in the epidermal layer of the integument.

In 2002, resistance in the tropical bed bug (*C. hemipterus*) to permethrin and α-cypermethrin was also recorded for the first time with a modern field infestation in Tanzanian villages where pyrethroid impregnated nets are used on beds (Myamba et al., 2002). Resistance in *C. hemipterus* has since been further confirmed with studies demonstrating resistance variously to the organochlorines, organophosphates, carbamates and pyrethroids in Sri Lanka (Karunaratne et al., 2007) and Thailand (Suwannayod et al., 2010; Tawatsin et al., 2011).

It should perhaps not be surprising that modern populations of both *C. lectularius* and *C. hemipterus* have developed resistance, particularly to the pyrethroids, given that prior to their near eradication as a public health pest in the 1960s and 1970s, bed bugs had begun to develop resistance to many insecticidal compounds (Brown, 1956; Busvine, 1958; Feroz, 1969; Gaaboub, 1971; Johnson and Hill, 1948; Sharma, 1963) and particularly those from the organochlorine family which can infer cross-resistance to the pyrethroids. There were even early reports of resistance to the pyrethrins; Busvine (1958) found two strains of bed bugs, one each of *C. lectularius* and *C. hemipterus* that had reduced susceptibility to this insecticide.

It is essential that resistance be monitored in order to detect and manage its effects for the improvement of eradication procedures and best practice in pest control. Until recently, the susceptibility status of field infestations in Australia remained unclear and could only be assumed based on anecdotal reports from pest control companies of poor product performance (Doggett and Russell, 2008) and generally low efficacy of several formulated products when screened in laboratory trials (Lilly et al., 2009a, 2009b). Here we examine and discuss insecticide susceptibility of a field collected strain of *C. lectularius* compared to an imported known-susceptible strain in response to exposure to a wide range of insecticide groups.

MATERIALS AND METHODS

Cimex lectularius Strains

Two strains were used in this study; the Sydney strain, where the founder specimens were collected from various locations in Sydney during 2004-5 and is suspected to be resistant, and the 'Monheim' strain, a known susceptible strain that was obtained from Bayer, Germany, and originated from around the late 1960s.

Dose Response Assays

Five compounds were selected for the trial; bendiocarb, deltamethrin, permethrin, imidacloprid and pirimiphos-methyl (Table 1). These reflected the major classes of insecticides registered for bed bug control in Australia at the time of the study. The compounds were serially diluted to obtain results that produced 0-100% mortality for each insecticide and bed bug strain. Bed bugs were temporarily immobilised by securing the dorsal surface to a small strip of double-sided tape and a 1μL drop of the diluted insecticide applied to the ventral surface. The insecticide was allowed to dry, the bed bugs removed and mortality recorded at 24 hours. For each product tested, there were four replicates of ten bed bugs each and an equivalent number of controls (which received the diluent only). LD_{50} values were calculated by Probit analysis.

RESULTS AND DISCUSSION

When profiled, all compounds tested against the Monheim strain returned clear dose responses and overall high levels of insecticidal activity.

Carbamates

Bendiocarb was highly efficacious against the Monheim strain, but not against the Sydney strain. The absence of a full dose response for the Sydney strain resulted in a high level of variance within the confidence intervals but, nonetheless, the factor of difference between the calculated LD_{50} values was determined to be 239.

Pyrethroids

Permethrin and deltamethrin both performed extremely poorly when tested against the Sydney strain, with neither compound reliably achieving >60% mortality at the highest dose of 100μg/μL with the Probit estimations subsequently returning highly heterogeneous results. This was in contrast to the Monheim strain, where both compounds returned highly efficacious results. The resistance factors separating the two strains for these compounds were consequently large (1.4 million and 432,042 respectively).

Organophosphates and Neonicotinoids

Pirimiphos-methyl was efficacious against both the Sydney and Monheim strains, although the results proved significantly different. The results for imidacloprid mimicked the degree of difference (≈2.7x) between the Sydney and Monheim strains for pirimiphos-methyl. The result of our study conforms to, and extends, previous resistance studies undertaken against field and laboratory strains of *C. lectularius*.

Table 1. Insecticides selected for topical susceptibility testing against *Cimex lectularius*

Compound	Group[a]	Products Registered in Australia	Resistance Internationally	Resistance in Australia
Pirimiphos-methyl	1B	Yes	Yes (Denmark)	No
Bendiocarb	1A	Yes	Yes (UK)	Yes
Permethrin	3A	Yes	Yes (UK, US)	Yes
Deltamethrin	3A	Yes	Yes (UK, US)	Yes
Imidacloprid	4A	No[b]	No	No

[a]Group according to the Insecticide Resistance Action Committee, www.irac-online.org
[b]At the time of testing. Temprid75® (β-cyfluthrin + imidacloprid, Bayer, Melbourne, Australia) has since been released

Insecticide Resistance in Australia

Despite the above results, the full extent of resistance in bed bugs in Australia remains uncertain from this investigation. The data derived for this study was based on a strain collected between 9-10 years ago from infestations around Sydney, and hence only provides a limited 'snap-shot' of the breadth of the problem as it does not include any specimens collected from other major cities across states or territories. It is also known from quarantine interceptions that new potential populations of bed bugs have been introduced from outside Australia on a regular basis (Doggett et al., 2004), and hence obtaining a full understanding of how widespread resistance is will always be difficult. However, recent information has revealed that *kdr* mutations in *C. lectularius* are widespread across the country and all modern bed bug strains appear resistant (see Dang et al. 2014 in these proceedings). A further complicating factor is that Australia is one of the few countries to host both the common (*C. lectularius*) and tropical (*C. hemipterus*) bed bug (Doggett et al., 2003). As noted above, resistance to several insecticide classes has been previously confirmed in *C. hemipterus*, although limited susceptibility and *kdr* testing has been conducted against this species in Australia. Hence, susceptibility of the bed bug species that is likely to predominate across the northern half of the continent, and efficacy of the products available for use against them, is not fully known.

The Drivers of Insecticide Resistance in Bed Bugs

Failure of pyrethroid products to control an infestation due to underlying insecticide resistance may facilitate or increase the spread of resistant bed bugs. Bed bugs have been reported to be repelled by pyrethroids and susceptible bugs will avoid resting on areas with residual levels of deltamethrin (Kramer, 2004; Romero et al., 2009). Resistant populations may, however, still find harbourages previously populated by bed bugs attractive due to the presences of aggregation pheromones. Resistant bed bugs will also exhibit increased activity upon exposure to a sub-lethal dose and are likely to disperse or, at the very least, spend more time away from their harbourage area (Romero et al., 2009). If the levels of pyrethroid resistance confirmed within this study are encountered in a field situation, this potential response must evidently be taken into account. Repeated applications of insecticide will already be required due to poor residual efficacy of most pyrethroid products (Lilly et al., 2009a, 2009b). Consequently, treatments must extend to potential access points and areas where bed bugs may disperse, either in avoidance of the applied insecticide or in search of a new host. In the event of continual sub-lethal exposure, resistance beyond the levels already documented are likely to develop.

There is no doubt that *kdr*-type mechanisms are contributing to the high degree of resistance. Similarly, evidence from earlier work on formulated products (Lilly et al., 2009a, 2009b) and deltamethrin (Romero et al., 2009) indicated the inclusion of piperonyl butoxide has some appreciable effect on pyrethroid efficacy, thereby suggesting the presence of cytochrome P450 monooxygenase enzymatic detoxification mechanisms (Hodgson and Levi, 1998).

In addition to these factors, supplementary mechanisms such as reduced penetration and behavioural resistance are likely to be present and acting as intensifiers of the other (potentially multiple) resistance mechanisms as above.

Reduced penetration of a toxicant through the cuticle has been known to be a resistance mechanism since it was first established in the 1960's with the pyrethrin (Fine et al., 1963), organophosphate (Forgash et al., 1962; Matsumura and Brown, 1961, 1963), carbamate (Georghiou et al., 1961), and organochlorine insecticide groups (Plapp and Hoyer, 1968; Vinson and Brazzel, 1966). It has since also been established with the pyrethroids in a wide variety of insect pests (Ahmad et al., 2006; Delorme et al., 1988; DeVries and Georghiou, 1981;Gunning et al., 1991; Noppun et al., 1989; Valles et al., 2000; Wood et al., 2010;). Ordinarily, reduced penetration does not, by itself, impart a high degree of resistance (Plapp and Hoyer, 1968; Yu, 2008), although it may nonetheless have importance by way of conferring a level of cross-resistance to a wider variety of insecticides (Oppenoorth, 1984; Plapp and Hoyer, 1968; Yu, 2008), increasing the efficiency of metabolic detoxification (Ahmad et al., 2006; Mamidala et al., 2012; Sawicki, 1970) or delaying the onset of knockdown (Scott, 1990; Wood et al., 2010) and thus has important direct implications for effectiveness of formulated products in field situations and the eradication of resistant bed bug infestations.

The status and methodology for detecting the presence or degree of reduced penetration in bed bugs is currently advancing and there is considerable scope for investigation of the structural or functional differences in resistant bed bug strains. Evidence from very recent molecular analysis of several *C. lectularius* strains point toward changes in protein-expression and manifestation of other resistance mechanisms in the cuticle being strongly indicative of reduced penetration and/or cuticle thickening (Adelman et al., 2011; Koganemaru et al., 2013; Mamidala et al., 2012; Zhu et al., 2013).

Utilising the framework set by the above molecular research on *C. lectularius* it is axiomatic that research specifically examining cuticle thickness and the dynamics of insecticide penetration should be undertaken on resistant bed bug strains. Changes in cuticle thickness, the lipid composition and passage of insecticides, if combined with expression of detoxifying enzymes or target-site insensitivity, may have important consequences for the use of formulated insecticides in control of infestations.

A final mechanism, behavioural resistance, similarly needs to be considered as it may enable insects to avoid a lethal dose through stimulus-dependent hypersensitivity or hyperirritability. As a resistance mechanism, behavioural resistance is less well understood than the physiological resistance, although it has been demonstrated that behaviourally resistant insects respond to lower concentrations of insecticide than resistant strains (Silverman and Bieman, 1993; Young and McMillian, 1979), indicating a potential physiological change in receptor sensitivity. Common bed bugs have been found to be repelled and irritated by pyrethroid residues, but will otherwise overcome this repellency in the presence of a host, harbourage, and/or aggregation pheromone. Thus, there is the potential that if bed bugs have attained the tendency to be hypersensitive to such residues, that such a behavioural response may also intensify other pre-existing physiological resistance mechanisms.

CONCLUSION

The evolution of insecticide resistance is the expected result of their repeated use. A high degree of resistance in *C. lectularius* has now been confirmed in Australia, and an early warning provided that resistance has developed in the previously effective carbamate insecticide group. The inability to control bed bugs with existing products and insecticides will necessitate a rethinking of control methodologies employed against this resurgent pest. Integrated pest management in the form of a solid understanding of the ecology, biology, risks and effective treatment options associated with a bed bug infestation will be required for successful eradication. Products that employ new modes of action, or the re-labelling and reformulation of existing products will be needed, along with greater development and utilisation of efficacious non-chemical methods such as mattress encasements, steam, vacuuming and monitoring devices, and the adherence of pest managers to industry standards or codes of practice on bed bug management. Further susceptibility research and exploration of resistance mechanisms, combined with the inclusion of *C. hemipterus* in such testing, is fundamental to both a better understanding of the causes of the resurgence and our ability to effectively counter infestations. Without an integrated approach control of bed bugs will continue to be difficult.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

DETECTION OF KNOCKDOWN RESISTANCE (*KDR*) IN *CIMEX LECTULARIUS* AND *CIMEX HEMIPTERUS* (HEMIPTERA: CIMICIDAE)

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Abstract: Worldwide, there are many reports of pyrethroid resistance in bed bugs (*Cimex* spp.). Previous studies on the Common bed bug, *Cimex lectularius* L., have identified two mutations (V419L and L925I) in the voltage-gated sodium channel (VGSC) gene responsible for knockdown resistance (*kdr*). However, nothing is known on possible *kdr* mutations in Australian strains of *C. lectularius* or on the Tropical bed bug, *C. hemipterus* F. This study aimed to identify the status of *kdr* mutations in Australian *C. lectularius* strains, and *C. hemipterus* in Australia and international strains. Samples of *C. lectularius* were obtained from 24 sites across Australia, while the *C. hemipterus* were sourced from Australia, as well as Africa, India, Malaysia and Thailand. DNA was extracted, purified and examined for the *kdr*-related genes by PCR and Sanger sequencing. In *C. lectularius* field populations, the haplotypes A (neither V419L nor L925I), B (L925I only), and C (V419L and L925I) were found, with most (88%) of the field populations being haplotype B. A novel mutation, I936F, was identified in an 'Adelaide' strain (initially identified as haplotype A), which may be linked with *kdr*-type resistance. In *C. hemipterus*, the V419L and L925I mutations were not detected, however, three novel mutations, M918I (*Methionine* to *Isoleucine*), D953G (*Aspartic acid* to *Glycine*) and L1014F (*Leucine* to *Phenylalanine*), were identified. Samples from Thailand have the three mutations, while samples from Australia and India have both M918I and L1014F mutations. Only the L1014F mutation was evident in the samples from Malaysia and Africa. The M918I and L1014F were assumed to be *kdr* mutations and contribute to the high pyrethroid resistance in *C. hemipterus*. Further studies are in process to determine the non-*kdr* type resistance mechanisms.
Key words *Cimex*, insecticide resistance, pyrethroids, novel mutations.

INTRODUCTION

Over the last 15 years, as per other regions of the world, infestations of bed bugs (from the Common bed bug, *Cimex lectularius* L. and the Tropical bed bug, *Cimex hemipterus* F.) have undergone a dramatic reappearance in Australia (Doggett et al., 2003, 2004; Doggett and Russell, 2008; Doggett et al., 2011, 2012). An often integral part of the control strategy in bed bug eradication is insecticide application and the pyrethroids have been widely employed (Doggett and Russell, 2008). However resistance to this class of insecticides has been well documented, both in *C. lectularius* (Boase et al., 2006; Gangloff-Kaufmann et al., 2006; Moore and Miller, 2006; Romero et al. 2007; Kilpinen et al., 2008; Lilly et al., 2009), and in *C. hemipterus* (Myamba et al., 2002; Karunaratne et al., 2007; Tawatsin et al., 2011).

It is now known that multiple insecticide resistant mechanisms exist in *C. lectularius* against the pyrethroids, and other insecticide groups (Mamidala et al., 2011; Zhu et al., 2013; Dang et al., 2013). Resistance mechanisms documented in bed bugs against the pyrethroids included behavioural resistance (Romero et al. 2009), cuticular resistance (Koganemaru et al. 2013), metabolic resistance (Zhu et al. 2013), and target-site insensitivity resistance due to substitute mutations at the DNA level (Yoon et al. 2008).

Pyrethroid insecticides are widely used due to their low mammalian toxicity and high efficacy for killing insect pests. Like DDT, they target the Voltage-Gated Sodium Channel (VGSC) of insects, resulting in paralysis, an effect known as *knockdown,* which eventually leads to death. However, an important resistant mechanism evolved to combat the pyrethroids (and DDT) is known as knockdown resistance (*kdr*). This stems from various mutations on the VGSC gene that results in substitutions of the amino acid sequence of the VGSC protein. The result is to reduce the sensitivity of VGSC to pyrethroid insecticides. In previous studies, the mutations V419L and L925I were identified in *C. lectularius* and found to contribute to pyrethroid resistance (Yoon et al., 2008). Subsequently these mutations were detected widely across the USA (Zhu et al., 2010, 2013). Four mutation haplotypes were defined; haplotype A (no target site mutation), haplotype B (L925I mutation only), haplotype C (L925I and V419L mutations), and haplotype D (V419L mutation only).

To date, insecticide resistance profiling in Australian *Cimex* has been undertaken on one laboratory colony strain; the 'Sydney' *C. lectularius* strain (Lilly et al., 2009), which has been shown to be highly resistant to the pyrethroids and moderately resistant to the carbamates. However, no studies have been undertaken at the molecular level examining the basis of the resistant mechanisms in Australia. Therefore, the purpose of this study was to examine for such resistance, beginning with the status of *kdr* mutations in Australian *C. lectularius*, and *C. hemipterus* collected from around the world.

METHODS AND MATERIALS

Samples of *C. lectularius* were obtained from 24 sites across Australia, while the *C. hemipterus* were sourced from Australia, as well as Africa, India, Malaysia and Thailand (Table 1). The pyrethroid-susceptible 'Monheim' *C. lectularius* strain was used as a control. No insecticide susceptible strain of *C. hemipterus* was available. All bed bug samples were identified to species according to the key of Usinger (1966) prior to any testing.

DNA was extracted, purified and examined for the *kdr*-related genes by PCR and Sanger sequencing. Two gene regions were amplified and sequenced (Zhu et al., 2010); the fragment (~800bp) encodes the domain IIS4–IIS6 region of the VGSC gene, which contains six putative mutation sites previously associated with *kdr* pyrethroid resistance in a large range of insect pests, namely M918, L925, T929, L932, I936 and L1014. Another region (~500bp) encodes the domain IS6 and front part of domain I–II linker region which contains four putative mutation sites, namely V410, V419, V421 and E435. All sequences were aligned by ClustalW using BioEdit and MEGA5.

RESULTS AND DISCUSSION

kdr mutations in *Cimex lectularius*

In *C. lectularius* field populations (Table 1), the haplotypes A (no V419L or L925I mutation), B (L925I only), and C (V419L and L925I) were identified, with most (88%) of the field populations showing haplotype B. No haplotype D (V419L only) was detected. A novel mutation, I936F, was identified in an Adelaide strain, which was otherwise haplotype A. Interestingly, a mutation (I936V) was previously identified in *Drosophila melanogaster* Meigen, which had been found to contribute to significant pyrethroid resistance (Usherwood et al., 2007). This is circumstantial evidence that I936F is potentially linked to pyrethroid resistance in *C. lectularius*, however further work is required to confirm this.

For locations within the United States that exhibited high proportion of *kdr* haplotypes, the haplotypes B and C were more prevalent than the others (Zhu et al., 2010). In comparison, a study from France reported a high incidence of *kdr* with all being haplotype B (Durand et al., 2012). The variable frequencies of *kdr* haplotypes in disparate countries would probably relate to the types that were initially introduced and subsequently spread throughout the respective nation. Although studies are limited, to date all investigations examining *kdr* have revealed a high proportion of the mutations suggesting that they are probably widespread in bed bugs across the world. The continuous use of pyrethroid insecticides (despite not being very efficacious) may be contributing to the maintenance of high *kdr* mutation frequencies.

kdr* mutations in *Cimex hemipterusIn initial testing of the 'North Queensland' laboratory strain of *C. hemipterus*, the V419L and L925I *kdr* mutations were not present. Except for synonymous substitutions, however, two novel mutations were found; M918I and L1014F.

Table 1. *kdr* mutations found in *Cimex lectularius* and *C. hemipterus* used in this study.

Species	Strain	Collection Year	Mutation					
			V419L	L925I	I936F	M918I	D953G	L1014F
C. lectuarius	Monheim colony	1960's						
	Sydney colony	2004		*				
	St Ives, SYDNEY	2007		*				
	Redfern I, SYDNEY	2007		*				
	Glebe, SYDNEY	2007		*				
	Byron Bay I	2007		*				
	Byron Bay II	2007		*				
	Darlinghust I, SYDNEY	2008		*				
	Redfern II, SYDNEY	2011		*				
	Abbotsford, SYDNEY	2011		*				
	Darlinghust II, SYDNEY	2011	*	*				
	Auburn, SYDNEY	2012		*				
	Parramatta, SYDNEY	2012		*				
	Northbridge, SYDNEY	2013		*				
	Maryland, NEWCASTLE	2013		*				
	Northbridge, PERTH	2007		*				
	Nedlands, PERTH	2007		*				
	Cottesloe, PERTH	2013		*				
	Southbank, MELBOURNE	2007		*				
	Ripponlea. MELBOURNE	2013		*				
	Moonee Ponds, MELBOURNE	2013	*	*				
	South Yarra, MELBOURNE	2013		*				
	West Melbourne	2013		*				
	Alice Springs, NT	2013		*				
	Narangba, BRISBANE	2007		*				
	Semaphore Park, ADELAIDE	2013			*			
C. hemipterus	Australia	2007				*		*
	India	1997				*		*
	Malaysia	2005						*
	Africa	2010						*
	Thailand	2011				*	*	*

Analysis of the other strains showed that samples from India, like the Australian strains, have both M918I and L1014F mutations, but only the L1014F mutation was present in colonies from Malaysia and Africa (Table 1). No other *kdr* mutation in either the domain IS6 and part of domain I–II linker, and the domain IIS4–IIS6 regions were detected in *C. hemipterus*. However, another novel mutation, D953G (GAT-to-GGT) located on DIIS5-S6 linker of the VGSC gene, was subsequently identified in the samples from Thailand. So far, there have been no published reports of the D953G mutation in other insects.

The L1014F mutation is reported as a critical *kdr* mutation in several insect species (Davies and Williamson, 2009). Additionally, the mutation at M918 site has been confirmed to be associated with *kdr* or *super-kdr* type resistance to the pyrethroids (Rinkevich et al., 2013). The M918I+L1014F *kdr* mutations were identified in the diamondback moth, *Plutella xylostella* (L.) and are responsible for resistance to the pyrethroids (Sonoda et al., 2008). Hence, both the M918I and L1014F mutations in *C. hemipterus* are assumed to be *kdr* mutations, contributing towards pyrethroid resistance. The study herein appears to confirm the suggestion of Karunaratne et al. (2007) that high tolerance to both DDT and the pyrethroids was due to a '*kdr*'-type resistance mechanism. The D953G mutation is probably associated with the resistance to the pyrethroids, however further studies are required to verify this. Studies are ongoing to determinate non-*kdr* type insecticide resistance mechanisms in *C. hemipterus* (and *C. lectularius*) in our laboratory.

CONCLUSIONS

It is noteworthy that different *kdr* mutation types (V419L and L925I in *C. lectularius*, and M918I and L1014F in *C. hemipterus*) have evolved independently in the two bed bug species, which are both closely related. In contrast, similar *kdr* mutation types have evolved in other insects that are related (Rinkevich et al., 2013). The evolutionary mechanisms of the respective *kdr* mutation types between the bed bug species are unknown and further research is required to elucidate these mechanisms.

In light of the widespread resistance and resistance mechanisms (including *kdr*), the control of bed bug infestations should follow an integrated management approach encompassing non-chemical means of control and a reduced reliance on the pyrethroid insecticides. It is important that pest managers follow 'best practice' as defined in the various bed bug management industry standards to reduce the risk of treatment failure.

ACKNOWLEDGEMENTS

We would like to thank Mark O'Brien (Termitedoc Pest Control. PO Box 425, Cardiff NSW 2285), Matthew McDonald (Acacia Pest Control, 1/19 Capital Drive, Grovedale VIC 3216), Mark Wilson (Elders Pest Control, 64 Cobb & Co. Drive, Oxenford QLD 4210), Steve Matheson (Steve Matheson Pest Services, 10 Chatsworth Close, Port Macquarie NSW 2444), Maureen Koegel (Sleep Tight Services, PO Box 579, Central Park VIC 3145) and Ecolab Pty Ltd (Pest Elimination, Macquarie Park NSW 2113) that provided samples of *C. lectularius*.

The 'Monheim' *C. lectularius* strain was imported (Permit IP060247782) from Bayer CropScience, Monheim, Germany and continued to be stored in accordance with Australian Quarantine and Inspection Service (AQIS) requirements. Storage and culturing of the bed bug strains was conducted as approved by the Westmead Hospital Animal Ethics Committee (WHAEC Protocol N°. 1008) and in accordance with NSW Animal Research Review Panel (ARRP) *Guidelines for the Housing of Rats in Scientific Institutions*. This study was partly supported by the Chinese Scholarship Council (No. 201206200048).

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

THE CITIZEN SCIENCE PROJECT 'MÜCKENATLAS' SUPPORTS MOSQUITO (DIPTERA, CULICIDAE) MONITORING IN GERMANY

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Abstract Owing to a neglect of mosquito research for several decades, basic data on the occurrence and distribution of mosquitoes are absent for Germany. However, without having available the status quo, it is particularly difficult to detect invasive species and monitor the spread of potential vector species, topics which have gained special attention recently. Alarmed by cases and outbreaks of mosquito-borne diseases in other parts of Europe during the last few years, a mosquito monitoring programme was initiated in Germany in 2011. In addition to mosquito traps operated all over the country, an instrument for passive monitoring by community participation was launched in 2012, the "Mückenatlas". Private people are requested to collect mosquitoes and send them to the monitoring scientists to assist in mosquito research. The Mückenatlas is a typical citizen science project which has substantially added to the analysis of the spatiotemporal mosquito distribution in Germany and, in particular, has led to the discovery of new populations of the invasive Asian bush mosquito *Aedes japonicus*.
Key words Citizen science, Culicidae, passive monitoring, climate change, mosquito distribution.

INTRODUCTION

Germany's ecosystems are undergoing biodiversity alterations as a consequence of increasing globalization and continuing environmental and climatic changes. Among others, alterations pertain to the culicid fauna as non-native mosquito species actively invading or passively introduced succeed to establish. Whether previously endemic species have disappeared recently is not known because respective investigations are missing. Except for the Upper Rhine Valley, where mosquito data have been collected for more than 30 years (Becker and Ludwig, 1983), there are only out-dated local or regional studies on the occurrence of these insects. During the past decades no longitudinal nation-wide monitoring programme was carried out to assess where and when which culicid species occurs.
Until a few years ago, 46 mosquito species were considered indigenous to Germany (Dahl et al., 1999) although this figure is nothing but an addition of all species ever demonstrated in Germany. After the discovery of some recently invaded and a newly recognized species (Kronefeld et al., 2012; Werner et al., 2012), the number of species supposed to occur in Germany increased to 50. Although with West Nile, Sindbis, Tahyna, Batai, Lednice and Inkoo viruses it became evident that at least six facultatively pathogenic mosquito-borne viruses circulate in Europe (Lundström 1999), there have been no studies yet on mosquito-borne pathogens possibly occurring in Germany. Respective cases of disease have not

been recognized but might have appeared and been mis-diagnosed as a flu due to lacking differential diagnostic examinations.
While comprehensive knowledge exists on the biological characteristics of tropical mosquitoes demonstrated to be vectors of disease in their native areas, little is known on the indigenous species. No data are available on whether indigenous mosquitoes are able to transmit imported tropical disease agents. Studies in the recent years demonstrated Sindbis virus, Batai virus and the animal pathogenic Usutu virus in mosquito pools collected in Germany (Jöst et al., 2010 2011a, 2011b). In other European countries, cases of West Nile fever, chikungunya fever, dengue fever and malaria recently occurred (Rezza et al., 2009; Danis et al., 2011; Sousa et al., 2012; Papa, 2012). A spread of mosquito-borne dirofilariae (*Dirofilaria repens*, *D. immitis*) to North and Northeast Europe, including Germany, has been observed (Genchi et al., 2011; Kronefeld et al., 2014).

MATERIALS AND METHODS

The Leibniz Centre for Agricultural Landscape Research and the Friedrich-Loeffler-Institut (German Federal Research Institute for Animal Health) have been commissioned by the Robert-Koch-Institut (German National Public Health Institute) and the German Federal Ministry of Food and Agriculture to carry out a nation-wide study on the occurrence and spatiotemporal distribution of culicids in Germany. For this purpose, mosquito traps have been operated since spring 2011 at more than 120 locations over the country. To complement the trap collection of species poorly attracted to traps, rare species, and species occurring in ecologically particular and remote areas, and to obtain data from many more locations than can be covered by the trap grid, the citizen science project Mückenatlas was initiated in 2012. People are invited to participate in mosquito research by catching mosquitoes in their surroundings and to send them to the research institutions, with some details of the collection. Detailed instructions are provided on the project's homepage: www.mueckenatlas.de. Mosquitoes are to be collected by putting a closable container over them. These containers are to be put in the freezer to kill the mosquitoes. The dead mosquitoes are then transferred to the research institutions together with a questionnaire. In the laboratory, the mosquitoes will be identified morphologically or genetically, and added to the reference collections of pinned specimens or DNA samples. The identification plus information coming with the mosquito will be entered into the German mosquito database Culbase where data from research projects and corresponding collections are brought together. The submitter of the mosquito will receive the identification result together with biological details of the species and may request to appear as a spot on an interactive collection map of the Mückenatlas homepage.

RESULTS AND DISCUSSION

Since its launch in 2012, the Mückenatlas has experienced popularity and has generated several interesting and surprising results. In 2012, 2,020 postal items were submitted to the Mückenatlas of which 1,564 (77.4%) contained culicids. The remainder consisted of spiders, beetles, grasshoppers, bugs, and various flies. The number of submitted culicids amounted to 6,127. In 2013, the number of postal items received increased to 2,409 of which 1,838 (76.3%) contained mosquitoes. A portion similar to 2012 comprised other arthropods, this consisted of significantly more mosquito-like insects such as chironomids, trichocerids and anisopodids and less non-mosquito-like insects as compared to 2012. The number of mosquitoes submitted in 2013 was 11,273. Figures 1 and 2 showt he locations where submitted mosquitoes were collected in 2012 and 2013. Submitted mosquitoes belonged to 38 species in 2012 and 36 species in 2013, with *Cx. pipiens* accounting for the major part of the submissions. In total, 4 *Aedes*, 6 *Anopheles*, 1 *Coquillettidia*, 5 *Culex*, 5 *Culiseta* and 17 *Ochlerotatus* species were registered (Table 1). Widely distributed species, such as *Cx. pipiens*, *Cx. torrentium*, *Cs. annulata*, *Ae. vexans* and *Oc. sticticus* were regularly sent in substantial numbers. Less frequent and rare species such as *Cs. glaphyroptera* and *Cs. ochroptera* were collected (Kampen et al., 2013).

Figure 1. Mosquito collection sites in 2012

Figure 2. Mosquito collection sites in 2013 (status January 2013)

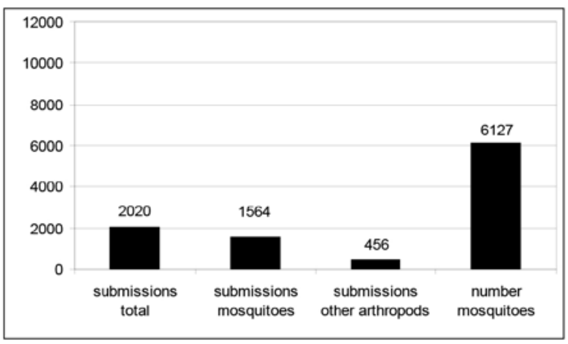

Figure 3. Mosquito submissions: 2012.

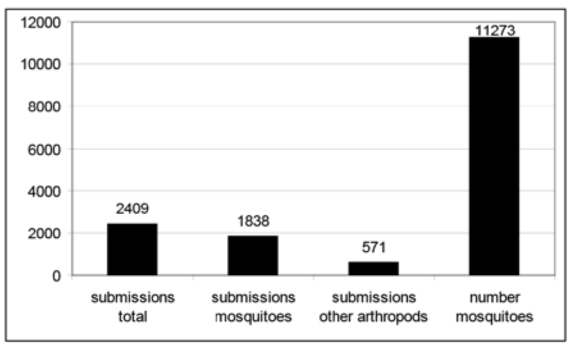

Figure 4. Mosquito submissions: 2012.

The Mückenatlas has gained relevance as an instrument to recognize hitherto unknown distribution areas. This became obvious when submissions of mosquitoes led to the detection of two established populations of the Asian bush mosquito *Ae. japonicus* in western and northern Germany (Kampen et al., 2012; Werner and Kampen, 2013).

In conclusion, the Mückenatlas has turned out an excellent tool for large-scale passive mosquito monitoring. It is successful with regard to both sides a citizen science project is meant to serve, i.e. the scientific one and the public one. The natural sciences are supported by data input from interested and cooperative citizens whilst the scientists make their expertise available to the community and contribute to their education.

Table 1. Species list of submissions to the Mückenatlas in 2012 and 2013

Gattung *Anopheles*	**Gattung *Aedes***	**Gattung *Coquillettidia***	**Gattung *Culex***	**Gattung *Culiseta***	**Gattung *Ochlerotatus***
An. claviger *An. atroparvus* *An. daciae* *An. maculipennis* *An. messeae* *An. plumbeus*	*Ae. cinereus* *Ae. geminus* *Ae. rossicus* *Ae. vexans*	*Cq. richiardii*	*Cx. hortensis* *Cx. modestus* *Cx. pipiens* (*pipiens/ molestus*) *Cx. territans* *Cx. torrentium*	*Cs. annulata* *Cs. subochrea* *Cs. glaphyroptera* *Cs. morsitans* *Cs. ochroptera*	*Oc. annulipes* *Oc. cantans* *Oc. caspius* *Oc. cataphylla* *Oc. detritus* *Oc. dorsalis* *Oc. excrucians* *Oc. flavescens* *Oc. geniculatus* *Oc. intrudens* *Oc. japonicus* *Oc. leucomelas* *Oc. pullatus* *Oc. punctor* *Oc. riparius* *Oc. rusticus* *Oc. sticticus*

Citizens obtain individual feedback about their data and additional information, which can increase their knowledge of wildlife and nature and bring about a change in attitudes. The material processing procedure still guarantees the highest possible quality of data since mosquito identification and data analysis and interpretation is performed by the scientists. As all citizen science projects, the Mückenatlas demands high quality communication between all participants. Scientists, citizens, communicators and the media are all involved. On a meta-level, the four main drivers for successful citizen science communication are realized within the Mückenatlas: dialogue at eye level, transparency, commitment and relevance.

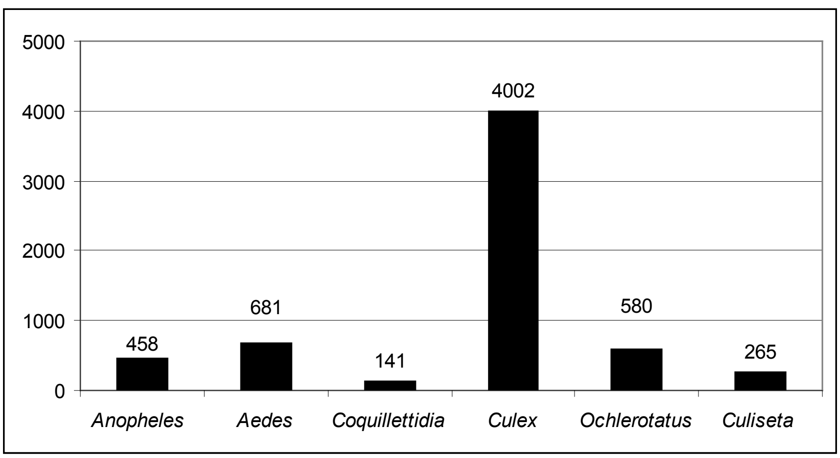

Figure 5. Mosquito submissions in 2012 according to genera

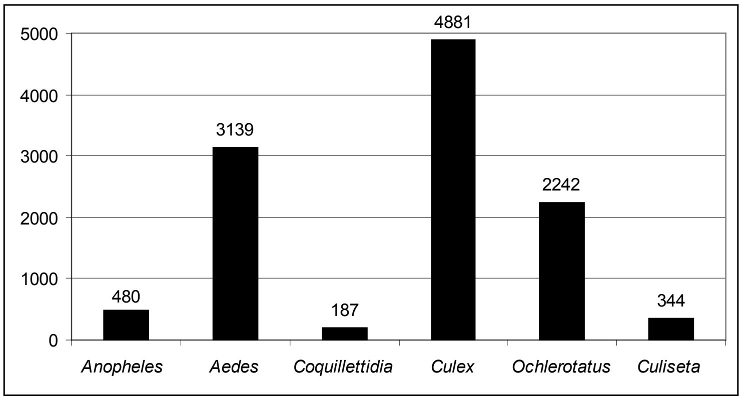

Figure 6. Mosquito submissions in 2013 according to genera

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

DISTRIBUTION OF *AEDES ALBOPICTUS* MOSQUITOES IN AN INLAND CLIMATE MOUNTAIN AREA, NAGANO PREFECTURE, JAPAN

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Abstract Nagano Prefecture, an inland climate mountain area of Japan, extends widely north and south, and the altitude varies greatly with the location. Mosquitoes transmitting infectious disease have a diversity of habitats in Nagano, and many species can be expected. There have been few reports about mosquito fauna, and there is little information on mosquitoes such as *Aedes albopictus*. In this study, we clarify the distribution patterns by investigating the fauna of mosquitoes, focusing on *A. albopictus*, in 8 areas (including Nagano, Ueda, Karuizawa, Matsumoto, Suwa and Okaya, Ina and Minamiminowa, Komagane and Miyata, and Iida) from July to September in 2012 and 2013. We set up CDC traps in each area and also collected mosquito larvae in temple and shrine precincts in each area. Although *A. albopictus* was not collected in the Karuizawa Area (altitude 950 m, annual mean air temperature 8.5° C), it was captured in the other 7 areas (annual mean air temperature 11.2 to 13.1° C). Kobayashi et al. (2002) reported that *A. albopictus* can inhabit areas with an annual mean air temperature above 11.0° C, and our results agreed with theirs.

Key words Altitude, annual mean air temperature, CDC trap, habitat, larval survey.

INTRODUCTION

Aedes albopictus (Skuse) ranks second only to *Aedes aegypti* (Linn.) in importance to human as a disease vector of dengue and dengue hemorrhagic fever (Knudsen, 1995; Knudsen et al., 1996). They affect at least 2.5 billion people living in urban and suburban environments in more than 100 countries in tropical and subtropical regions around the world (European Centre for Disease Prevention and Control, 2009; Guzman et al., 2010). According to Hotta (1953), Japan experienced endemic dengue outbreaks in several coastal cities in 1942-1945. Although dengue hemorrhagic fever has not been prevalent in Japan for 50 years, the possibility of dengue hemorrhagic fever outbreak exists because of the common distribution of the vector *A. albopictus* (Hotta, 1998). Vector control is an essential measure for controlling the outbreak of viral disease. Thus, collecting information about distribution and ecology of vectors might be the most important and essential/effective method of vector control.

Nagano Prefecture, an inland climate mountain area, extends widely north and south, and the altitude varies with the location in Japan. Therefore, mosquitoes transmitting an infectious disease have a diversity of habitats in Nagano, and many species can be expected (Kamimura, 1968; Uchikawa, 1977). However, there have been few reports about mosquito fauna, in particular; little information on mosquitoes such as *Aedes albopictus* has been distributed (Kurihara et al., 2000; Shirai et al., 2002).

Kamimura (1968) reported 19 mosquito species including *A. albopictus* in Nagano Prefecture in July-August 1964 and in August, September-October 1965 (qualitative data). However, he did not describe the collecting sites and densities, so we do not know which area *A. albopictus* inhabited and how many individual *A. albopictus* were collected. From 1972 to 1974, Uchikawa (1977) investigated the mosquito fauna in Matsumoto and its environs by light trap and the collected larval survey from June to September. He reported that 11 species were caught by light traps and 9 species of mosquito larvae were collected by a dipping method. *A. albopictus* was not collected during the investigation periods. Kurihara et al. (2000), reported many individual *A. albopictus* adults were collected from Nagano, Matsumoto and Ueda Cities. Shirai et al. (2002) reported *A. albopictus* larvae were not collected from Matsumoto and its environs in August of 2000 and July of 2001.

In this study we attempted to clarify the distribution patterns by investigating the fauna of mosquitoes, especially focusing on *A. albopictus*, in 8 areas from the end of July to early September in 2012 and 2013. CDC traps were set in each area and mosquito larvae were collected in several temple and shrine precincts in each area.

MATERIALS AND METHODS

Mosquito surveys were carried out from the end of July to early September 2012 and 2013 in 8 areas (Nagano, Ueda, Karuizawa, Matsumoto, Suwa and Okaya, Ina and Minamiminowa, Komagane and Miyata, and Iida; including 8 cities, 1 town and 2 villages) in Nagano Prefecture (Figure 1). Adults and larvae were collected at 57 sampling sites. Table 1 lists the study area, sampling site, locations (longitude; 137°48’46.32”E - 138°38’10.77”), and environmental conditions (annual mean air temperature in each area from 2003 to 2012; 10 years) of collecting sites and investigation periods/days. Figure 2 shows the relationship between the altitude latitude of sampling sites in this study. The latitude range was from 35°30’21.24”N to 36°39’42.95”N, and altitude was 353 - 996 m, respectively.

For adult collections, CDC traps without a bulb, baited with 1 kg dry ice were used and operated continuously from 15:00 to 10:00. Mosquitoes in the traps were collected every morning and taken to the laboratory for identification. Species was identified following the morphological keys of Tanaka et al. (1979). *A. albopictus* samples were picked up and counted. CDC traps were set up at 37 sites (a total of 47 nights) at 1-2, 7-8, 30-31 Aug. 2012 and 3-4 Sep. 2012 at 2 sites in the Karuizawa Area, 3-4 Sep. 2012 at 1 site in the Ueda Area, 29-30 Jul. 2013 at 7 sites in the Matsumoto Area, 12-13 Jul. and 6-7 Aug. 2013 at 12 sites in the Suwa and Okaya Areas, 4-5 Sep. 2013 at 8 sites in the Ina and Minamiminowa Areas, and Komagane and Miyata Areas, and 3-4 Sep. 2013 at 7 sites in the Iida Area. Collection sites of larvae were selected from each sampling area. The larvae were collected at a total of 31 sites (76 points), using a glass pipette, from artificial containers, such as flower vases in graveyards of Buddhist temples, wash basins in shrines, used tires stacked in the backyard or along roadsides, used cans or plastic containers, and bamboo stumps. We collected larval samples at 5 Sep. 2012 at 7 sites (25 points) in the Ueda Area, 6 Sep. 2012 at 6 sites (15 points) in the Nagano Area, 30 and 31 Aug. 2012 at 1 site (4 points) in the Karuizawa Area, 29 and 30 Jul. 8 Aug. 2013 at 7 sites (22 points) in the Matsumoto Area, 7 Aug. 1 site (1 point) in the Suwa and Okaya Areas, 4 Sep. 2013 at 6 sites (6 points) in the Ina and Minamiminowa Areas, and Komagane and Miyata Areas, 3 Sep. 2013 at 3 sites (3 points) in the Iida Area. The larvae were collected and placed in small polypropylene containers (ca. 100 ml volume), which were kept at room temperature in our insectary. Identification was based on emerged adults.

RESULTS AND DISCUSSION

A total of 255 individual *A. albopictus* (93 adult mosquitoes by CDC traps and 162 individuals from larvae) were collected during the investigation periods (Table 1).

Although *A. albopictus* was not collected in the Karuizawa Area, it was captured in the other 7 areas in Nagano Prefecture. For adult collections, a large number of *A. albopictus* were collected from the Iida Area, the total of 49 individuals; average 7.0 individual numbers / night / CDC trap, followed by the Ueda Area, 14 individuals; average 14 individual numbers / night / CDC trap. On the other hand, for larval collections, a large number of *A. albopictus* were collected from the Matsumoto Area, the total of 84 individuals; average 3.8 individual numbers / sampling point, followed by the Ueda Area, 57 individuals; average 2.3 individual numbers / sampling point.

According to Kurihara et al. (2000), *A. albopictus* adults were collected from Nagano, Matsumoto and Ueda Cities. They also reported that the density of *A. albopictus* might be increased from ca. 1992/1993. In our study, *A. albopictus* was newly confirmed in 17 sites (adult collecting at 16 sites, larval collecting at 2 sites, and both collecting at 1 site) at 4 study areas (southern part of Nagano Prefecture; i.e., Suwa and Okaya, Ina and Minamiminowa, Komagane and Miyata, and Iida Areas).

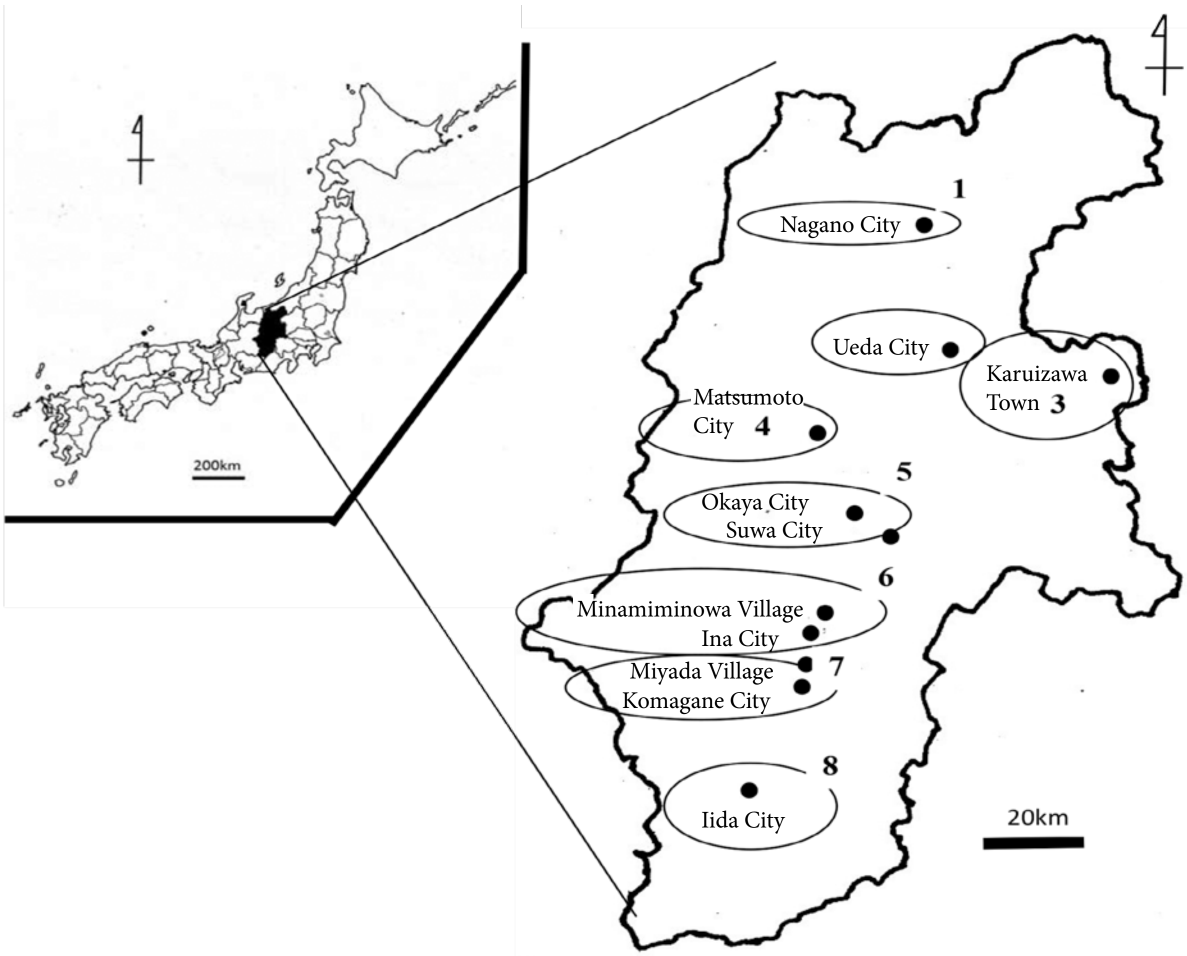

Figure 1. Distribution of studied 8 areas in Nagano Prefecture: 1. Nagano Area, 2. Ueda Area, 3. Karuisawa Area, 4. Matsumoto Area, 5. Suwa and Okaya Areas, 6. Ina and Minamiminowa Areas, 7. Komagane and Miyada Areas, and 8. Iida Area.

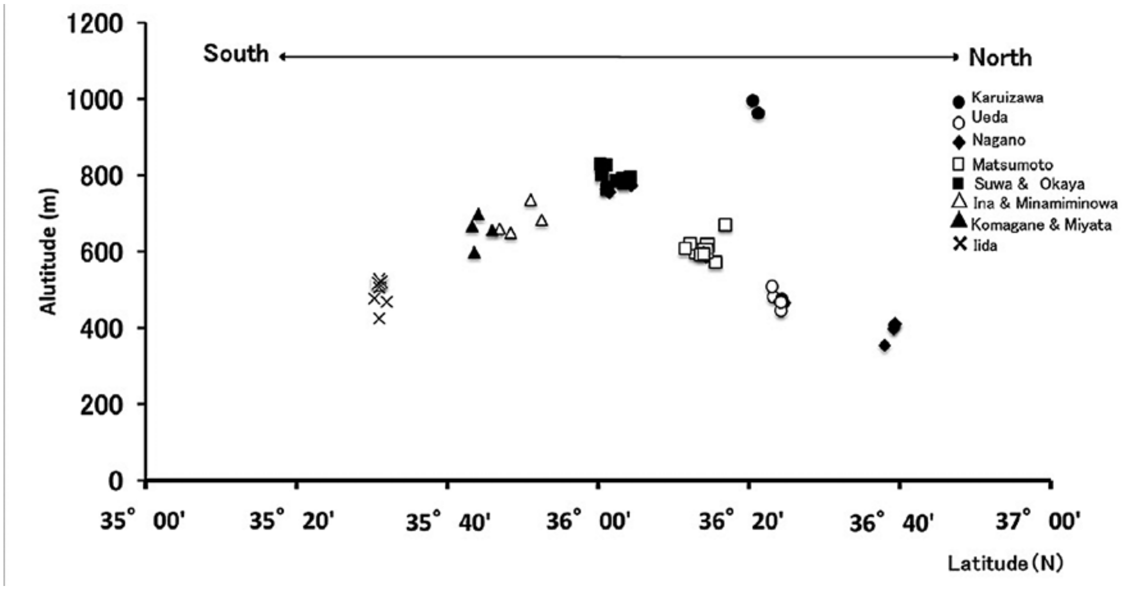

Figure 2. Relationship between altitude and latitude of sampling sites.

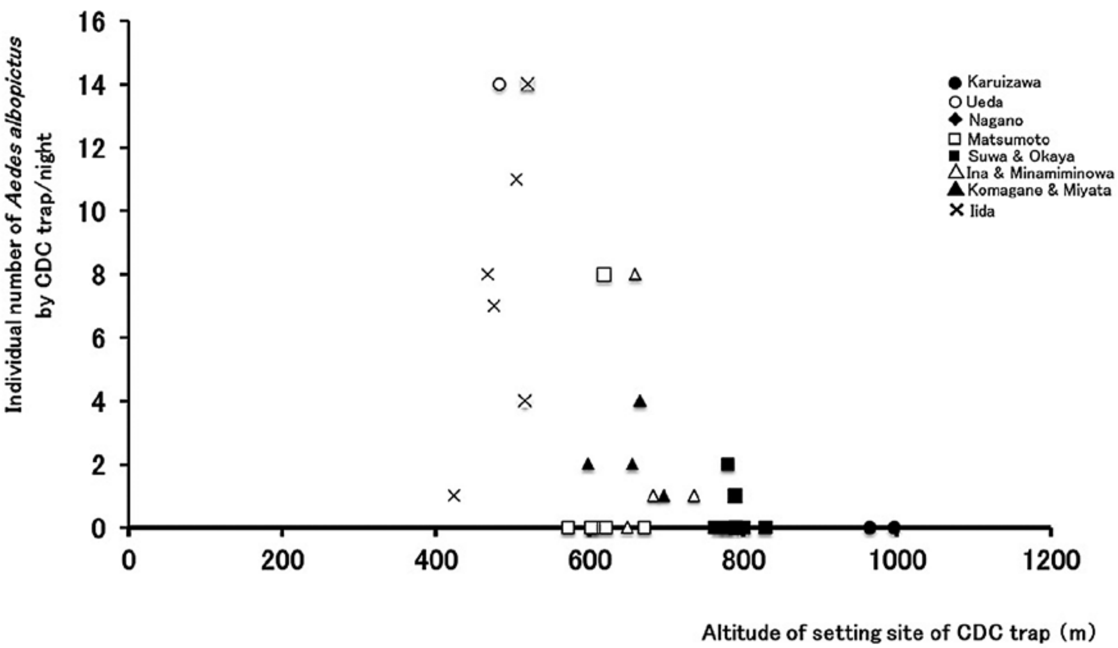

Figure 3. Relationship between individual number of *Aedes albopictus* by CDC trap/night and altitude of CDC trap site.

Figure 3 shows the relationship between the individual number of *A. albopictus* by CDC trap / night and the altitude of the CDC trap site. The individual number of *A. albopictus* decreased with increasing altitude. A strong negative correlation was found between the altitude of the meteorological station in this study area and the annual mean air temperature at each area during the 10 years ($r^2 = 0.72$). According to Kobayashi et al. (2002), there was a strong correlation between the *A. albopictus* mosquito-infested areas and annual mean air temperature above 11 degrees Celsius. In our study, *A. albopictus* was not collected in Karuizawa, located in a mountainous area, with an altitude of 964-996 m, and an annual mean air temperature of 8.6±9.0 degrees Celsius. In the other 7 areas, (annual mean air temperature; ranging from 11.2 to 13.1 degrees Celsius), *A. albopictus* was captured. Thus, our results agreed with those of Kobayashi et al. (2002).

This is, to our knowledge, the first report on the quantitative data of *A. albopictus* by CDC traps in Nagano Prefecture. Further follow-up field investigations are necessary to collect more adults by several combined sampling methods may well further improve our understanding of the mosquito fauna in Nagano Prefecture.

ACKNOWLEDGEMENTS

We would like to express sincere thanks to Dr. Kamimura K. and Dr. Kurihara T., for their valuable taxonomical advice. We express our thanks to Mr. Nagai Y., for his field work support.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

SURVEILLANCE AND CONTROL OF *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE) IN SWITZERLAND

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Abstract In 2003 the invasive Asian tiger mosquito, *Aedes albopictus* arrived in Southern Switzerland for the first time. It has since then been continuously surveyed and controlled using ovitraps for monitoring alongside targeted applications of larvicides (*Bti* and diflubenzuron) and larval source reduction. Until recently the focus of activities was on surveillance and control in the Canton of Ticino (Southern Switzerland) and less attention was drawn on its potential introduction to the northern parts of the country. As this mosquito has been reported from neighbouring Southern Germany and Northern France we examined whether *A. albopictus* is also being introduced north of the Alps. Here, mosquitoes were monitored by ovitraps placed along motorways, ports and major airports across Switzerland. Collected mosquito eggs were identified using recently developed protein profiling methodology. This study shows that *A. albopictus* is occasionally introduced to Switzerland even north of the Alps along motorways though the results suggest no firm establishment of the mosquito in those areas.
Key words Vector entomology, Asian tiger mosquito, MALDI-TOF MS, ovitraps.

INTRODUCTION

The Asian tiger mosquito, *Aedes (Stegomyia) albopictus* (Skuse, 1894), originally native to South-East Asia has been passively spread across the globe mainly through the used tyre trade to the United States, Latin America, Europe and several Pacific and Indian Ocean islands (Reiter and Sprenger, 1987; Paupy et al., 2009). Within continental Europe the mosquito is mainly passively dispersed through adults trapped in vehicles. Since 2003 *A. albopictus* is also present in Switzerland (Flacio et al., 2004).

According to the Global Invasive Species Database *A. albopictus* is among the 100 of the world's worst invasive alien species (ISSG, 2013) and a competent vector of chikungunya and dengue virus and several other disease agents, such as West Nile virus, encephalitis and dirofilaria worms (Paupy et al., 2009). *A. albopictus* poses a threat to human health also in continental Europe as the chikungunya outbreak in Italy (Rezza et al., 2007) and autochthonous cases of dengue in metropolitan France (La Ruche et al., 2010) and Croatia (Schmidt-Chanasit et al., 2010) have shown. Systematic surveillance and control of *A. albopictus* is, therefore, needed to keep mosquito populations at densities below risk of disease outbreak.

In Switzerland surveillance and control has been restricted to the geographic area of the Canton of Ticino (Wymann et al., 2008), a region located south of the Alps at the border to Italy. Data from France suggest, however, that *A. albopictus* is progressively migrating along the Rhone valley to the North (Anonymous, 2012) and it is only a matter of time for it to arrive in Geneva, Switzerland, where the climate would be favourable enough for the mosquito to settle (Neteler et al., 2013). *A. albopictus* has also been reported from Southern Germany, to where it has likely been introduced by cars coming from southern Europe (Pluskota et al., 2008; Werner et al., 2012).

Switzerland being at the northern tip of *A. albopictus* distribution in Europe has motivated us to initiate a pilot study to monitor the introduction of this highly invasive species. We set up traps to collect eggs from gravid females – so called ovitraps - along the main traffic routes across the country at motorway service stations, airports and ports during the summer of 2013 and determined present eggs to species level using matrix assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF MS). Here, we demonstrate, for the first time, the introduction of *A. albopictus* to Switzerland north of the Alps.

MATERIAL AND METHODS

We set up traps at 30 locations at motorway service stations, major airports (Basel-Moulhouse-Freiburg, Geneva and Zürich) and at the Rhine ports in Basel between June and September 2013, covering the peak season of *A. albopictus*. We used ovitraps to collect eggs as these tend to be more sensitive than traps for sampling adult mosquitoes (ECDC, 2012). Ovitraps mimic breeding sites, attracting gravid females to deposit their eggs. Our traps consisted of 1.5 litre, black plastic flower pots, filled with tap water. The eggs were then collected on a wooden slat that was partially submerged and partially stuck out of the water. *A. albopictus* females would naturally deposit their eggs near the water surface. In order to prevent the ovitraps from becoming potential breeding sites we added larvicide granules of *Bacillus thuringiensis* var. *israelensis*. The slats were then replaced every fortnight. At the motorway service stations we set up three traps on each serviced side of the road and six traps at each airport and port. Detected eggs were examined visually for morphological species specific features under a stereo microscope and matrix-assisted laser desorption-ionisation time of flight mass spectrometry (MALDI-TOF MS) similar to Kaufmann et al. (2012) and Müller et al. (2013) using reference spectra that were established at Mabritec AG in collaboration with the Institute of Parasitology in Zürich, Switzerland. Where ovitraps were positive for *A. albopictus* we also set up BG sentinel traps with BG lure (Biogents, Germany) in an attempt to also attract adult mosquitoes.

RESULTS AND DISCUSSION

A. albopictus eggs were present at all trapping locations in Ticino (Figure 1) which is not unexpected giving the results of the ongoing local surveillance and control programme (data not shown). In August we also detected two eggs at the motorway service station A1 Raststätte Grauholz in the Canton of Bern and 22 eggs at A2 Raststätte Gotthard in the Canton of Uri, north of the Alps. In September we identified another 72 eggs north of the Alps at the service station A13 Raststätte Heidiland in the Canton of St. Gallen (Figure 1).

This study is the first account of *A. albopictus* introduction into Switzerland north of the Alps. All cases outside Ticino were, however, singular. The results fit well together with earlier accounts of *A. albopictus* in southern Germany and underline the continuous introduction of this invasive species through motorway traffic. Interestingly, while *A. albopictus* has repeatedly been found in Germany just across the border from Basel (Werner et al., 2012) none of the traps set up in and around Basel have been found positive in this study.

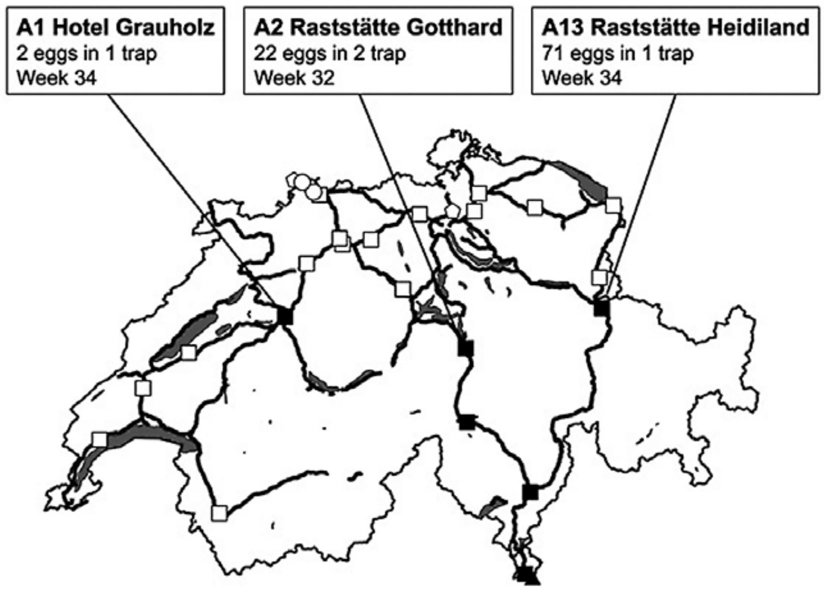

Figure 1. Locations positive for *Aedes albopictus* during the 2013 Swiss national survey. Filled squares (motorway service stations) and the triangle (train station) show the locations with ovitraps positive for *A. albopictus*. Empty symbols represent locations that were sampled but negative for *A. albopictus*.

The presence of *A. albopictus* in Ticino, with the exception of the positive site at the motorway service station A2 San Gotthardo, located north in the canton, are not unexpected and corroborate the patterns being repeatedly observed from the on-going local surveillance programme.

CONCLUSION

The present study shows that *A. albopictus* is being introduced to Switzerland also north of the Alps along the motorways. Encouragingly, the results from this study suggest no firm establishment of the mosquito in those areas yet. It is recommended to continue the surveillance program and establish a national action plan for its control, particularly for areas such as Geneva where environmental conditions for an establishment of *A. albopictus* are already favourable.

ACKNOWLEDGEMENTS

We thank Seraina Vonzun, Tobias Suter and team members of the Vector Control Group at Swiss TPH in Basel and GLZ in Lugano and Corinne Jacquelin who helped setting up and replacing traps or counted eggs, Prof Peter Lüthy, Nicola Patocchi and Dr Basil Gerber for valuable discussions and support. We also thank Dr Valeria Guidi for laboratory analysis. We thank all authorities and private persons who gave us access to the sites for placing our traps. This study received funds from the Swiss Federal Office for the Environment.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

AEDES ALBOPICTUS RESTISTANCE STATUS AND POPULATION DYNAMICS ACROSS THE SWISS-ITALIAN BORDER

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Abstract *Aedes albopictus* is a vector for over 20 viruses, including chikungunya and dengue and where present may pose a threat to human health. Since 2003 *A. albopictus* is also present in Southern Switzerland in the Canton of Ticino and has since then been continuously surveyed and controlled, mainly by larviciding and larval source reduction. The Italian communities just across the border, however, lack such a surveillance and intervention programme. Here, we examined the seasonal and spatial abundance of *A. albopictus* in forest and urban areas across the Swiss-Italian border during the mosquito's active season in 2012 and 2013, and measured the susceptibility of the local mosquito population against larvicides in use. We found that mosquito densities peaked between end of August and early September and that they were higher in Italy and in the urban areas. Laboratory bioassays showed that the local mosquito population is still susceptible to the applied larvicide, *Bacilus thuringiensis* var. *israelensis (Bti)*. Together the results support the hypothesis that the intervention programme in Ticino successfully lowers the local *A. albopictus* population.
Key words Culicidae, Asian tiger mosquito, vector control, *Bacilus thuringiensis* var. *israelensis*

INTRODUCTION

The Asian tiger mosquito *Aedes (Stegomyia) albopictus* (Family Culicidae; Skuse, 1894) originates from Southeast Asia. Over the recent decades *A. albopictus* has spread to the United States of America, Europe, Latin America and Africa, primarily by transport of dormant eggs in used tyres and trade of "lucky bamboo", *Dracaena sanderiana* (Scholte et al., 2007). At regional level, ground transport (i.e. cars and lorries) further added to the passive dispersal of the mosquito. Once the vector is established in a new area, *A. albopictus* may then also disperse actively to suitable habitats (Straetemans, 2008).
A. albopictus is a competent vector for at least 22 arboviruses, notably dengue, chikungunya, yellow fever, West Nile fever and *Dirofilaria* (Gratz, 2004). Establishment of this mosquito species has led to autochthonous cases of chikungunya in Ravenna, Italy with over 200 confirmed cases in 2007 (Angelini et al., 2008; Sambri et al., 2008) and dengue in Croatia and metropolitan France in 2010 (La Ruche et al., 2010; Gjenero-Margan et al., 2011).
In Switzerland, the first *A. albopictus* mosquitoes were detected in the Canton of Ticino in 2003 (Flacio et al., 2004) and was since then sporadically re-introduced to Ticino until the situation changed significantly in 2007 when in Chiasso, at the border to Italy, the monitoring network

detected a dramatic increase in positive mosquito traps, indicating that a local mosquito population had then been established (Wymann et al., 2008). In response to this increase the monitoring network in Ticino has been expanded and interventions including larviciding were implemented. Today, over 1,200 ovitraps (traps collecting the eggs of container breeding *Aedes* mosquitoes) are used for surveillance in Ticino. In contrast to Ticino no such systematic programme exists across the border in Northern Italy.

In the present study, we set out to investigate whether we find evidence that the Ticino model may have a an impact on the local mosquito population and whether *A. albopictus* would be more present in human settlements as compared to the forested surroundings. In addition, the susceptibility of the local *A. albopictus* population to *Bti* was studied in order to assess the efficacy of the microbial larvicide that is part of the overall intervention strategy.

MATERIALS AND METHODS

Spatial and Temporal Distribution of *A. albopictus*

Surveys of relative mosquito abundance across the Swiss-Italian border were carried out in July – November 2012 and May - November 2013. For the surveys an area of 118 km^2 has been chosen from each of the adjacent side of the Swiss-Italian border (65 km^2 on the Italian side and 53 km^2 on the Swiss side). Using the ArcGIS version 10.0 (ESRI Inc., USA) geographic information system (GIS) software the study area was divided into grid squares, each measuring 250 m by 250 m. The grid squares were then stratified into "urban" and "forest" areas. A grid square was classified as forest area if 50% or more of the area within that square was covered with trees, and vice versa. From the total grid 70 squares (35 urban area squares and 35 forest area squares) were randomly selected in each country. Within a selected grid square relative *A. albopictus* density was then sampled by placing two ovitraps at a distance of at least 50 m between them to avoid interference. All traps were geo-referenced.

Ovitraps mimic breeding sites, attracting gravid females to deposit their eggs. Our traps consisted of 1.5 litre, black plastic flower pots, filled with tap water. The eggs were then collected on a wooden paddle that was partially submerged and partially stuck out of the water. In order to prevent the ovitraps from becoming potential breeding sites we added larvicide granules of *Bti.* The paddles were replaced biweekly and eggs, where present, counted with the aid of a stereo microscope.

Larval Susceptibility Assays

For the larvicide susceptibility bioassays, colonies were established from eggs collected in ovitraps and reared at the insectarium of the Department of Entomology-CPqAM at 26±1 °C temperature, 70±5 % relative humidity and a light:dark photoperiod of 12:12 hours. Larvae were kept in trays filled with tap water and fed with cat chow (Friskies – Seafood Sensations, Nestlé Purina Pet Care). Adults were fed with 10% sucrose solution and females given chicken blood.

The susceptibility of two *A. albopictus* colonies and the *A. aegypti* Rockefeller, an insecticide susceptible reference colony, were assessed against the *Bti* standard IPS-82 reference strain from the Institute Pasteur, Paris (containing a mixture of crystals and spores). The two *A. albopictus* colonies were the "RecAlb_Lab" colony, a laboratory reared *A. albopictus* colony founded from mosquitoes collected in Recife, Brazil and "Switzerland", a colony established from field-caught *A. albopictus* of the 2012 field survey in Switzerland.

Larval bioassays were conducted following the guidelines for larval susceptibility tests of the World Health Organization (WHO) Pesticide Scheme (WHO, 2005). Data were reported as the lethal concentration that would kill 50% or 90% of the larvae (i.e. LC_{50} and LC_{90}, respectively).

RESULTS AND DISCUSSION

Spatial and Temporal Distribution of *A. albopictus*

In total, 6,440 paddles containing 225,120 eggs of *A. albopictus* were recovered during a trapping period of 48 weeks (20 weeks in 2012 and 28 weeks in 2013). Number of eggs per trap ranged between 0 and 1,537 eggs. Egg counts reached a maximum around end of August, beginning of September in both years (Figure 1), in accordance with previous studies (Carrieri et al., 2011; GLZ, 2013).

Throughout the whole season egg densities were lower in Ticino as compared to Italy and lower in the forest than in the urban areas (Figure 1). At the peak, the ratio of the total egg counts between the Italian and Swiss urban was 2.2 and 2.6 in 2012 and 2013, respectively. The ratio in peak egg counts between urban and forest areas was also rather similar across the two countries and years, being 3.6 (2012) and 2.3 (2013) in Switzerland and 3.2 (2012) and 2.3 (2013) in Italy.

The results are consistent with the hypothesis that the intervention programme in Ticino has an impact on mosquito densities although other reasons such as potential differences in breeding site quality, mosquito species competition etc. might also explain the differences observed in this study.

Susceptibility to *Bti*

All colonies tested showed LC_{50} and LC_{90} values typical for *Bti* susceptible mosquitoes (Kamgang et al., 2011; Liu et al., 2004) albeit the Switzerland colony was slightly less susceptible than the Rockefeller *A. aegypti* reference strain and the RecAlb_Lab laboratory colony (Table 1).

Table 1. Susceptibility in third instar larvae of *Aedes* spp. against *Bacillus thuringiensis* var. *israelensis*.

Species	Colony	No. larvae exposed	LC_{50} (95% CI) [mg/l]	LC_{90} (95% CI) [mg/l]
Aedes aegypti	Rockefeller	1,620	0.008 (0.007-0.009)	0.026 (0.021-0.036)
Aedes albopictus	RecAlb_Lab	1,080	0.009 (0.008-0.011)	0.028 (0.023-0.037)
	Switzerland	1,440	0.015 (0.012-0.018)	0.036 (0.030-0.060)

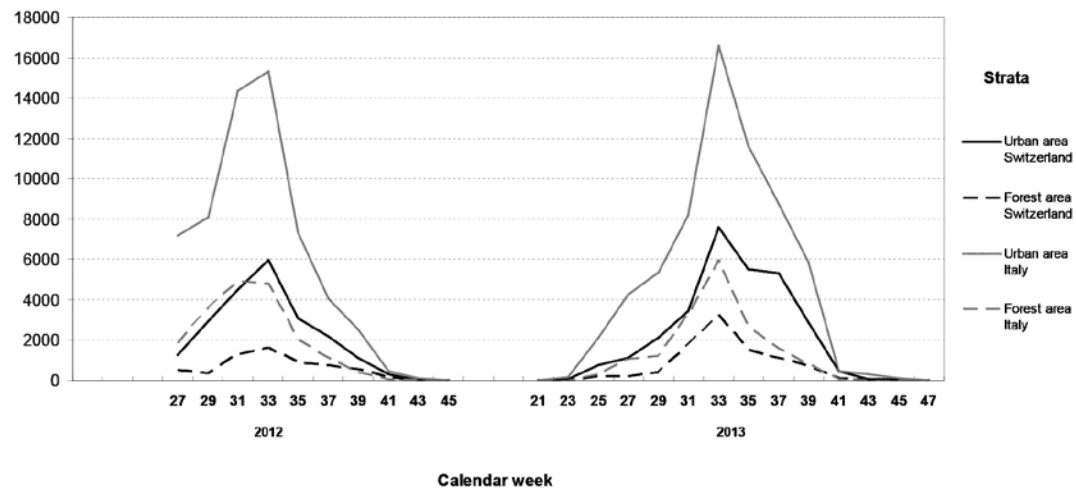

Figure 1. Temporal and spatial distribution of *Aedes albopictus* across the Swiss-Italian border. Numbers show total egg counts for each strata and time point.

CONCLUSIONS

The larvicide, *Bti* currently in use against *A. albopictus* in Ticino, Switzerland is still effective and, although not conclusively, the data support the hypothesis that the current intervention programme in place significantly reduces mosquito densities in the control area.

ACKNOWLEDGEMENTS

We would like to thank Federica Apruti for field assistance. We are also grateful to Drs Lêda Regis, Constância Ayres and the Department of Entomology of the Centro de Pesquisa Aggeu Magalhães-FIOCRUZ in Recife for the excellent collaboration. We are also grateful to Prof Christian Lengeler and the Gruppo cantonale di Lavoro Zanzare in Ticino for fruitful discussions and support. This study received funds from the Brazilian Swiss Joint Research Programme and the Emanuel Burckhardt Stiftung, Basel.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

DENGUE VECTOR DISTRIBUTION AND ABUNDANCE IN LOWER RIO GRANDE VALLEY, TEXAS USA

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Abstract Dengue virus has been classified as a worldwide emerging disease. South Texas is potentially at risk for dengue virus introduction due to proximity to Mexico, a country with endemic dengue transmission. Previous research has identified two dengue vectors, *Aedes aegypti* and *Aedes albopictus* in the region. Both species share similar habitats and often compete as larvae. Understanding the distribution and relative abundance of these species will aid in the process of determining risk for dengue virus transmission. We examined the relative abundance of these two species in a six sites extending from the Gulf of Mexico inland approximately 60 miles. Three different habitats were surveyed at each field site: a tire store, a cemetery, and a residential location. Mosquitos eggs were collected weekly using oviposition traps. Temperature and humidity were recorded during each collection. We predicted we would find a greater abundance of *A. aegypti* further away from the shoreline where the humidity was lower. We also predicted we would find greater abundance of *A. aegypti* in tire shops, where there is little cover. Preliminary analysis indicates *A. albopictus* were more common in cemeteries, while *A. aegypti* were more common in tire shops. Both appeared to be equally abundant in residential area. There is no correlation between species abundance and distance from the Gulf of Mexico. These data provide insight into the potential for dengue virus introduction and transmission in the region.
Key words Dengue virus, *Aedes albopictus*, *Aedes aegypti*.

INTRODUCTION

Dengue virus is a vector borne disease that is increasing in prevalence in North and South America (Ehrenkranz et al., 1971; Gubler, 1998; Guzman and Kouri, 2003; Siqueira et al., 2004; World Health Organization, 2009; Jansen and Beebe, 2010). Dengue has recently been classified as an emerging pathogen due to increased prevalence worldwide. Dengue virus historically has been transmitted in Northern Mexico; however there is an extreme disparity between the infections rates of dengue virus and Mexico. From 1980 to 1999, over 60,000 dengue cases were reported in Mexican border states, while Texas only had 64 cases (Reiter et al., 2003). Endemic dengue activity was reported in 2013 in South Texas, suggesting that despite a lack of reported cases in the past, dengue is an ongoing concern (Oliveres, personal communication).

Aedes aegypti is the primary vector of dengue, although *Aedes albopictus* has been shown to be capable of transmitting the disease as well (Ibanez-Bernal et al., 1997; Gratz, 2004; Kurane, 2007; Erickson et al., 2010). Both mosquitos are commonly found in habitats that are associated with human presence and activity. *Aedes aegypti* and *Aedes albopictus* share similar biological characteristics, are both container-breading mosquitos and often compete with each other for recourses (Sota and Mogi, 1992; O'Meara et al., 1995; Alto et al., 2008; Frances et al., 2011; Richards et al., 2012). The result of their competitive actions influences the distribution of both species. Many studies have been conducted focusing on container breeding mosquito interactions, but the majority of these studies have focused on the Florida environment (Harper and Paulson, 1994; O'Meara et al., 1995; Daughtery et al., 2000; Juliano and Lounibos, 2005; Leisnham and Juliano, 2009).

While studies in Florida, a subtropical region in the United States, have suggested that *A. albopictus* may outcompete *A. aegypti* in more humid areas, they have also suggested that in drier, hotter areas *A. aegypti* may be able to persist (Juliano et al., 2001). South Texas, and specifically the Lower Rio Grande Valley, is a subtropical region as well. However, this region of Texas that is generally hotter and drier than Florida. The Lower Rio Grande Valley is adjacent to Northern Mexico where endemic dengue is present. Understanding vector distribution and potential disease transmission dynamics is of increasing importance in this region.

Our research focused on examining habitat preference between *A. aegypti* and *A. albopictus*. Determining habitat preference can assist in controlling these vectors, possibly limiting or preventing potential outbreaks of dengue virus in the lower Rio Grande Valley. We conducted a field survey for *A. aegypti* and *A. albopictus* to examine habitat preference and abundance based on habitat type, as well as changes in preferences moving closer to the shoreline. We hypothesized we would find a greater abundance of *A. aegypti* further away from the shoreline and in tire shops, and a greater abundance of *A. albopictus* closer to the shoreline and in cemeteries.

MATERIALS AND METHODS

Oviposition Trapping. From May to August 2013 field collections for the mosquitos *Aedes aegypti* and *Aedes albopictus* were conducted weekly from trapping sites within the cities of McAllen (3 sites), Weslaco (1 site), Mercedes (2 sites), Los Fresnos (2 sites), Laguna Vista (1 site), and Port Isabel (2 site). The cities were chosen based on their relative proximity to the Gulf of Mexico. Trapping sites were classified as cemeteries, tire shops, and residential areas. Cemeteries were well shaded with well-tended lawns but little understory vegetation. Tire shops had little to no shade but a high number of potential oviposition sites in used tires that were scattered around the locations. Residential areas were well shaded, often with many shrubs or bushes, and many hand well-tended lawn. Ten to fourteen oviposition traps were placed in shaded or protected regions in the sites depending on the size of the site. Oviposition traps consisted of a black cups filled with 300mL of deionized water and a scraped wooden tongue depressor (stake) that was secured to the cup with a binder clip. Each week the stakes were collected and replaced. Water still left in cups were inspected for mosquito larvae and replaced. Missing or damaged cups were replaced as needed. Temperature and humidity for each site was recorded at each collection. All stakes and water containing larvae were returned to the laboratory for inspection.

Egg and Larvae Collection. All larvae collected from the field sites were counted and recorded. Larvae were placed in rearing pans at low densities (50 larvae per small pan or 150 larvae per large pan). For each wooden stake the total number of unhatched and hatched or damaged eggs was recorded. All stakes containing unhatched eggs were hatched in an aerated 1.0g/L nutrient broth and stayed submerged for 24 hours as described by Vitek and Livdahl (2006). After the eggs were hatched, the larvae were counted and placed in rearing pans at the previously mentioned densities and reared to adulthood. Pupated larvae were transferred into smaller containers, 10 pupae per container, and left to emerge to adults. Adult mosquitoes were frozen and identified to species and recorded as *A. aegypti, A. albopictus*, or other. After hatching, wooden stakes were inspected again and total number of hatched, viable, and nonviable eggs were recorded.

RESULTS AND DISCUSSION

Both vectors species were found in all locations, although there were clear habitat preferences between the two species. The total number of eggs per cup was calculated to use a common measure for analysis. A multiple factor ANOVA was used to analyze the total eggs collected per cup, with site location, habitat type, and week as potential explanatory variables (non-significant interactions were removed). Week was not significant and removed as a potential variable ($F = 0.923$, $p = 0.339$, df = 1). Habitat type was significant in the multi-factor ANOVA ($F = 4.222$, $p = 0.017$, df = 2), with cemeteries having significantly more mosquitoes than residential or tire shop locations. In addition, a significantly larger number of mosquitoes were collected in two field sites (Figure 1, $F = 5.732$, $p = 0.00001$, df = 5) although this was not correlated with distance from the coast.

Eggs that were collected and hatched showed a high level of survivorship to adulthood. Overall, 87.4% of the eggs that were hatched were successfully reared to adulthood, suggesting an estimated abundance of both species based on adult identification would be accurate. The percentage of *A. aegypti* adults to *A. albopictus* adults was calculated from hatched eggs to determine a relative abundance of both species, with week, longitude, and habitat type as potential variables. A multiple factor ANOVA was used to calculate the differences between the potential variables, and non-significant interactions were removed from the analysis. Habitat type was significant ($F = 93.451$, $p < 0.0001$, df = 2). A Least Squares Means Students t-test was used to compare sites, and a significantly higher proportion of *A. aegypti* mosquitoes in tire shops and a significantly smaller proportion in cemetery sites. Longitude significantly interacted with habitat type ($F = 17.918$, $p < 0.0001$, df = 2). A regression line for longitude using each habitat site was developed, with significant positive linear relationship for residential sites ($F = 24.1967$, $p < 0.0001$, $r^2 = 0.35$, Figure 3). The regression lines for cemetery and tire shop sites were non-significant, indicating the relative proportion of *A. aegypti* did not change relative to longitude.
Both of these vectors have a short flight range (Reiter et al. 1995, Harrington et al. 2005) suggesting the localized risk estimates based on specific local habitat or conditions may be critical to estimate risk of exposure. These results highlight the role local conditions may play in vector abundance, including the abundance of multiple species. Leisnham and Juliano (2009) reported densities of *A. aegypti* differed among land categories, being more abundant in residential areas compared to industrial and commercial areas, whereas densities of *A. albopictus* did not differ. The change in proportion of *A. aegypti* in residential areas suggests that risk of dengue transmission may vary based on the specific geographic location and conditions rather that overall estimate of mosquito abundance.

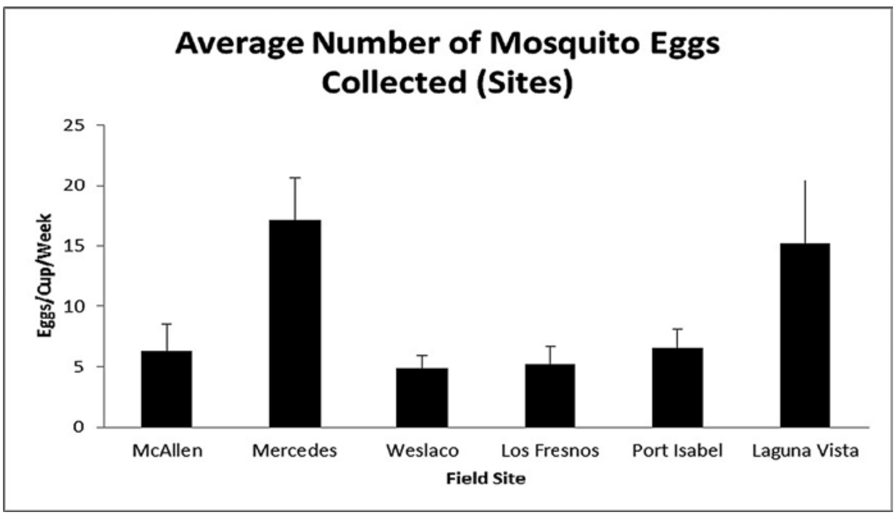

Figure 1. Average number of eggs per cup per week among field sites.
Sites with different letters are significantly different from each other. Sites arranged furthest from the coast (left) to closest to the coast.

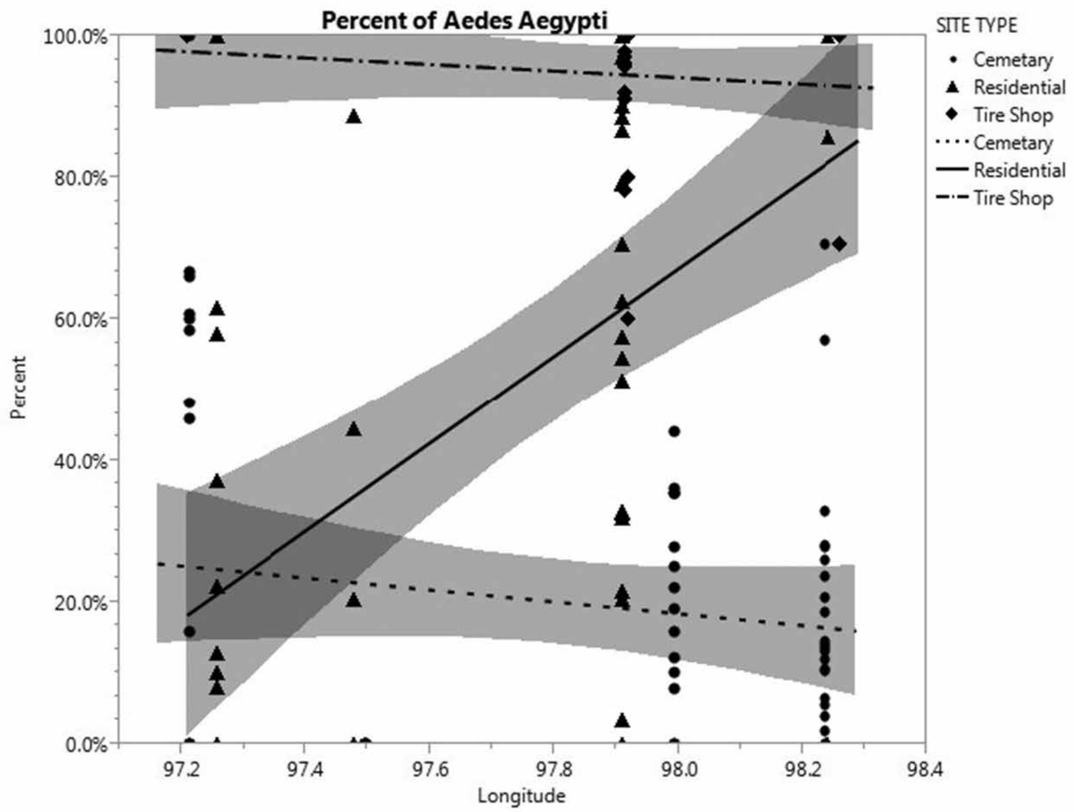

Figure 2. Abundance of *Aedes aegypti* by site and longitude. Moving inland, the percentage of *A. aegypti* is significantly different in residential sites, no change in tire shops and cemeteries with longitude (non-significant regression lines). Lines drawn with 95% confidence intervals.

Previous work also suggested a temporal change in relative abundance of *A. aegypti* to *A. albopictus*. While week by itself was not significant in the multi-factor ANOVA ($F = 0.281$, $p = 0.597$, $df = 1$), week did interact significantly with longitude ($F = 9.815$, $p = 0.0021$, $df = 1$). Interestingly, of the six sites, only McAllen (furthest inland) showed any significant relationship between week and percentage of *A. aegypti* ($F = 6.032$, $p = 0.019$, $r^2 = 0.137$). The McAllen area was where the previous collection was conducted, showing the same negative relationship.

The Lower Rio Grande region of South Texas represents an at-risk location that has historically been neglected as a potential focal point of vector-borne disease introduction and transmission. The increased poverty level, including the rural poor often living in substandard housing locally referred to as *colonias*, may represent both an at-risk population as well as a potential reservoir population for diseases like dengue virus and chikungunya virus.

Previous epidemics in South Texas have been linked to corresponding outbreaks in Northern Mexico (Rawling et al., 1998; Brunkard et al., 2007; Brunkard et al., 2008; Adalja et al., 2012). However, the recent identification of 4 locally acquired cases in late 2013, suggest that dengue is more prevalent than previously accepted and maybe a continual thread (Oliveres, personal communication). These results indicate the common presence of both vector species, as well as specific habitat preferences between the two species, further highlight the importance of coordinated research and control efforts to assess and minimize risk of vector-borne disease in the region.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

MOSQUITO ASSEMBLAGE DIVERSITY IN URBAN PARKS, SÃO PAULO, BRAZIL

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Abstract Due to the scarcity of information about the fauna of mosquitoes in the city of São Paulo, Brazil, an investigation was done with the aim of describing the diversity of species present in green areas of municipal parks in the city. Between March 2011 and February 2012, adult and immature mosquitoes were collected in seven parks in the municipality. The collections were once a month, in each park. The species richness was analyzed, Simpson's diversity index (1 - D), abundance of the species of epidemiological interest and similarity of assemblages by Sorensen's qualitative similarity index. Richness ranged between 11-36 species per park. Simpson index ranged between 0.014-0.833. *Culex quinquefasciatus*, *Cx nigripalpus*, *Aedes fluviatilis*, *Ae. scapularis* and *Ae. albopictus* were collected in parks showing relatively high abundance in some. *Ae. aegypti* were scarce or absent in these areas. The similarity between sessions ranged from 0.32 (Anhanguera - Ibirapuera) to 0.8 (Chico Mendes - Ibirapuera).

Key words *Culicidae*, green areas, biodiversity.

INTRODUCTION

Brazil has a considerable richness of *Culicidae* (about 486 described species (WRBU, 2013). Many species, especially those of the genera *Aedes*, *Anopheles* and *Culex*, are important vectors of pathogens in different regions of Brazilian (Forattini, 2002). Currently, there is a great concern among public health agencies such as the emergence or re-emergence of diseases transmitted by culicids and their dispersal in an urban environment (Weissenböck et al., 2010; Weaver, 2013).

In the city of São Paulo, located in southeastern Brazil, there are several fragments of green areas to conserve native or introduced populations of species of flora and fauna, besides conserving springs, streams, lakes and water reservoirs. Much of these fragments are in the urban parks, constantly visited by the population as leisure environments. Some of these parks have vestiges of the Atlantic Forest in the secondary stage of development the remaining parks have gardens area and groves with native or exotic flora species, reflecting different historical interventions (São Paulo - SVMA, 2012).

Studies have indicated relatively high richness and abundance of mosquitoes in urban parks showing that many native and exotic species are able to dwell in these "green islands" as shelter and maintenance of their populations (Taipe-Lagos and Natal, 2003; Montes, 2005; Medeiros-Sousa et al., 2013). Despite these contributions, there are now over a hundred public parks and protected areas scattered throughout São Paulo's city, of which nothing is known about its culicid fauna, either from an ecological or epidemiological perspective.

Due to the scarcity of information about the fauna of mosquitoes in urban parks of the city of São Paulo, and its importance in the dissemination of pathogens to humans, it was developed an investigation with the aim of describing the diversity of species present in urban parks in the city.

MATERIAL AND METHODS

Characterization of the Study Areas

Collections were performed in the urban parks of the city of São Paulo (23.54° W 46.63° S) inserted in the Atlantic forest of southeastern Brazil. Currently, the city has 100 municipal parks, two Areas of Environmental Protection and a Private Reserve of Natural Heritage administered by the municipal government (São Paulo, 2012). Besides these, ten other protected areas lie within the city, administered by the state. For this study selected seven municipal parks: Alfredo Volpi, Anhanguera, Carmo, Chico Mendes, Ibirapuera, Santo Dias and Shangrilá (Figure 1).

Collection and identification of specimens

The Collection has performed in each park during the period March 2011 to February 2012. Adult mosquitoes were captured in the parks using an electric aspirator 12 volt battery (Natal and Marucci 1984), CDC and Shannon traps (Bustamante and Pires, 1951, Gomes et al. 1985). Aspirations were performed at three predetermined areas of the park each lasting about 20 minutes. CDC traps were installed at two points in each park, one in an open area, preferably near the administrative offices or recreational areas, and another in the forest. At each point two traps were placed, one at about 1 meter above the ground and one at 5 meters or more in height. These traps were triggered at the beginning of the twilight and maintained for about three hours. Shannon trap with light gas lamp were installed inside the forest, for two hours after dusk, mosquitoes that landed on the surface of the tent were collected by manual electric aspirator batteries (6 volts battery).

Immature forms were collected using entomological shells (400 ml) in natural or artificial breeding sites as puddles, ponds and water tanks. Removable breeding sites had their content poured into plastic trays and the specimens were collected with pipettes. In the case of vegetable breedings, such as bromeliads, tree holes and bamboo nodes contents were extracted by using a suction pump. All samples obtained at immature stage were maintained in the laboratory until adult emergence.

Morphological identification of specimens was performed at the Laboratory of Entomology of Public Health, School of Public Health, University of São Paulo (LESP/FSP/USP).

Data analysis

We have compared observed and estimated richness (Jackknife 1), Simpson's diversity index (1 - D), abundance of the main species of epidemiological interest and similarity of assemblages by qualitative index of Sorensen. Adults and immature were considered for analysis of total richness and qualitative index of Sorensen, but for the analysis of Simpson's diversity index and abundance of the main species only adults were considered, since the aggregate distribution of immature of many species of culicids could cause biases in this type of analysis.

RESULTS AND DISCUSSION

Based on samples taken at each park culicid richness observed ranged from 11 species in the Ibirapuera park to 36 species in the park Anhanguera. The estimate of the total richness, based on Jackknife 1, showed that only in Anhanguera and Shangrilá parks not get an approximation of the asymptote, unlike other parks surveyed (Figure 2). The park Anhanguera has a much larger area than other parks, with 9.5 million square meters, with different types of forest formations like Atlantic forest remnants, dry fields,

Figure 1. Map of the city of São Paulo (23.54° W - 46.63° S) indicating the location of the seven urban parks where the culicid collections were performed.

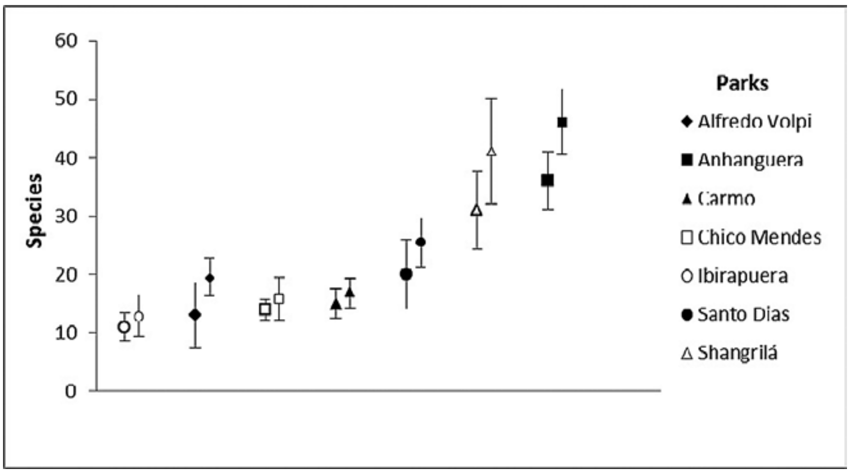

Figure 2. Observed richness (larger symbols) and estimated richness by Jackknife 1 (smaller symbols), with a confidence interval of 95%, to culicids of seven urban parks of the city of São Paulo, in sampling from March 2011 to February 2012.

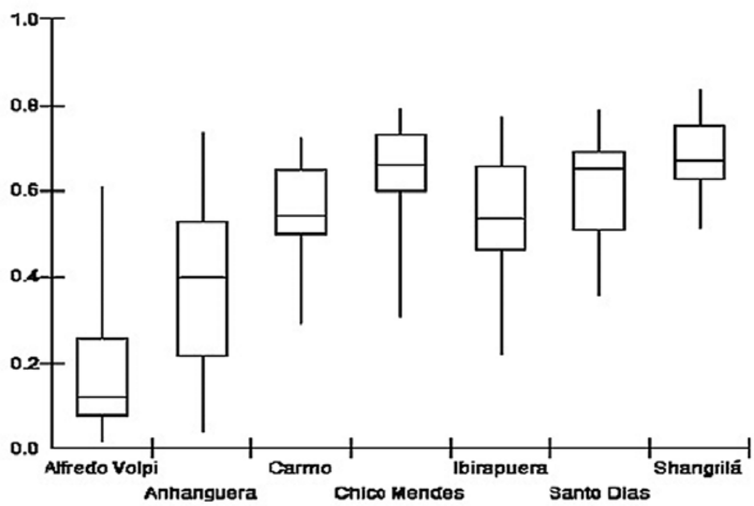

Figure 3. Box plot containing the median, first and third quartiles and variation (whisker) on values of the Simpson's diversity index (1-D) from culicid adults in seven urban parks of the city of São Paulo in samples taken from March 2011 to February 2012.

swamps, forests of *Eucalyptus* sp. and garden areas, this heterogeneity can provide various breeding sites and allow a greater number of species occur in this park. In turn, the Shangrilá Park, although smaller in extent, with 75,000 square meters, is located on the edge of the water reservoir Billings, within an area of environmental protection with over 90 million square meters. These characteristics may be responsible for greater culicids richness in these two parks. Taipe- Lagos and Natal (2003) and Montes (2005) had already demonstrated high mosquito richness in peripheral green areas of the city.

The Simpson's diversity index showed values ranging from 0.014 to 0.833 in the Alfredo Volpi and Shangrila parks respectively, these two values were obtained in December 2011. Shangrilá park showed the highest diversity and lower variation values over the year of collection (0.511-0.833). The most significant differences were observed in the Anhanguera park (0.035-0.733), low values of Simpson's index in this park are due to a high abundance of *Culex nigripalpus* compared to other species, mainly between the months September to December 2011. Alfredo Volpi showed low values of diversity because of the presence of few species per collection and because the high abundance of *Aedes fluviatilis* (87.4% of total samples in the park). Other parks showed intermediate values with a relative influence of the dominant species (Figure 3). Regarding the Sorensen's qualitative similarity index, the values ranged from 0.32 (Anhanguera - Ibirapuera) to 0.8 (Chico Mendes - Ibirapuera) (Figure 4). The Anhanguera and Shangrilá parks showed lower similarity to other parks possibly due to the already mentioned features on these two areas. The other parks are more inserted into in the urban area and probably this characteristic influences the greater similarity in composition and lower species richness.

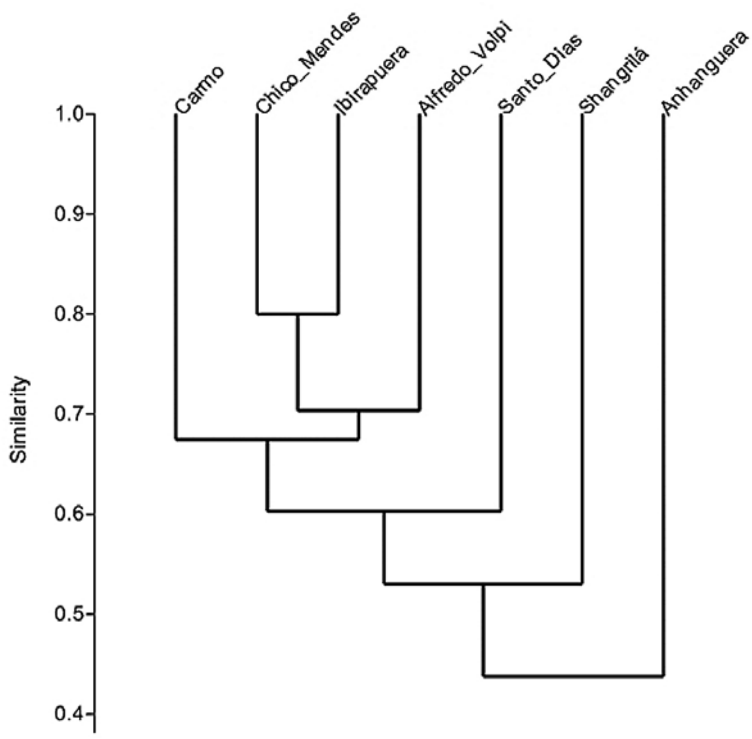

Figure 4. Dendrogram showing the similarity (Sorensen's qualitative similarity index) between the species composition of culicids from seven urban parks in the city of São Paulo in samplings from March 2011 to February 2012.

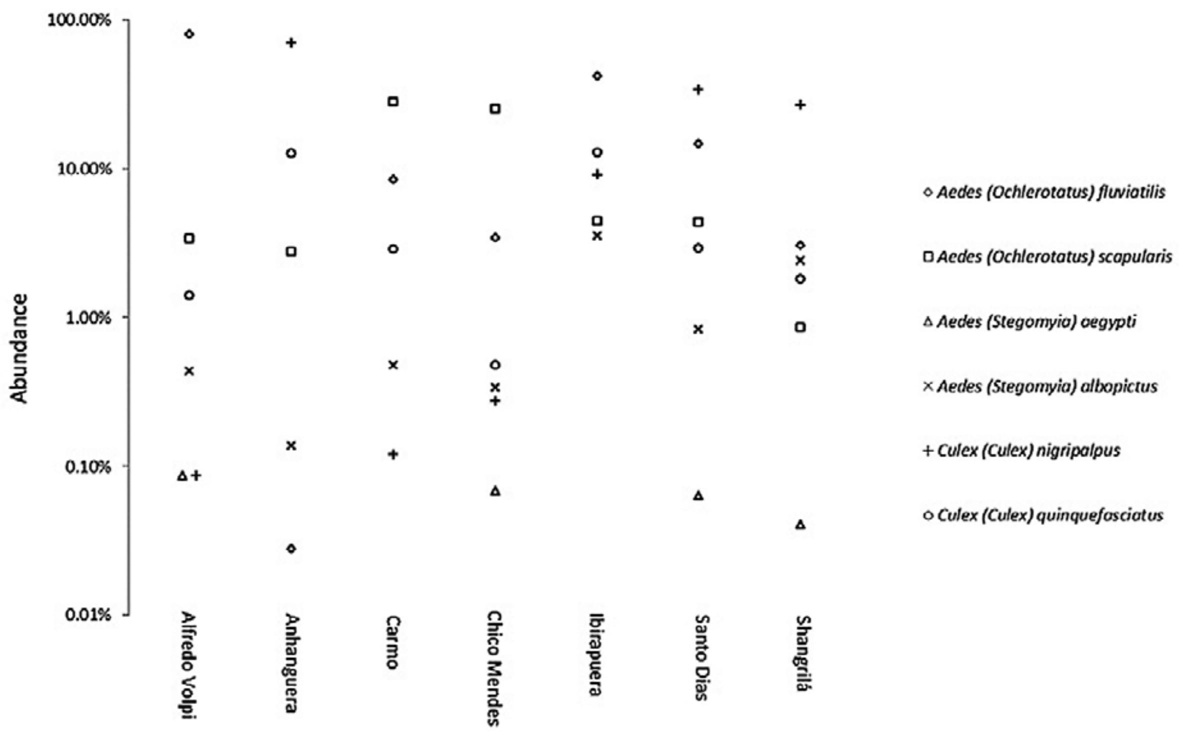

Figure 5. Relative abundance of culicids adults from six species of epidemiological interest collected in seven parks in the city of São Paulo from March 2011 to February 2012.

Some of the species of the epidemiological interest collected in these parks also shown to be quite abundant and frequent, like *Aedes fluviatilis*, *Ae. scapularis*, *Ae. albopictus*, *Cx nigripalpus*, *Cx quinquefasciatus* (Figure 5). These species, besides causing nuisance to people due to their bites, are recognized vectors competent for the transmission of many pathogens, mainly arboviruses (Forattini, 2002; Weissenböck et al., 2010; Fontes et al., 2012; Weaver, 2013). *Ae. Aegypti* presented low abundance or absent in these areas, possibly because this species is highly anthropophilic, preferring the domestic environment of the surroundings (Forattini, 2002).

There is a permanent risk that visitors, employees and the people who live or work in the surrounding of the parks come into contact with emerging or reemerging pathogens circulating in these environments. The knowledge of the Culicidae fauna and its diversity in urban parks can contribute to the effectiveness of monitoring and control methods, preventing transmission cycles of pathogens established in this environment. Future analyzes based on these data will help to understand the role that urban fragments of green areas have much to preserve biodiversity on the one hand and host species of epidemiological interest on the other side.

ACKNOWLEDGEMENTS

We thank Fundacão de Amparo à Pesquisa do Estado de São Paulo (BIOTA Program: Project 2010/51230-8) for funding and to all other members of the Department of Epidemiology of the School of Public Health, University of São Paulo, Zoonosis Control Center, and Department of Parks and Green Spaces São Paulo, who contributed during the field and laboratory works.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

EFFICACY OF A NOVEL AREA-REPELLENT AEROSOL FORMULATION AGAINST MOSQUITOES AND FLIES (DIPTERA: CULICIDAE, MUSCIDAE)

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Abstract Field studies of two area repellent aerosol formulations with transfluthrin and permethrin active ingredients were carried out against mixed freshwater mosquitoes near Humpty Doo, Northern Territory and against flies (a mixed population of house flies, *Musca domestica* and bush flies *Musca vetustissima*) in Bell, South West Queensland, Australia. The mosquito test site was adjacent to a breeding area, with a large population of biting mosquitoes. Fly repellency testing was conducted in the milking shed of a dairy farm with a large resident fly population. Non-absorbent (glazed tile) and absorbent (plywood) substrates were sprayed with the formulations and exposed to the ambient conditions. For the mosquito study, substrates were located adjacent to seated human test subjects. The test subjects counted all mosquito landings on the visible areas of their body during a 5 minute period every 2 hours for 6 hours. Fly evaluation was conducted in a similar manner, except that human assessors counted all fly landings on surfaces within a 1 metre radius of where they were seated. There were no significant differences in efficacy between the high and low active level formulations for both mosquitoes and flies. The lower active level formulation (0.5% transfluthrin and 0.05% permethrin) gave a reduction of 96.3% over 6 hours for glazed tiles, compared to the untreated controls, and a reduction of 80.0% for plywood. For flies, the respective reductions for the same rate were 81.4% and 79.0%. These studies demonstrated that both formulations provided effective area protection from mosquitoes and flies for up to 6 hours after application, on both absorbent and non-absorbent surfaces.
Key words Transfluthrin, area repellent, *Musca domestica, Musca vetustissima, Anopheles bancroftii, Culex annulirostris*

INTRODUCTION

An area or spatial repellent has been defined as: 'an inhibiting compound, dispensed into the atmosphere of a three dimensional space which inhibits the ability of mosquitoes to locate and track a target such as a human or livestock' (Nolen et al., 2002). This study addresses the area repellency of a novel aerosol product against biting mosquitoes and nuisance flies.

Outdoor area repellents are a relatively new concept in consumer pest control. Formats include mosquito coils, lanterns, metered aerosols, thermal devices and candles (WHO, 1998). The majority of these products are designed exclusively for mosquito repellency and are generally ineffective in repelling flies. In Australia, the House fly, *Musca domestica* and the Bush fly, *Musca vetustissima* are important nuisance pests (Gerozisis et al., 2008). The bush fly is a particularly annoying pest over much of Australia in the summer months because of the habit of landing on people (Hughes et al., 1972). Therefore, there is a need for an area repellent that is efficacious in repelling both mosquitoes and *Musca* flies.

Treating an inert surface with a vapour-active repellent is an appealing concept. This idea is not new, however. The US Army tried spraying a repellent on the ground in 1943 to repel mosquitoes, with limited success (Moore and Stage, 1943). For the concept to work well a chemical which vapourises at ambient temperatures is needed. The pyrethroid transfluthrin has a vapour pressure of 9 x10^{-4} Pa at 20°C (WHO,2014) and is a good candidate for this use. Ogoma et al. (2012) demonstrated the efficacy of this repellent against *Anopheles arabiensis*, when sprayed onto hessian strips. Permethrin, the second pyrethroid included in our test product, has also been used as a repellent spray on clothing and was shown to be effective (Debboun et al., 2007).

Australian consumers embrace the aerosol format for domestic pest control. This drove the development of an aerosol based area repellent that can be conveniently applied to a variety of substrates to provide area repellency against flies and mosquitoes.

MATERIALS AND METHODS

Aerosol Formulations and Application To Substrates

All aerosol formulations in this study were provided by Pascoe's Pty Ltd, , 40-46 Fairfield Street, Fairfield East, NSW 2165, Australia. Aerosol formulation AS01-37a contained 1.0% transfluthrin and 0.1% permethrin and AS01-37b contained 0.5% transfluthrin and 0.05% permethrin.

For the mosquito study, 30.0g of aerosol formulation AS01-37a was sprayed from a distance of 200mm onto 4 x 500mm x 500mm glazed floor tiles (total area 1.0m^2) and another 30.0g was applied as above to a piece of unpainted plywood 1,200mm x 800mm (total area 0.96m^2). The application was repeated to give 5 sets of approximately 1.0m^2 area of each of the above substrates. All the above was repeated for aerosol formulation AS01-37b. Once the substrates had been treated they were left for 30 minutes to dry outdoors, away from the test site.

For the fly study, 60.0g of aerosol formulation AS01-37a was sprayed from a distance of 200mm onto 9 x 450mm x 450mm glazed floor tiles (total area 1.82m^2) and another 60.0g was applied, as above, to 2 pieces of unpainted plywood 1,200mm x 800mm (total area 1.92m^2). The application was repeated to give 5 sets of approximately 1.0m^2 area of each of the above substrates. All the above was repeated for aerosol formulation AS01-37b. Once the substrates had been treated they were left for 30 minutes to dry outdoors, away from the test site.

Mosquito Study

Mosquito species were identified primarily as *Anopheles bancrofti, Culex annulirostris* and *Mansonia uniformis*. A large level cleared section of ground in close proximity to a mosquito breeding area at Thomsen's farm, Thomsen Road, Humpty Doo, NT, Australia, was selected as the study site. Test subjects selected for the study were males and females between the ages of 18 and 70 years. All test subjects were required to wear full body Elite Edition Original Bug Shirt□ mosquito suits (trousers and jackets with hoods) disposable latex gloves and covered shoes for the duration of the study.

There were 5 test subjects, 4 of whom evaluated the product and the other acted as the untreated control. All test subjects sat on folding camping chairs during the pre-count and evaluation periods. To avoid experimental bias, the control was a different subject on every evening of the study. Test subjects sat a minimum of 10m apart from one another, with the control sited at one end of the test area to minimize aerial contamination from the treated subjects.

The study commenced when biting mosquitoes were first noticed each evening (approximately 7pm). All subjects simultaneously conducted a pre-count of 5 minutes duration, counting all mosquito landings of greater than one-second duration on the visible parts of their body (primarily the abdomen, legs and arms). Product evaluation was then started if each subject recorded a minimum of 10 landings during the 5-minute assessment (i.e. 2 per minute).

Non-absorbent (glazed tiles) and absorbent (unpainted plywood) substrates were used to replicate typical domestic outdoor surfaces. These were sprayed with one or other of the coded formulations detailed above. Glazed tiles and plywood for the 4 and 6 hour assessments were aged under ambient conditions (27-35ºC) prior to the study commencing, while substrates for the 0 and 2 hour assessments were aged during the study. These were placed adjacent to the test subjects, 10 minutes prior to the start of each assessment.

For each evaluation, a 5-minute count of mosquitoes landing for more than 1 second was conducted. Landings were defined as the mosquito resting on the subject, not merely touching them. After mosquitoes had settled, movement of the subject disturbed them.

***Musca* Fly Study**

Fly species were the House fly, *Musca domestica* (approximately 80% of the population) and the Bush fly, *Musca vetustissima* (approximately 20% of the population). A large milking shed located on a dairy farm on Walkers Creek Road, Bell, Darling Downs District, Queensland, Australia was selected as the study site. The milking shed provided protection from strong winds and rain, but was open on one side and therefore exposed to the elements (sun and breeze), making it suitable for testing outdoor pest control products. This shed had a very high resident fly population, which was attracted to the cows (which were milked there twice daily) and to the many cowpats (cow faeces) deposited around the shed.

Human assessors were used to count the flies. There were 5 assessors, 4 of whom evaluated the product, while the other counted the untreated control. All assessors sat on folding camping chairs during the pre-count and evaluation periods. To avoid experimental bias, the control was a different subject on every day of the study. Assessors sat a minimum of 5m apart from one another, with the control assessor sited at one end of the test area to minimize aerial contamination from the treated subjects.

The study was commenced when flies were first noticed each morning (approximately 9 am). All assessors simultaneously conducted a pre-count of 5 minutes duration, counting all fly landings of greater than one second within their assessment area (an area with an outer perimeter 1m from the sprayed surfaces placed around the seated assessor). Product evaluation was then started if each subject recorded a minimum of 10 landings during the 5 minute assessment (i.e. 2 per minute).

Non-absorbent (glazed tiles) and absorbent substrates (unpainted plywood) were used to replicate typical domestic outdoor surfaces. These were sprayed with one or other of the coded formulations detailed above. All glazed tiles and plywood were aged *in situ* during the study under ambient conditions (14 - 26ºC). These test substrates were exposed to sunlight and wind during the study, to replicate domestic outdoor conditions. Substrates were placed adjacent to the test subjects, 10 minutes prior to the start of each assessment. For each evaluation, a 5 minute count of flies landing for more than 1 second was conducted. After the flies had settled, movement of the assessor was then used to disturbed them.

Calculation of Percentage Repellency

The following formula was used to calculate the percentage repellency for each treatment:

$(1 - C_0/C_1 \times T_1/T_0) \times 100$ = Percentage Repellency

C_0 = Control subject count for pre-treatment assessment

C_1 = Control subject count at a given assessment time

T_0 = Test subject count for pre-treatment assessment

T_1 = Test subject count at same given assessment time

Data Analysis

The data were analysed by analysis of variance (ANOVA) general linear model using SPSS® for Windows™ Version 20 (SPSS Inc. 2011). The assumption of normal distribution was checked using P-P plot and homogeneity of variance using Levene's test of equality of error variances.

RESULTS AND DISCUSSION

Mosquito Repellency

Table 1 gives the percentage repellency of each treatment at each of the time points during the study, as well as the average repellency over the six-hour assessment period. Landing inhibition using a glazed tile substrate was 93.6% for AS01-37a and 96.3% for AS01-37b, averaged over the 6 hour study. For plywood, the corresponding figures were 80.4% and 80.0%, respectively.

The mean numbers of mosquito landings at various post treatment times on a glazed tile substrate are presented in Figure 1. Table 2 shows a statistical analysis of the mean number mosquito landings on test subjects at various time point post-treatment on the same substrate. These data show no significant differences between the two treatments at most time points post-treatment.

The mean number of mosquito landings at various post treatment times on a treated plywood substrate is presented in Figure 2. Table 3 shows a statistical analysis of the mean number mosquito landings on test subjects at various time point post-treatment on the same substrate. These data show no significant differences between the two treatments at most time points post-treatment.

The weather conditions prevailing during the mosquito study were: temperature: 24.0 – 31.4°C; relative humidity: 74 – 89%; wind speed: 0 – 9 km/h; rainfall: 0.2 mm.

Table 1. Percentage mosquito landing inhibition of test formulations on two surfaces over 6 hours

Formulation	Surface	Ageing Time (h)	Landing Inhibition (%)	Number of Replicates
AS01-37a	Glazed Tiles	0	81.4	4
		2	96.8	4
		4	97.3	4
		6	99.1	4
		Average	**93.6**	**4**
	Plywood	0	76.1	4
		2	95.4	4
		4	58.2	4
		6	91.9	4
		Average	**80.4**	**4**
AS01-37b	Glazed Tiles	0	98.4	4
		2	98.5	4
		4	90.4	4
		6	98.0	4
		Average	**96.3**	**4**
	Plywood	0	81.5	4
		2	86.2	4
		4	59.8	4
		6	92.3	4
		Average	**80.0**	**4**

***Musca* Fly Repellency**

Table 4 gives the percentage repellency of each treatment at each of the time points during the study, as well as the average repellency over the six-hour assessment period. Landing inhibition using a glazed tile substrate was 85.1% for AS01-37a and 81.4% for AS01-37b, averaged over the 6 hour study. For plywood, the corresponding figures were 80.9% and 79.0%, respectively.

The mean numbers of fly landings on a glazed tile substrate at various post treatment times are presented in Figure 3. Table 5 shows a statistical analysis of the mean number fly landings on test subjects at various time point post-treatment on the same substrate. These data show no significant differences between the two treatments at most time points post-treatment.

Table 2. Summary of the statistical analysis for the average number of mosquito landings at various post treatment times in the presence of a treated glazed tile surface

Treatment	Pre-treatment[1]	F-test	0 hours post treatment	F-test	2 hours post treatment	F-test	4 hours post treatment	F-test	6 hours post treatment	F-test
AS01-37a	42.25* (12.39)	a**	2.25* (0.63)	b**	0.25* (0.25)	a**	0.50* (0.29)	a**	0.50* (0.50)	a**
AS01-37b	31.00 (5.99)	a	0.25 (0.25)	a	0.25 (0.25)	a	1.50 (0.65)	a	0.50 (0.50)	a

* Values are mean number of mosquito landings (n=4). Standard errors of mean are in parentheses. ** Treatments with same letter do not differ significantly from each other. [1]Data were ln(x+1) transformed prior to analysis.

The mean numbers of fly landings on a treated plywood substrate at various post treatment times are presented in Figure 4. Table 6 shows a statistical analysis of the mean number fly landings on test subjects at various time point post-treatment on the same substrate. These data show no significant differences between the two treatments at most time points post-treatment. The weather conditions prevailing during the fly study were: temperature: 13.7 – 26.1°C; relative humidity: 21 – 62%; wind speed: 0 – 19 km/h; rainfall: 0 mm.

Table 3. Summary of statistical analysis for average number of mosquito landings at various post treatment times in the presence of a treated plywood surface

Treatment	Pre-treatment[1]	F-test	0 hours post treatment[1]	F-test	2 hours post treatment	F-test	4 hours post treatment	F-test	6 hours post treatment	F-test
AS01-37a	31.00* (5.99)	a**	12.25* (4.11)	b**	1.00* (0.41)	a**	5.75* (0.85)	a**	1.50* (0.87)	a**
AS01-37b	31.50 (6.89)	a	2.25 (0.48)	a	2.00 (0.71)	a	7.00 (0.71)	a	1.50 (0.96)	a

* Values are the mean number of mosquito landings (n=4). Standard errors of mean are given in parentheses.** Treatments with the same letter do not differ significantly from each other.[1]Data were ln(x+1) transformed prior to analysis.

Table 4. Percentage fly landing inhibition of test formulations on two surfaces over 6 hours

Formulation	Surface	Ageing Time (h)	Landing Inhibition (%)	Number of Replicates
AS01-37a	Glazed Tiles	0	81.7	4
		2	88.0	4
		4	87.7	4
		6	80.9	4
		Average	**85.1**	**4**
	Plywood	0	79.7	4
		2	85.0	4
		4	82.8	4
		6	76.2	4
		Average	**80.9**	**4**
AS01-37b	Glazed Tiles	0	65.2	4
		2	80.1	4
		4	90.0	4
		6	90.4	4
		Average	**81.4**	**4**
	Plywood	0	66.9	4
		2	81.1	4
		4	91.2	4
		6	76.9	4
		Average	**79.0**	**4**

Table 5. Summary of the statistical analysis for the average number of fly landings at various post treatment times in the presence of a treated glazed tile surface

Treatment	Pre-treatment	F-test	0 hours post treatment	F-test	2 hours post treatment	F-test	4 hours post treatment	F-test	6 hours post treatment	F-test
AS01-37a	23.50* (2.26)	a**	7.00* (3.00)	a**	2.50* (0.65)	a**	5.50* (0.87)	a**	4.25* (1.70)	a**
AS01-37b	51.00 (12.80)	a	11.50 (4.09)	a	6.75 (1.32)	b	3.25 (0.63)	a	3.25 (0.95)	a

* Values are mean number of fly landings (n=4). Standard errors of mean are given in parentheses.

** Treatments with the same letter do not differ significantly from each other.

Table 6. Summary of the statistical analysis for average number of fly landings at various post treatment times in the presence of a treated painted plywood surface

Treatment	Pre-treatment	F-test	0 hours post treatment[1]	F-test	2 hours post treatment	F-test	4 hours post treatment	F-test	6 hours post treatment	F-test
AS01-37a	23.50* (2.26)	a**	4.50* (1.04)	a**	4.50* (1.71)	a**	7.00* (2.52)	a**	6.25* (2.02)	a**
AS01-37b	51.00 (12.80)	a	17.50 (5.72)	b	4.00 (1.23)	a	3.25 (1.38)	a	7.75 (3.61)	a

* Values are the mean number of fly landings (n=4). Standard errors of mean are given in parentheses. ** Treatments with same letter do not differ significantly.[1]Data were ln(x+1) transformed prior to analysis.

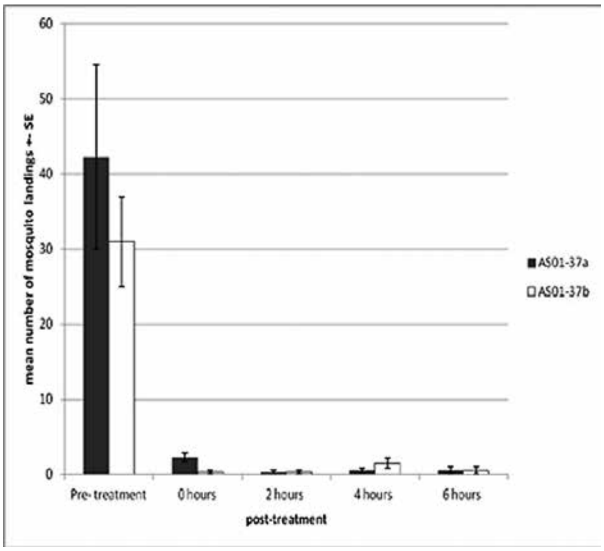

Figure 1. The average number of mosquito landings at various post treatment times in the presence of a treated glazed tile surface (bars) with their standard errors of mean

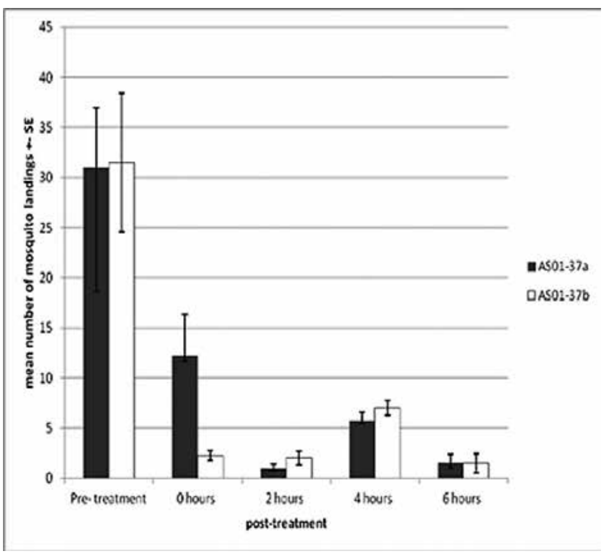

Figure 2. The average number of mosquito landings at various post treatment times in the presence of a treated plywood surface (bars) with their standard errors of mean

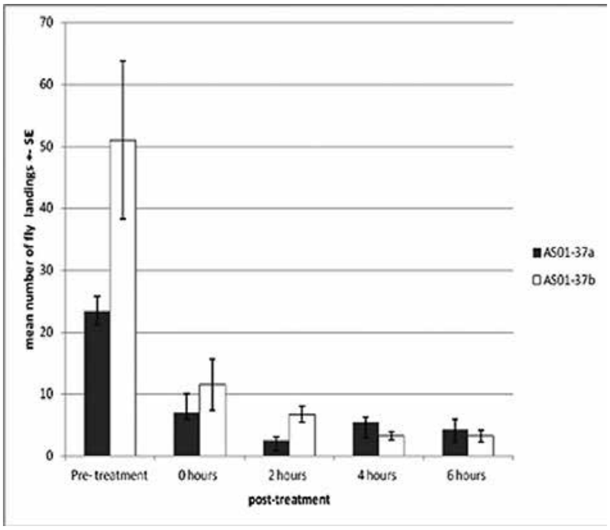

Figure 3. The average number of fly landings at various post treatment times in the presence of a treated glazed tile (bars) with standard errors of mean

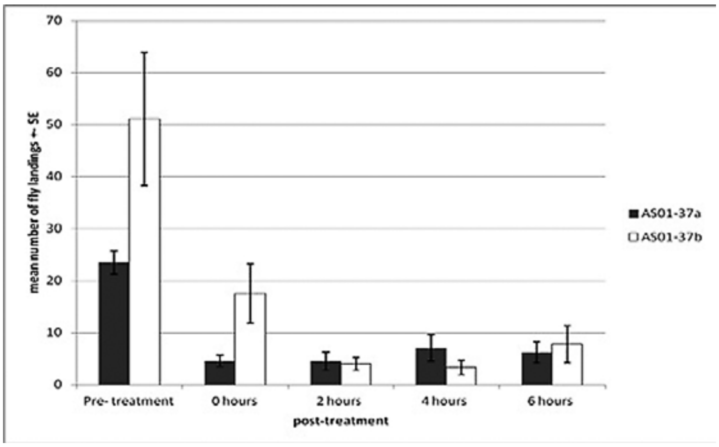

Figure 4. Average number of fly landings at post treatment times in the presence of treated plywood (bars) with standard errors of mean.

CONCLUSIONS

The mosquito and fly field studies showed that there were no significant differences in efficacy between the high active rate and the low active rate. Both formulations provided effective protection from biting mosquitoes and nuisance flies on both absorbent and non-absorbent substrates. The low active rate can be used, without compromising efficacy. A sprayed surface area of $1m^2$ was sufficient to provide continuous protection for at least 6 hours against biting mosquitoes, $2m^2$ was required for adequate fly protection. This product functions by vapour action from sprayed substrate, and is dependent on ambient conditions for emanation and consequently for mosquito and fly efficacy. The product remained effective over a range temperature and humidity and both still and windy conditions. The product is suitable for use in repelling flying nuisance insects in domestic environments.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

EVALUATION OF TOPICAL MOSQUITO REPELLENTS AND INTERPRETATION OF EFFICACY DATA: A SYSTEMATIC LITERATURE REVIEW

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Abstract Under the European Union Biocidal Products Regulation repellents are classified as biocides, and are subject to efficacy studies. To facilitate testing of repellents for product registration the EU has provided technical guidelines. The guidelines leave uncertainties as to how efficacy should be examined and how data may be evaluated in terms of label claim. The question is how do laboratory arm-in-cage bioassays relate to real conditions? We conducted a systematic literature review to examine published laboratory and field repellent studies that measured protection time against biting mosquitoes in humans with one of the four active ingredients: DEET, icaridin, citriodiol/PMD or EBAAP. Out of 871 publications identified with the search term mosquito repellents only nine studies met inclusion criteria. The data were insufficient to make a quantitative comparison between laboratory and field studies, which indicates the need for studies to support authorities in making evidence-based decisions on label claims for product registration.
Key words Travel medicine, European Union biocides regulation, Culicidae.

INTRODUCTION

Biting mosquitoes (Diptera, Culicidae) are important vectors of several diseases including malaria, filariasis and viral infections, including dengue, West-Nile or chikungunya, particularly in the tropical and subtropical regions but also increasingly in Europe as recent autochthonous cases of dengue and chikungunya show (Tomasello and Schlagenhauf, 2013).

Topical repellents for application on the skin provide good protection against mosquito bites. Under the European Union Biocidal Products Regulation (No 528/2012) repellents are classified as biocides, product type 19, and as such are subject to rigorous efficacy studies. In order to facilitate testing of formulated repellent products for product registration the European Union has also provided technical (draft) guidelines. The guidelines leave uncertainties as to how efficacy should be examined and how data from such studies may be evaluated in terms of label claim, that is, how protection time measured in laboratory studies compares to protection time under end-user conditions.

In an attempt to address comparability between laboratory tests and field application we carried out a systematic literature review following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) principles (Liverati et al., 2009). Our objective was to identify and compare data from laboratory and field efficacy studies in humans that report protection time of at least one of the four United States of America Environmental Protection Agency approved active ingredients for topical repellents against mosquitoes (DEET, PMD, icaridin and EBAAP). Here, we report the results of the systematic literature review and give recommendations for future studies.

MATERIAL AND METHODS

The systematic literature review followed the principles outlined in PRISMA (Liverati et al., 2009). We queried five literature retrieval data bases (Table 1) with the search term "mosquito repellents" for publications between 1953, the discovery of DEET, and end of March 2013.

Table 1. Data bases included in the literature review

Data base	URL
Cochrane library	http://www.thecochranelibrary.com
ISI Web of Science	http://apps.webofknowledge.com
LILACS	http://lilacs.bvsalud.org
PubMed	http://www.ncbi.nlm.nih.gov/pubmed
ScienceDirect	http://www.sciencedirect.com

We included only studies that met the following criteria: 1) The study reports on repellents against biting mosquitoes; 2) The repellents were tested in humans; 3) The tested repellents contained one of the following active ingredients: a. ethyl-butylacetylaminopropionate (EBAAP); b. hydroxyethyl isobutyl piperidine carboxylate (icaridin); c. *N,N*-diethyl-meta-toluamide (DEET); d. *para*-menthane-3,8-diol (PMD) or citriodiol. 4) The study reports the protection time, either complete protection or relative protection above definedthreshold, e.g. ≥95%.
Studies that did not mention the applied dosage, described mixtures of repellents and literature reviews without original data were excluded from the analysis.

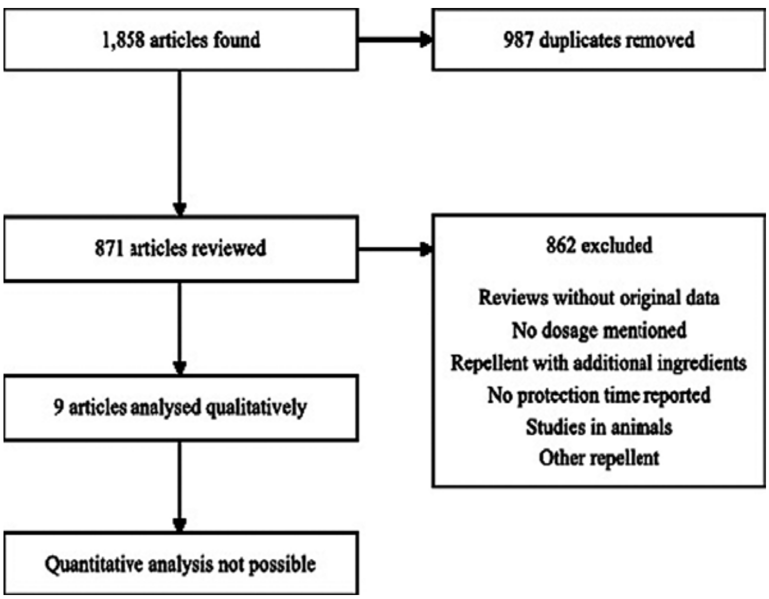

Figure 1. Selection process of available publications included in the systematic literature review.

RESULTS

After removing duplicates, the search term "mosquito repellents" yielded 871 unique hits yet only nine publications met our selection criteria (Figure 1).

In two out of the nine included publications, field and laboratory data were compared side-by-side (Carroll and Loye, 2006; Frances et al., 2009). From the remaining seven studies, one was a field study (Copeland et al., 1995) and six were performed under laboratory conditions (Ali et al., 2012; Barnard and Xue, 2004; Cilek et al., 2004; Drapeau et al., 2011; Logan et al., 2010; Obermayr et al., 2010). In addition of being only a total of nine publications, the formulations and concentrations, not even mentioning mosquito test species, varied widely across the studies (Table 2).

Table 2. Overview of studies included in the systematic literature review.

Repellent	Concent-ration (%)	Laboratory	Field
DEET	5	-	Copeland et al., 1995
	7	Barnard and Xue, 2004	-
	10	Carroll and Loye, 2006; Cilek et al., 2004; Logan et al., 2010	-
	15	Barnard and Xue, 2004	-
	20	Frances et al., 2009; Barnard and Xue, 2004; Cilek et al., 2004; Stanczyk et al., 2010	Frances et al., 2009; Carrol and Loye, 2006
	30	Carroll and Loye, 2006	-
	97	Ali et al., 2012; Drapeau et al., 2011	-
Icaridin	10	Barnard and Xue, 2004	-
	20	Obermayr et al., 2010	-
EBAAP	7.5	Barnard and Xue, 2004	-
	10	Cilek et al., 2004	-
	20	Cilek et al., 2004	
PMD	10	Carroll and Loye, 2006	-
	13	Drapeau et al., 2011	-
	20	Carroll and Loye, 2006; Drapeau et al., 2011	Carroll and Loye, 2006
	26	-	Carroll and Loye, 2006

In one of the two laboratory-field comparisons Frances et al. (2009) judged a 20% DEET against a 20% SS220 (a piperidine compound) formulation. The field study was done in Queensland, Australia. In their study DEET showed a longer protection against *Anopheles farauti, Aedes aegypti* and *Culex annulirostris* in the laboratory, while SS220 outperformed DEET under field conditions. In laboratory experiments the complete protection time of SS220 was between 18 and 180 minutes. DEET showed a complete protection time of 82 up to more than 360 minutes. However, the low sample size casts some doubts about the validity of the authors' conclusions. The study included only one and three human volunteers in the laboratory and field tests, respectively.

The other laboratory-field comparison (Carroll and Loye, 2006) measured protection time of DEET at 10% and 30% alongside PMD at 10% and 20%. Here, ten volunteers took part in the field study and even 24 volunteers were included in the laboratory study. DEET and PMD showed a similar efficacy against mosquitoes in the field- and laboratory experiments.
From the remaining studies it is also difficult to draw any conclusions. There was only a matching set of field and laboratory studies for 20% DEET (Table 2) and only for *Ae. aegypti* (The remainder of the studies listed in Table 2 and 20% DEET refer to other mosquito species prohibiting a fair comparison.) Intriguingly, for 20% DEET, Frances et al. (2009) measured a mean protection time of 195 minutes against *Ae. aegypti* in the field which is very close to the 180 minutes previously observed in the laboratory study by Cilek et al. (2004).

CONCLUSIONS

Though some studies suggest comparability between laboratory and field repellent studies, the resulting data were insufficient to make a quantitative comparison, highlighting the need for informative studies in order to support authorities in making evidence based decisions on label claims for product registration.

ACKNOWLEDGMENTS

We thank Anita Gassner for helping us in searching literature. This study received funds from the Swiss Federal Office of Public Health.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

REDESCRIPTION OF *AEDES AEGYPTI* AND DETECTION OF DENGUE VIRUS FROM FIELD COLLECTED LARVAE FROM LAHORE, PAKISTAN

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Abstract Dengue virus is transmitted by female mosquito *Aedes aegypti* and it consists of the most common *flavivirus* infection. Pakistan is also at high risk of dengue epidemics. The present study focused on the redescription of *Aedes aegypti* and the detection of dengue virus from 685 field collected larvae of *A. aegypti* from different areas of Lahore. No morphological change, as compared to previous work, was observed. Temperature and humidity have direct effect on the distribution, population density and growth rate of *A. aegypti.* Population and growth rate increases during rainy season and decreases during dry season. High number of larvae (99) was found in July, 2012. Bhati Chowk area is more receptive to dengue transmission as compared to other areas because high percentage of larvae (23%) was found from there. A rapid identification of dengue virus from larvae of *A. aegypti* was done by RT-PCR. RNA extracted from each pool was tested by RT-PCR for detection of dengue virus. It was shown that dengue serotype 2 was prevalent in Lahore, Pakistan. DV-2 was found in 26 pools, DV-3 in 9 pools and DV-4 was present in 3 pools. DV-1 was not found in any pool.
Key words RT-PCR, serotype, morphology, population.

INTRODUCTION

In Pakistan *Aedes. aegypti* is considered as the most important vector related to the dengue outbreaks. Dengue fever and dengue hemorrhagic fever, commonly known as break-bone fever, is caused by dengue virus (DV). There are four different serotypes of the virus, belonging to genus *flavivirus*: DENV-1, DENV-2, DENV-3 and DENV-4 (Mitchell, et al., 1987), which are closely related to each other antigenically. The difference in the nucleotide sequences of all serotypes is about 24-36% (Fauquet et al., 2005). From all serotypes, DV-4 is the most different. As far as literature is concerned, no thorough study is available to describe the morphology of *A. aegypti* from Pakistan. The present study was carried out from July 2012 to September 2013 to redescribe *A. aegypti* and to know whether any change has occurred in it due to climatic conditions during past few years or correlate the abundance of *A. aegypti* with temperature and relative humidity. Another objective was to detect dengue virus from field collected larvae of *A. aegypti*.

MATERIALS AND METHODS

More than 600 larvae were collected from different localities of Lahore. *A. aegypti* larvae were redescribed by using key (Harrison, 2005).Seasonal population of *A. aegypti* in different localities, Sex ratio, and relation with environmental factors like temperature and relative humidity were noted during study also. Detection of Dengue virus (DV) was done by RT-PCR. Minimal infection rate and viral infection rate were also calculated.

RESULTS AND DISCUSSION

As described in materials and methods, 685 larvae of *A. aegypti* were collected from different areas of Lahore during July, 2012 to September, 2013. Larval and pupal skin was taken and studied to redescribe *A. aegypti.* As *A. aegypti* was not described morphologically from Pakistan so during this study, morphological features of *A. aegypti* were studied, hairs present on head, thorax and abdomen of larvae and pupae were also counted. Larvae of *A. aegypti* were studied previously by (Puri, 1931; Belkin, 1953; Christopher, 1960). Recently, Andrew and Bar (2013) described the morphology of *A. aegypti* adult mosquitoes from India. The presence of 12-15 hairs on the head region, 1-14 hairs on thorax region and 10-15 hairs on abdomen were observed. These studies also witnessed the presence of 8-12 Comb teeth present in a single row. The results of current study are identical to the previous work and no significant change has been observed. Pupae of *A. aegypi* were also studied 10-12 hairs on cephalothorax and 10-14 hairs on each segment of abdomen were counted (Belkin, 1953). A single hair on each pupal paddle was also observed (Christopher, 1960). Pupal body of *A. aegypti* consisted of a large and somewhat rounded cephalothorax with 10-12 hairs. Like larvae of *A. aegypti,* pupal abdomen is also having 8 segments with 10-14 hairs each segment. It was seen that 8th segment contains circular paddles which have stubbles on them.

Results also revealed that the highest percentage of *A. aegypti* larvae were found in the Bhati Chowk area (23%). Lowest percentage (4.5%) was found in the Raj Gerh area. It can be depicted from results that the area of Bhati Cowk is at high risk with the highest population (157) of *A. aegypti* larvae. Female larvae were found in great abundance (63.5%) as compared to male larvae (36.5).As the ecology of *A. aegypti* is concerned, the number of collected larvae were correlated with the temperature and relative humidity (RH). The population of *A. aegypti* larvae were most abundant in the months of hot and humid weather. At the temperature of 32ºC and 93% RH (July, 2012), maximum number of *A. aegypti* larvae was found. The minimum number of larvae were found at the temperature of 14ºC and 86% RH in the month of January 2013. Previous studies also show that high population density of *A. aegypti* is mostly concentrated in densely populated areas (Chakravarti and Kumaria, 2005). Environment in densely populated areas predisposes large number of prone hosts to the bite of *A. aegypti* (Gibbons and Vaughan, 2002). *A. aegypti* is highly adapted to urban areas (Honorio, et al., 2003). Female *A. aegypti* lays eggs 1 km radius from their feeding sites, so they increase their boundaries (Chun et al., 2007; Weaver and Reisen, 2010). In addition to increase in population density, mass production of non-biodegradable plastic containers provide breeding sites for mosquitos (Kyle and Harris, 2008). A survey report (2009) of Lahore described that larvae of *A. aegypti* were prevalent in areas of Data Gunj Baksh and Samanabad as these areas were having artificial containers. The prevalence of *A. aegypti* was fewer in other areas that were not having artificial breeding sites.

Numerous environmental parameters like temperature and relative humidity were recorded during the study. All these factors have a strong association with mosquito abundance and development (Manimegalai, 2010; Devi and Jauhari, 2007; Leisnham et al., 2006). During extreme winters and summers, the larvae of *A. aegypti* do not survive. An average increase in daily temperature may have a major biological or morphological impact on *A. aegypti* (Tun-Lin et al., 2000; Mohammad and Chadee, 2011). Optimum temperature for larval growth is 32°C (Bar and Andrew, 2012) and optimum relative humidity is 80 ± 15% (Walker et al*.,* 2011). The same results were found for mosquito abundance associated with temperature in the present study. Mosquitoes were found highly abundant in the summer season. *A. aegypti* larvae were plenty in number during the month of July and August of both years when the temperature was 32ºC and humidity was between 80 to ≥ 90%.

About 300 larvae of *Aedes aegypti* were used for the detection of dengue virus. 30 pools of larvae were made to run the PCR. All serotypes (1-4) of dengue virus were detected. Out of 30 pools, Serotype 1 was not present in any pool. Agarose gel for serotype 2 with the band size 403 kb. 26 pools were positive with serotype 2. Serotype 3 was present in 9 pools. In serotype 3, bands (453 kb) were seen. Serotype 4 (401 kb) was also amplified. Out of 30 pools, serotype 4 was found in 3 pools. Viral Infection rate (VIR) and Minimal Infection Rate (MIR) were also calculated .Serotype 2 reveals the highest VIR and MIR i.e. 8.7 and 87, respectively. Species which transmit dengue virus transovarially or vertically in their next generations are *A. albopictus, A. aegypti, A. mediovittatus* (Freier and Rosen, 1988), *A. alcasidi, A. cooki* etc. (Rosen, 1988; Rohani et al*.,* 2005). Dengue virus persists in several generations of *Aedes aegypti* through transovarial transmission (Joshi et al., 2002). Thenmozhi et al*.* (2007) studied the vertical transmission of dengue virus in *Aedes* mosquitoes in India. It was concluded that vertical transmission also occurs in nature. Vertical transmission via the transovarial route in female *Aedes* mosquitoes also occur in nature. (Mulyatno et al., 2012).

CONCLUSIONS

The established method in this study included two-step RT-PCR for the detection of dengue virus. This is a rapid and very sensitive technique to detect the viruses. Present study showed that dengue virus serotype 2 was dominant in the larvae of *Aedes aegypti*. The other serotypes present were serotype 3 and 4. Serotype 1 was absent. The current research work will have an intense influence on dengue control programs and further epidemiological studies. The molecular detection of dengue virus will help to have more precise virus surveillance in the areas where there is a large population of vectors. Detection or isolation of different serotypes of dengue virus from endemic areas will be easier by using molecular.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

ISOLATION AND CHARACTERISATION OF BACTERIA ASSOCIATED WITH *MUSCA DOMESTICA* (DIPTERA: MUSCIDAE) IN HOSPITALS

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Abstract This study sought to determine the role of *Musca. domestica*, sampled from UK hospitals, as reservoirs of pathogenic bacteria. *M. domestica* were collected from pre-existing ultra-violet light flytraps located throughout the hospitals. External washings and macerates of *M. domestica* were prepared and inoculated onto agars and following incubation bacterial colonies identified by biochemical tests. Fourteen batches of *M. domestica* (n = 67) were sampled from 6 hospitals between March 2010 to August 2011 and 28 bacterial isolates (21 different species) were obtained. Bacterial isolates were recovered from all 14 *M. domestica* batches. There were 15 occurrences of Enterobacteriaceae (53%) (12 species), 7 Bacilli (25%) (4 species), 3 Clostridia (11%) (1 to genus level, 2 other species) 2 Staphylococci (7%) (*S. aureus*) and 1 Streptococci (4%). Bacterial species recovered multiple times were *Bacillus subtilis* Group, *Klebsiella pneumoniae* ssp *pneumoniae* and *Enterobacter cloacae*. The significance of these data is that *M. domestica* may carry pathogenic bacteria in the healthcare environment. This study highlights the potential for *M. domestica* to contribute to persistence and spread of pathogenic bacteria in hospitals and the need to consider pest control as part of infection control strategies.
Key words House fly, disease, pest control, infection control, healthcare.

INTRODUCTION

The house fly *M. domestica* is a synanthropic fly and the endophilic, communicative behaviour of such flies coupled with their potential for exposure to pathogenic bacteria presents a significant threat to public health (Gracyzk et al., 2001). House flies can transmit such pathogenic bacteria by mechanical transmission (Lane and Crosskey, 1993) and bioenhanced transmission (Kobayashi et al., 1999).
M. domestica sampled from hospitals in Nigeria, India, and Senegal harboured pathogenic bacteria, including *Bacillus* spp, (Adeyemi and Dipelou, 1984), *Eschericia coli* (Fotedar et al., 1992), antimicrobial resistant *Klebsiella pneumoniae* ssp *pneumoniae* (Fotedar et al., 1992a), *Salmonella* sp. (Nmorsi et al., 2007), Methicillin resistant *Staphylococcus aureus* (MRSA) (Rahuma et al., 2005), and MRSA with a sensitivity profile and phenotype of resistance identical to patients (Boulesteix et al., 2005). In Europe, house flies sampled from a hospital in the Czech republic harboured antimicrobial resistant *Enterobacter* spp., *Klebsiella* spp., *Citrobacter* spp, *Staphylococcus* spp and *Enterococcus* spp (Sramova et al., 1992). Laboratory models are also showing that house flies, *M. domestica* are able to transfer *Clostridium difficile*, one of the so-called 'hospital superbugs' (Davies et al., 2011).
The aim of this study was to isolate and characterise bacteria associated with *M. domestica* in hospitals, to understand the relevance of pest control as a component of infection control in healthcare facilities.

MATERIALS AND METHODS

M. domestica were collected from pre-existing ultra-violet light flytraps in the form of electronic fly killers (EFK's) and professional sticky traps located throughout 6 health care facility sites from March 2010 to August 2011. The contents of the EFK's were tipped into sterile bags. The glue boards from the sticky traps were removed and covered with a sterile plastic bag. The samples were stored at 4°C in a domestic refrigerator, pending identification and microbiological analysis. External washings of *M. domestica* were serially diluted and inoculated onto CCFA+Tc, Nutrient Agar, Mannitol Salt Agar and Violet Red Bile Glucose agar (all Oxoid Ltd, Basingstoke, UK). The flies were then macerated and the above process repeated for the macerates.

Nutrient agar, Mannitol Salt agar and Violet Red Bile Glucose agar plates were incubated at 37°C for 24 hours in aerobic conditions. CCFA+Tc agar and a set of Nutrient Agar plates were incubated in anaerobic conditions at 37°C (Don Whitley Anaerobic cabinet) for 48 and 24 hours respectively. Bacterial colonies were identified by macroscopic morphology, Gram staining, microscopic examination of morphology, oxidase and catalase tests (National Standard Methods BSOP TP 26 and BSOP TP 8) API 20E test kits, API Staph test kits, rapid ID 32A API test kits (bioMérieux, Marcy l'Etoile, France) and Bacillus-ID test kits (Microgen Bioproducts Ltd, Camberley, UK).

RESULTS

Fourteen batches of *M. domestica* (n = 67) were sampled microbiologically from 6 hospitals from March 2010 to August 2011 and 28 bacterial isolates (21 different species) were obtained (Table 1). Bacterial isolates were recovered from all *M. domestica* batches. Table 1 shows that of the bacteria isolated from *M. domestica*, there were 15 occurrences of Enterobacteriaceae (12 species), 7 isolates of *Bacillus* spp (4 species), 3 Clostridia (1 to genus level, 2 other species) 2 Staphylococci (both *S. aureus*) and 1 Streptococci. Species of bacteria recovered multiple times were *Bacillus subtilis* Group (x 4), *Klebsiella pneumoniae* ssp *pneumoniae* (x 3) and *Enterobacter cloacae* (x 2).

Figure 1 shows the proportion of isolates belonging to certain bacterial groups that were identified from *M. domestica*. Figure 1 shows that the majority of bacterial isolates taken from *M. domestica* sampled from hospitals were of the family Enterobacteriaceae (53%), followed by *Bacillus* spp (25%), Clostridia (11%), Staphylococci (7%) and Streptococci (4%). *M. domestica* carrying this variety of microorganisms were sampled from a number of locations, including hospital catering areas, ward kitchens, wards, hospital food stores and a mortuary (Table 1). To our knowledge, this study provides the first example of *B. licheniformis*, *B. pumilus*, *C. beijerinckii* / *C. butyricum*, *C. clostridioforme* and *R. terrigena* isolation from *M. domestica* (Table 1).

DISCUSSION

The clinical significance of many of the species of bacteria isolated from *M. domestica* in this study is well known, as is the role of house flies in the dissemination of these microorganisms, much of which is discussed in the review by Graczyk et al., (2001). As a result, the focus of the discussion of this study is on the significance of the bacterial species isolated for the first time from *M. domestica*.

B. licheniformis was isolated from house flies sampled from a hospital mortuary (Table 1). This is of importance, because over half of bloodstream infections with *Bacillus* spp have been attributed to *B. licheniformis* where the cause was the use of non-sterilised cotton wool for skin disinfection and in one case, the patient died following infection (Ozkocaman et al., 2006). In this outbreak, *B. licheniformis* showed some antibiotic resistance, caused pneumonia and fever and was classed as a ‘new’ pathogen causing serious infection in patients with neutropenia (Ozkocaman et al., 2006).

Bacillus pumilus was isolated from *M. domestica* collected from a hospital food store (Table 1). The significance of this is that catheter infection in children due to *B. pumilus* has been recorded in the

Table 1. Bacteria isolated from *M. domestica* sampled from hospitals. Key: The hospital areas sampled: catering (HC), ward kitchens (WK), wards (W), food stores (HS), mortuary (M). *Isolated from *M. domestica* for the first time, to our knowledge.

Bacteria isolated from *M. domestica*	Area	Significance
***Bacillus* spp**	HC	Resistant neonatal sepsis
Bacillus lentus	M	Septicaemia
**Bacillus licheniformis*	HS	Catheter-related bacteraemia
**Bacillus pumilus*	HC	Fatal brain and lung infection
Bacillus subtilis Group	WK	
Bacillus subtilis Group	HC	
Bacillus subtilis Group	W	
Bacillus subtilis Group		
Clostridia	HC	Neonatal necrotizing enterocolitis
**Clostridium beijerinckii/butyrricum*	HC	Bacteraemia
**Clostridium clostridioforme*	HC	
Clostridium sp		
Enterobacteriaceae	HC	Haemolytic uraemic syndrome
Citrobacter freundii	W	Wound infection
Enterobacter asburiae	HC	Resistant neonatal bacteraemia
Enterobacter cloacae	HC	
Enterobacter cloacae	HC	Haemolytic uraemic syndrome
Eschericia coli	HC	Catheter-related bacteraemia
Eschericia hermannii	HC	Haemorrhagic colitis
Klebsiella oxytoca	HC	Pneumonia
Klebsiella pneumoniae ssp *pneumoniae*	HC	
Klebsiella pneumoniae ssp *pneumoniae*	HC	
Klebsiella pneumoniae ssp *pneumoniae*	HC	Fatal neonatal septicaemia
Pantoea sp	HC	
Pantoea species 1	HC	
Pantoea spp 3	HC	
Pantoea spp 4	W	Resistant neonatal sepsis
**Raoultella terrigena*		
Staphylococci	W	Resistant infection of blood, skin, urine,
Staphylococcus aureus	HC	respiratory tract
Staphylococcus aureus		
Streptococci	HC	Endocarditis
Streptococci		

literature (Bentur et al., 2007). The *B. pumilus* infection was only eradicated following catheter removal and antibiotic use (Bentur et al., 2007).

C. beijerinckii / *C. butyricum* was isolated from houseflies *M. domestica* sampled from a hospital catering area (Table 1). Clinically significant *C. butyricum* strains have been isolated from the faeces of newborn babies suffering from Neonatal Necrotizing Enterocolitis (NNE) and those experiencing haemorrhagic colitis and an adult with peritonitis, while *C. beijerinckii* has been detected in dairy products (Popoff and Dodin, 1985).

C. clostridioforme was isolated from *M. domestica* sampled from a hospital catering area (Table 1). There appear to be no records in the literature of *C. clostridioforme* isolation from insects. To our knowledge, this study reports for the first time, isolation of *C. clostridioforme* from insects, specifically *M. domestica*. *C. clostridioforme* infection has been identified in cases of bacteraemia, intra-abdominal abscess, peritonitis, wound infection and other infections (Finegold et al., 2005).

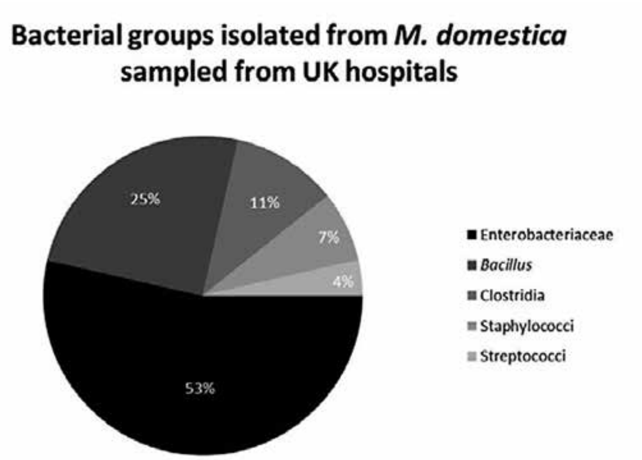

Figure 1. Bacterial groups isolated from M. domestica sampled from UK hospitals.

R. terrigena was isolated for the first time from *M. domestica*, which were sampled from a hospital ward (Table 1). Multi-drug resistant strains of *R. terrigena* have been described in over 25% of blood cultures taken from neonates, who were suffering with sepsis due to this microorganism (Elamreen, 2007). Neonatal enteral feeding tubes can be a source of bacteria and one study showed that 10% of isolates from such tubes were *R. terrigena*, representing an important risk factor for infection in neonates (Hurrell et al., 2009).

Based on 'read-across' from studies on the transmission of bacteria by *M. domestica* (Kobayashi et al., 1999), it follows that house flies in hospitals may act as a mobile reservoir and vector of clinically significant *B. licheniformis*, *B. pumilus*, *C. beijerinckii* / *C. butyricum*, *C. clostridioforme* and *R. terrigena*, which were isolated from them for the first time in this study, emphasising the importance of pest control as a component of infection control in hospitals.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

FLIGHT BEHAVIOR OF *LIMNOPHYES NATALENSIS* (DIPTERA: CHIRONOMIDAE) WITHIN A FOOD INDUSTRY

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Abstract We investigated the seasonal abundance and flight activity of adult *Limnophyes natalensis* within a food industry. A total of 43,163 insects were automatically counted with a special light trap during the study period. The automatically counted numbers reflected the abundance of *L. natalensis,* because other insect abundance was negligible. The daily total *L. natalensis* abundance showed the highest peak in mid June (716 individuals/day), some adults were collected during winter. Daily abundance of *L. natalensis* showed a significant positive correlation with daily mean room air temperature. The diel flight activity of *L. natalensis* showed two peaks (morning and evening) from spring to autumn, with one peak in winter. Adult *L. natalensis* were collected on days when mean air temperature was higher than 7.4°C. Results suggest that thermal conditions influence daily abundance and diel flight activity.
Key words Chironomid midges, control, diel flight activity, light trap, seasonal abundance

INTRODUCTION

The Chironomidae -non-biting midges- are one of the largest groups of the order Diptera, containing over 10,000 species and having a worldwide distribution (Langton and Pinder, 2007). The immature majority of the species live in aquatic habitats, although some species of Orthocladiinae are semi-aquatic or terrestrial (Pinder, 1995). Massive chironomid swarms have been observed near their habitats. The swarms often limit human activities and lead to severe nuisances. In addition, the adults may cause economic problems, such as contaminating the final products of food processing industries. Nearly 100 nuisance species have been reported to emerge in massive swarms (Ali, 1995). As Chironomidae is not listed as a household pest (Ito, 1982), places indoors are generally invaded by adults from outdoor habitats (Matsuzaki and Buei, 1993; Hattori and Moriya, 1996).

Recently, the abundance of *Limnophyes natalensis* (Kieffer) indoors has been reported (Tanikawa et al., 2009). Kimura et al. (2010) also reported emerging and swarming of *L. natalensis* within a food industry by species such as *Limnophyes* sp. (Kimura, unpublished). *Limnophyes natalensis* is not listed as a nuisance species (Ali, 1995). Methods for controlling *L. natalensis* have not been established, because the species' biological and ecological characteristics are poorly understood (Sæther, 1990). However, chemical control has a tendency to be avoided, and biological control is not realistic in indoor environments. In this study, we investigated the seasonal abundance and flight activity of adult *L. natalensis* within a food industry in order to establish an ecological basis for the physical control of *L. natalensis*.

MATERIALS AND METHODS

Study site

A certain food industry is located in Chiba City, Chiba Prefecture, Japan. The industry usually operates from 8:00 to 18:00. Lights-out is from 18:00 to 8:00.

Collection of *L. natalensis*

Limnophyes natalensis were collected by a light trap (Figure 1, OptCounterFIS-003, IKARI Corp.) in the industry. The light trap, equipped with a 20-W black fluorescent lamp and a sticky sheet, was hung on the wall 1.8 m above the floor. The most important feature of the light trap is its automatic counting and collecting of insects more than 0.7 mm in length with photoelectric sensors. The seasonal abundance of *L. natalensis* was monitored from February 1, 2010 to January 31, 2011. A black fluorescent lamp and a sticky sheet were usually replaced every three months and every month, respectively. All insects except *L. natalensis* that were collected by the sticky sheets were identified as several other taxa and counted separately under a binocular dissecting microscope.

Environmental Condition

Room air temperature was measured every hour with a thermo-recorder (Ondotori TR-72U, T and D Corp.) during the study periods. In addition, outside air temperature during the study period was measured at the nearest public observatory, Chiba Station (Japan Meteorological Agency, 2010, 2011), and used for the environmental data. Sunrise and sunset were obtained from the database of the Ephemeris Computation Office Public Relations Center (National Astronomical Observatory of Japan, 2010). In the present study, morning and evening flight periods were defined as 4:00 to 9:00 and 16:00 to 21:00, respectively. Flight periods included the sunrise or sunset regardless of the season.

Data Analysis

Statistical tests were performed in SPSS version 11.5.1.J for Windows (SPSS Japan Inc.).

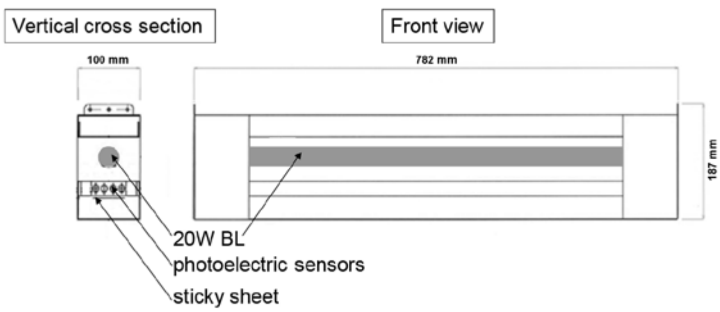

Figure 1. Structure of automatic counting light trap.

RESULTS

Daily Abundance

The daily mean room air temperature ranged from 11.1°C (January 16) to 22.2°C (August 17), averaging 17.1 ± 2.5°C during the study period (Figure 2). On the other hand, the daily mean outside air temperature ranged from 2.1°C (February 13) to 30.9°C (August 17), averaging 16.6 ± 8.4°C during the study period. The monthly value of daily mean room air temperature was significantly higher than the monthly value of daily mean outside air temperature ($p < 0.001$, Wilcoxon signed-rank test), except for May ($p = 0.192$, Wilcoxon signed-rank test) and October ($p = 0.432$, Wilcoxon signed-rank test). A total of 43,163 insects were automatically counted in the study period. The sticky sheet collected *L. natalensis* and other insects, such as Psychodidae (481 individuals), Diptera excluding Psychodidae (471 individuals) and other insects except for Diptera (70 individuals). In particular, only two individuals of the dipteran species other than *L. natalensis* were collected in January and February. The automatically counted numbers reflected the abundance of *L. natalensis*, because other insects were in the minority (1022 individuals, 2.4%) and their abundance was negligible. The daily number of adults evidenced a repeated short-term fluctuation. The daily abundance of adults reached a maximum on June 14 (714 individuals/day; room temperature 18.1°C; outside temperature 18.8°C) and minimum on January 28 (3 individuals/day; room temp. 12.0°C; outside temperature 4.2°C), averaging 118.3 ± 101.8 individuals/day during the study period. There was no day in the year when an adult was not collected. Daily abundance of *L. natalensis* showed a significant positive correlation with daily mean room air temperature ($r = 0.530$, $p < 0.001$, Pearson's correlation coefficient).

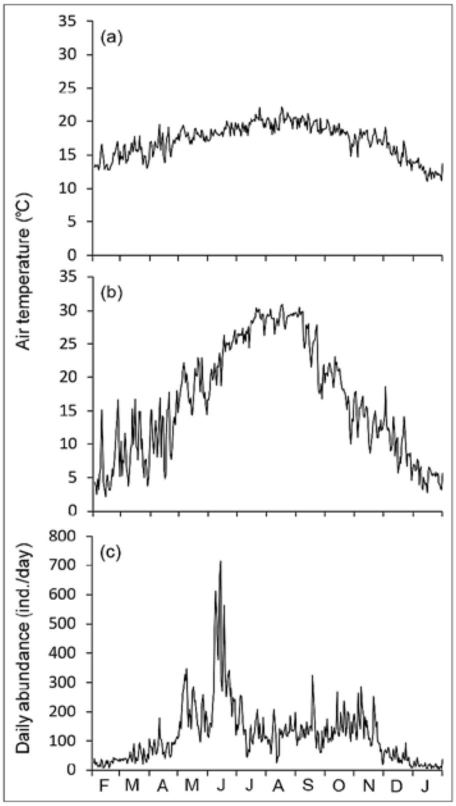

Figure 2. Thermal condition and daily abundance of Limnophyes natalensis in a food industry; showing (a) daily mean room air temperature, (b) daily mean outside air temperature (Japan Meteorological Agency, 2010, 2011), and (c) daily abundance of Limnophyes natalensis.

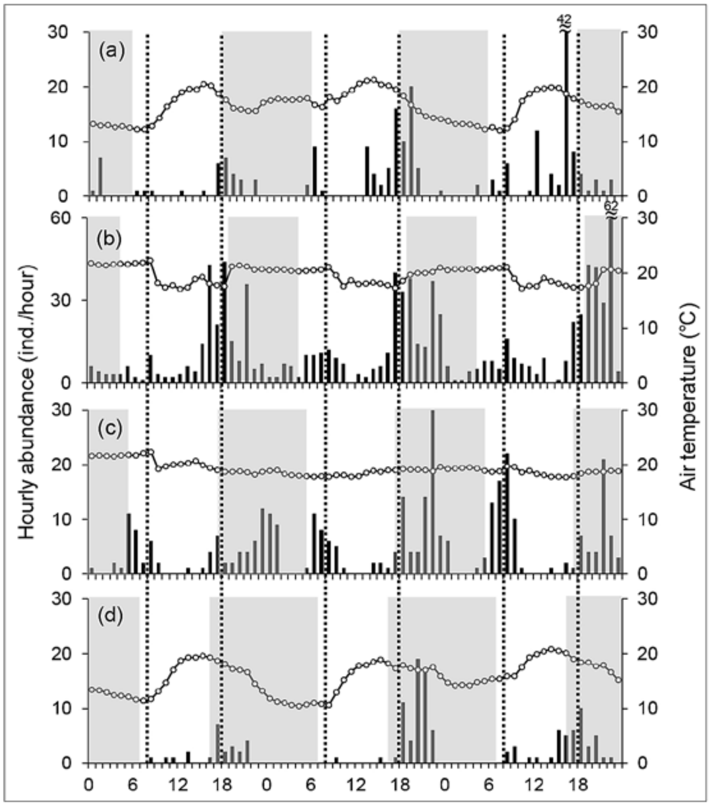

Figure 3. Diel activity of Limnophyes natalensis (black bars) and thermal condition (open circles) in a food industry; (a) spring (March 20-22, 2010), (b) summer (June 20-22, 2010), (c) autumn (September 22-24, 2010), and (d) winter (December 20-22, 2010). Shaded area and dashed line indicate nighttime and lighting (8:00) and lights-out (18:00), respectively.

Diel Activity

Figure 3 shows a typical diel flight pattern of *L. natalensis* in each season. The number of adults was large at morning and evening, but small in the day and at midnight from spring to autumn. Adults were collected only at dusk in winter. The room air temperature changed with the industry's operation. The air temperature of the evening flight period did not change between seasons, but the air temperature at the morning flight period was affected by outside temperature. The morning abundance of *L. natalensis* showed a significant positive correlation with mean room air temperature ($r = 0.640$, $p < 0.001$, Pearson's correlation coefficient). *L. natalensis* were collected in the morning when the mean air temperature was higher than 7.4°C.

DISCUSSION

A total of 28 species from Japan are recognized in the genus *Limnophyes* (Yamamoto, 2004; Nihon Yusurika Kenkyu-kai, 2010). However, *L. natalensis* are not included in this list of Japanese *Limnophyes*. The first record of *L. natalensis* in Japan was made in an indoor drain pit (Tanikawa et al., 2009). In addition, *L. natalensis* was collected on the outdoors of the western and southern parts of Japan (Kawai et al., 2011). As *L. natalensis* is very rare in Japan, their biological and ecological characteristics have scarcely been studied. In addition, Sæther (1990) indicated that all previous world records of *L. natalensis* need to be reexamined due to misidentification.

A large number of adult *L. natalensis* were collected throughout a year indoors. The automatically counting light trap is useful for capturing simple fauna. Genus *Limnophyes* is eurytopic (Wiederholm, 1983; 1989), and *L. natalensis* have been reared from rivers and streams (Sæther, 1990). Organic (materials of products) residue from drains and machines in a certain food industry contained *L. natalensis* larva (Kimura, unpublished). Although larva *L. natalensis* may only be able to distribute in water, they also have lentic and/or semiterrestrial habitats. Thermal conditions determine the flying activity, larval development, emergence period and number of generations per year, all of which govern the seasonality of insects (Gullan and Cranston, 1994). Except for some Orthocladiinae species, nuisance midges are not collected by light trap outside of winter (e.g., Sasa and Nishino, 1995; Tanaka et al., 2003), much less throughout a year in Japan. On the other hand, room temperature was fixed during the operation of this food industry by air conditioning. It is known that household pests are able to emerge at fixed temperatures caused by air conditioning throughout the year (Matsuzaki and Buei, 1993; Hattori and Moriya, 1996). Our results suggest that there was enough warm air temperature in this industry in winter for the emergence of *L. natalensis*.

The diel flight activity of *L. natalensis* showed two peaks (morning and evening). Flight activity was from spring to autumn, with one peak in winter. Morning room air temperature in winter limited the flight of *L. natalensis*. Environmental cues for the timing of emergence have been attributed to changes in light intensity (Armitage, 1995). Diel flight activity is affected not only by light intensity but also air temperature. *L. minimus*, the most abundant species of genus *Limnophyes* in Japan (Yamamoto, 2004), was collected from midnight to dawn in summer (Kawai et al., 2002). The diel flight activity of *Limnophyes* may differ from species to species, but that is unclear because Kawai et al. (2002) studied only one night.

Physical and cultural control methods for chironomidae are divided broadly into three categories: mechanical control, habitat management and ecological manipulation, and behavioral manipulations of adult midges (Ali, 1995). In this study, adult *L. natalensis* were collected on days when mean air temperature was higher than 7.4°C. Therefore, keeping daily mean room air temperatures below 7.4°C may serve to reduce the contamination of the final industrial products by the flight of *L. natalensis.* In addition, daytime production also reduces the contamination of *L. natalensis*. Further research on the flight behavior of this species should be undertaken for us to better understand how to control the species in the food processing industries. Increased knowledge of population dynamics in ecosystems outdoors would also assist in preventing the invasion and colonization indoors coming from the outside.

ACKNOWLEDGEMENTS

We would like to express sincere thanks to Mr. Takashi Kurosawa, President of IKARI Corporation, for their generous support. We express our thanks to Dr. Masaru Yamamoto, for his valuable taxonomical advice.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

OUTBREAK OF FLIES, RATS, AND OTHER PESTS AFTER THE GREAT TSUNAMI IN JAPAN

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Abstract More than a half million ton of frozen fish, fresh fish and processed sea foods were strewed around the Tsunami disaster areas from fishing port and huge freezing storehouses located along the saw-toothed coastline, approximately 35 minutes after the Great East Earthquake on March 11, 2011. Large populations of the black blow fly, *Calliphora nigribarbis* larvae were found under rotten fish in the early May. Adult flies invaded residential areas from the end of May to early June. *Phormia regina* and other blow flies were replaced by the *C. nigribarbis* after middle June. The pest control operators from all over Japan were requested to spray rubble heaps and rotten fish in the affected areas from May to September. Etofenprox emulsion was selected as an insecticide due to its efficacy and low avian toxicity. With integrated efforts of the insecticide spraying, replacement of rubble heaps or dried-up rotten fish, fly population declined from the end of July. In 2012, no fly problem was observed in the Tsunami affected area. Rat infestation became a public health concern as 87% rubble heaps were positive with rats. Since these rats were likely to escape from rubble to residents nearby, we recommend controlling the rats with rodenticides by local administrations and pest control operators in Iwate and Miyagi prefectures.
Key words Tsunami disaster, *Calliphora nigribarbis, Phormia regina,* fly control, *Rattus novegicus.*

INTRODUCTION

The Great Tsunami raided into the saw-toothed coastline in the Northern Pacific Japan in the afternoon of March 11, 2011. The coastline is stretched about 300 km from Miyako-city, Iwate prefecture to Ishinomaki-city, Miyagi prefecture. Along the coastline, fishing ports, seafood processing plants and the freezer warehouses were destroyed. The devastated news suggested that the blow fly problems would occur in the following months. The Annual Marine Industrial Statistic 2010 indicated that approximately 443,000 tons of fish productions were estimated in these areas including cattle fish, salmon, tuna, Pacific saury, mackerel, and bonito. In addition, about 100,000 tons of fish processing products were reported. Almost all of these products were scattered around the affected area immediately after the Great Tsunami. Fishing nets, oyster rafts and scallop breeding nets were also washed up around seashore. We dispatched warning to city offices in the concerned cities, informing that fly problems would be inevitable in the coming warm season. We also advised them that vast fly problem would difficult to control by them, and we could help by inviting pest control operators all over Japan when deemed necessary. Rat survey in rubble heaps was conducted in the following year of the Tsunami (2012). We settled adhesive rat traps, rat cages or bounce traps on the skirts of rubble heaps.

Some parts of these studies were published in the Japanese Journal of Medical Entomology and Zoology in Japanese with English summary (Tabaru et al., 2012; Tabaru et al., 2013). This paper reports chronologically what happened on the medically important pests in the Tsunami affected area.

SURVEY SITES AND METHODS

Fly survey was carried out at 33 sites (The Northernmost point is N 38, 24’,01,88”, 141,18’,53,75” and the southernmost point is N 39, 41’ 45,96, 142,00’,39,04”) in 5 cities and two towns in two prefectures (Iwate and Miyagi) from early May through November (Figure 1). The survey sites were selected on the basis of rubble heaps, fishing ports, freezer houses, warehouses and residence reporting fly problems. Fly survey were varied such as sweeping collection by nets, sticky papers, and monitoring larvae under rotten fish. The exposure time of

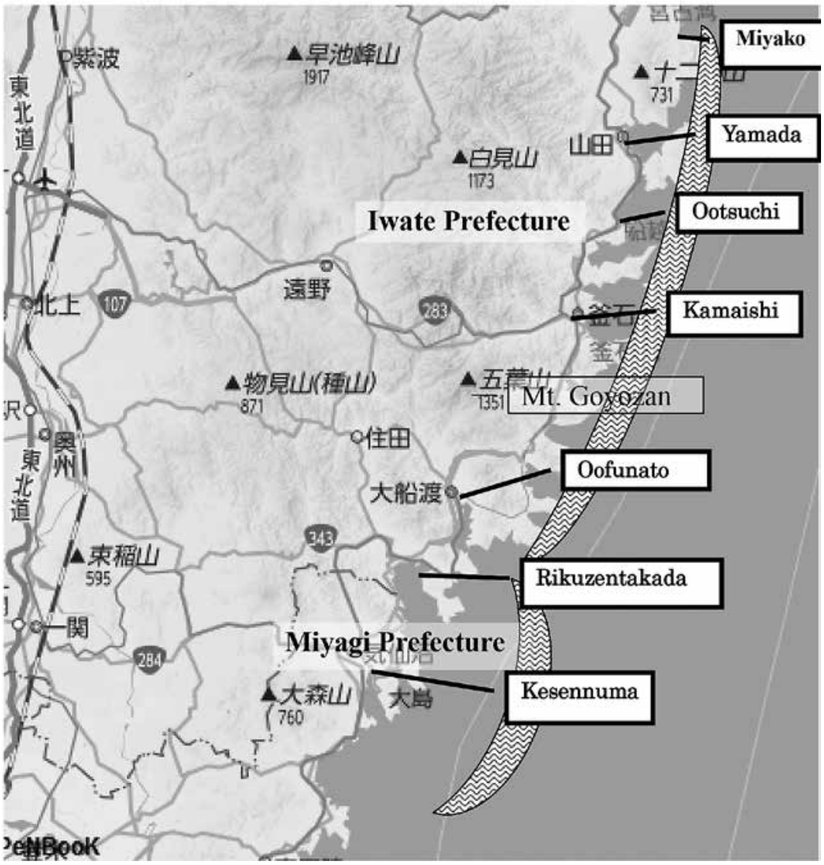

Figure 1. Fly survey sites (Cities and towns) in Iwate and Miyagi Prefecture, 2011

traps was depended on fly numbers in view; 20 min. in case of big number without bait and 24 hrs in small number with baits. The collected flies were identified. Fly control was carried out by the employee of the members of the Japan Pest Control Association. We selected Etofenprox emulsion according to the efficacy, avian low toxicity and low fish toxicity as insecticides, and some places with Fenitrothion EC. Fly survey was also carried out in the following year (2012) in the limited sites. Rat survey was also conducted at the same places except limited in the rubble heaps using adhesive rat traps, rat cages or bounce traps. There were no rat problems in the previous year of 2011. The captured rats were examined pathogen.

Figure 2. Larvae of Calliphora nigribarbis developed in a damaged food freezer in May in Ootuchi town.

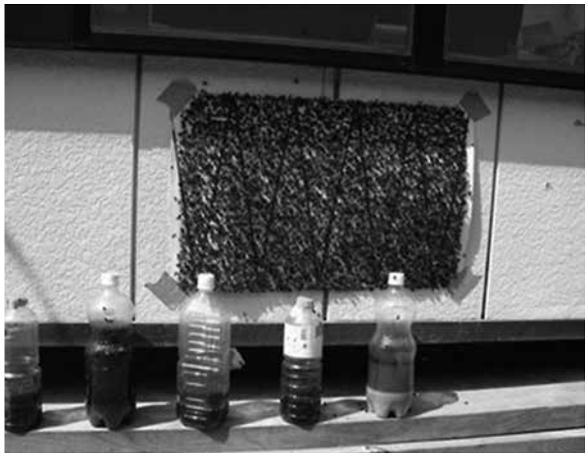

Figure 3. Adult Phormia regina captured in 24 h. Sticky paper (44 mm x 32 cm) and bottles with attractants mixed with vinegar, sugar and rice wine.

RESULTS AND DISCUSSION

Fly Condition in Early May, 2011

We surveyed medical important flies in the Tsunami affected area in May 5 through 8, 2011; just 55 days after the catastrophe. Fly larvae were wriggled in mess under strewed rotten fish at that time; outside temperature was below 14°C (abb. C.) in day time (Figure 2). The larvae were developed to the final stage, and the larvae were identified as *Calliphora nigribarbis*. This large blow fly is common in the early spring and late autumn in Japan. Adult flies were a few at early spring. Takei (1964) reported that the blow flies deposit their eggs in eyes, gills, and anal organ of dead fish from the following day of their death. Outdoor temperature in early spring in Kesennuma city varied as 1.6°C. (March), 8.2°C. (April) and 13.5 C. (May) by the Sendai Meteorological Station in 2011. Other micro-diptera, such as seaweed fly, *Coelopa frigid* and *Coproica vagans*, manure fly, *Leptocera fuscipennis*, and seashore fly, *Fucellia apicalis* were observed in washed up algae on sea-food breeding nets. However, these flies do not invade human residents. In late May the Tsunami affected area was infested with fly larvae around destroyed warehouses, freezers (Figure 3), food processing plants and residential area. Housewives frightened watching fly larvae invading their yards one after another. We felt that fly outbreaks were coming instantaneously.

Fly Condition in Early June, 2011

The adult flies invaded residents nearby with great numbers. The city offices received telephone calls every minute from morning to evening by residents, informing fly infestations and asked to control them. Housewives could not open windows and entrances were surrounded by the extraordinary number of swarming flies. Figure 4 shows adult flies on sticky paper in a day at a resident in the Kesennuma city, where rice fields spread in front of the house, leaving rotten fish in the fields. Rotten fish were washed from freezing plants located 2 km away from seashore. Housewives used plastic bottles to collect flies with attractants; mixed with vinegar, sugar, and rice wine in it. From late June *Phormia regina* was replaced by *C. nigribarbis*. We encountered crowded flies like carpets in a fishery village while driving in Ootsuchi-town

Figure 4. Swarming of Phormia regina on a street in Ootsuchi town, July 2011.

Figure 5. Insecticide spraying on rubble heaps, July 2011.

in June 12 (Figure 4). These flies developed in destroyed freezers nearby. Table 1 shows the flys captured in 20 minutes on the surface of sticky traps in a several locations, July, 2011. *P. regina* was the major, followed by *Musca domestica,* and *C. nigribarbis* was few. *P. regina* was captured 2,564 individuals in the fishing port of Kesennuma city and 2,364 flies in the fish processing plant in Ootsuchi town. In middle September *P. regina* and *C. nigribarbis* were quite a few. Fly emergence might have been enhanced by washed away natural enemies such as small birds, reptiles, and predatory insects by the Tsunami.

Table 1. Results of sticky trap (44 cm x 22 cm, no attractant) collection for 20 min in mid July and September.

		Fly species					
Survey sites	**Pref.**	**Mid July**			**Sept.**		
		M. domestica	***P. regina***	***Calliphora spp***	***Spaerocerdae***	***Coleopidae***	***Calliphora spp***
Ootsuchi A	Iwate	304	2,364	0	0	0	3
Miyako B	Iwate	78	7	0	25	0	0
Yamada B	Iwate	14	98	0	7	1	0
Oosawa C	Iwate	0	61	0	169	0	11
Oofunato D	Iwate	41	135	0	140	0	2
Rikuzen D	Iwate	29	24	0	34	0	3
Rikuzen D	Iwate	141	154	0	7	0	0
Kesennma B	Miyagi	252	2,564	0	175	0	0
Kesennma A	Miyagi	60	401	0	128	0	7

Fly Control Activities

In the Tsunami affected public municipalities, there were no insecticides and spray equipment at the time. The Japanese Pest Control Association (Headquartered in Tokyo) invited pest control operators from all over Japan. The operators began to spray garbage, rotten fish, warehouses and residential areas from early June. Each pest control team was consisted of 2 people and the spray machine was laden in track with wide range nozzle, 1000 little tank and 100 m long hose. The total man-day was tallied up to 2,500 man-days from late May to mid September (Figure 5). The most important issue was how to obtain fresh water for dilution. After spraying in freezers, fish processing plants, rubble heaps and residences dead flies were accumulated in mess. However, new borne flies flocked in residences within short days for a while.

Fly population dramatically declined at the end of August due to fly control, removed rubble, and dry-up rotten fish. Table 2 shows the fly condition at October in the Tsunami affected area. The medical important flies; *C. nigribarbis, P. regina M. domestica or Fannia* were a few in comparison with fly condition in July.

Table 2. Fly numbers collected by sticky traps baited with fish and sugar for 24 hours in October.

Prefecture	Sites	Baits	*Calliphora nigribarbis*	Other Calliphoridae	House fly	*Hydrotaea ignava*	Fannia sp.
Iwate	Osabe resident	Fish	1		1		
		Sugar			3		
	Osabe warf	Fish		1	2		
		Sugar	1	1	2		2
Miyagi	Benten-cho, freezing plant	Fish	0	6	1	15	
		Sugar				2	2
	Asahi-cho, grain warehouse	Fish					
		Sugar	3		7	5	6
	Fureai wafr	Fish	1			4	
		Sugar					
	Hajikami residence	Fish					
		Sugar					
	Akaiwa Plastic plant	Fish	1		2	1	
		Sugar					
	Restaurant	Fish					
		Sugar	3	1	3	0	

Table 3. Fly reduction rate in 5 hr and 24 hr after the application of three insecticide formulation in rubble heaps; reduction rate (%) = 100 (fly numbers in post treatment / fly numbers pre) x 100. Spray area varied from 800 to 10,000 square meters. Numbers in parenthesis in 24 h.

Insecticides	Mean reduction (%)		Research sites
	House fly	Blow fly	
Fenitrothion EC	43.8	69.9	12
	(33.1)	(78.6)	
Etofenprox EC	58.5	75.8	22
	(67.6)	(84.5)	
Propetamphos EC	76.3	86.7	16
	(77.3)	(95.5)	

We tried to collect the late autumn *C. nigribarbis* colonies, because the female of this species comes back to lowlands for winter hibernation from highland places where they spent the summer season (Arakawa, et al., 1991). But we failed to collect them in the late November in the Tsunami affected area. The fact indicates us that *C. nigribarbis* did no reach mountains nearby in the summer of 2011 due to control activities.

Fly Condition in 2012

Fly survey was conducted in the following year of the Tsunami in 20 sites in the two prefectures. Most of survey sites were the same as 2011. Fly numbers were very small even though the sticky traps were set for 24 hours with baits on them. The medically important flies might control in 2011, and rotten fish and fresh rubble were removed in 2011. Fly problems in residence were almost absent in 2012.

Table 4. Captured rats in September in Iwate and Miyagi prefectures.

Prefecture	Positive sites/ Survey sites	Captured rats/ Trap Nos.	Species	
			R. norveigicus	***R. rattus***
Iwate	9/10 (90%)	19/38(50%)	12	7
Miyagi	6/6(100%)	13/25(52%)	12	1

Rat Problem in 2012

Yabe and Ishikawa (2012) could not detect rat evidence in the rubble heaps in the year of 2011 in Tsunami affected area. However, some rat infestation evidences; such as feces, fur or foot prints were found on the surface of adhesive rat traps set around skirts of the rubble heaps and an abandoned school in May, 2012, but no rats were captured. Rat collections by sticky traps might be unsuccessful due to wet rat bodies. Sixteen Norway rats (*Rattus norveigicus)* and three roof rats *Rattus rattus* were captured by rat cages or bounce traps in the heaps of the Tsunami affected areas in the following year of the tsunami in the beginning of July, 2012. Nine rubble heaps out of 13 heaps were positive with Norway rats and a roof rat was captured in grass field of an abandoned school and two of rubble heaps in Rikuzentakada city. On the September survey, rat infestation was higher compared with the survey of July; 14 rubble heaps out of 16 were positive with rats, and 23 Norway rats and 8 roof rats were captured in 60 trap cages (Table 4). Sun flower seeds in road sides were eaten by roof rats in Oofunato city. The rats have tendency to break loose from the rubble heaps at the time of removing or collecting the refuse. Thus, we recommend controlling the rats with rodenticides by local administrations and pest control operators. The captured 32 rats were immediately killed by CO_2 and kept in dry ice. All captured rats were examined for micro pathogens in the National Institute of Public Health, but no evidence obtained. In the Tsunami affected zone no infectious disease was reported in the following 3 years.

Present Situation

Adults of *Calliphora nigribarbis* were collected at a mountain lodge (1,351 m sea level) located 12 km away from nearest coastline of Oofunato city in June 7, 2013. This indicates us the natural environment in the fishing ports recovered as a normal condition. However, fly problem in the Tsunami affected area did not occurred in 2013. By the end of 2013, 90% of rubble heaps were removed in Iwate and Miyagi prefectures and this condition resulted rat free environment. But, in the Fukushima prefecture rat problem are still serious due to keeping away from their residents. A scientist commented that roof rats (*Rattus rattus*) are abundant in vacant houses in Fukushima prefecture. Looking back on the fly control activities in 2011, fast response and mobility brought success.

ENTOMOLOGICAL INVESTIGATIONS

Field Evaluation of Pesticides

In the middle of July field evaluation of pesticides were carried out in the rubble heaps in Rikuzentakada city with area of 800 m^2 to 10,000 m^2 where fly numbers were abundant. At first we located sticky paper (22 cm x 22 cm) without baits for 20 minutes in rubble heaps, then we sprayed pesticide as 200 liter per 100 m^2 in 200 times dilution each of three insecticides; Fenitrothion 10% EC, Etofenprox 7% EC, or Propetamphos 5% EC in the different rubble heaps. In 5 and 24 hrs after spraying, we tried again fly collection by the same method. The test was carried out in 12 to 22 sites. The results are shown in Table 3. The reduction of blow flies was superior in Propetamfos EC, followed by Etofenprox EC and Fenitrothion EC. The effect against house fly showed inferior, especially with Fenitrothion EC. According to the fly species; dominant is *P. regina* in rubble heats, we recommend using Etofenprox EC.

Susceptibility Test

Susceptible tests were carried out in a rural vacant house in Rikuzentakada city, after collecting flies with sweeping nets from rubble heaps. In this test three insecticides were approved: Fenitothion 10% EC, Etofenprox 7% EC and Propetamphos 5% EC. We released them in cages (30 cm x 30 cm x 30 cm). Then, we put approximately 20 flies in a 500 ml plastic bottle with insecticide impregnated filter paper (4 cm x 14 cm), and counted mortality after 24 hours. All of the tested blow fly were dead at low concentrations 0.0125% (a.i.) of each insecticides. A few number of the house fly survived. The tests were repeated 4 times in each pesticide. The fact that the blow flies were very susceptible to any pesticides used resulted in excellent control in the field.

Repellent Test

Repellent test was conducted in fish processing plants where *P. regina* and *M. domestica* were observed. The filter papers (9 cm diameter) were absorbed in the insecticide solution (1% a.i.) of three different chemicals for 5 min. and dried. Then the paper was put on the center of a sticky paper (22 cm x 44 cm), and the sticky paper was placed on rubble heaps where fly activities observed for 20 min. Ikeda (1961) reported that repellent activity of the house fly could analyze using insecticide impregnated filter paper. The result indicated that these three insecticides did not show repellency against blow flies. Flies landed on the sticky papers located insecticide impregnated filter papers in the center of sticky papers. .

CONCLUSION

Fly problems after Great Tsunami occur even in developed countries. The fly problem is liable on depending to industries in relation with organic materials and residence nearby. Scientific pest surveys in early stage after the Tsunami helped following activities. The success of fly control could support recovery in the affected area. It is no doubt that fast response and mobility must have brought success in fly control. Rats invaded rubble heaps the following year and invaded residents. Microbe investigation of captured pests will be essential to prevent infectious disease around affected area.

FINANCIAL SUPPORT ACKNOWLEDGED

Fly control activities were financially supported by a Japanese NGO, Nippon International Cooperative for Community Development. The fund was used for labor expense, insecticides, pickup truck rentals and comestibles. Without NICCO's financial support fly control activities could not have been conducted. Rat and fly survey in 2012 was financially supported by the Japan Pest Control Association.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

GEOGRAPHIC INFORMATION TECHNOLOGY APPLICATIONS FOR URBAN INTEGRATED PEST MANAGEMENT

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Abstract Madrid City Council has always used maps for this purpose and in the last decade has taken a further step incorporating Geographical Information Technologies (GIS). As a result urban pest control has improved notably and integrated pest management principals have been straightforwardly accomplished. This technological development has required and is still requiring budget and staff effort, however it allows a very powerful data management and analyse in a unique way. Currently, city pest management is highly influenced by GIS analyse, allowing better decision making and resource optimization. This manuscript provides general information about the implementation process, results obtained and relevant conclusions.
Key words GIS, vector surveillance.

INTRODUCTION

Pest prevention and control operations require the location of pest problems (García-Howlett and Cámara, 2009). Traditionally this requisite has implicated the use of plans or maps depending on the scale of the problem. Additionally, specialists that work on pest control need to identify evaluate and manage all environmental factors that determine and interact with pest problems. To accomplish IPM strategies (Brenner et al., 2003; Lacey, 2006) it is fundamental to identify and acquire the biological, social, economical, geographical and environmental data that establish the different pest situations. Understanding the environmental determinants of vector-borne disease transmission is critical to predict and prevent pest/vector risks.

GIS are tools that permit a technical and scientific based collection, processing and management of data (Bosque-Sendra, 1997). Global Positioning Systems (GPS) and GIS are now essential vector-control tools (Bonnefoy et al., 2008). These technologies allow researchers and organizations to study and publish vector risks based on a cartographic approach (Lawson et al., 1999; Albert et al., 2000; Durr and Gatrell, 2004).

Health Madrid, as the municipal health administration of the City Council of Madrid manages pest prevention and control operations. For the Vector Control Unit (UTCV) GIS is not only a complex software solution for individual health problems, but a corporative and widespread strategy for the global processing of information. All municipal pest/vector programs and work interventions are planned and developed with the help of GIS (Cámara and Tamayo, 2008; Cámara et al., 2012; Tamayo, 2013).

MATERIAL AND METHODS

Pest and vector prevention GIS applications principally arise from experiences focused on singular vector problems (Traweger and Slotta-Bachmayr, 2005) and applied to large areas (Estrada-Peña, 1998). Translation of GIS applications to a more complex urban range and to several animal species implicates the need of a comprehensive approach.

In 2005, collaborating with the Computer Services of the City Council (IAM), Health Madrid (UTCV) started developing a global GIS scaled project based on two general approaches. Firstly designing and developing a "user friendly" corporative software, accessible for pest related municipal professionals that also allows to extract data; secondly a more powerful desktop GIS software solution, where internal and external information may also be incorporated and analysed. In this context, UTCV had to reorganize in order to adapt to this new technology which involved internal operation redesigning, improving data management and staff training.

The corporative GIS is the most challenging goal, because of its cost, complexity and project schedule. The decision of using previous GIS projects, derived from other municipal department (urban planning), proved to be a good option in order to reduce cost and promote collaboration. Desktop GIS solutions are easy to incorporate in any organization and have a lower cost but require higher qualified users. Although they can be a temporal solution for urban pest control units, UTCV considers that the combination of both corporative and desktop solutions is the best option (Cámara et al., 2012).

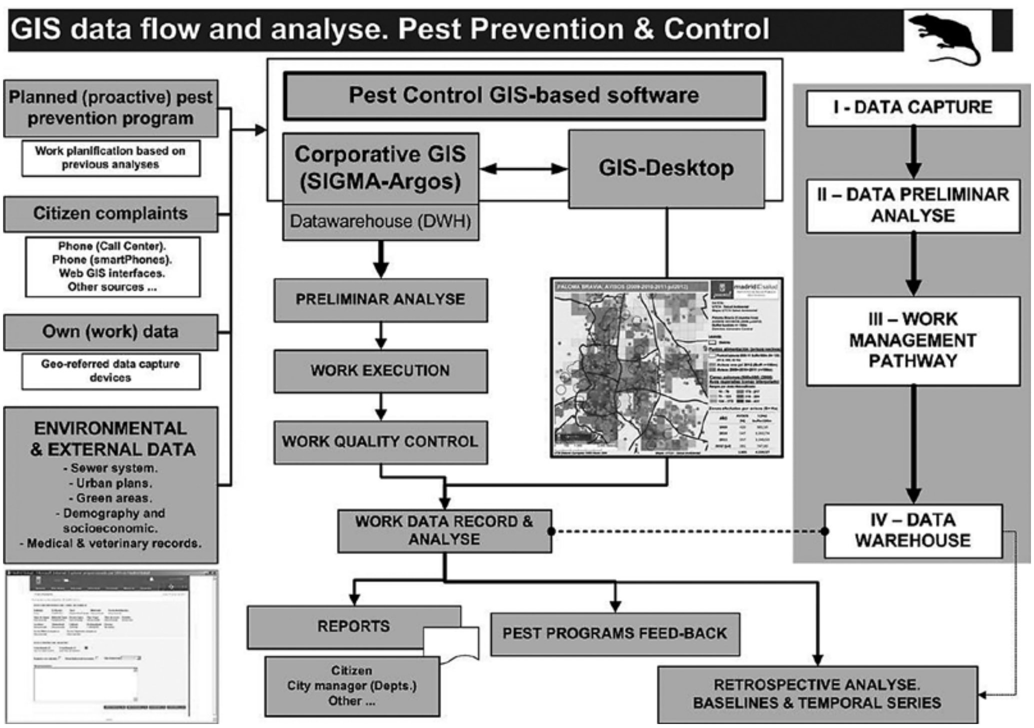

Figure 1. UTCV GIS process flow diagram.

Any municipal pest computer program usually is based on a general process in which citizen complaints, management operations, data analysis and reports are included. All of them are deeply affected by GIS capabilities. The most critical element is the precise geographical coordinates of events, an automatic process in UTCV corporative GIS as you can see in Figure 1. Next step implicates preliminary GIS analysis and event characterization (environmental factors, background reports), allowing to fulfil an accurate diagnoses (Tamayo, 2013). Afterwards the control measures are determined and carried out, where GIS helps to manage logistic and task assignments. Corporative software is designed to permit additional work data capture in order to improve future analysis. Last step includes report generation for both external and internal use. Map use associated to external reports is attractive and useful, however results essential for internal technical analysis, see Figure 2.

Accurate pest/vector risk management implicates extra performance (Estrada-Peña, 1998; Elliot et al., 2000; Moore and Frier, 2004; Tamayo, 2013). Prevention strategies implementation, continuous evaluation and vector monitoring are essential parts of IPM programs (Bonnefoy et al., 2008). All of them are improved by GIS and have proved so in Madrid (Cámara et al., 2012). GIS can manage huge spatial database and can carry out complex spatial data analysis and modelling (Smith et al., 2007) allowing mathematically based decisions for pest control and prevention operations.

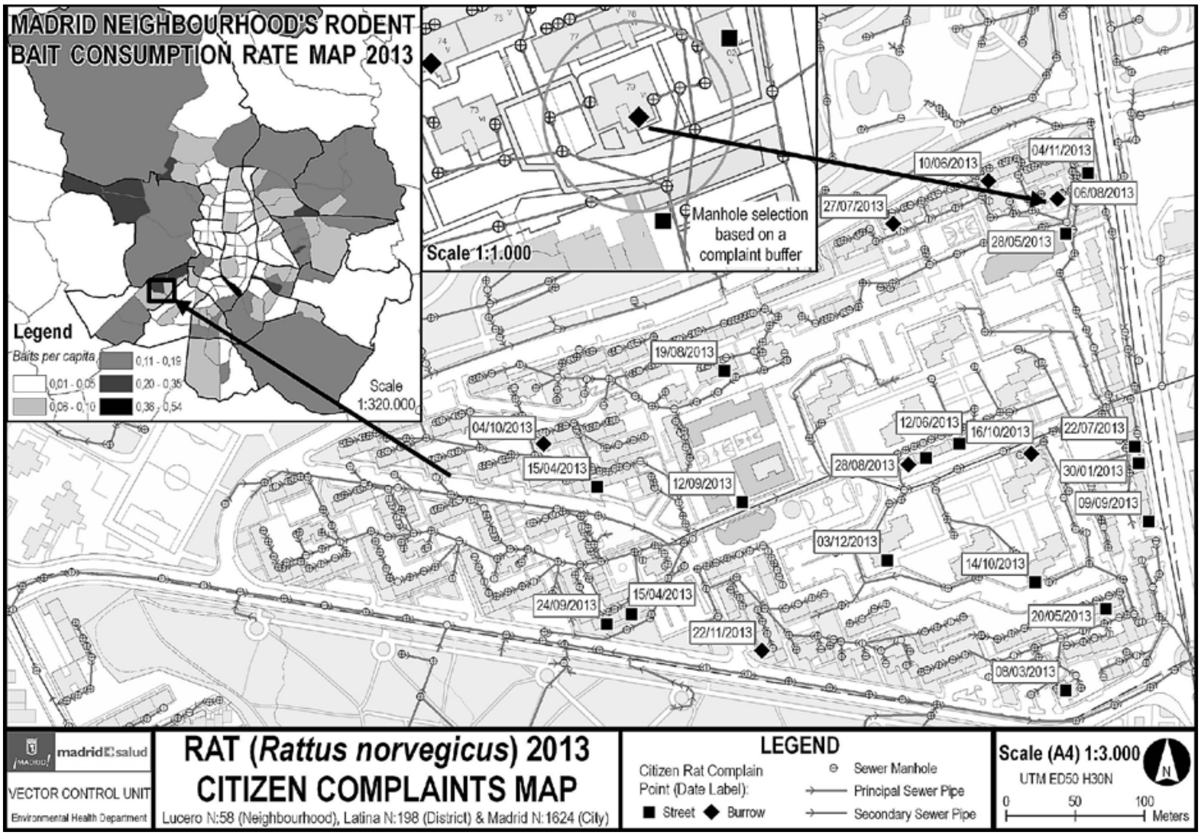

Figure 2. UTCV Example Map, Madrid neighbourhood rat (Rattus norvegicus) events.

Unexpected and extraordinary events, such as human and animal vector disease outbreaks (spatial epidemiology), animal abnormal mortalities, invasive animal management, natural or man borne catastrophes or hazards, are scenarios where GIS is an insuperable tool (Bosque-Sendra, 1997; Kitron, 1998; Elliot et al., 2000; Robinson et al., 2002; Moore and Freier, 2004; Durr and Gatrell, 2004).

RESULTS AND DISCUSSIONS

Almost ten years after GIS was introduced, this technology is globally implanted in all UTCV programs and activities as described in material and methods. Citizens can now notify pest related events directly in web maps linked to corporative software; which automatically realizes preliminary spatial analysis. Routine work planning and procedures are optimized by GIS analysis. Reports usually include thematic cartography so that citizen and city managers can access to map based information.

Spatial and temporal series of data are systematically checked, processed (Tamayo, 2013) and studied. Successive year programs are then influenced and replaned by previous data GIS analysis. State of art has allowed that in Madrid City Council spatial data sheets (GIS data layers) can be managed and provided by different municipal departments and also from other non municipal sources, in many occasions the information flow is bidirectional. Accessible data includes environmental and socioeconomic inputs, decisive to perform better descriptive and spatial health statistics (Gerin, 2003). As well, GIS tools have been systematically incorporated to pest monitoring, vector surveillance and animal disease outbreak, increasing the UTCV biological knowledge.

In a simplified approach, UTCV GIS assembles pest events (mostly citizen and public building complaints), environmental-socioeconomic factors and pest-vector monitoring results in a geodatabase and uses this data to carry out statistically based definition of pest threshold, relationship between pest and other variables, pest prediction models and program evaluations.

CONCLUSIONS

Pest control activities need to use spatial information (Bonnefoy et al., 2008). GIS technology incorporates a new perspective, allowing spatial data to perform a much more important function due to the quickness in fulfilling complex geographical analysis (Smith et al., 2007) and processes (Cauvin et al., 2007). This means better knowledge, efficiency and performance executing our role in local public health.

GIS has proved to be a helpful tool to succeed IPM strategies, thus pest prevention and vector-risk management can be substantially improved by its use. Urban environment are very complex scenarios where the access to spatial data and environmental information is critical (Gerin, 2003; Kanevsky, 2008). Systematic GIS use strengthen data analyze, internal evaluation practises and provide pathways to share information. GIS can be introduced in local pest control management in a different ways; under UTCV's point of view, a combination of a corporative and desktop solution has provided the best results.

Experience in Madrid has allowed detecting GIS intrinsical and operative problems, such as: poor data, analisis uncertainties, inaccurate map edition. All this can conclude in erroneous decision making, especially dangerous because maps are easily visualized and trustworthy.

Incorporating TIG to municipal pest management is a complex process with pre-established landmarks. UTCV has achieved access to technology, capable human resources and automatic georeferenced information, all of them defining a first landmark. The next landmark is the capacity to correlate spatial information, probably the most useful advantage of pest GIS, recently achieved by UTCV. Next phase opens technical opportunities concerning advanced geostatistics and remote sensing. In Madrid, GIS have improved municipal pest management based on better decision making and resource optimization.

ACKNOWLEDGEMENTS

Authors specially want to acknowledge Madrid City Computer Services (IAM - Javier Delgado) for their diligent implication and support.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

MOSQUITO-BASED ARBOVIRAL SURVEY IN WESTERN LOMBARDY, ITALY: 2009–2013

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Abstract A mosquito-based arbovirus surveillance was settled in "Parco Lombardo delle Valli del Ticino" and in neighbouring sites in Milano province (Lombardy). Nine sites were monitored between 2009 and 2013 sampling 82,106 mosquitoes, of which 68.6 % belonged to *Culex pipiens* L. species. Mosquito were sorted in pools according to species, data and place of collection and submitted to biomolecular analysis for detection of flaviviruses and orthobunyaviruses. Usutu virus was detected in three different pools of *Cx. pipiens*, Batai virus was detected in a pool of *Anopheles maculipennis s. l.* and in two pools of *Cx. pipiens*, and Tahyna virus in a pool of *Aedes vexans* (Meigen) and in one of *Ochlerotatus caspius* (Pallas). Obtained data give useful information on mosquito community in surveyed area and demonstrate the ability of this surveillance system to detect arboviruses circulating in mosquitoes.
Key words Arbovirus, surveillance, *Culex pipiens*, *Anopheles maculipennis*, *Ochlerotatus caspius*, Usutu virus, Tahyna virus, Batai virus

INTRODUCTION

Burden of arthropod-borne viruses (arboviruses) has been steadily increasing in recent years, creating health problems worldwide. Also in Italy diseases caused by these viruses have been increasingly reported, with relevant outbreaks, like the Chikungunya epidemic in Castiglione (Emilia-Romagna), and the 69 human cases of West Nile disease reported to the end of September in 2013 (ECDC data). Additionally, other arboviruses have been already detected in Italy, like Batai and Tahyna viruses (Nicoletti et al., 2008; Huhtamo et al., 2013), or seem to be recently introduced, like USUV. The pathogenic potential of these viruses is not well characterized and probably is underestimated (Gratz, 2006), and they cause diseases often considered neglected.

Health surveillance programs, also based on vectors screening, were established in Italy for monitoring presence of potentially pathogenic arboviruses. Results and characteristics of a entomological surveillance program adopted in western Lombardy, between 2009 and 2013, are described herein.

MATERIALS AND METHODS

From 2009 to 2011, mosquitoes were trapped in the "Parco Lombardo della Valle del Ticino" (Lombardy region), a 91,000 ha Regional Natural Park that protects the Italian stretch of Ticino River, an area that includes a variety of natural, agricultural and urban sites, although in one of the most densely populated areas of Italy. Stations (5 in 2009, of which one monitored also in 2010-2011) were located in 5 natural areas characterized by riparian vegetation, in close proximity to urban and agricultural environments, with a high number of rice fields. In 2012 and 2013 mosquito monitoring was conducted in a riding school located in a rural area, surrounded by rice fields, in Parona municipality (PV). Moreover in 2012 urban parks were monitored in the city of Milan (2), and Cusago Municipality (3). Mosquitoes were trapped using modified CDC traps baited with carbon dioxide (Bellini et al., 2002), and a BG sentinel plus carbon dioxide in 2012 in Cusago and Milan. Sites were monitored weekly from June to the end of September. The modified CDC traps worked one night per week from approximately 5:00 PM to 9:00 AM. BG sentinel traps worked during the day, from approximately 10:00 AM to 20:00 PM.

Collected mosquitoes were identified using morphological characteristics according to classification keys (Schaffner et al., 2001; Severini et al., 2009) than pooled by date of collection, location, and species (with a maximum number of 200 individuals per pool), ground and submitted to biomolecular analysis (Calzolari et al., 2010). For simultaneous detection of arboviruses, pools were tested by screening PCRs for detection of orthobunyaviruses (Kuno et al., 1996) and flaviviruses (Scaramozzino et al., 2001). Amplicons obtained by positive PCRs were sequenced by an automated fluorescence-based technique following the manufacturer's instructions (ABI-PRISM 3130 Genetic Analyzer, Applied Biosystems, Foster City, CA). These sequences were employed as tools for the identification of viruses by performing a BLAST search in the GenBank database.

RESULTS AND DISCUSSION

Mosquito Collection

Using CO_2-baited traps, from 2009 to 2013, a total of 82,106 mosquitoes were collected; more than 68% were *Culex pipiens* L. Other species abundantly sampled were *Aedes vexans* (Meigen) and *Ochlerotatus caspius* (Pallas), both in about 11%. Also a relevant number of anopheline mosquitoes were collected (more than 8%), the largest number of which belonged to *Anopheles maculipennis* complex. This complex includes different species in which adult females are morphologically indistinguishable, but have different competence for Malaria parasite transmission (Severini et al., 2009). The knowledge of species composition of this complex is important to assess the possible risk of reintroduction of the parasite. Specimens of other eight species, including the Asian Tiger Mosquito, *Aedes (Stegomyia) albopictus* (Skuse), were collected under the 1% of the total for species.

Virus Detections

Three different viruses were detected during the survey, the flavivirus USUV and the two orthobunyaviruses BATV and TAHV.

Usutu virus was detected in three different pools of *Cx. pipiens*, in 2009 and 2011. This virus was first confirmed in Europe in Vienna, in 2001, and it seems to spread to neighbouring countries from here (Pfeffer and Dobler, 2010), but it was retrospectively detected in birds sampled in 1996 in Italy (Weissenböck et al., 2013). USUV was abundantly detected in mosquitoes in Pianura Padana since 2009 (Calzolari et al., 2010). The main vectors of USUV are ornithophilic mosquitoes, mainly of the genus *Culex*; wild birds are principal reservoirs. The medical importance of USUV is not fully understood, but this virus appears to be pathogenic to humans (Pfeffer and Dobler, 2010).

Batai virus was detected in a pool of *Anopheles maculipennis s. l.* (in 2009) and in two pool of *Cx. pipiens* (one in 2009 and one in 2011). This virus, mainly spread by species *An. maculipennis* and *Anopheles claviger* (Meigen) in Europe, is also present in Asia and Africa (Gratz, 2006; Hubálek, 2008). Several serological surveys conducted in Europe showed a low prevalence of antibodies to BATV in humans and a higher prevalence of antibodies in other mammals (Lundström, 1999). BATV was recently isolated from mosquitoes in Piemonte (Huhtamo, et al., 2013). This virus has not been associated with clinical disease, only mild influenza-like symptoms were associated with seroconversion to BATV in humans (Gratz, 2006).

Tahyna virus was detected in a pool of *Ae. vexans* (in 2009) and in one of *Oc. caspius* (in 2010). This virus has been mainly isolated from *Ae. vexans* but was also detected in *Oc. caspius* and in other mosquitoes (Gratz, 2006; Hubálek, 2008). Several serological surveys and viral isolations reported the widespread occurrence of TAHV in humans and other mammals in many European countries (Lundström, 1999), but this virus is also present in Africa and Asia (Gratz, 2006; Hubálek, 2008). TAHV infection in humans causes influenza-like symptoms and, in some cases meningoencephalitis and atypical pneumonia (Graz, 2006). The detection of viral RNA in mosquitoes, confirmed the presence of this virus in northern Italy, as reported previously (Nicoletti et al., 2008).

This positive PCR detection demonstrates that wide range of arboviruses involved in human and animal diseases could be detected by entomological survey. The described survey allowed the detection of neglected virus, for which human cases are probably underestimated, pointing out important epidemiological data on temporal and geographic diffusion of these viruses, in addition to the description of mosquitoes present in the monitored area. This work demonstrates that entomological surveillance can provide useful data in assessing the risk of arbovirus spreading.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

CONDITIONS OF TICK (ACARI: IXODOIDEA) POPULATION PERSISTENCE IN THE URBAN ENVIRONMENT

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Abstract Tick pests and vectors of human and animal diseases are a permanent component of urban fauna. The present paper addresses the conditions required for persistence of tick populations in urban environment. All urban tick populations originate from populations in natural habitats around towns. Some of these can successfully persist under urban conditions without continuous replenishment by additional specimens brought by hosts from outside. Availability of 'green corridors' between natural habitats of ticks and urban localities with suitable vegetation creates a unique opportunity to support urban tick populations. Exotic tick species brought into towns by birds, with pets or by other means, have no chance for prolonged persistence since they have no resources for replenishment. The persistence of tick species (*Rhipicephalus sanguineus* and *Argas reflexus* s.l.) that can live in man-made structures depends on the availability of their main hosts. Both species are able to create independent urban populations.
Key Words Tick hosts, green corridors

INTRODUCTION

The problem of ticks and tick-borne diseases in urban areas has attracted attention of researchers for several decades (Korenberg et al., 1984; Daniel and Černý, 1990; Dautel and Kahl, 1999; Wilamowski et al., 1999), and new data concerning the occurrence of ticks and recognition of zoonoses in urban areas are continuing to accumulate. The sporadic and disparate nature of published observations calls for a thorough review and analysis in order to identify patterns and regularities determining tick persistence in our towns. In our previous papers (Uspensky, 2008a, 2014) a general picture of the problem of urban ticks was given and the main groups of ticks living in urban environment were described. The goal of the present paper is to analyze the suitability of various localities within urban environment for tick existence and to classify tick populations inhabiting these localities.

URBAN LOCALITIES POTENTIALLY SUITABLE FOR TICK EXISTENCE

Ticks which inhabit urban localities originate from tick populations persisting in wild natural habitats around cities and towns (Uspensky, 2014). The successful survival of ticks depends on optimal temperature and humidity, the main components of microclimate, in their habitats. Exophilic ticks which live in the lower parts of vegetation, litter and upper layer of the soil are especially dependent on microclimate. The more environmental conditions in urban localities correspond to those under which tick populations live in wild nature, the more suitable for ticks these localities are. The suitability of urban localities for tick persistence has to be maximal in suburban areas near the boundary to wild nature and minimal in the central parts of cities. The availability of appropriate tick hosts is the second requirement to an urban locality suitable to tick life. As a rule, the localities suitable to ticks are also suitable for many small mammalian hosts of immature ticks. However, the vulnerability of exophilic ticks to climatic conditions, especially humidity, is higher than that of their hosts. In our analysis we follow the classification of urban localities used by other authors to the extent possible, even though the exact status of a locality where surveys were carried out may be unclear in some published reports. Most reported tick surveys were carried out in urban forests exploited as recreational areas, while many other types of localities have been inspected rarely if at all. That is why in some cases we extrapolate possible tick existence from data on small mammals living in the given locality.

Urban Forests

Urban forests as a category consist of several types of forest localities. This category includes residential areas situated inside woodlands, parts of natural forests absorbed by developing towns, and those surrounded by built-up areas (forest parks). The two latter types of forests are often used as recreational sites for urban residents. Urban forests are usually characterized by a complex of small mammals typical for the adjacent wilderness as well as by one or more species of medium-sized mammals (hedgehogs, squirrels, raccoons, badgers, hares) and large mammals such as deer, wild boar, and foxes regularly migrated from the wilderness.

Residential areas in the U.S.A. are characterized by the same abundance of *Ixodes scapularis* as their forest surroundings (Falco and Fish, 1988; Maupin et al., 1991; Carroll et al., 1992). A high abundance of adult taiga tick *I. persulcatus* was found in spacious areas on the periphery of St. Petersburg covered with deciduous forests, connected with large forest tracts and exposed to low anthropogenic pressure (Gorbunova and Tretyakov, 2012; Tretyakov et al., 2012). Immature ticks were regularly found on several species of small mammals. These areas are favorable for medium-sized and large mammal hosts of adult ticks which either live there or migrate from the adjacent forest tracts. Localities similar with or smaller than previous ones, under anthropogenic pressure of different degrees and connected with adjacent forest tracts not so tightly were characterized by a lower abundance of adult *I. persulcatus*. Immature ticks were also found on small mammals. Urban forest parks are small in size, have rather weak or no connection with natural forests and are exposed to strong anthropogenic pressure; the abundance of adult ticks in such localities was very low and findings of immature ticks on small mammals were rare (Gorbunova and Tretyakov, 2012; Tretyakov et al., 2012).

I. ricinus ticks infected with *Borrelia burgdorferi* were found in two large forest parks of London (955 and 445 ha) close to the downtown and surrounded by roads and buildings (Guy and Farquhar, 1991). Herds of deer roaming free and other medium-sized and small mammals inhabit both parks. Numerous tick bites and symptoms of Lyme disease were reported in workers of these parks (Rees and Axford, 1994). *I. ricinus* persistence was observed in recreational areas of Helsinki where the hare was the only possible host for adult ticks (Junttila et al., 1999). Five species of ticks (with *Rhipicephalus turanicus* dominance) were found in a large forest park in Rome (740 ha), which is characterized by woods and bush strips and is linked with green zones outside the city (Di Luca et al., 2013).

Public Gardens, Boulevards, Cemeteries

The abundance of *I. ricinus* ticks in public gardens (cultivated parks) significantly varies being higher in the sites with forest-like vegetation (Maetzel et al., 2005). Ticks are absent in the localities where trees and undergrowth are scarce. Another condition of this species prosperous existence is a permanent thick layer of leaf litter which provides a stable humid microenvironment (Kahl et al., 1992). In city parks of Košice, Slovak Republic, the abundance of ticks correlated with the size of the park, the density of plant and bush cover and the tie with peripheral forests (Nadzamová et al., 2000). No ticks were found in urban parks of Lyon (Quessada et al., 2003), which is thought to be to good maintenance of park vegetation. Thousands of ticks, mainly *I. ricinus* but also *I. hexagonus*, were collected from hedgehogs at Margaret Island in central Budapest during two surveys (Földvári et al., 2011). The island (96.5 ha) is a landscape park and a popular recreational area. *I. ricinus* and *Dermacentor reticulatus* were found in parks in and near the central part of Kiev (Akimov and Nebogatkin, 2002).

D. variabilis has been established in many urban areas of the U.S.A. In 1978 it was collected from 153 outdoor locations in New York City (Committee.., 1980). Dozens of adult ticks were collected in a park of Bronx, New York City after several cases of Rocky Mountain spotted fever (RMSF) were diagnosed in residents of the area. Many adults of this species were also collected in a park of Brooklyn, whereas no ticks were found in 7 other parks of New York City (Salgo et al., 1988). Recently one more case of RMSF was diagnosed in Bronx and ticks from an urban park were incriminated (Community.., 2011). All stages of *I. scapularis* were found in a city park of New York City (Daniels et al., 1997) and in an inner-city park of Baltimore (Schwartz et al., 1991). In the latter case several workers of the zoo located in the park regularly removed ticks from themselves and one of them was diagnosed with Lyme disease. Both parks are rather large, surrounded by urban development and have small and medium-sized mammals as permanent inhabitants (several deer inhabit the park in Baltimore).

Cemeteries comprise a variable group of localities depending on their status (used or disused), size and localization in the town. Old heavily wooded cemeteries which are visited only sporadically might be considered as urban forests with low anthropogenic pressure. Such a cemetery in a Siberian town of Tomsk was characterized by very high abundance of ticks of several species (Romanenko, 2011). An interesting phenomenon was noted by Romanenko (2009): the heavier is anthropogenic pressure in public gardens and cemeteries, the higher is proportion of *I. pavlovskyi* as compared with *I. persulcatus*. The first species can survive in localities with a thin layer of leaf litter characteristic for intensively trampled areas while the second one needs in a permanent thick layer of leaf litter.

Private Gardens, Yards, Grass-Plots

Although the possibility of finding ticks in any urban grass-plot is often assumed, there are only single documented records of such events. Attachment of *D. silvarum* female to a boy in the central part of Omsk was recorded (Fedorov, 1968). High numbers of *I. ricinus* were found in some inner-city gardens of Hannover where hedgehogs were the only hosts for adults (Plate, 1993). Only sporadic findings of this tick were reported on ruderal vegetation in the central part of Košice (Nadzamová et al., 2000). In Bonn, these ticks were found in private gardens adjacent to forested areas (in some of the gardens deer were observed); in gardens which were not tied with forested areas no ticks were found (Maetzel et al., 2005). Green localities small in size surrounded by urban built-up areas were called 'urban isolates' (Sokolov et al., 1995) characterized by low numbers of small mammals. However, even in small urban localities (2.25 to 3.75 ha) of Berlin deer, wild sheep, foxes and hedgehogs can serve as hosts for adult ticks (Matuschka et al., 1990). An example of tick importation into urban isolates was given by Korenberg (personal communication). On a fenced-in property located near the central part of Sofia a case of Lyme disease was diagnosed in a boy who had not left the property for several weeks. *I. ricinus* adults were found on the lawn under cherry trees, and thrushes, the known hosts of nymphs of this tick, were observed migrating to the trees.

Railway Right-Of-Way Zones And River Banks

This category of landscape can be considered as 'green corridors' connecting wild nature with towns, sometimes with their central parts. The number of species of small mammals as well as their abundance can be rather high, especially on the river banks. Cases of dog piroplasmosis transmitted by ticks in Orenburg, Russia, were mostly registered in flood-lands of the Ural River (Khristianovsky, 2004). Migratory routes of birds are often determined by valleys of big rivers. River banks serve as stopovers for migrating birds where engorged immature ticks infesting birds drop off. An example of tick dispersal over river stream was given by Romanenko (2005, 2011). He presented data on appearance of populations of *I. pavlovskyi* in Tomsk, which are associated with the banks of the Tom' River. River banks are considered the area of *I. ricinus* and *D. reticulatus* survival on the islands of Dnepr River in the boundaries of Kiev (Nebogatkin, 2012).

CONDITIONS OF TICK POPULATION PERSISTENCE

From the above data one can see that tick persistence in a particular locality depends on many factors. The importance of a strong association of urban forest localities with wilderness for persistence of abundant tick populations was pointed out by Vershinsky et al. (1988) for *I. persulcatus* and by Nadzamová et al. (2000) and Supergan and Karbowiak (2009) for *I. ricinus*. Apparently, such association will be more common in the future because the idea of 'green corridors' is a key part of conservation strategy in Europe (Jongman et al., 2004). Another important condition for tick persistence is the availability of hosts for adult ticks (Korenberg et al., 1984; Daniel and Černý, 1986; Dautel and Kahl, 1999). As noted above, even one species of medium-sized hosts (hare, hedgehog) can support the tick population. The size of the locality, the type of adjacent territories, the scale of anthropogenic pressure over the locality, and the degree of its isolation from other potential tick localities are also of importance. Anthropogenic pressure can be controlled (landscape replanning, replanting and development) or uncontrolled (trampling understorey vegetation by visitors). When urban parks are visited by a high number of people who can move only over designated paths, such pressure has little influence on tick population persistence.

Urban forests that are large and tied with wilderness are characterized by independent populations of exophilic ticks [according to the classification proposed by Beklemishev (1960)] (Table 1). Independent populations are self-sufficient and can persist and flourish without constant replenishment by specimens from other populations. The migrations of medium-sized and large mammalian hosts provide constant exchange by tick specimens between urban forests and adjacent forest tracts. In some cases independent tick populations can persist in public parks even of small size having no direct ties with natural woodlands. Proper vegetation covering and constant presence of adult tick hosts support persistence of the tick population. When these conditions are not met, the tick population finds itself in dependence on additional tick specimens brought by hosts from outside. Such populations may be semi-dependent or dependent. The existence of a number of ticks in a locality unsuitable for prolonged persistence is characteristic of temporary populations. This can happen in any type of localities with either poor microclimate or the absence of hosts. Occasional (even regular) findings of ticks in gardens, yards and grass-plots reflect an existing possibility of importing new tick specimens by their hosts.

Table 1. Classification of tick populations according to Beklemishev (1960).

Populations	Conditions of populations persistence
Independent	Self-sufficient populations which can persist and flourish without replenishment.
Semi-dependent	Populations can persist but cannot flourish without replenishment.
Dependent	Populations cannot persist without constant replenishment.
Temporary	Short-lived populations in unfavorable habitats periodically appearing after importation of tick specimens by their hosts from outside.

EXOTIC TICKS IN URBAN ENVIRONMENT

Chances of ticks brought outside of their geographic range establishing a viable population are rather low. When ticks are brought to the north or to the south of their reproductive range they are found themselves under unfavorable climatic conditions. Regular findings of *I. persulcatus* adults in Yakutsk and surrounding areas, far north from the northern limit of their reproductive range, were reported and discussed by us previously (Uspensky et al., 2003). When ticks move in zonal direction (west-east), they may find themselves under unusual climatic conditions with a number of unusual hosts. It is important that in the great majority of cases, artificial introduction of exotic ticks occurs when a single specimen or, in rare cases, a small number of specimens are delivered to a single locale, usually a point of animal quarantine or another place that is under observation. Single specimens of exotic ticks may be of danger when they carry human or animal pathogens but they cannot initiate even a temporary population. The only exception is the brown dog tick (or kennel tick) *Rhipicephalus sanguineus* (see below).

CONSTANT INHABITANTS OF URBAN ENVIRONMENT

There are tick species that are highly adapted to life in urban environment, specifically the brown dog tick *R. sanguineus* and the pigeon tick *Argas reflexus* s.l. (Uspensky, 2008a, 2014). The persistence of their populations absolutely depends on the availability of their main hosts, dogs and pigeons, respectively. Both species are able to create independent urban populations. *R. sanguineus* is enabled to create independent urban populations mostly due to the close association of all developmental stages of the tick with dogs (Uspensky, 2008c), whereas in argasid ticks this quality is based on their ability to survive prolonged starvation (Uspensky, 2008b). The initiation of new populations of both species is a result of tick specimens being brought by hosts from other sites. There are numerous examples of appearance of *R. sanguineus* populations when infested dogs returned home after being for some time in endemic countries (see Uspensky, 2014). All stages of these ticks actively attack humans in urban conditions and in dwellings. *A. reflexus* provokes severe allergic responses while *R. sanguineus* transmit several pathogens to humans and dogs.

CONCLUSION

Thus, each locality in urban environment can be inhabited by a certain type of tick population depending on a number of factors. Our attempt to find some patterns and regularity in tick existence under urban conditions should be considered as a preliminary one because of the scarcity of material. The main part of publications concerning urban ticks has been devoted to estimating infection rate of these ticks with various pathogens but important biological details have been ignored. The fragments of information which were possible to be extracted from numerous publications gave an initial picture that might create a basis for purposeful studies of the problem. The better understanding of the regularity of tick existence is a necessary condition for the development of protective measures for urban residents against ticks living near them. This is precisely the independent populations of ticks that should attract primary attention of researchers and public health managers.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

INSECTICIDE SUSCEPTIBILITY AND RESISTANCE MECHANISMS IN BODY LICE IN RUSSIA

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Abstract The majority of body lice colonies examined in Moscow were permethrin resistant and susceptible to organophosphorus (fenthion, malathion) and neonicotinoid insecticides. Metabolic resistance of body lice to permethrin was investigated using synergists piperonyl butoxide (PBO), S,S,S-tributylphosphorotrithioate (DEF) and diethylmaleate (DEM). Synergism with enzyme inhibitors in permethrin-resistant body lice demonstrated that in some cases enhanced metabolism was involved in pyrethroid resistance. Resistance to permethrin was partially suppressed by` PBO, DEM and DEF, which suggests possible involvement of monooxygenases, esterases and glutathion-S-transferases in the resistance mechanism. Since the use of synergists did not lead to full suppression of resistance, it appears that lice also posses knockdown resistance (*kdr*). Body lice were studied by real-time PCR to detect *kdr* mutations (T917I and L920F) in the para-orthologous voltage-sensitive sodium channel gene, which are associated with permethrin resistance. The frequency of occurrence of the pyrethroids resistance gene was measured in 153 lice. Of these, 101 (66.0%) turned out to be homozygous resistant, 18 (11.8%) homozygous susceptible, and 34 (22.2%) heterozygous.
Key words *Pediculus humanus,* pyrethroid resistance, synergist, knockdown and metabolic resistance, sodium channel mutations.

INTRODUCTION

Body lice (*Pediculus humanus humanus* L.) is a hematophagous ectoparasite that lives on the body and in the clothing or bedding of its human host. They transmit such human diseases as epidemic typhus, relapsing fever, and trench fever. Body lice infestation is usually found only in persons who have no access to clean clothes or bathing facilities (e.g. the homeless population). In 1990, permethrin was introduced for the control of *P. humanus* in Russia but by 2008 it was found ineffective. Research conducted in 2009-2012 revealed that resistant insects are present in over 90% of lice micropopulations collected on the homeless. Permethrin-resistant lice also show cross-resistance to d-phenothrin and DDT (Lopatina and Eremina, 2011, 2013). Permethrin-resistant body lice also have been found in France (Drali et al., 2012).

The main mechanism of pyrethroid resistance in arthropoda involves lower sensitivity of their nervous system to this group of insecticides because of a mutation in the gene responsible for voltage-sensitive sodium channels. Point mutations in □-subunits of the para-orthologous sodium channel gene of the head and body louse (M815I, T917I, and L920F) are associated with permethrin and DDT resistance (Lee et al., 2003; Gao et al., 2003; Clark, 2010). *Kdr* type resistance may be combined with changed activity of enzyme systems. Pyrethroid resistance, however, was investigated in head lice only. It was demonstrated that permethrin-resistant head lice show the most pronounced increase in the activity of monooxygenases (MO), but a smaller one in that of esterases (Est) and glutathion-S-transferases (G-S-T) (Hemingway et al., 1999; Piccolo et al., 2000; Bartles et al., 2001; Audino et al., 2005). Only limited information on the resistance mechanism in head lice is available (Drali et al., 2012). The present work is a summary of our insecticide resistance monitoring in body lice from 2009 to 2013. Mutations associated with permethrin resistance in body lice have been detected. Evidence of possible involvement of monooxygenases, esterases, and glutathion-S-transferases was also obtained in the course of synergism studies.

MATERIALS AND METHODS

Body lice colonies. Body lice were collected from the clothing of homeless persons in two shelters in Moscow, Russia. The homeless were given new clothes, and their lice-infested clothes were delivered to the laboratory. Clothes of about 1500 homeless persons were examined. Isolated lice samples were also taken in St. Petersburg and Tambov. A susceptible strain of human body lice maintained on rabbits and never exposed to permethrin was used as control. Toxicological experiments were performed only on lice from large micropopulations (samples from single hosts were from 500 to over 6000 insects).

Chemicals. Technical grade insecticides used for baseline susceptibility tests and synergistic studies were permethrin (93.0%), DDT (76.0%) and malathion (92.8%). In the investigation of lice enzyme system technical grades synergists were also used. These were piperonyl butoxide (PBO) (99.0%), a monooxygenases inhibitor, S,S,S-tributylphosphorotrithioate (DEF) (98.3%), an esterase inhibitor, and diethylmaleate (DEM) (100%), a glutathion-S-transferases inhibitor. All technical grade insecticides were diluted in analytical grade acetone. Emulsifiable concentrates of insecticides were diluted in water. These were permethrin (20%), d-phenothrin (10%), malathion (57%), fenthion (20%), and a water soluble concentrate of imidacloprid (20%).

Bioassays. The percentage of resistant lice was established by the method proposed by B.C. Zeichner (Zeichner, 1999) that consisted of placing insects on filter paper impregnated with insecticides (permethrin 0.02 mg cm^{-2}, DDT 1.0 mg cm^{-2}, malathion 0.2 mg cm^{-2}). Knockdown rate was established after six hours of exposure. The sensitivity of older larvae and mature lice to water insecticide emulsion was evaluated by dipping lice into emulsion for 15 minutes with subsequent rinsing in fresh running water. Lice mortality was assessed after 24 hours. For synergism studies, lice were sprayed with synergist solution in acetone (PBO – 0.1%, DEF – 0.1%, DEM 1.0% a.i.) for 30 min. prior to permethrin susceptibility test by a 15 min. immersion in an aqueous emulsion of insecticide. Each experiment was replicated three times.

Genetic Methods. Body lice were studied by real-time PCR to detect the *kdr* mutations (T917I and L920F) in the para-orthologous voltage-sensitive sodium channel gene, which are associated with permethrin resistance. Insects stored prior to DNA extraction in 95% ethanol. Genomic DNA was extracted from individual specimens, using the AmpliSens Riboprep Kit (Central Institute of Epidemiology, Moscow, Russia) according to manufacturer's instructions. The *P.humanus VSSC* – specific forward and reverse primers were, at 360 nmol/L, Ped-F TGG GTC GAA CTG TTG GAG CTT and Ped-R CCA TAA CGG CAA ATA TGA ATA TGA T, respectively. The corresponding dye-labeled probes (final concentration 100 nmol/L) were Ped-S FAM-TGG GTA ATT TAA CAT TCG TCC TTT GCC-BQH1R6G-CCTGGGGA and Ped-R R6G-TGG GTA ATT TAA TAT TCG TCT TTT GCC-BQH1. The PCR conditions were 95°C for 15 min followed by 5 cycles at 95°C for 10 sec., 60°C for 25 sec., and 72°C for 15 sec.; then by 40 cycles at 95°C for 10 sec., 56°C for 25 sec., and

72°C for 15 sec. (Rotor-Gene 6000, Qiagen). The fluorescence signal was recorded at the 56°C step for the last 40 cycles. Each run included negative control and positive recombinant control DNA of the *P. humanus* VSSC *kdr*-resistant and *kdr*-sensitive alleles of gene fragment as a standard. Absence of false-positive PCR results was confirmed direct sequencing of two genomic regions of the sodium channel α-subunit (exon1 and exon3) with primers 5'QSMI and 3'QSTILF (Kwon et al., 2008).

Data Analysis. Data were pooled and subjected to probit analysis using software program (Probit Analysis, v.1.0). Resistance ratio (RR) was calculated as the relation the CL_{50} value of the resistant strain to the same of the susceptible laboratory body lice colony. Synergistic ratio (SR) was calculated as the relation the CL_{50} value for permethrin to CL_{50} value for the synergist and permethrin combination.

RESULTS AND DISCUSSION

Body lice were found in 30% of clothing from homeless persons. Similar figures were obtained in other countries. In Marseille, for instance, body lice were found in 22% of the homeless (Brouqui et al., 2005).

Lice from various locations (Moscow, St. Petersburg, Tambov) were resistant to pyrethroids and DDT, and sensitive to malathion. Generally permethrin-resistant lice comprised 40-60% of the sample while the average was about 45%. Samples of lice with 100% susceptibility to permethrin are rare, and their share dropped during the observation period. In Moscow it was 9.1% in 2009, 3.3% in 2010, 5.4% in 2011, 1.9% in 2012, and none in 2013.

In 2012 studies conducted in St. Petersburg the situation was similar to that in Moscow. Permethrin-resistant lice were found in all samples from St. Petersburg, and their share was about 60%. In 2013 permethrin-resistant lice were found in Tambov too but constituted 22.5%.

Body lice show resistance to permethrin and d-phenothrin in the dipping test. Sensitivities of different lice samples however vary greatly (Table 1 and 2). It was not possible to obtain the 95% death rate in the part of high RR populations. Toxicity increased linearly at higher doses but showed a plateau of 25-50% mortality at permethrin concentrations of 0.005-0.5%. This type of response is typical for mixed populations containing resistant individuals.

Permethrin resistance of lice is unrelated to their sensitivity to organophosphates (fenthion, malathion) and neonicotinoids (imidacloprid). Synergism with enzyme inhibitors in permethrin-resistant body lice indicates that in some cases enhanced metabolism is involved in pyrethroid resistance. Synergistic ratios were 3.3 to 52.0 for MO inhibitors, 5.2 to 7.4 for Est, and about 4 for G-S-T (Table 2). Slight synergism of permethrin combined with detoxifying enzyme inhibitors indicates that there is another resistance mechanism in addition to increasing enzyme activity (MO, Est and G-S-T). Since the use of synergists did not led to the full inhibition of resistance, lice probably posses knockdown resistance (*kdr*) as well. Lack of correlation between resistance and synergistic ratios supports this view. Cross-resistance to pyrethroids (permethrin, d-phenothrin) and organochlorine insecticide DDT also provides indirect evidence for *kdr*-resistance.

In Moscow the resistant haplotype was present in all body lice samples. The frequency of the pyrethroid resistance gene (T917I, L920F) was measured in 153 insects using RT-PCR. Of these, 101 (66.0%) were homozygous resistant, 18 (11.8%) homozygous susceptible, and 34 (22.2%) heterozygous. No colonies of exclusively permethrin-susceptible (SS) insects were found. Sequestering of the 1st and 3rd exons of the Vssc gene confirmed the specificity of the developed method of detecting T917I, L920F mutations in real time using PCR. The three mutations, M815I, T917I, and L920F, were detected only en bloc.

Table 1. Body lice susceptibility to insecticides.

Insecticides	RR at CL_{50} permethrin	n	Slope± SE	CL50 (95% CI) % a.i.	CL95 (95% CI) % a.i.
			Pyrethroids		
permethrin	17	360	1.80±0.19	0.017 (0.013-0.022)	0.09 (0.07-0.12)
	50	360	2.42±0.26	0.05 (0.038-0.065)	0.25 (0.19-0.33)
	80	360	1.73±0.17	0.08 (0.062-0.104)	1.0 (0.76-1.31)
	320	360	2.90±0.28	0.32 (0.25-0.42)	>1.0
	360	360	2.29±0.24	0.36 (0.28-0.47)	>1.0
d-phenothrin	90	360	2.19±0.76	3.0 (2.3-3.9)	>5.0
	320	360	-	>5.0	>5.0
			Organophosphates		
malathion	17	360	2.5±0.20	0.0045 (0.0035-0.0059)	0.022 (0.017-0.029)
	320	360	3.03±0.26	0.0050 (0.0038-0.0065)	0.025 (0.019-0.033)
fenthion	17	360	3.38±0.32	0.00012 (0.00009-0.00016)	0.00037 (0.00028-0.00048)
	320	360	3.47±0.73	0.00015 (0.00012-0.00020)	0.00040 (0.00031-0.00052)
			Neonicotinoids		
imidacloprid	25	360	2.71±0.55	0.0035 (0.0029-0.0041)	0.015 (0.012-0.020)
	360	360	2.59±0.27	0.0046 (0.0040-0.0052)	0.024 (0.018-0.031)

The data obtained indicate that the mechanism of lice permethrin resistance involves complex adaptation to pyrethroids. It includes at least two factors: *kdr* mechanism and non-specific resistance through the increase in the activity of detoxifying enzymes. Different lice populations may have the same overall resistance ratio, but the ability of their enzyme systems to increase their activity may differ.

Table 2. Effect of synergists on potency of permethrin in various body lice populations

Colony	n	Insecticide + synergist	CL_{50} (95% CI) (% a.i.)	SR	RR
Susceptible strain	420	permethrin	0.001 (0.0007-0.0013)	-	0
M158	420	permethrin	0.017 (0.013-0.021)	-	17
	420	+ PBO	0.009 (0.007-0.012)	1.89	9
	420	+ DEM	0.0045 (0.0035-0.0059)	3.78	4.5
	420	+ DEF	0.002 (0.0015-0.0026)	8.50	2
M109	420	permethrin	0.045 (0.035-0.059)	-	45
	420	+ PBO	0.054 (0.042-0.070)	0.83	54
M214	420	permethrin	0.19 (0.14-0.25)	-	190
	420	+PBO	0.20 (0.15-0.26)	0.95	200
	420	+DEM	0.21 (0.16-0.28)	0.90	210

M147	420	permethrin	0.23 (0.18-0.30)	-	230
	420	+ DEM	0.10 (0.07-0.12)	2.30	100
	420	+ DEF	0.18 (0.14-0.23)	1.28	180
M206	420	permethrin	0.30 (0.23-0.39)	-	300
	420	+ PBO	0.09 (0.07-0.12)	3,33	90
	420	+ DEM	0.075 (0.058-0.098)	4.00	75
	420	+ DEF	0.058 (0.045-0.075)	5.17	58
M36	420	permethrin	0.36 (0.28-0.47)	-	360
	420	+ PBO	0.23 (0.18-0.30)	1.57	230
	420	+ DEM	0.13 (0.10-0.04)	2.77	130
	420	+ DEF	0.24 (0.18-0.31)	1.50	240
M74	420	permethrin	0.37 (0.28-0.48)	-	370
	420	+ PBO	0.09 (0.07-0.12)	4.11	90
	420	+ DEM	0.35 (0.27-0.46)	1.06	350
M168	420	permethrin	0.50 (0.38-0.65)	-	500
	420	+ PBO	0.40 (0.31-0.52)	1.25	400
	420	+ DEM	0.51 (0.40-0.67)	0.98	510
M169	420	permethrin	0.51 (0.40-0.68)	-	510
	420	+ PBO	0.31 (0.23-0.39)	1.65	310
M207	420	permethrin	0.52 (0.40-0.68)	-	520
	420	+ PBO	0.01 (0.008-0.013)	52.00	10
	420	+ DEM	0.12 (0.09-0.16)	4.33	120
	420	+ DEF	0.07 (0.05-0.09)	7.43	70

CONCLUSIONS

Because resistant lice were detected in all colonies, it is speculated that resistant lice are spread extensively in Russia, both in large cities and smaller towns. Toxicological and genetic data indicate that the principal mechanism of body lice resistance to permethrin is nervous system insensitivity as a result of mutations in the *Vssc1* gene. A weak enzyme-based permethrin resistance mechanism was identified. Our results indicate multiple mechanism of pyrethroid resistance in lice populations.

ACNOWLEDGMENTS

This work was in part financially supported by the Russian Foundation for Basic Research (grant № 13-04-01657).

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

URBAN SCORPION POPULATIONS AND PUBLIC HEALTH IN BRAZIL

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Abstract Intoxications due to venomous animals are considered a neglected disease in Brazil. Out of the others poisonings, scorpionism became the most important urban plague spread through different regions. Scorpions are ancient arthropods and the bio-ecological features of some dangerous species and their abilities to colonize areas under strong antropic pressure led to a great and unexpected raise in human intoxications and deaths in Brazil and other countries from the America continent. Despite many laboratory reports showing high efficacy of different chemical tools on scorpions control, there are only few and/or poor field assays on this subject. Brazilian government strategies for prevention and control are based on education, systematic capture and environmental correction. In other hand, many insecticides are registered and used for scorpions and spiders control. Private services are also available in many cities of the country. This scenario is confuse and demands rapidly efforts to alignment of actions. The present paper discuss the technical and scientific criteria to establish minimal conditions of safety and efficacy of potentials chemicals or naturals tools for scorpion control, the mandatory needing of a field protocol for evaluation of insecticides on scorpions urban population and also the positive and negative aspects of public campaigns based on interventions on human habits and culture involved on scorpion proliferation around the world.
Key words Scorpionism, public health, environmental education.

INTRODUCTION

Some scorpion species from Buthidae family represents serious public health problem in different parts of the world (Chippaux, 2008). Asia, North Africa, Middle East, South and North America are the main regions where dangerous animals from *Leiurus*, *Androctonus*, *Mesobuthus*, *Tityus* and *Centruroides* genera are responsible for thousands envenomings and hundreds of deaths every year (Borges, 1996; Chippaux, 2012). Potent neurotoxic venom and ability to occupy human modified rural and/or urban areas are common characteristics of those species considered plague (Souza, 2012). As neglected disease, few resources on research of new treatments, medicines, pesticides and control methodologies; unsecure epidemiological data, difficult access on antivenom therapy for endangered poor human population results in a complex and underestimated global scenario of scorpionism. Since the begging texts addressing scorpions control, association of environmental interventions and appliance of chemical insecticidal substances (natural or synthetic) are the main strategy frequently proposed, with different levels of success or failure (Ramsey et all, 2002; Brites-Netto and Brasil, 2012). In Brazil The criteria for selection of chemicals are the availability of insecticide, its cost, or prior experience with the use to control another pest, excluding bio-ecological aspects of scorpions (Souza, 2012). In this study our objective is to assess the applicability and reliability of laboratory methods for evaluating the effectiveness of insecticides in the chemical control of scorpions and propose their standardization, discuss the effectiveness of control initiatives based on environmental education and collecting scorpions and the general outlines of a field trial for chemical control of scorpions.

MATERIALS AND METHODS

Experimental and control groups were composed of 10 scorpions *Tityus serrulatus*. Each experiment was repeated 3 times. We used the microencapsulated insecticide lambda-cyhalothrin 10% CS (37.5 mg i.a./m^2) as chemical tool model. Topical toxicity assessment was in terms of Lethal Dose 50% (LD_{50}) from lethality recorded at 72 h, 168 h and 216h after application of insecticide dosages (1-25 ug/ind) fixed in a volume of 5 ul on the back of scorpions. Pre-treated (24 h) discs of filter paper (154 cm^2) and baked bricks (551 cm^2) served as substrate to evaluate the residual effect of the active principle on scorpions by exposure to continuous contact for 24 h or 168 h at T□; 30 days, 60 days and 90 days. The dislodge effect was seen as the percentage of scorpions that drops a crevice between two bricks in a given time interval (0-15 min) after application of insecticide. The results were analyzed with Student's T test, full probit analysis test and X^2. We also show the records of scorpion stings official epidemiological information system of the Ministry of Health of Brazil between 2001 and 2013.

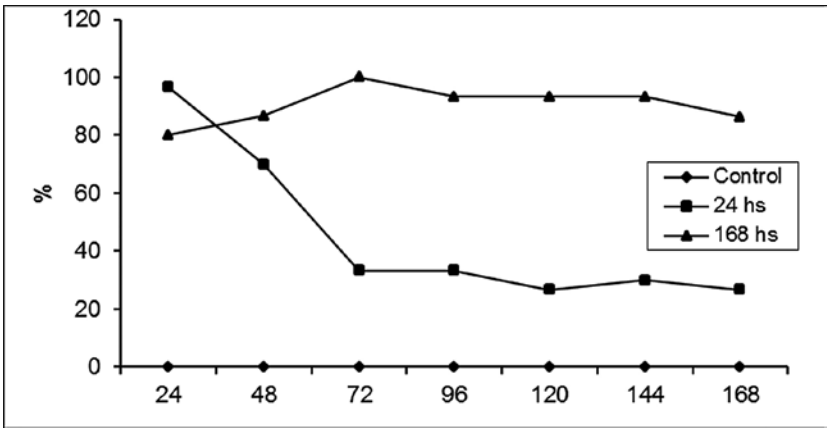

Figure 1. Filter paper impregnated with lambda-cyhalothrin 10% CS. Animals exposed for 24 h showed signs of intoxication, at 168 h lethality decays to 25% ($p < 0.05$). Scorpions exposed for 168 h continuously showed 100% lethality ($p < 0.05$).

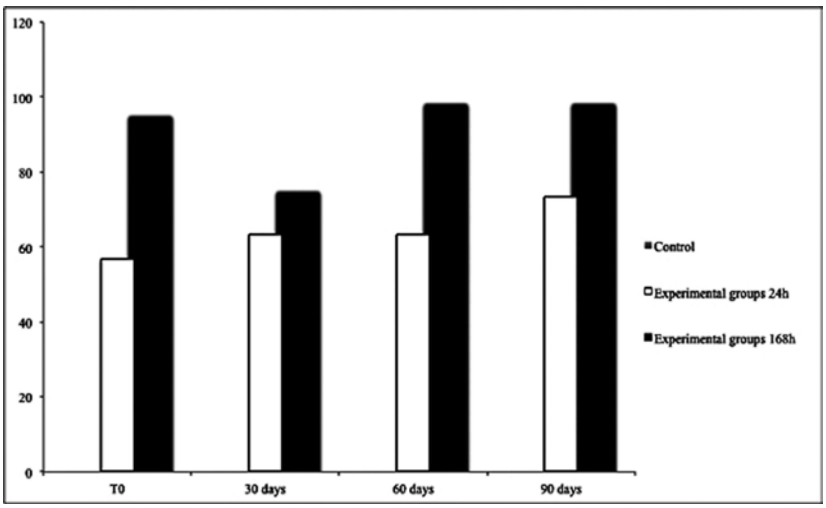

Figure 2. Bricks treated with lambda-cyhalothrin 10% CS. White bars show 168 h percentage lethality after 24 h continuous contact. Black bars show lethality for continuous exposure for 168 h to bricks.

RESULTS AND DISCUSSION

Acute Toxicity

Unlike many opinions (Brasil, 2009) *Tityus serrulatus* scorpions are highly sensitive to the insecticide tested as shown in Table 1, indicating the use of this tool for scorpions control. The animals showed severe signs of acute intoxication and the final results in terms of mortality LD_{50} exceeded the limit of validity stated by WHO (Zaim, 2006) for control of insects with behavioral characteristics and hideaway to similar sized scorpions.

Table 1. Lethal Dose 50% of lambda-cyhalothrin 10% CS against *Tityus serrulatus* (mean weight 1.2 g, n=30). Lethal effect observed up to 216 h after treatment, indicating evidence of residual effects.

Dose	**Lethality** **72 h**	**168 h**	**216 h**
1 ug/ind	4 / 30 (13.3%)	8 / 30 (26.6%)	11 / 30 (36.6%)
5 ug/ind	9 / 30 (30.0%)	15/ 30 (50.0%)	17/ 30 (56.6%)
25 ug/ind	19/30 (63.3%)	24/ 30 (80.0%)	26/ 30 (86.6%)
Lethal Dose 50% (probit analysis test)	13.00061 µg/ind (0.01083 µg/mg)	4.305473 µg/ind (0.00358 µg/mg)	2.498498 µg/ind (0.00205 µg/mg)

Treated Surfaces

Tests for different surface treatment (Figures 1, 2) showed that the habits of rest and shelter of the scorpion *Tityus serrulatus* allow contamination of its bodily surface with concentrations of insecticide capable of inducing lethality. The differences observed between the groups exposed for 24 h or 168 h to the treated surfaces (bricks) indicate that lethality is proportional to the contact time with the active ingredient, indicating the formulation and persistence of a product as essentials to effective scorpion control.

Dislodge Effect

The dislodge effect of insecticides used to control scorpion is extremely important due to urban feature of this public health problem. Our results, shown in Figure 3 indicate that the active principle tested induces important level of dislodge effect, however 24 h all scorpions that left the slit or not were dead.

While conducting the experiments, animals exposed to low doses of the insecticide tested showed signs of detoxification and full recovery after up to 216 h, indicating the quality of correct dosage of the application as critical to the effectiveness of an insecticide to control scorpions. In tests with other insecticides, scorpions showed “high heels” behavior indicating the capacity of the sensory system to perceive the treatment and prevent intoxication of the animal. The irritant effect is really a major concern in planning strategies for chemical control of scorpions, as it may aggravate the problem in very dense urban areas. All results in the laboratory indicate the use of insecticides for control of scorpions, after due execution of large field trial.

The initiatives of scorpion control based on environmental intervention, environmental education and collecting consolidated and implemented since 2009 in Brazil, have not yet shown the ability to influence the crescent curve of scorpionism in the country (Figure 4). Perhaps this maybe explained by the high and fast rate of parthenogenetic reproduction of the main problem species, *Tityus serrulatus* and *Tityus stigmurus* and the high operating costs of maintenance teams for systematic collection of these animals, as proposed in the National Program for Scorpion Control. Another important point is the high colonizing ability of these species in areas with high standard of urbanization and income

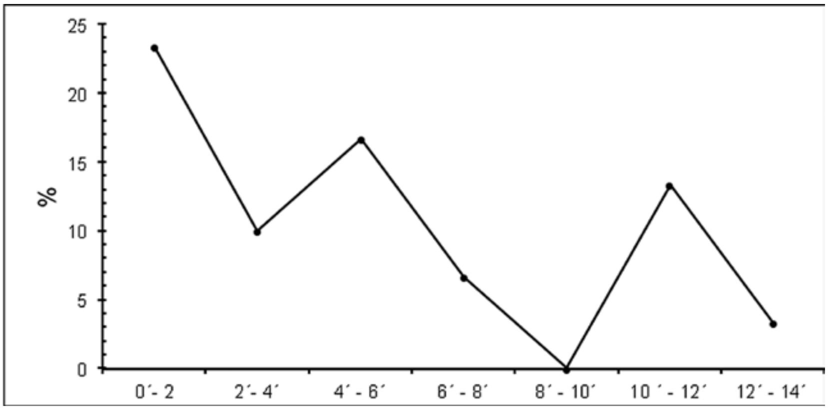

Figure 3. Dislodge effect of lambda-cyhalothrin 10% CS on T. serrulatus scorpions. In 15 min, about 70% of the animals left the crevice after application of insecticide. 100 % lethality after 24 h.

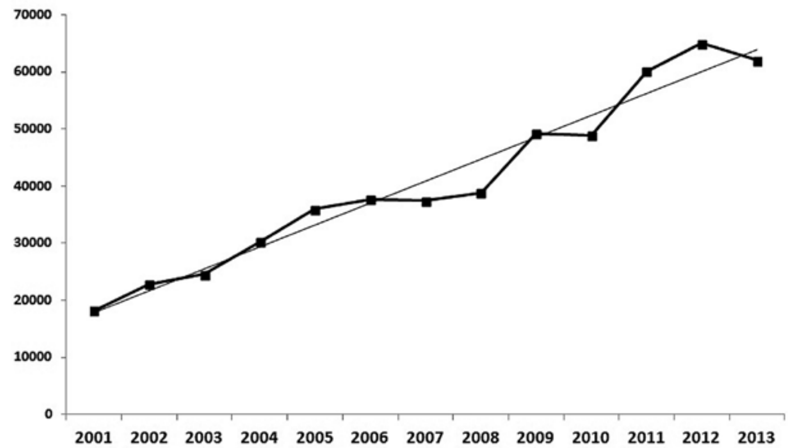

Figure 4. Evolution of the epidemiology of scorpions stings recorded by Ministry of Health from Brazil 2001-2013.

(Brites Neto and Galassi, 2012; Fernandes, 2012), indicating that only measures of environmental correction are not able to make the environment inhospitable to such animal. The justified fear of broad indication of insecticides to control scorpions in Brazil, should be the need for careful evaluation methodologies in the field of application, suitable formulations, schedules, statistical models and effects of candidate insecticides on behavior of urban populations of scorpions, environment and human population health exposed as the Mexican experience (Ramsey, 2002; Conyer, 2003), since the actual indications of the manufacturers for insecticide use in scorpions control, is based only on the known behavior of theirs products to control pests with very different biological characteristics of scorpions.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

LASIUS NEGLECTUS (HYMENOPTERA: FORMICIDAE) IN THE UK: STATUS, IMPACT AND MANAGEMENT

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Abstract The Invasive Garden Ant, *Lasius neglectus*, was first recognised in Europe in 1990, and has now been recorded from at least ten European countries. It was first found in the UK in 2009 in Hidcote Manor Garden, Gloucestershire, UK, where the ant is believed to have been present for several decades. A survey of the garden shows that the ant currently occurs over an area of c.12 ha, and is found in the formal garden, buildings, adjacent woodlands, pasture, and around the edges of arable fields. Within this area, native ant species appear to have been almost entirely displaced. In buildings, the ant is a nuisance to residents, a pest in food catering facilities, and can cause damage to electrical installations. A number of measures have been put in place to reduce the risk of onward transmission of the ant to other locations, including holding all garden and building wastes on site, cessation of sending Hidcote-grown plants to other gardens, and an ant control programme using insecticide baits. Experimental treatments with 0.03% imidacloprid ant gel within the cottages at Hidcote resulted in a c. 90% reduction in ant numbers. Surveys and investigations elsewhere in the UK have not found any other locations of this species, so an eradication programme at Hidcote is being considered.
Key words Invasive garden ant, imidacloprid.

INTRODUCTION

Invasive species are one of the most important drivers of biodiversity loss, and have been calculated to cause damage costing 12 billion Euros/year in Europe (EEA, 2012). Managing this threat requires an integrated approach involving prevention, detection and response.

Lasius neglectus (Van Loon, Boomsma and Andrásfalvy) is a native of Turkey, Iran, the Black Sea area, and other areas of Asia Minor. Outside of its native range, the first colony was detected in Budapest, Hungary where it had been known since the 1970s, but was not identified as *L. neglectus* until 1990 (Van Loon et al., 1990). Colonies have since been identified in ten European countries (Espadaler and Bernal, 2010), and most recently in the UK, at Hidcote Manor Garden in Gloucestershire. This species is considered by Rey and Espadaler (2004) to have the potential to become as serious a problem in Europe as the Argentine ant (*Linepithema humile*).

Lasius neglectus worker ants are superficially similar to the black garden ant *Lasius niger*, but are slightly smaller and paler. They may be found within buildings, on the ground outdoors, and trailing up the stems or trunks of plants. Where they are well established, *L. neglectus* is noticeably at a higher density than would normally be the case for *L. niger* (Fox, 2010). Differentiation of *L. neglectus* from similar species requires examination of a number of morphological characters, including the presence or absence of short erect hairs on the scapes and tibia (Espadaler and Bernal, 2010; Fox, 2010).

The nests of *L. neglectus* are typically in protected locations, such as under stones, paving slabs, logs, or in the soil around plants with a high population of aphids (Espadaler and Bernal, 2008).

Individual nests are normally occupied by several queens (Van Loon et al., 1990), unlike *L. niger.* Nests may separate or coalesce depending on local factors, and the ants in any one area become in effect a 'super-colony', comprising large numbers of interlinked nests. In Spain, one super-colony covering 14 ha was estimated to contain 2,500 queens and 8 million worker ants per hectare (Espadaler et al., 2003). Some super-colonies are known to cover several hundred hectares (Espadaler et al., 2007). The queens mate within the nest (Van Loon et al., 1990), and as there is no nuptial flight; new nests are typically founded by budding (Espadaler and Bernal, 2008). A single queen ant is sufficient to establish a new colony (Espadaler and Rey, 2001). The rate of colony expansion ranges from c.3 to 90m/year (Espadaler et al., 2007).

This ant is dependent feeds on the aphid honeydew and other sap-sucking insects, and most activity is concentrated on plants and trees, that have large aphid populations (Espadaler and Bernal 2008). Ants have been seen carrying small insect prey, such as collembolans (Espadaler and Rey, 2008). In buildings the ant feeds on carbohydrate-rich foodstuffs. In continental Europe, most colonies are found in disturbed urban and suburban areas. Outdoor habitats include grass beside roads, parks and gardens, waste ground, and patches of woodland in city areas (Espadaler and Bernal, 2008).

The only known colony of this species in the UK is in Hidcote Manor Garden in Gloucestershire, southern England. The estate consists of c. 10 ha of ornamental garden, ten cottages, a manor house and farm buildings, together with some areas of woodland and farmland. The garden was created in the early 20th century by Lawrence Johnston, an American horticulturist, with plants obtained from several continents, including Asia (Pearson, 2007). The garden is of international significance owing to its design, and is open to the public, attracting around 160,000 visitors per year. It is in the care of the National Trust, a charitable organisation that manages and protects many sites of high historical or natural importance in England, Wales and Northern Ireland.

Residents in the cottages reported that the ants have been present on the estate for several decades. Their continual presence within their homes, even in winter, is a nuisance, while staff in the catering facilities have to take care to avoid food contamination. This ant species is attracted to electrical installations, and in some buildings light switches, power sockets and electrical security systems have been damaged by its activity. In 2009 the ants were identified as *Lasius neglectus* - the first colony of this ant to be identified in the UK (Fox, 2010).

The objectives of the work reported here were to establish the extent of the ant colony, identify effective techniques for its control, identify and implement measures to reduce the risk of spread of the ants to other locations, and to determine if this ant was present at other locations in the UK. Part of the work reported here was funded by the National Trust, and part by Bayer Environmental Science.

METHODS AND MATERIALS

Ant surveys. To establish the extent of the area colonised by *Lasius neglectus*, the garden and surrounding farmland were systematically surveyed for ants in July and August 2013. Any ants seen under stones, on the ground, tree trunks, or walls etc were collected and placed into labelled tubes for identification. In addition, honey-baited insect detectors (AF Crawling Insect Detector, Killgerm Ltd) were used to detect ants present in areas where they were not visible on inspection. The monitors were placed in likely locations, collected 48 h later, and any ants identified.

Hidcote Manor Garden had from time to time donated plants from its gardens to other large ornamental gardens elsewhere in the UK. Three such gardens were identified, and ant surveys using honey-baited detectors was carried out in the areas of these gardens where plants obtained from Hidcote Manor Garden had been planted. Finally, to try to establish whether pest control companies were aware of any other infestations of this ant, announcements were placed in two of the UK pest control magazines, these requested companies who were aware of any unusual black ant infestations, to make contact.

Ant management at Hidcote Manor Garden. Once it became apparent that the garden may hold the only UK colony of this ant species, the various activities at the site were assessed in order to identify those that were at high risk of inadvertently spreading ants to other locations, and how that risk could be minimised.

Additionally, to identify the effectiveness of insecticidal control measures against this ant, a trial of an insecticidal bait was carried out in the ten residential cottages. Honey-baited crawling insect monitors were used to assess ant numbers, with between five and seven used in each cottage. Monitors were placed in situ for 48 h, and then collected and examined. Monitor assessments were carried out twice prior to treatment, and then for four consecutive weeks after treatment, and finally at 19 weeks after treatment. Maxforce Quantum (0.03% imidacloprid gel) was applied using a B&G gel applicator, set to deliver c. 0.2g of gel at each pull of the trigger. Gel was applied at the label rate of 0.2g/m^2, with beads of gel being applied either directly into cracks and crevices, or into small plastic bait stations. All rooms and hallways within the cottages were treated. In addition, treatments were also applied around the external footings of the buildings to create a ‘buffer zone’ to reduce the re-invasion from outside. In these outdoor areas, some gel was also applied into cracks and crevices and some into bait stations. At 3 weeks after the initial treatment, a partial re-treatment with imidacloprid gel was carried out in areas where ants were still active. In all, eight cottages were treated, while two were left as untreated controls.

RESULTS AND DISCUSSION

Distribution and Spread at Hidcote Manor Garden

The survey found that *L. neglectus* occupied a broad range of habitats in the garden such as flower-beds, lawns, paths, rock gardens, kitchen gardens, green-waste composting areas and log piles. However it was also present in numbers in adjoining damp and shaded woodland areas, on the edges of arable fields, in permanent pasture up to 50m from the garden, and on farm tracks and around farm buildings. The greatest numbers of ants were usually seen on the trunks of trees, where dense columns were sometimes found, presumably accessing aphids and other sap-feeding insects in the foliage. In total, *L. neglectus* was found to occupy an area of c. 12 ha (Figure 1).

Natural budding of ant nests is likely to be responsible for the spread of the ants in some areas, for example where they have colonised the pasture to the west of the garden. Human activity is also likely to have contributed to the spread of ants through the gardens. The ants were present in numbers in the composting area, and it is likely that the frequent movement of infested compost onto the garden had contributed to its spread. In several peripheral areas of the estate, ants were found on rough tracks. Investigation revealed potholes in the tracks filled with loose rubble, which had been obtained from piles of broken stone within the garden, in which ants were active.

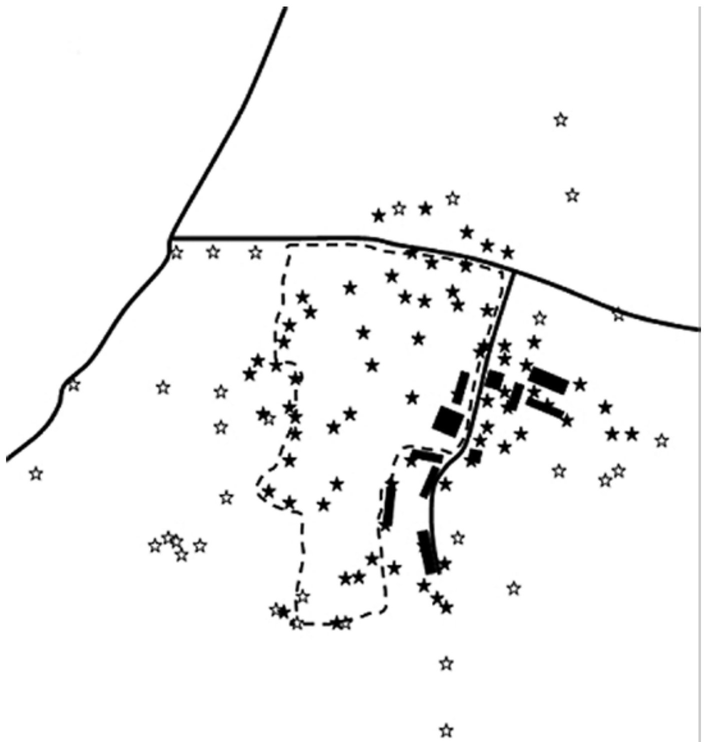

Figure 1. Diagram showing distribution of ants in and around Hidcote Manor Garden. Solid line = road or track; solid rectangle = building; dashed line = garden boundry; open star = native ant species; solid star = Lasius neglectus.

Interaction Between *L. neglectus* and Native Ant Species

At Hidcote Manor Garden, outside the main area occupied by *L. neglectus*, several native ant species were present, including *L. niger* (widespread), *L. brunneus* (mainly associated with old oak trees), *L. flavus* (in grassland), and a *Myrmica* species. No native ant species were present within the central area occupied by *L. neglectus* in the garden (Figure 1). At a nearby (1km distant) and similar garden, where *L. neglectus* has not been found, native ant species were present. Around the boundary of the area colonised by *L. neglectus*s, the distribution of invasive and native species overlapped slightly at several locations. Aggressive behaviour between *L. neglectus* and other ant species has been reported (Cremer et al., 2006).

Other *L. neglectus* Colonies in the UK

Surveys of gardens which had received plants from Hidcote Manor Garden, did not reveal any colonies of *L. neglectus*. There was one independent introduction of *L. neglectus*, in 2010. Natural tufa rock imported from Italy to Stowe Landscape Gardens in Buckinghamshire was found to harbour a small number of *L. neglectus* individuals. This colony was destroyed by fumigating the tufa with phosphine, while the surrounding area was monitored and treated with imidacloprid ant gel. No more *L. neglectus* have been seen at this location since. A few pest control organisations have made contact regarding unusual ant infestations, but none of these have been *L. neglectus*.

Preventing Spread Outside Hidcote Manor Garden

A number of activities in the garden were identified that were high risk in terms of exporting *L. neglectus* to other locations. To reduce the risk of dispersal, a number of changes in procedures were made (Table 1).

Table 1. Changes to procedures at Hidcote Manor Garden, to reduce the risk of dispersion of the Invasive Garden Ant

Risk	Mitigation measure
Sales of garden-grown plants to the public.	Cease selling garden-grown plants.
Sales of bought-in plants to the public. (These plants are cultivated elsewhere, and are brought to the garden for onward selling only.)	Control ants in plant sales and stock holding area.
Transfer of garden-grown plants to other professional gardens.	Cease transfer of plants from the garden.
Movement of plants grown in cottage gardens on the estate.	Cease removal of plants from the cottage gardens.
Disposal of soil and building waste off-site.	Retain soil and building waste on-site.
Disposal of green waste off-site.	Compost and use all green waste on-site.

Control at Hidcote Manor Garden

Examination of the bait stations showed that the imidacloprid bait was readily consumed by the ants. Monitoring data showed that at one week after the initial treatment, ant activity within the cottages was reduced by 91% (Figure 2). Large accumulations of dead ants were visible in some places. At the 19 week post-treatment assessment, ant numbers per monitor within the cottages still showed a 91% reduction compared to pre-treatment counts. Around the external footings of the buildings, although there was a substantial kill of ants in places, the overall reduction in numbers was not as great as seen indoors. This is likely to be a result of re-invasion of ants from nests outside the treated area.

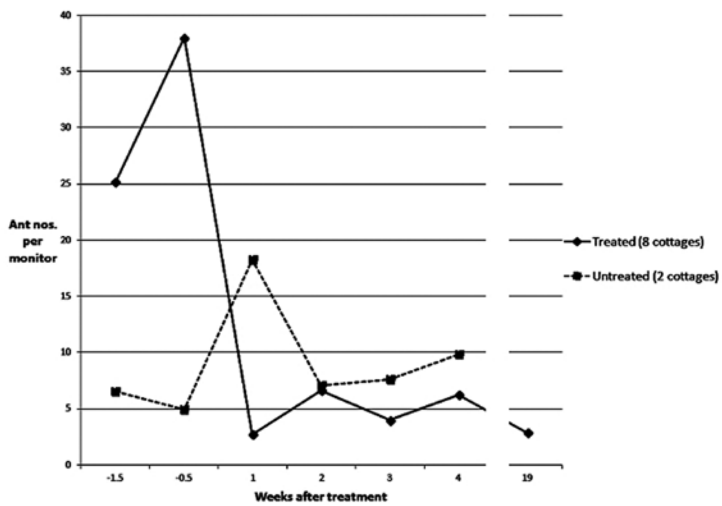

Figure 2. Effectiveness of imidicloprid bait against Lasius neglectus in cottages at Hidcote Manor Garden

Future Strategy for Ant Management at Hidcote Manor Garden

Control of *L. neglectus,* at Hidcote Manor Garden, is underway, not only to prevent damage and nuisance to the estate itself, but to reduce the risk of its spread. The overall control strategy depends on whether this is the only UK colony. The feasibility of eradication at Hidcote is being considered. Area-wide ant eradication programmes have been carried out against *Linepithema humile* in New Zealand (Harris et al., 2002), and *Wasmannia auropunctata* in the Galapagos Island (Causton et al., 2005). The treatment technique, effectiveness, and environmental impact will be important considerations for an eradication project at Hidcote Manor Garden. A pilot study in part of the infested area will be essential to enable some of these issues to be tested and understood better.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

EVALUATION OF ANT BAITS FOR CONTROL OF *PHEIDOLE MEGACEPHALA* (HYMENOPTERA: FORMICIDAE) ON LORD HOWE ISLAND, AUSTRALIA

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Abstract Lord Howe Island is a small sub-tropical island off the east coast of Australia. African bigheaded ant *Pheidole megacephala* (F.) was discovered on the island in 2003 and been subject to ongoing attempts at eradication resulting from concerns over its impact on native biota. In 2006-2007 a trial was conducted to evaluate various commercial and experimental ant baits, including Amdro™ Granular Ant bait (hydramethylnon) and Distance® Plus Ant Bait (pyriproxyfen), for the control of *P. megacephala* on Lord Howe Island. Pyriproxyfen eliminated *P. megacephala* within 33 weeks, as did a combined pyriproxyfen/hydramethylnon experimental bait, whereas formulations containing hydramethylnon alone reduced numbers to low levels but did not entirely eliminate *P. megacephala* from those blocks. Abundance of *P. megacephala* in blocks treated with Hydramethylnon-based baits returned to normal by 91 weeks after treatment whereas abundance remained low in the block treated with pyriproxyfen.
Key words Pyriproxyfen, hydramethylnon, Lord How Island, African bigheaded Ant, *Pheidole megacephala*

INTRODUCTION

Pheidole megacephala is a common tramp ant around the globe. It is present in many environments in Australia, the south Pacific and southeast Asia (Loope and Krushelnycky, 2007; Wetterer, 2007). This species is unicolonial, forms supercolonies is highly aggressive, and quickly dominates natural arthropod communities (Hoffmann, 1998; Wetterer, 2007). It was first recorded in Australia approximately 100 years ago but on Lord Howe Island in 2003. It is believed to have been introduced in soil imported before 2003 (Anonymous, 2007). While *P. megacephala* is a nuisance species in dwellings, there is concern for the ecological impact it may have on native biota (Anonymous, 2007).

There are very few control options available for *P. megacephala* in Australia. There are several hydramethylnon-based granular baits registered for use on this species and more recently a pyriproxyfen based granular bait (Distance Plus) was approved for use on *P. megacephala* in Australia. Otherwise there are only a few consumer products that are unlikely to be viable options on a larger scale. *P. megacephala* is currently the target of an eradication campaign on the Lord Howe Island (Hoffmann, pers. comm.). This study was conducted to evaluate candidate products for control of *P. megacephala*.

MATERIALS AND METHODS

Distribution of *P. megacephala* in the Study Area.

Prior to establishing treatment areas, a survey was conducted to define the extent of the infestation of *P. megacephala* in the Ned's Beach area on Lord Howe Island. *P. megacephala* was abundant in the narrow strip of coastal rainforest between the sea-cliffs and the adjoining private properties to the south (Figure 1) and extended into the adjacent private properties. The heaviest density was within the five blocks marked in Figure 1. A single transect was placed in the area adjacent to the carpark but density was relatively low and it was not suitable as an untreated control transect. Similarly, at the top end of the trail through the reserve, density was lower and in fact this proved to be the boundary of the infestation where *P. megacephala* co-existed with various other species including *Rhytidoponera victoriae* (Smith), *Paratrechina obscura* gp., *Paratrechina minutula* gp., *Tapinoma melanocephalum* (F.) and *Monomorium nigrium* gp. Three transects were placed in that area to monitor the interaction of the species present and hopefully detect any expansion of the infestation, but again they were considered unsuitable as untreated control transects.

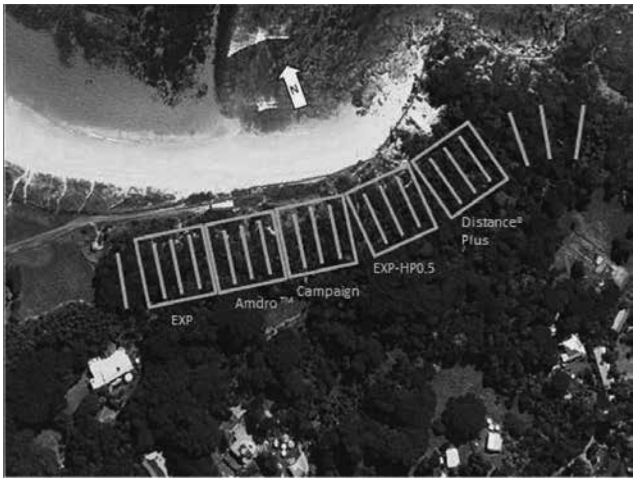

Figure 1. Location of trial site at Ned's Beach. Each block contains three roughly parallel transects. Four transects are located outside of the designated blocks and are considered pseudo-controls only.

Trial Design

Fourteen 50 m transects were marked out over the area of the apparent infestation. The walking track up the headland was the centre line through each transect. Each transect was perpendicular to the track and lures were positioned every 3 m either side of the track giving 10 lures per transect. At the end of each transect there was a ca. 10 m buffer to the edge of the apparent infested area (usually the cliff face on one side and a boundary fence on the other). After the pre-treatment assessment, five adjoining blocks of 50 x 50 m (0.25 ha) containing two transects each were marked out over the most seriously infested area, allowing for five different treatment blocks. The size of the infestation did not allow for replication because smaller treatment plots would be too small to eliminate the confounding effects of wide foraging territories which are common in species with large and co-operative colonies like *P. megacephala* (Warner et al., 2008; Vega and Rust, 2003). No control block was assigned due to low abundance or sporadic distribution of *P. megacephala* outside of the 5 blocks assigned active treatments. The transects placed either end of the five assigned blocks provided useful information on both temporal abundance of *P. megacephala* and their interaction with other species at the boundary of the infestation. These transects were not included in any statistical analysis. Later in the trial (week 9) a third transect was added to each block equidistant between the two existing transects to provide a better view of the distribution of *P. megacephala* within each block. This third transect in each block was excluded from statistical analysis to be consistent with previous assessments. However it showed that distribution of *P. megacephala* was relatively even across each of the blocks.

The results from four of the treated blocks are reported here. Block 1 (EXP) was treated with a new experimental ant bait. Block 2: Amdro™ Granular Ant Bait (7.3g/kg hydramethylnon) manufactured by BASF. Block 3: Campaign Ant Bait (7.3g/kg hydramethylnon) manufactured by Sumitomo Chemical. Block 4: EXP-HP0.5 (2.5g/kg pyriproxyfen plus 3.65g/kg hydramethylnon, Sumitomo Chemical). Block 5: Distance Plus Ant Bait (5g/kg pyriproxyfen, Sumitomo Chemical).

At the time of the trial, Amdro (along with some other equivalent hydramethylnon-based baits) was the only bait registered for use on *P. megacephala* in Australia. Distance Plus has subsequently been registered for this purpose based partly on the results of this trial. Both Campaign and EXP-HP0.5 are experimental baits and remain under development for use in Australia. They both currently have approved uses under special permit in Australia in government-sponsored eradication programs for yellow crazy ant (*Anoplolepis gracilipes*) and little fire ant (*Wasmannia auropunctata*).

Amdro and Campaign were applied at the currently approved Amdro rate of 2.5 kg/ha. Distance Plus was applied at the now approved rate of 2 kg/ha as was EXP-HP0.5, for which 2 kg/ha is the proposed use rate.

Bait was broadcast by hand evenly over the treatment area using a swath width of 5 m and travelling in two opposite directions and covering the area twice with bait. Bait was applied twice, first on 13 March 2006 and again on 5 April 2006 (3 weeks later). The decision to treat twice was based on the very high density of *P. megacephala* and the need to ensure that adequate bait reached all nests.

Ant abundance was assessed using lures comprising honey and tinned fish (ca. 1-2 g of each) placed on a small 8 x 6 cm sheet of paper at each designated point on each transect. Due to the large number of ants present at the lures, counting was not feasible. Ant abundance was assessed at 10 minutes and 1 hour after placement using the following rating system: 0 = no ants, 1 = 1-5 ants, 2 = 6-25 ants, 3= 26-50 ants, 4 = 51-100 ants and 5 = >100 ants. This rating system has been used previously in similar high density situations (Webb, 2011; Webb and Hoffmann, 2013). Recruitment to lures prior to treatment (utilizing all lures in the five blocks plus the four additional untreated transects) was rapid with almost full recruitment achieved within 10 minutes. Abundance ratings at the two assessment times were highly correlated prior to treatment (Pearsons correlation $R=0.65$, $P<0.001$) and this did not change following treatment. Hence only the 1 hr assessments are reported here.

Ant samples were identified by Dr. Ben Hoffmann of CSIRO Sustainable Ecosystems, Darwin.

Statistical Analysis

As 5 treatment regimes were superimposed over the core infestation, the four untreated transects established on the periphery of the infestation could not be considered untreated controls. Statistical analysis was limited to comparisons between treated blocks within each sample period using all 20 lures as replicates. The third transect at week 9 was not included in the analysis. Non-parametric Kruskal-Wallis one-way ANOVA (Statistix ver. 9, Analytical Software) was used to compare treatments.

RESULTS

All three baits containing hydramethylnon caused a decline in abundance of ants attending lures within 3 weeks, whereas the first evidence of decline in the Distance Plus block was at 9 weeks after first treatment (Figure 2). However, at 33 weeks after treatment, no ants were present at lures in the Distance Plus and EXP-HP0.5 blocks while ants still remained in the Campaign and Amdro™ blocks (ratings of 0.4 and 1.0 respectively). As there were no appropriate control transects available it is not possible to be certain that the declines in abundance in the treated blocks were entirely due to baiting. However, *P. megacephala* remained present in the untreated transects throughout the trial (see Figure

3) and abundance in all treated blocks, with the exception of Distance Plus, returned to close to pre-treatment abundance by week 91 (Figure 2). The decline in abundance from week 9 to week 33 in the untreated transects (Figure 3) is difficult to explain. It is possible that some unknown environmental factor caused a general decline in numbers during winter (between weeks 9 and 33) but there were no obvious perturbations in rainfall or temperature. It is also possible that baiting in the blocks adjoining the untreated transects caused a broader decline in abundance.

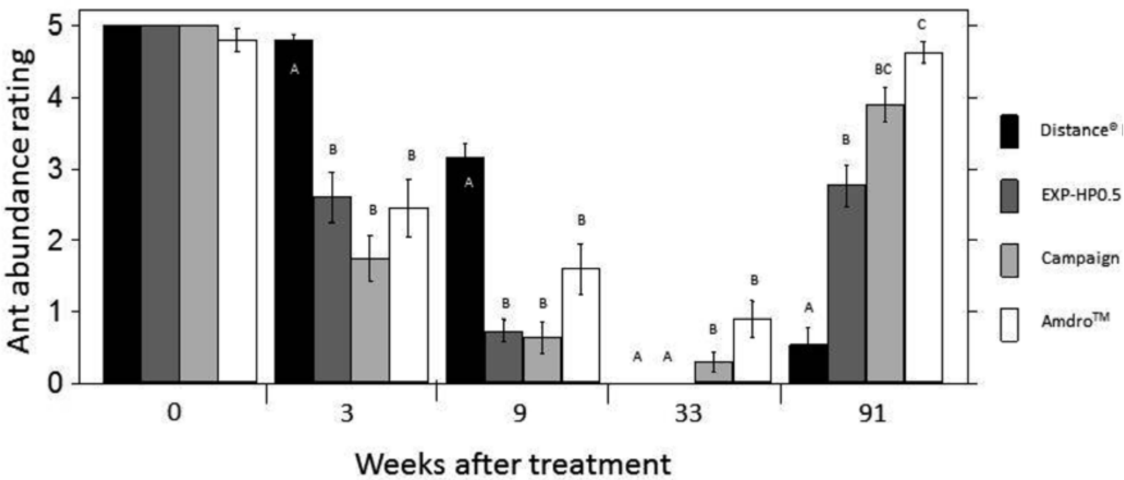

Figure 2. Changes in *Pheidole megacephala* abundance following baiting. For each time period bars with the same letter are no significantly different using Kruskal Wallis non-parametric ANOVA. Error bars are calculated using raw data.

Of the three untreated transects at the top of the headland, the most dramatic decline in *P. megacephala* occurred in the transect most adjacent to the Distance Plus block (mean rating of 4.86 at week 9 declining to 0.6 at week 33 and finally zero at week 91). All blocks adjoined heavily infested semi-rural private property and it is likely that re-establishment occurred over the period of the study. This is indicated when data for each side of the walking track are considered separately (Figure 3). In the untreated transects, abundance of *P. megacephala* was higher on the RHS of the track adjoining the private property, as it was also for the Amdro, Campaign and EXP-HP0.5 treated blocks. This appeared not to be the case for the Distance Plus block. On both sides of the track in the Distance Plus block abundance of *P. megacephala* was reduced to zero by November 2006. However, no rebound in abundance was evident on the RHS at the December 2007 assessment whereas a small rebound occurred on the LHS (Figure 3). This was the result of a small pocket of infestation on the left hand side of one transect but the other 2 transects remained clear of ants. It is not evident whether this was a focal point for recovery of an existing colony or a new introduction. Aside from one lure in the EXP-HP0.5 block where *Paratrechina* sp. occurred at week 91, the block treated with Distance Plus was also the only block where species other than *P. megacephala* occurred during later assessments, mostly *Paratrechina* spp. These were only detected in the block from week 33 onwards. The pyriproxyfen treated block adjoined the area at the top of the headland where other species co-existed with *P. megacephala*.

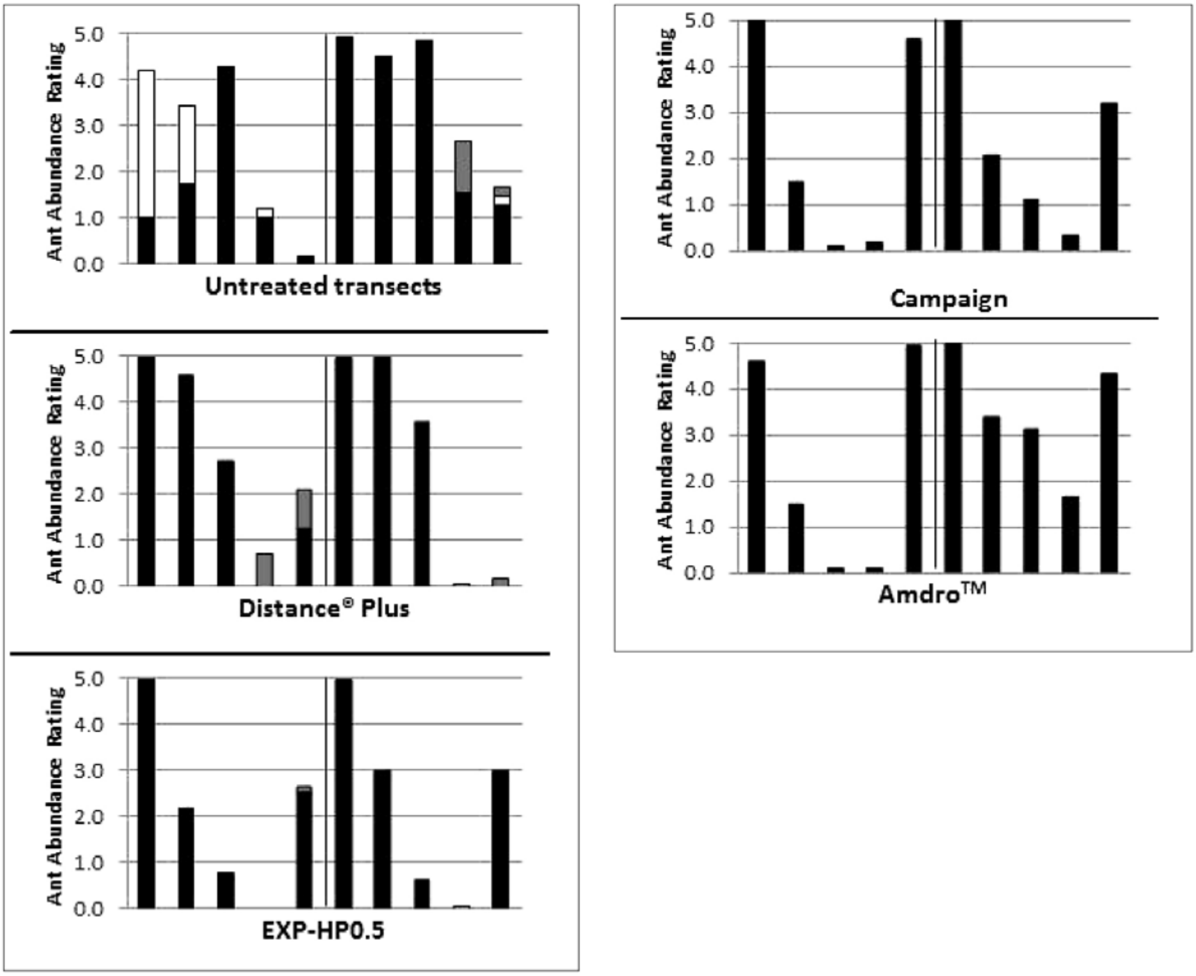

Figure 3. Abundance ratings for *Pheidole megacephala* in black, *Rhytidoponera victoriae* in white, various ant species in grey. 5 bars for left and right represent assessment: pre, 3 wks, 9 wks, 33 wks, 91 wks, for left and right-hand sides of main track.

DISCUSSION

All treatments substantially reduced abundance of foraging *P. megacephala* at lures over time. The reduction in numbers in the Amdro, Campaign and EXP-HP0.5 treated blocks was more rapid than for Distance Plus, which was to be expected given the nature of the two active ingredients. Amdro and Campaign did not achieve full elimination from lures at any point in time, and by the end of the study abundance had returned to near pre-treatment levels. While the decline in abundance in the EXP-HP0.5 block generally mirrored that of the Amdro and Campaign blocks, EXP-HP0.5 achieved zero abundance at lures by November 2006 (week 33) as did Distance Plus. At the final assessment, 12 months later, abundance in all treated blocks, with the exception of Distance Plus, had returned to close to pre-treatment levels. Abundance in the Distance Plus block remained at less than 20% of pre-treatment levels and largely this resulted from one small patch within the block and not on the boundary. The rest of the lures were either free of ants or occupied by a range of other ant species.

Amdro and other hydramethylnon-based baits have commonly been used to control or eradicate *P. megacephala* and other invasive ants (Apperson et al., 1984; Causton et al., 2005; Hoffmann, 2011; Hoffmann et al., 2011; Hoffmann and O'Connor, 2004; Klotz et al., 2000; Krushelnycky and Reimer, 1998). Amdro has been used in the eradication campaign on Lord Howe Island (Hoffmann, pers. comm.). There is a need for a wider range of options because hydramethylnon is unstable in ultraviolet (UV) light. Unless bait is harvested by ants rapidly, the active ingredient can be degraded under UV exposure (Chakraborty et al., 1993; Malipudi et al., 1986; Vandermeer et al., 1982). The speed of action may be too rapid to ensure thorough colony integration of the active ingredient, allowing colony resurgence (Hooper-Bui and Rust, 2000; Klotz et al., 1996; Knight and Rust, 1991; Kruschelnycky and Reimer, 1998; Oi et al., 1994, 2000). Ant baits utilizing slow acting insect growth regulators such as methoprene, fenoxycarb and pyriproxyfen have been successfully used in control programs against *Solenopsis invicta* Buren in the U.S., Australia, Taiwan (Hwang, 2009; Vanderwoude et al., 2003; Williams et al., 2001) and have reproductive and developmental effects on *P. megacephala* (Banks and Lofgren, 1991; Edwards, 1975; Glancey et al., 1990; Hsieh and Su, 2000; Lim and Lee, 2005; Reimer et al., 1991; Reimer and Beardsley, 1990; Vail and Williams, 1995). In recent times, pyriproxyfen granular bait has been used in the field against *P. megacephala* and *Monomorium destructor* (Jerdon) in Australia (Webb and Hoffmann, 2013) and *Wasmannia auropunctata* Roger in Hawaii (Souza et al., 2008). Pyriproxyfen has also been shown to be stable under normal environmental conditions (Sullivan and Goh, 2008; Webb et al., 2011) and a good candidate for effective residual control.

Webb and Hoffmann (2013) reported on the results of 4 field trials in northern Australia using Distance Plus against *P. megacephala*. In all cases there was a decline in abundance of worker ants at lures over a period of ca. 3 months. Although there was a large gap in assessment between 9 and 33 weeks in this trial (coincident with winter), there was a dramatic decline in abundance between these two assessments. It is possible that reduction occurred closer to the 9 week assessment than the 33 week assessment.

In all of the 3 blocks treated with hydramethylnon-based baits, abundance had returned to close to pre-treatment levels by the final assessment (ca. 18 months after initial treatment). It is not clear whether this represents resurgence of existing colonies which were not completely eliminated or the result of re-infestation from the adjoining private properties, or both. Persistently higher abundance adjacent to private property suggests that re-invasion has occurred. Abundance in the Distance Plus block remained low even after 18 months and despite adjoining infested private property. Similar effects were evident for *S. invicta* in Texas where abundance increased more dramatically in the plots treated with Amdro alone than in the Esteem Ant Bait (5g/kg pyriproxyfen) only plots (Drees et al., 2005). Collins et al. (1992) suggested that reduced resurgence in abundance of *Solenopsis invicta* with fenoxycarb-based bait relative to hydramethylnon-based bait may be the result of retention of the insect growth regulator (IGR) within the colony particularly by the longer lived major workers, which continue to transfer the active ingredient to any remaining reproductives and brood, or to newly adopted queens. This might also be exacerbated by the propensity of IGR (in this case methoprene) to induce higher production of major workers (*Pheidole bicarinata* Mayr) (Wheeler and Nijhout, 1983). An alternate theory is that the persistence of these major workers in IGR-treated areas long after reproductive capacity is lost may still prevent the establishment of new incursions through active interference (Barr, 2002; Barr et al., 2002).

A formulated blend of hydramethylnon and pyriproxyfen (EXP-HP0.5) did not appear to provide the expected blending of the biological activity of the two active ingredients.

Although zero abundance was achieved at 33 weeks, the same as pyriproxyfen treated block, by 91 weeks abundance had returned close to pre-treatment levels, although still lower than both hydramethylon-alone treatments. Similar results have been obtained for *S. invicta* in the U.S. using either formulated blends of hydramethylnon and IGRs, or blends of the two individual products (Barr and Best, 2002; Drees and Calixto, 2010; Drees et al., 2005). It is possible that the lower loadings of each active ingredient may be below the threshold for effective sustained control by one or other of the active ingredients. The balance between attractancy and repellency is delicate because it is known that formulated mixtures utilizing full dose levels of the two active ingredients (ie. 5 g/kg pyriproxyfen and 7.3 g/kg hydramethylnon) are repellent to some ant species (Webb, unpubl. data). Repellency was evident for yellow crazy ant and Argentine ant (*Linopithema humilis* (Mayr)) but not for *P. megacephala* or tropical fire ant (*Solenopsis geminata* (F.)). Similarly, for *S. invicta*, Barr et al. (2002) found no difference between full dose and half dose blends of S-methoprene and hydramethylnon.

Application of IGR-based ant baits may have an effect beyond the treatment boundary through resource sharing in some species. Oi et al. (2000) suggested that the delayed action of pyriproxyfen on pharoah ant (*Monomorium pharaonis* (L.)) relative to hydramethylnon may facilitate the wider distribution of the toxin among adjoining colonies through resource sharing and trophyllaxis. Using dye stained pyriproxyfen bait, Vail et al. (1996) showed contamination of *M. pharaonis* in untreated regions of an apartment block indicating interaction between widely dispersed colonies presumably involving resource sharing. Drees et al. (1992) found that spot treatment of *S. invicta* mounds with fenoxycarb resulted in reduction in mounds 7 m away. In this study there appeared to be reduced abundance of *P. megacephala* in an untreated area immediately adjacent to the Distance Plus block which may be evidence of this phenomenon.

Distance Plus has been shown to be effective on *P. megacephala*. There appeared to be some benefit in combining hydramethylnon and pyriproxyfen in a single bait for control of *P. megacephala*. The combined bait provided effective control mid-term, similar to pyriproxyfen alone, but did not prevent resurgence entirely, although more so than hydramethylnon-only bait formulations.

ACKNOWLEDGEMENTS

Terry Wilson and Meg Lorang (Lord Howe Island Board) provided assistance during the establishment of the trial. Ben Hoffmann (CSIRO Darwin) kindly identified the ant species recorded during the trial and provided comments on earlier drafts.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

EXPERIMENTAL DESIGN FOR EFFICACY TESTING OF BAITS AGAINST *MONOMORIUM PHARAONIS* (HYMENOPTERA: FORMICIDAE)

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Dedicated to the memory of our friend and colleague, Gabi Schrader

Abstract In the European Union, baits against pharaoh ants (*Monomorium pharaonis*) have to be authorized as biocidal products. The dossier to be submitted for authorization of the product has to include a proof of product efficacy. No consistent test standards have been established by the European Commission. A test method for efficacy testing of baits against Pharaoh ants was published by the Federal Environment Agency Germany in 1998 and is in use for efficacy tests according to the German Infectious Diseases Protection Act. The test system is a test arena of interconnected test boxes (each 25 cm x 25 cm). In our study, this test system was compared with a dish (diameter 23 cm) as test arena. Test duration was 50 days for both arena types (boxes and dish) for choice and 20 days for no-choice tests. The results for choice tests revealed that in the box system more colonies were eradicated (boxes: 75%, dishes: 0%) within 50 d, and more queens died (average number of surviving queens was 0.5 in boxes and 29.7 in dishes).
Key words Pest control, insecticides, Pharaoh ant, proof of efficacy

INTRODUCTION

Monomorium pharaonis is an ant species of major importance as a health pest organism, since it is a mechanical vector for human pathogens such as salmonella (*Salmonella* sp.), *Pseudomonas* sp., *Klebsiella pneumoniae*, *Streptococcus* sp., *Proteus mirabilis*, *Enterobacter* sp. (Beatson, 1972). Moreover, pharaoh ants can also cause respiratory allergies (Kim et al., 2007). Due to global transport, pharaoh ants are distributed worldwide and can be found on all continents except for Antarctica. The ability to distribute is facilitated by their small size, as they can nest in numerous small transportable objects. Pharaoh ants are polygynous with many queens and hence, new colonies containing only a few workers with queens and brood (Peacock et al., 1955; Petersen and Buschinger, 1971) can be founded easily through fission or budding (Vail and Williams, 1994). Pharaoh ants prefer humid and warm conditions and occur mainly indoors. They infest apartment and public buildings, zoos, restaurants and hospitals.

For control of *Monomorium* the best method is the use of oral baits. Not all of the ants in a colony forage outside of the nest, and thus insecticides sprays or dust hardly achieve eradication. Oral baits are distributed to queens, workers and brood in the nest through trophallaxis (Edwards and Abraham, 1990).

In the European Union, biocidal products and active substances are regulated under the EU Biocides Regulation (EU BPR) 528/2012. Biocide products have to be authorized before being used or sold on the EU market. Since the pharaoh ant is a health pest, insecticidal baits for their control are categorized as biocidal products. For authorization of bait products, the applicants have to submit a dossier which has to include a proof of efficacy. The Technical Notes for Guidance (PT 18) give only general information about testing conditions for baits controlling pharaoh ants. Up to now, valid international standards are lacking, and test duration, size of test arena or size of colony used for testing are not standardized.

A German test guideline (Iglisch, 1998) for efficacy tests has been published by the Federal Environment Agency Germany for efficacy tests according to the Infectious Diseases Protection Act (Infektionsschutzgesetz § 18). The test system prescribed by the guideline is a multi-compartment system which tries to simulate the situation of infestation and control of pharaoh ants. In case of an infestation the bait would be placed on an ant trail somewhere between the nest and a food source. Thus, the test system consists of three compartments, a nest compartment connected to a bait compartment which again is connected to a compartment containing the food source, and the ants have to pass the bait to reach the food source. The assumption of this test system is that if the bait is sufficiently attractive, ant foragers prefer it over the non-toxic food source since it can be acquired with lower foraging costs.

Although this test system simulates the practical situation in which a bait product is used, it is expensive to build and consumes larger areas of laboratory space, thus limiting the number of replicates in an experiment. Small ready-made test arenas made from laboratory material already available for other purposes may allow a larger number of replicates with lower costs. Without valid standards for test designs it is challenging to evaluate efficacy tests for authorities. There is little information about the influence of test design (e.g. position of the bait in relation to the food source, distance between nest and bait) on test outcome, which makes an objective assessment of test data difficult.

In this study the test system used for testing the efficacy of baits against pharaoh ants by the German Infectious Diseases Protection Act was compared with a single-compartment system to get information about the of the test design´s impact on the test outcome.

MATERIALS AND METHODS

Study Animals. Pharaoh ants were taken from the laboratory colony in the Federal Environment Agency. Ants were kept in cylindrical glasses (300 mm high) coated with polytetrafluoroethylene (PTFE) at the sidewalls to prevent escape. Small plastic containers with cellulose tissues served as nest boxes. Ants were fed dead *Periplaneta americana* and a drinking trough consisting of a small petri dish with cotton ball soaked with water (66%), honey (30%) and multivitamin syrup (Sanostol®) (4%). Climate conditions were 25 ± 1.5°C and 60 ± 10 % humidity.

Transfer of Ants and Nesting Boxes. Small glass tubes were placed in an empty cylindrical rearing glass and nest boxes containing queens, workers and brood from the laboratory colony were poured out into the glass. The ants used the tubes as nest shelters and moved into the tubes with brood and queens. The tubes were used as a means of assembling and transferring the ants into the test arenas. Before placing the tubes in the test systems the number of queens was visually estimated and about 30 queens were introduced in each arena. In the arena wood cubes (30 mm x 30 mm x 13 mm) with holes (diameter 25 mm, height 6 mm) served as nesting boxes. At each of the four sides a 1 mm diameter hole was drilled as a nest entrance. Nest boxes were covered with glass slides and the slides were covered with grey plastic to keep the interior dark. In every test arena two nesting boxes were placed.

Bait. The insecticide agent of the bait used in the choice and no-choice tests was borax. Borax and boric acid baits are used for the control of pest ant species (Vobrázková et al. 1976, Klotz and Moss 1996; Klotz et al. 2000, Rust et al. 2004, Sola et al. 2013). Borax has not been notified in the EU as a biocidal insecticide (PT 18). We used this active ingredient to avoid possible conflicts with biocide authorisation, as the aim of our study was the evaluation of the test system rather than the insecticide.

We used a bait composition similar to that described by Klunker et al. (1990): whole egg powder (63 %), honey (23 %) and borax (14 %). Bait was offered in small petri dishes (diameter 3 cm).

Test Systems. All of the test arenas were placed on a plate surrounded by double walls filled with paraffin oil between the two walls in order to prevent ant escapes. Two types of test arenas were investigated: two or three boxes connected with tubes (multi-compartment system) and crystallizing dishes (single-compartment system) (Figure 1).

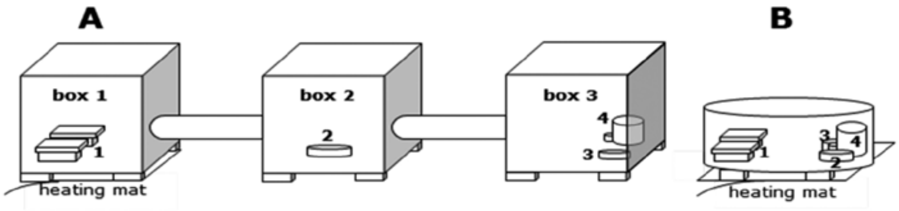

Figure 1. Test design of the multi-compartment system (A) and single-compartment system (B). 1 = nest boxes, 2 = bait, 3 = non-tox food, 4 = trough

Multi-compartment system. The system consisted of three connected test boxes for choice trials and two connected test boxes for controls and no-choice tests, respectively. The test boxes were connected with tubes. Boxes as well as tubes were made of acrylic glass. Each box had a size of 25 cm x 25 cm x 20 cm, the tubes were 25 cm long, and had an inner diameter of 4 cm. To avoid ants from escaping, the upper part of the walls was covered with insect glue. One test box/compartment was used as nesting compartment in which the nesting boxes were placed. The bottom of this box was heated to 30 °C with a heating mat. The nesting compartment was directly connected to a box which either contained bait and drinking trough in no-choice tests or food and a drinking trough for the control group. For choice tests, the box next to the nest box contained bait and was connected to a third box which contained non-tox food (dead oriental cockroaches, *Blatta orientalis*) and a drinking trough. The ants started foraging in box 1 (nest compartment), had to pass the ant bait in box 2 (bait compartment) before they reached non-poisoned food and the drinking trough in box 3 (food compartment).

Single-compartment system. The single-compartment system consisted of a crystallizing dish as a test arena. The dishes were made of glass and had a diameter of 23 cm and a height of 15 cm. The walls were coated with polytetrafluoroethylene (PTFE) to prevent ants from escaping. In choice tests the nesting boxes were placed on one side of the crystallizing dish, and the drinking trough and the bait were placed side by side on the opposite side of the dish. In no-choice tests only the bait and water and in the control only nontoxic food and water were offered. Heating mats were placed under the crystallizing dishes to achieve a temperature of 30°C like in the nesting compartment of the multi-box system. In multi-compartment systems the distance between nest and food source was about 120 cm, and from nest to bait about 50 cm. In single-compartment systems the distance to both food and bait was about 15 cm.

Test Protocol. The ant colonies were acclimatized in the respective test arena for one week prior to the introduction of poison baits. During this period, they were fed with dead oriental cockroaches and a mix of water and honey (for exact composition, see above).

In the multi-compartment system, at the beginning of the experiments the bait was placed in the bait compartment (box 2) in choice tests. In no-choice tests the non-toxic food (cockroaches) was replaced by the bait. The mix of water with honey was replaced with water. Water and non-toxic food replenished when needed. The baiting period was scheduled after Iglisch (1998) with 50 days in choice tests and 20 days in no-choice tests in experiments with both types of test arenas (multi-compartment as well as single-compartment). A trial ended either at the end of the baiting period or when a colony in an arena was eradicated. Once a week a photograph was made of every test arena and ants were counted. In the nests the number of queens was estimated by visual inspection and the test arenas were inspected for dead queens. At the end of all trials, test arenas with surviving ants were frozen and queens and workers were counted.

RESULTS AND DISCUSSION

To evaluate the two test designs the parameters for comparison were the number of workers counted on the photographs and the number of dead queens collected during the experiment, the number of the survived workers and queens after the trial period and if an eradication (100% mortality) was achieved. In none of the tests systems was eradication of all colonies achieved (Table 1). This was due to the limited duration of the tests (50 and 20 days). Results indicate that longer test durations are more realistic for bait efficacy tests. Eradication was achieved in 75 % of the colonies after 50 days of bait exposure in the multi-compartment system in choice tests. 50 % of the colonies were eradicated after 20 days in no-choice tests. In the single-compartment system no eradication was achieved in choice tests and 25 % of the colonies were eradicated in no-choice tests (Table 1).

The number of surviving queens during the experiments differed markedly between the arena designs. In multi-compartment systems, nearly no queens survived (0.5, SD 1) in choice as well as no choice tests. In the single-compartment system queen survival was highest in choice tests (29.7, SD 9). 20 days after the beginning of the experiment the number of queens was approximately half in no-choice tests in the single compartment system (Table 2). In multi-compartment systems also almost no surviving workers were found in contrast to the single-compartment system (Table 1, Figure 2).

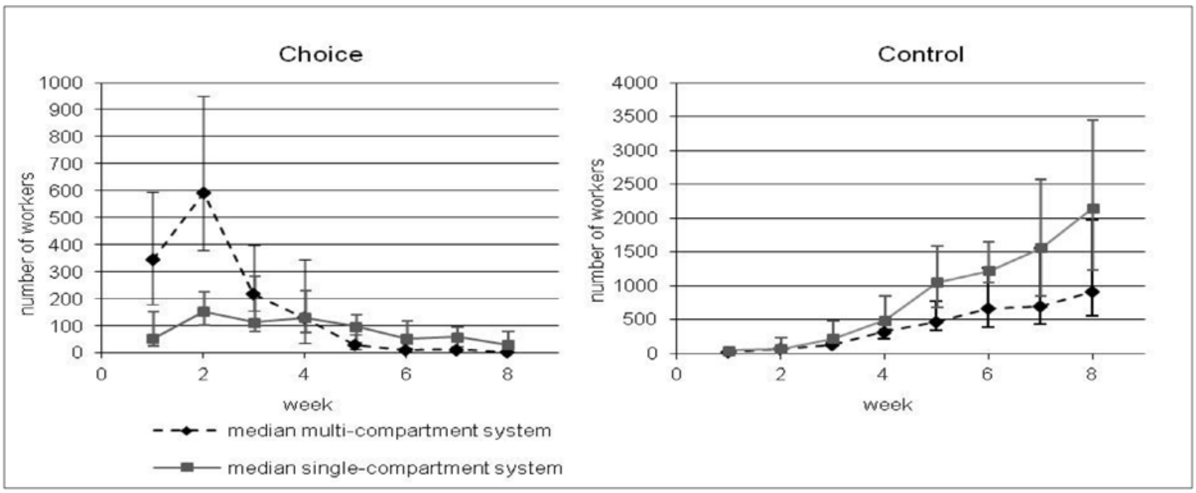

Figure 2. Number of workers in multi-compartment systems (n = 4) and single-compartment systems (n = 12) counted weekly. Squares denote the median of number of workers, bars denote the 1. and 3. quartile.

More workers foraged in the multi-compartment system than in the single-compartment system at the beginning, but this changed in week 4 (Figure 2). An explanation for this would be that the longer distance between nest and food sources in the multi-compartment system increased time and energy expenditure for foraging. Hence, in the multi-compartment system, ant workers had to be more active to meet the food and energy demands of the colonies, which were of equal size in both test systems. The difference in colony development between the multi- and single-compartment system could also be observed in the development of queen numbers in the controls: although the number of queens was approximately identical in both test systems at the beginning of the experiment, it increased about 3.5 fold in the multi-compartment system and about 7.5 fold in the single compartment system (Table 2).

We hypothesize that the loss of workers due to the action of the insecticide had a more profound effect on colonies with a higher energy demand due to greater foraging distances. After approximately 4 weeks we observed that workers in multi-compartment systems stayed in the food and bait boxes and did not return to the nest to feed the queens and brood. This could be another reason for the differences in the number of surviving queens between both test systems, as the queens in the multi-compartment system probably died due to starvation. In single-compartment systems the workers took care of the queens until the end of the test period.

A second factor which could explain the differences in mortality and queen survival in choice tests is the arrangement of food and bait. In the single-compartment system, food and bait were placed side by side. In the multi-compartment system the bait was placed at half the way to food and trough. The decision to take food or bait can be influenced by their distance to the nest or their arrangement. Fewell (1988) could show that in the harvester ant *Pogonomyrmex occidentalis,* saving time is more important than direct energy costs in the choice of the food source. Hence, in the multi-compartment systems the shorter time needed for foraging of the bait compared to the non-toxic food biased the ant decisions towards the bait. This decision had not to be made in the single-compartment system, since food and bait were placed side by side at an equal distance to the nest. Energy or time costs were not crucial factors for foraging decisions. The bait has to be much more attractive than the non-toxic food. In our experiments cockroaches were the non-toxic food. Cockroaches are attractive to pharaoh ants and are preferred over otherwise very attractive and effective baits (Sy, 1987; Adams et al., 1999). Although different bait choice behavior related to bait position may has an influence on test outcome, this holds only good for choice tests. The differences in queen survival and time for colony eradication were observed in no-choice tests in the absence of a non-toxic food source, when ant foragers obviously could not make a decision which bait to prefer.

Table 1. Control and colony survival in efficacy tests of an insecticide in two different types of test arenas.

Type of test	n	Bait exposure time (d)	Minimum time for eradication (d)	Eradicated colonies (%)	Number of surviving workers		
					Median	1. quartile	2. quartile
Choice							
Multi-compartment	4	50	28	75	0	0	1
Single-compartment	12	50	-	0	25.5	14.3	55.3
No-choice							
Multi-compartment	4	20	16	50	1	0	8.3
Single-compartment	4	20	19	25	10	3.8	16

Table 2. Queen survival in efficacy tests of an insecticide in two different types of test arenas.

Type of test	**Test design**	**n**	**Queens inserted (estimated)** Mean (Standard deviation)	**Dead queens (counted)** Mean (Standard deviation)	**Surviving queens (counted)** Mean (Standard deviation)
Choice	Multi-compartment	4	28.3 (3.3)	33.8 (2.8)	0.5 (1)
	Single-compartment	12	25.6 (5.1)	2.5 (4.1)	29.7 (9)
No-choice	Multi-compartment	4	27.8 (7.2)	31.5 (11.4)	0.5 (1)
	Single-compartment	4	25 (5.4)	1 (17.9)	13.3 (16.3)
Control	Multi-compartment	4	23.3 (4.8)	1 (1.2)	82.5 78.4
	Single-compartment	4	20 (4.1)	0.25 (0.5)	151.3 163.5

In conclusion, the absolute distance between bait and nest had an influence on test outcome in all tests, and the relative distance of bait and non-toxic food may have had an influence on the test outcome in choice tests. Our study shows that the test design, size of test arenas, and test duration have to be taken into account for evaluation of efficacy studies. In contrast to our initial expectation, it may be more challenging to achieve an eradication of an ant colony in small test arenas than in large test systems. Further studies are needed to identify the impact of parameters like bait position and distance to the nest.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

MANDIBULAR WEAR IN *CAMPONOTUS CHROMAOIDES* WORKERS (HYMENOPTERA: FORMICIDAE)

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Abstract *Camponotus* colonies have a worker subcaste, consisting of major and minor workers. This designation is based on the width and height of the head (cephalic index, CI). Performance of tasks by workers results in wear of the mandibular teeth. A sample of workers was taken from a laboratory colony of *Camponotus chromaoides*. The CI was calculated to determine the major (CI 106.9) and minor (CI 83.6) workers. The objective of the study was to document the characteristics and extent of mandibular wear, and to divide the wear into distinct categories. Grade 1: little or no wear, Grades 3 and 5: moderate wear of the teeth, and Grade 7: absence of mandibular teeth. The absence of incisor and molar teeth may benefit rather than diminish foraging tasks of workers.
Key words Mandibular teeth, cephalic index, worker subcaste, foraging, carpenter ants.

INTRODUCTION

The mandibular teeth of insects wear during normal feeding as an immature and adult (Chapman and Boer 1995). Wear can be extensive and the original structure of the mandible may be worn away. This can result in a change in foraging behavior and other tasks. Excessive mandibular wear has been reported in grasshoppers (Chapman, 1964), leaf feeding and predaceous beetles (Raupp, 1985; Wallin, 1988), plant-piercing bugs (Roitberg et al., 2005; DePieri, 2010), leaf-cutter ants (Schofield et al., 2011), and aquatic insect larvae (Arens, 1990). Studies of mandibular wear in wood-infesting insects include investigations of the concentration of metals (Zn, Mn, Ca) in the mouthparts of termites (Stewart et al., 2011). There is little or no information on the mandibular wear sustained by carpenter ants (*Camponotus* spp.).

Camponotus is the largest ant genus with about 1,000 species worldwide; they are an economically important pest in tropical and temperate regions (Dukes and Robinson, 1982; Robinson, 2005). These large ants establish nests in wood or soil, and colonies can have several thousands of individuals (Pricer, 1908). Nest cavities and galleries in wood are excavated by the workers using their large mandibles. Fibrous shavings are removed and pushed out of galleries through openings, or packed into an unused cavity. Galleries are generally excavated in the direction of the wood grain, and in the sapwood portion of the wood. Walls of *Camponotus* galleries are smooth, which is the origin of the common name.

The objective of the research presented here was to document the characteristics and extent of mandibular wear in worker ants of *C. chromaoides*, and to divide the wear into distinct categories or grades based on the absence of mandibular teeth. The potential link between extensive mandibular and changes in foraging tasks is considered.

MATERIALS AND METHODS

Major and minor worker ants were randomly selected from a laboratory colony of *C. chromaoides*; 40 individuals from each of these castes were selected for a total of 80 workers.

Cephalic Index. The head of each worker was removed and the width across the occiput and the height (excluding the mandibles) were measured with an ocular micrometer. These measurements were used to calculate the Cephalic Index (CI) according to the formula: CI = Head width x 100 / Head height. The index presented here is the mean.

Mandibular Wear. The mandibles of each worker were removed and the amount of wear was assessed and graded on a scale of 1, 3, 5, or 7. Grade 1 has no noticeable wear and all the teeth are intact, and grade 7 has extensive wear with the absence of all the teeth on the mandible.

RESULTS AND DISCUSSION

Mandible Wear and Cephalic Index

An analysis of variance of the amount of wear indicated that major workers (CI 106.9; range 97.0 to 126.4) had significant more than minor workers (CI 83.6; range 77.0 to 90.7). The right and left mandibles of major workers had significant ($P<0.05$, $P<0.001$, respectively) wear to their distal margin.

Mandibular wear has been documented for a variety of plant feeding, scavenging, and predaceous insects. Adult *Camponotus* species feed primarily on liquids. They use their large mandibles to crush solid food to extract liquids, and the mandibles are also used to excavate a nest cavity in soil and/or wood. While performing these tasks the major and minor workers in the colony wear the teeth on their mandibles. The extent of the wear can be graded on a scale based on the loss of mandibular teeth.

Mandible Structure

The mandibles of *C. chromaoides* workers are elongate and uniformly pigmented (Figure 1, A). The length of the mandible from the dorsal condyle to the tip of the incisor tooth (Fig. 1, It) is about twice the width. There are a series of molar teeth (Figure 1, Mt) along the distal margin. The two incisor teeth (Figure 1, It) are prominent and slightly longer than the three molar teeth below. The angle between the incisors and between them and the first molar tooth is about 30°. Right and left mandibles are nearly symmetrical. Because they are not used for grinding food the two sides do not fit into each other and are usually held apart at rest.

The sequence of mandible wear usually begins with changes in the molar teeth; the upper incisor is often the last to show significant wear. This tooth may have a higher level of hardness than the other teeth. Hillerton et al. (1982) reported the incisor tips can have nearly twice the hardness as other parts of the mandible, and Hillerton and Vincent (1982) reported the presence of zinc (Zn), a strengthening metal, in the mandibles of several ant species. Schofield et al. (2002) reported Zn in the mandibles of leaf-cutter ant (*Atta* sp.) workers, and in the mandibles of the odorous house ant, *Tapinoma sessile* (Schofield et al., 2003). The stiffness and thickness at the top edge of *C. chromaoides* mandibles may provide strength and resistance to wear or breaking of the upper incisor.

Mandibular Wear Scale

The wear of the mandibles of the major and minor workers was divided into four categories based on loss of the incisor and molar teeth. The amount of wear was graded on a scale: 1, 3, 5, or 7.

Grade 1 (Figure 1, A). These mandibles show little or no wear of the incisor or molar teeth. There is a 30° groove between the second incisor and the first molar tooth. Individuals with this amount of wear may be recently emerged workers that have not excavated galleries in wood or soil.

Grade 3 (Figure 1, B. These mandibles show wear on all the mandibular teeth. Most or all teeth have rounded tips, some have broken tips. Angle at the base of the incisor teeth is wider than 30°.

Grade 5 (Figure 1, C). Mandibles of these workers have sections without distinct teeth; there are gaps in the row of molar teeth and one or both of the incisor teeth is worn or broken off.

Grade 7 (Figure 1, D). There is little or no evidence of teeth on either mandible. When the incisor tooth is present it is rounded and without a cutting angle. The distal face of these mandibles is straight or a curved ridge, which seems more suited for cutting than for gauging substrates.

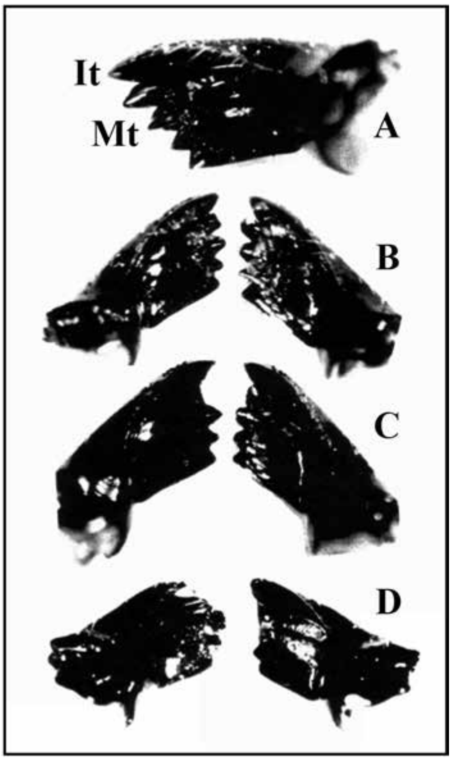

Figure 1. Mandibles of major and minor workers of Camponotus chromaoides. It – Incisor teeth; Mt – Molar teeth. Mandible wear scale: A, Grade 1; B, Grade 3; C, Grade 5; D, Grade 7 (major worker).

Mandible Function

The structure of the mandible is generally linked to the type of food the insect eats (Chapman and de Boer, 1995). Predatory species have long mandibles with pointed incisors, and a molar region with teeth for tearing apart prey. Phytophagous species have short mandibles and reduced molar and incisor teeth, which are suited for gouging leaf surfaces and chewing plant fibers (Chapman and de Boer, 1995). The mandibles of some saturniid caterpillars have a combination of teeth and ridges that tear away small fragments of leaf (Bernays and Jansen, 1988). The mandibles of *C. chromaoides*, and probably most *Camponotus* species, are between the predaceous and plant feeding forms. This intermediate form has short incisor and molar teeth that provide the capacity to gouge the surface of wood to create the smooth galleries and the fragments of wood (frass) characteristic of carpenter ant nests (Robinson, 2011).

The extreme mandible wear, Grade 7 (Figure 1, D) in workers in this study, may provide an advantage in dismembering insects and returning parts back to the primary nest site. Grade 7 mandible wear often results in a smooth edge along the molar margin after the teeth have eroded or broken off. This type of continuous ridge enables *Heterocampa obliqua* caterpillars to cut segments out of leaves, and enables the carabid, *Elephrus cupreus* to cut and remove fragments of prey.

Tasks and Tools

Pricer (1908) reported that the initial tasks of the major workers of *C. pennsylvanicus* and *C. chromaoides* (as *C. ferrugineus*) were primarily inside the nest and involved caring for brood and perhaps gallery excavation. However, Fowler and Roberts (1980) observed major and minor workers foraging away from a *C. pennsylvanicus* nest. They reported that minor workers primarily tended aphids for honeydew, while major workers foraged on the ground and in trees for insects. This change in task from housekeeping to foraging may reflect a change in the tools (mandibles) the workers have. Mandibles may not be effective in maintaining and extending galleries in wood when they are worn and lack sharp teeth. Schofield et al. (2011) reported that leaf-cutter ant (*Atta cephalotes*) workers with 10% mandibular wear switched their foraging task from leaf cutter to leaf carrier.

Instead of wear resulting in reduced utility of major and minor workers, it may increase foraging efficacy when they transition from being inside the nest to outside. When wear reaches Grade 5 and 7 (Figure 1, C, D) mandibles may be better suited for slicing pieces of insect prey than gouging wood in galleries. While forage on the ground or in trees they may encounter disabled insects and can carve out pieces of flesh to be returned to the nest.

Foraging worker from a nest of *C. pennsylvanicus* carried parts of cicadas, plant bugs, beetles, and wasps (Fowler and Roberts, 1980). Cutting the cuticle and flesh of these insects may be more effective using mandibles with an edge than mandibles with teeth.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

DIVERSITY OF PONERINAE ANTS (HYMENOPTERA: FORMICIEDAE) USING URBAN AREAS FOR REPRODUCTION: THEIR POTENTIAL THREAT TO HUMAN HEALTH

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Abstract Workers and mainly winged females of Ponerinae ants have been causing painful stings on people in the city of São Paulo, Brazil. The species live in small colonies, comprised from a few dozen to a few hundred individuals with monomorphic workers. Ponerinae ants are predators, although some species feed on plant nectaries, fruits and honeydew. In order to register which are the species that reproduce in the city, a survey was conducted for 13 months with light and Malaise traps that were placed in two different areas with the aim at collecting alate ants. The following Ponerinae ants were registered: *Odontomachus haematodus, O. bauri, Pachycondyla laevigata, P. striata, P. unidentata,* 10 morphospecies of Hypoponera and one species of Anochetus. Alate specimens were captured throughout the year. Peaks of alate capture are distinct among the species, from 1 to 655 specimens. With the exception of *P. striata* that reproduces in the dryer seasons, most Ponerinae species show reproductive flights in the warmer and rainy months.
Key Words Nuptial flights, alate, sting

INTRODUCTION

Most ant species reproduce and disperse by nuptial flights, and reproductive period varies according to the species and place they occur (Holldobler and Wilson, 1990). Some ant species have their nuptial flights synchronized (Pfeiffer and Linsenamair, 1997). The importance of synchronization for reproduction is essential in nearby colonies of the same species, once both sexes suffer high losses due to predation (Wilson, 1975), in most species of ants the males live only a few days, due to low investment in single individuals during development, and to achieve exogamous pairing, male and female reproductives of different colonies have to be synchronously active to meet at the mating places.

Ants show at least two mating strategies: (i) male aggregation, males gather in large numbers in a certain place that are often reused annually, marking these places with pheromone; females join these flights aggregation and aggregation of males tend to be highly synchronized (Wilson, 1957), and (ii) female calling syndrome, females attract males through pheromones. In contrast with male aggregation, the female calling syndrome seems to be not well synchronized and do not take place in a fixed site. Some authors evaluate such strategy for those species that show nuptial flights for several months (Kaspari, 2001; Dunn, 2007).

Besides biological behaviors, abiotic factors may influence the reproductive time. In the neotropical region the daily photoperiodic alteration within the year is limited, thus giving hardly any guide to the season, which could serve as a proximate releaser for physiological activity. Since most other environmental clues, like temperature or rainfall, are either constant or unpredictable, exact *zeitgebers* seem to be lacking near the equator. However, the more aseasonal the environmental conditions become, the lower is the need for close synchronization with them. (Pfeiffer and Linsenamair, 1997).

In urban areas ant nuptial flights are rarely observed by people, once most species are small and alate specimens do not sting. Some ant species even do not produce alate reproductives and mating occurs inside the colony (Passera, 1994). But many Poneromorph ants (Bolton, 2003) may cause serious nuisance due to the alate females´ size and nuptial flights are promptly noticed by citizens, especially those who have already had the experience of being stung by them.

Accidents from alate ant stings are reported from the São Paulo citizens to Instituto Biológico, and most species belong to the Ponerinae subfamily. This is a primitive group of ants which is primarily tropical in distribution and shows a high diversity in the Atlantic rain forest (Feitosa and Ribeiro, 2005). It is unknown which are the Ponerinae species that reproduce in the city of São Paulo and their nuptial flight periods. This work surveys for reproductive Ponerinae forms in the city of São Paulo, to understand their diversity and flight periods.

MATERIALS AND METHODS

A survey was conducted in the city of São Paulo. The city has a humid subtropical climate (Cfa/Cwa), according to the Köppen classification, characterized by a dry winter and a rainy summer. Light and Malaise traps were placed in two different areas to collect nocturnal and diurnal alate ants, respectively. Site 1 was located in a 3 ha park in the Southeast region of the city, surrounded by a residential urban area, 16 Km from an Atlantic Forest reservoir, and the Site 2 away 7 Km from it, in the Northern region of the city. Site 2 is surrounded by residential and commercial areas lacking green areas around it.

The Atlantic Forest reservoir, called Serra da Cantareira (Cantareira Hills), is comprised of 64,800 hectares, and is considered one of the biggest urban forests in the world. It is located in the Northern region of the city. Light and Malaise traps were left on each site for 13 months (October 2012 to October 2013). Light traps were attached to a photoelectric cell in order to turn them on at dusk and off at dawn. Specimens were weekly collected and identified to species, whenever possible. As most identification keys focus on the workers not all species could be identified to species. For analysis purposes, the occurrence of each captured species was registered in the month, independent if it was collected in only one week in a given month. Data from the two collecting sites were grouped and the number of captured alate specimens was registered.

Temperature and rain fall data were obtained at the National Institute of Meteorology located in the Northern region of the city of São Paulo.

RESULTS AND DISCUSSION

Only light traps captured reproductive Ponerinae ants. It was registered sixteen species belonging to four genera, *Anochetus* (one species), *Hypoponera* (ten species) *Odontomachus* (two species), and *Pachycondyla* (three species) (Table 1). Feitosa and Ribeiro (2005) collected 14 Ponerinae species (workers) in Serra da Cantareira, in leaf litter samples. These authors collected the same genera that were collected in this work, except for *Odontomachus*.

The number of captured alate species is higher in site 1, in an urban park (Table 1). Urban green areas are known for the maintenance of biodiversity (Sandströma et al., 2006). Few studies have demonstrated in Brazil the biodiversity of ants in urban green areas (Pacheco and Vasconcelos, 2007; Munhae et al., 2009; Ribeiro et al., 2012) and no work surveyed for reproductive ants, only workers. The data show how rich is the Ponerinae biodiversity that uses urban areas for reproduction.

Alate specimens were captured throughout the year. Peaks of alate capture are distinct among the species (Table 2), from 1 to 655 specimens. *Anochetus* sp. and *Hypoponera* sp. 10 were captured in only one month, and only 1 and 2 specimens were recorded for each species, respectively, in contrast with *Pachycondyla striata* Fr. Smith that a huge number of alate ants were captured, in winter (August and September 2013). All other captured specimens concentrate their nuptial flights in spring, summer and autumn (Table 2), in the higher temperatures (Figure 1). This is a very large period, agreeing with Pfeiffer and Linsenamair (1997) statements that predicted that gain independence from seasons may develop shorter reproductive cycles, and thus benefit in the reproductive competition with their inter-specific seasonal rivals.

Table 1. Alate Ponerinae in light traps, in São Paulo, Brazil: October 2012 to October 2013.

Species	**Site 1**	**Site 2**
Anochetus sp.	X	
Hypoponera sp. 1	X	X
Hypoponera sp. 2	X	
Hypoponera sp. 3	X	
Hypoponera sp. 4	X	X
Hypoponera sp. 5	X	X
Hypoponera sp. 6	X	X
Hypoponera sp. 7	X	
Hypoponera sp. 8	X	X
Hypoponera sp. 9	X	
Hypoponera sp. 10	X	
Odontomachus bauri	X	X
Odontomachus haematodus	X	
Pachycondyla laevigata		X
Pachycondyla striata		X
Pachycondyla unidentata		X

From all captured Ponerinae species, some are known for promoting anaphylaxis in humans, especially those from the genus *Odontomachus* and *Pachycondyla*. Some ant species were captured along the months, even with few alate specimens. *Odontomachus bauri* Emery, for example was captured along 10 months, in all seasons, with peaks of reproductive specimens in spring and summer (Table 2).

Odontomachus species are often common and conspicuous insects. They are about 8 mm, with elongate mandibles Deyrup and Cover, 2004). In spite of these formidable jaws, they have a sting strong enough to elicit a definitive reaction in humans. Urticaria was reported in Venezuela produced by a flying *O. bauri* (Rodriguez-Acosta and Reyez-Lugo, 2002).

Alate specimens of *Pachycondyla* were not collected in site 1, but three *Pachycondyla* species were captured in site 2 (Table 1). The three species were collected in several months, but *P. striata* showed a peak of alate production in winter. *Pachycondyla laevigata* and *Pachycondyla unidentata* make nuptial flight in all seasons but alate specimens were collected throughout the year (Table 2). The light traps captured a high number of *P. striata* reproductive males and females (655 in August 2013 – Table 2). Silva-Melo and Gianotti (2010) excavated *P. striata* nests in Rio Claro, SP, 180 Km from the

Table 2. Number of alate specimens of Ponerinae ants collected with light traps, in two regions in São Paulo, Brazil, from October 2012 to October 2013.

Species	2012			2013									
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct
Anochetus sp.													
Hypoponera sp. 1													
Hypoponera sp. 2													
Hypoponera sp. 3													
Hypoponera sp. 4													
Hypoponera sp. 5													
Hypoponera sp. 6													
Hypoponera sp. 7													
Hypoponera sp. 8													
Hypoponera sp. 9													
Hypoponera sp. 10													
Odontomachus bauri													
Odontomachus haematodus													
Pachycondyla laevigata													
Pachycondyla striata						2		2		22	655	467	
Pachycondyla unidentata													
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct
	Spring			Summer			Autumn			Winter			Spring

city of São Paulo, and collected 80 males in one nest in April 2006. The high number of alate *P. striata* captured in the light traps indicate a synchronization of nuptial flights of several nests in São Paulo in the dry season (Figure 1). *P. striata* is one of the largest *Pachycondyla* species (Head Width > 2.8 mm), and known for their pugnacity with which they defend their nests, and their painful stings (Wild, 2002). The genus *Pachycondyla* is responsible for most cases of anaphylaxis (Klotz et al., 2005).

The species of *Hypoponera* varied their nuptial flights periods, from a few number of specimens captured for several months (*Hypoponera* sp. 1 and sp. 2), concentrating their flight activities in winter and summer (*Hypoponera* sp. 4) or in summer and autumn (*Hypoponera* sp. 5, sp. 6, sp. 7, sp. 8, and sp. 9) (Table 2). Members of the genus *Hypoponera* have a vast geographic range and are prevalent on a global scale (Wilson, 1976). Colonies are small (<100 individuals) and nest in soil or rotten wood (Wheeler and Wheeler, 1986). They are occasional pests because female winged reproductives may sting during their mating flights (Klotz et al. 2005). The captured *Hypoponera* alate specimens were 3.5 to 4.5 mm in length.

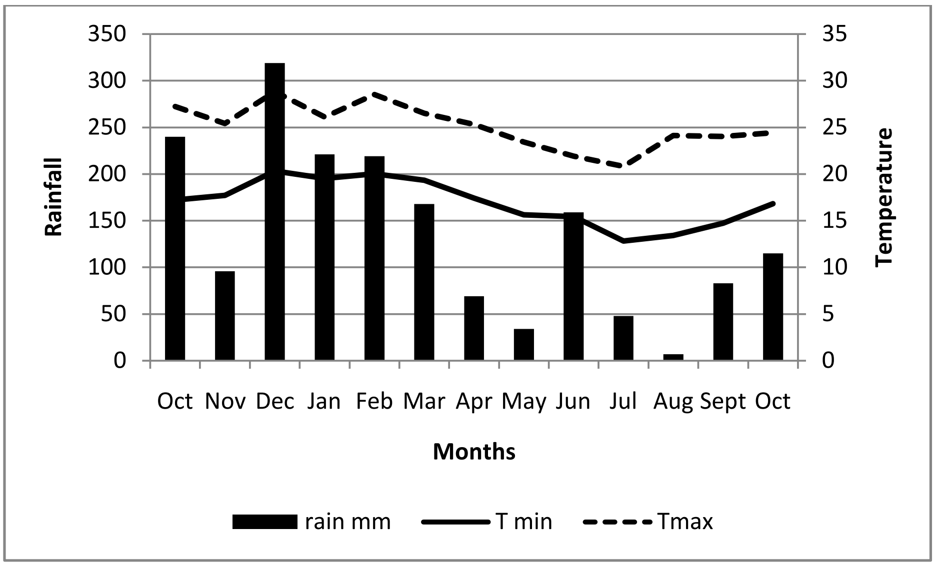

Figure 1 – Temperature and rainfall in the city of São Paulo from October 2012 to October 2013.

CONCLUSIONS

Data registered in this work help to comprehend the Ponerinae flight activities in the city of São Paulo and serve for the purpose of educating citizens for preventing Ponerinae stings. The most critical months are July to September, when the number of *P. striata* winged forms increases, and from October to February, in the peak of *O. bauri* alate production. Both species are known for their painful stings and for causing anaphylaxis in some humans.

The diversity of Ponerinae reproductive ants in the city is high and similar to surveys conducted in the Atlantic rain forest reservoir previously made by other authors. *Hypoponera* species were collected in the urban area, showing that even in a highly disturbed habitat like the city of São Paulo, such species can reproduce.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

CHALLENGES FOR SUCCESSFUL MANAGEMENT OF *PACHYCONDYLA CHINENSIS* (*HYMENOPTERA: FORMICIDAE*)

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Abstract *Pachycondyla chinensis* (Emery), the Asian needle ant, is an invasive, stinging ant established in the United States. It is known to displace both native and invasive ant species such as *Linepithema humile* (Mayr), the Argentine ant. It can be medically important to people who are allergic to arthropod stings and may cause anaphylaxis in hypersensitive individuals. Successful management of *P. chinensis* has proven to be difficult using traditional treatment methods with liquid insecticides or bait products. Although *P. chinensis* readily consumed toxic bait products in a laboratory study, it was not effectively managed in the field during seasonal periods of peak activity. Perimeter and targeted treatments using liquid insecticides were variable. Although targeted treatments were more successful, *P. chinensis* colonies were not eliminated. The potential reasons for inadequate population reduction are based on *P. chinensis* behavior and nest site distribution.
Key words Formicidae, treatment strategies, Ponerinae, Asian needle ant.

INTRODUCTION

Pachycondyla chinensis (Emery), commonly known as the Asian needle ant is an invasive species in some areas of the United States. It usually does not enter structures in large numbers, but rather its urban pest status is based on its ability to sting which may cause mild to severe allergic reactions including anaphylaxis (Nelder et al., 2006). Sting reactions are well documented in Korea and other areas in the native range of this species (Kim et al., 2001; Cho et al., 2002). *Pachycondyla chinensis* also has an ecological pest status based on its ability to displace key native species in woodland habitats (Paysen 2007; Guénard and Dunn, 2010). In a study in an urban setting, the Argentine ant, *Linepithema humile* (Mayr), which normally displaces other species within a habitat, was itself displaced by the Asian needle ant. Although *L. humile* colonies are often larger and more aggressive than *P. chinensis* colonies, they were displaced early in the season before populations reached an overwhelming capacity (Rice and Silverman, 2013). *Pachycondyla chinensis* forms small colonies in rotting logs often associated with termite colonies, or in the soil beneath stones, wood debris, or structural objects including logs, rocks, subterranean tree roots, landscape objects, bricks, lumber, or other human-made debris. Paysen (2007) found *P. chinensis* nests around buildings and landscaping in urbanized areas, on the forest edges, and interior forests. However they are not known to nest in open areas and consequently do not compete with the red imported fire ant, *Solenopsis invicta* Buren. In forest areas, *P. chinensis* workers forage on the ground and on tree trunks. With generally small populations of several hundred to several thousand, nests tend to be small and discrete, but if circumstances provide a large area of contiguous, suitable habitat, colonies may fill the void. They opportunistically feed on decaying fruit, and prey on live arthropods, and dead organisms including terrestrial invertebrates, birds and mammals. Recruitment of

P. chinensis does not result in trails of workers going to a food source as with *L. humile* or *Tapinoma sessile* Say. They forage individually or recruit cohorts by tandem carrying to a food source (Guénard and Silverman 2011). For this reason, control using bait may be effective in some instances, but success has been variable. In studies reported here based on Mo (2013), targeted application of ant bait was often not a reliable strategy for control, nor were targeted applications of liquid insecticides. These variable results are likely because of the cryptic nature of *P. chinensis* nest entrances. This paper provides a discussion of considerations and challenges of *P. chinensis* management in light of results of laboratory and field trials with an emphasis on why success may be elusive.

MATERIALS AND METHODS

Choice / No Choice Bait Laboratory Study

A choice/no choice bait study was conducted using field-collected *P. chinensis* challenged with seven bait products including granular, gel and liquid formulations. Detailed procedures, analysis, and results for the study are found in Mo (2013). The products included were Advion® fire ant bait, Advion® Insect Granule, Advion® ant gel (DuPont™, Nemours and Company, Wilmington, Delaware, USA), Advance® 375A Select Granular Ant Bait (BASF Corporation, St. Louis, Missouri, USA), Optigard® Ant gel Bait (SYNGENTA CROP Protection, Greensboro, North Carolina, USA), Maxforce® Complete Brand Granular Insect Bait, and Maxforce® Quantum Ant Bait (Bayer Environmental, Research Triangle Park, North Carolina, USA).

A choice test was performed by providing food and bait in a treatment dish in which ants were offered termites and a bait product. Control ants were offered termites only. Five replicates were performed using five colonies. In a no-choice test, ants were offered only bait in the treatment and only termites in the control. Six replicates were performed using four colonies. Ants within each replicate were from the same colony. Bait products were refreshed every three days. Every day food and water were refreshed and mortality was recorded for 14 days.

Analysis of variance was performed on arcsine square root transformed data in the study comparing the daily mortality of each treatment (PROC GLM, SAS institution 9.3 2011). Fisher's Least Significant Difference test was used to compare the difference in mean daily mortality between each two treatments.

Bait Field Study

Evaluation of bait products in the field was conducted in urban areas where actively foraging ants and potential nesting sites were located. Detailed procedures, analysis, and results for the study are found in Mo (2013). Each site was identified by non-overlapping foraging resources. Pitfall traps were placed in the ground to monitor *P. chinensis* populations at each site. Population change was calculated by comparing weekly *P. chinensis* pitfall trap catches with the number collected before treatment was applied.

Perimeter/Targeted Applications Compared with Perimeter- Only Treatments

A comparison of perimeter and targeted (P/T) insecticide spray applications, and perimeter-only (P-only) spray treatments against *P. chinensis* was conducted around homes in the area of Clemson, SC in August and September. In the P/T replicates, sites targeted beyond the perimeter were located within 6 m of the treated structure. Tempo Ultra SC (Bayer Environmental, Research Triangle Park, North Carolina, USA) at 16ml/93m^2 was applied 0.3 m up the foundation wall and up to 3 m out using a Solo (Solo™, Newport News, VA, USA) back-pack sprayer at 25 psi. Three homes were identified for each treatment including the control. Pre- and post treatment data were collected using pitfall traps at 0, 1, 2, 3 and 5 weeks. A pitfall trap consisted of a 2x15 cm test tube filled about one third its length with propylene glycol and fitted into a PVC sleeve. Traps were placed flush with the soil at 6 m intervals around the perimeter of each structure and adjacent to previously identified nest sites. This placement resulted in 14-20 pitfall traps per replicate.

Data were evaluated using analysis of variance with a least significant means test applied where appropriate at $P = 0.05$.

RESULTS

Choice/No Choice Bait Laboratory Study

In both choice and no-choice laboratory tests, the control groups were provided food (termite workers) and water daily, and had survival rates of 90-95%. Food also was provided in the choice test in each treatment dish. A detailed discussion of laboratory results can be found in Mo (2013).

Advion® fire ant bait, Advance, and Maxforce® Complete achieved 100% mortality within the 14 day period in both the choice and no-choice studies. Advion® gel provided approximately 90 and 96% mortality in the both studies and was not significantly different from products reaching 100% mortality after 14 days. When there was a choice of a natural food source, Optigard® was less effective than in a no-choice test in which it was not significantly different from Advion® gel. Optigard® was not significantly different from Maxforce® Quantum in the choice test achieving a mortality of 43%. Advion® granule was the least effective bait in both choice and no-choice tests at 22 and 34% respectively, and was not significantly different from the control.

Bait Field Study

Field results at 10 weeks showed the most effective bait products in the field differed from the most effective bait products in the laboratory tests. Only Advion® gel reduced the field population over 10 weeks. Advion® fire ant bait achieved 70% reduction in the field population during the first three weeks; however the population increased during week 5 and 6. After reapplication, the population was reduced again. Advance® was effective in the first seven weeks and had no effect on the population reduction in the last three weeks even after reapplication. Maxforce® Complete was not as effective as in the laboratory, and the field population increased during most weeks of the study. Optigard® and Maxforce® Quantum had similar trends in population change during the 10 weeks. The population increased during weeks when there also was an increase at control sites and decreased in weeks when there was a decrease in the *P. chinensis* population at control sites.

Perimeter/Targeted Applications Compared with Perimeter-Only Treatments

Both P/T and P-only treatments had significantly reduced pitfall trap counts compared with controls at weeks one and two, but were not significantly different from each other. There were 72 and 74%, and 64 and 71% reduction in the number of *P. chinensis* collected in pitfall traps at weeks one and two for P/T and P-only treatments, respectively. By weeks 3 and 5 there were no significant differences between these two treatments and the control.

DISCUSSION AND CONCLUSIONS

Results for the laboratory and field bait studies were variable in achieving mortality of *P. chinensis.* Bait products, which cause high mortality of *P. chinensis* in laboratory studies, were not consistently effective in the field during seasonal periods of peak activity. Rice et al. (2012) reported effective control of *P. chinensis* using hydramethylnon bait in both clumped and scattered application. By 28 days after treatment, *P. chinensis* was increasing, although the control sample was decreasing.

Over a ten week period, management with bait products in our field study varied weekly. Advion fire ant bait, Advion gel, and Advance were more efficacious than other products tested in this study, but no product achieved acceptable results over the study period and results were variable week to week, although products were reapplied according to label directions.

The results of the spray applications against *P. chinensis* also did not achieve sufficient, sustained control over the study period. Significant differences compared to the control were documented only for weeks one and two after treatment.

The studies discussed here offer insight about the many potential considerations and challenges which may hamper successful *P. chinensis* control. We speculate that among the most important is that *P. chinensis* has many often disjunct nest sites. Without recruitment by trailing disconnected or very large populations may not locate bait or be affected by insecticide applications that do not directly target each nest.

Bait products may be depleted before reapplication can occur with a larger *P. chinensis* population or when other arthropods are attracted to the site of application. In the bait field study crickets, pill bugs and millipedes were found weekly in many pitfall trap samples. When bait products are applied and reapplied according to label rates the amount of product may not be sufficient to reduce populations, even when they are moderately-sized and contiguous. During periods of heavy rainfall or climatic periods of high temperature and humidity, baits may quickly become unpalatable. This would not be as problematic if recruitment strategies for *P. chinensis* were more similar to *L. humile or S. invicta*, but rapid, large-scale recruitment to a food source does not occur.

Inadequate, sustained control with applications of liquid insecticides, even when many nest sites are targeted, may be due to similar factors. Because nest sites are not always contiguous and because interaction of ants from one nest site to another is not dependable, a treated area may have limited impact on untargeted sites. In the P/T treatments of our study we did not treat targeted nest sites beyond 6 m from the structure leaving open the possibility of recolonization of treated sites over time. Insecticides also are impacted by climatic and weather-related causes which can reduce their effectiveness.

A combination of integrated strategies may improve treatment success especially if careful inspection is conducted prior to treatment. *Pachycondyla chinensis* will move their nest very rapidly after a disturbance (personal observation). Insecticide applications should be made at the time of inspection to impact the most ants. After activity is reduced, introducing attractive bait with timely reapplication may offer longer-term control of *P. chinensis*. Regular inspection is necessary after treatment to time reapplication and prevent resurgence in the population. It also may be advantageous to begin treatment early in the season before *P. chinensis* populations increase. After successful control is achieved, reducing preferred nesting habitat will be important to prevent possible immigration of *P. chinensis* from untreated areas.

ACKNOWLEDGEMENTS

We thank the staff of Woodburn Historical Plantation and homeowners for providing study sites including Mr. Rick Willey, Nora Stewart, Bob and Jackie Carek, and Paul and Jane West. We also thank Bob Bellinger, Terry Pizzuto, and Hamilton Allen for technical assistance.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

CONTROL OF *BLATTA ORIENTALIS* (BLATTODEA: BLATTIDAE) IN ZURICH, SWITZERLAND

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Abstract The oriental cockroach (*Blatta orientalis)* occurs in several buildings of two different blocks in the city of Zürich. Pest control companies were unable to control the infestation, and reported cockroaches in the backyards of these blocks during the warmer periods of the year. The Urban Pest Advisory Service (UPAS) conducted an indoor cellar and outdoor backyard monitoring of the cockroaches in tenement block I (21 buildings) during the warm season, and outdoor backyard monitoring in tenement block II (7 buildings). The UPAS required the house owners to have professional pest control operators treat the problem, and the UPAS coordinated treatments to occur within a week. Monitoring the following spring showed that the treatment was successful; the infestation was reduced to the outside of one building in block I and to one outdoor spot in the backyard of block II. UPAS continues monitoring in both blocks. This demonstrates that coordinated pest control treatment can achieve reduction and possibly eradication of cockroach infestations.
Key words Monitoring, oriental cockroach control

INTRODUCTION

The main goal of the Urban Pest Advisory Service (UPAS) is to survey, control and eliminate health hazards posed by insects and rodents in the city of Zurich. It is the only non-commercial and official pest advisory service in Switzerland. Our main duties are described in Landau et al. (2008). Based on the 'cantonal decree on public and private living hygiene' (Kanton Zürich, 1967), we can enforce the control of pests of public health importance, and impose the costs of these measures upon the owners of infested buildings.

In 2009 an established pest control company reported to the UPAS that they could not eliminate an infestation of the oriental cockroach *Blatta orientalis* (L.) (Blattodea: Blattidae), and that there might be a vast infestation in the neighboring buildings. Research in our specifically designed SQL-database (Apel and Köhl, 2002) showed that oriental cockroaches had been reported before in this area: 1993 a house in the same tenement block was reported to be infested, and there was also a record from 1995 when cockroaches were seen in the backyard during the night. We found four further records between 2006 and 2008 when oriental cockroaches had been a problem both in- and outside. The whole block consists of 21 apartment buildings and includes a large backyard (Figure 1). Since oriental cockroaches are known to live outside during the warm season (Thoms and Robinson, 1986; Le Patourel, 1993; Mallis, 2011), we decided in 2010 to check not only all buildings but also the streets and the backyard. Our decision to force all involved house owners to join in the coordinated control measures was motivated by an experience that German colleagues made a few years earlier in the region of Damme (Nordrhein-Westfalen, Germany) in an area of more than 100 km^2 with oriental cockroaches walking in the streets during daytime in the summer of 2004. This included parts of the city but also food industry plants and rural areas with pig farmers. When it became apparent that the control actions had to be paid by the house owners or the farmers, many of them cheated with the monitoring or even refused cooperation. Because the first control failed, the responsible authorities decreed a compulsory control that finally worked out when the area-wide control actions were coordinated (Freise, 2005, 2006).

In 2012, we performed a second coordinated control effort in another block (tenement block II) consisting of 7 buildings (Figure 2). The decision was taken because the pest control technician of one of the restaurants had seen oriental cockroaches in the back yard and because the food inspector was not satisfied with the results of the previous pest control actions in the restaurant. In our database we had already two buildings in that block with a record of oriental cockroaches in 2003 and 2011 (Figure 2).

MATERIALS AND METHODS

Tenement block I. The block consists of 21 apartment buildings, most of them built together, with 4 stories and one or two cellar levels. Most of the back yard is accessible by car. A former underground parking deck has been converted to a second-hand shop. There is no connection between any of the houses. In July 2010 UPAS placed Catchmaster® insect monitors with a lure pill (Agrisense Ltd) on the ground floor and in the cellars of all the houses. In the backyard and in front of the houses we placed clear AF® insect monitors with a sticky trap and lure pill and checked them one week later (Figure 1).

Tenement block II. This block consists of seven buildings (Figure 2). Three of the buildings feature large grocery stores on the ground floor, in two other buildings there are restaurants. The front of the buildings of the whole block is all paved sidewalks with no hiding possibilities for the cockroaches, so monitoring was restricted to the backyard. The outside was treated by spraying with suspension of Cislin® CS (deltramethrin 25g/l) or Ficam® W (bendiocarb 800 g/kg). At some places on the face of the buildings and in cracks Goliath® gel (fipronil 0.5 g/kg) was additionally applied.

RESULTS AND DISCUSSION

Tenement Block I

In spring 2010 the house owners of all involved buildings were informed by UPAS that a coordinated control action of the oriental cockroach in and outside the houses was planned in order to eradicate the local population. In July traps were placed both in- and outside, and the presence of cockroaches on the glue traps was checked a week later (Figure 1). The second-hand shop in the former underground garage in the middle of the backyard had no problems with cockroaches. We informed all house owners that we had found cockroaches everywhere and that they had to have a pest control company do the treatments. As the whole block was heavily infested, we had no difficulties convincing any of the house owners. In August the sewage water system in the streets was inspected by descending into the pipe and walking all the way through. All three sewer pipes were accessible: the narrowest pipe height was 90 cm. Since only 2 cockroaches were found in the whole length of 440 m sewage, we decided not to take control measures in the sewage system. In August the 5 involved companies started the treatments in the cellars of all the houses and the monitoring in all apartments. In September a coordinated control action outside took place. Before the treatment we discussed the control measures with the involved companies. Only in three houses apartments were infested (Figure 1), and there the gel method was used. In the corridors and cellars surfaces were sprayed, sometimes also gel was used. Some companies treated the drains in the floor with insecticidal varnish. Outside insecticide spray was applied for the lower face of the buildings, the paved and unpaved ground, and gel placements were put into cracks of the buildings.

The springs of 2011, 2012 and 2013 UPAS surveyed the buildings again by placing traps outside (Figure 3). Based on experience, knowledge of the geographical situation and control success, the UPAS could reduce the monitoring time. We needed 6 hours in 2011, we only took 2.5 hours in 2012 and 2 hours in 2013. Where necessary, the pest control companies did another treatment. We plan to conduct another monitoring as soon as the temperatures are high enough, probably in May 2014.

Tenement Block II

End of May 2012 we placed 15 clear AF® insect monitors with a sticky trap and lure pill along the buildings and checked them five days later. 12 of the 15 traps were positive with cockroaches sticking to them. One of the traps was empty and 2 others were missing (Figure 2). All house owners had already required the services of a pest control company for cockroach control, four buildings were permanently monitored. All buildings had a control action done within the last two years, with four involved companies. After the instruction by UPAS, control measures were undertaken in the middle of July 2012 within the houses and in the backyard.

At the beginning of August, 14 new traps were placed. Cockroaches were only found in a concrete framed flower bed (0.5 x 10 m) with low vegetation and one tree in the middle of the backyard (Figure 2). In September / October a second outdoor (and where necessary indoor) treatment was applied by the companies. Subsequent monitoring at the end of April 2013 showed that the traps at the same two spots in the plant bed had caught oriental cockroaches (Figure 4).

DISCUSSION

In temperate climates oriental cockroaches often live outside the houses during the warm season and even seem to be able to over-winter in hiding places (Thoms and Robinson, 1986; Le Patourel, 1993), such as behind marble blocks glued to the face of buildings, and in wall cracks. Cockroaches can also hide in the vegetation of a back yard, in soil with small bushes or untidy places, in some distance from the houses. In tenement block II the plant bed is 5 meters away of the nearest house and can act as a reservoir for the cockroaches at least during the warm season.

To eradicate cockroach populations in buildings and backyards with or without vegetation belonging to various house owners, the dimension of the infestation must be carefully identified. All possible refuges have to be identified and checked. This requires pest control technicians that have experience with the habits of oriental cockroaches, and all involved parties have to coordinate their control actions. It is a challenge for the authorities to persuade all the house owners that a professional is needed for the control. Some owners tried to convince us that they did not have any cockroaches, others thought that they would not require a control company and wanted to control the 'roaches' with own measures. The house owners have to be persuaded within short time because coordinated outside control measures (including second treatment after 8 to 10 weeks) have to be performed during the warm season and before night temperatures fall in autumn. Thoms and Robinson (1987) recommend treatments against populations of oriental cockroaches in May or June to be most effective, before the adult population peak.

A possible problem is the loss of time due to stubborn house owners who seek a legal dispute. The best solution is to explain and discuss the problem and appeal to the house owner's responsibility to convince them that the cockroach problem can only be solved by working together.

The sewer system of tenement block I was not infested at the time of heavy infestation of the backyard and the buildings. UPAS had checked the sewer system on two other occasions without finding any infestation, making measures unnecessary. During the very high cockroach infestation in

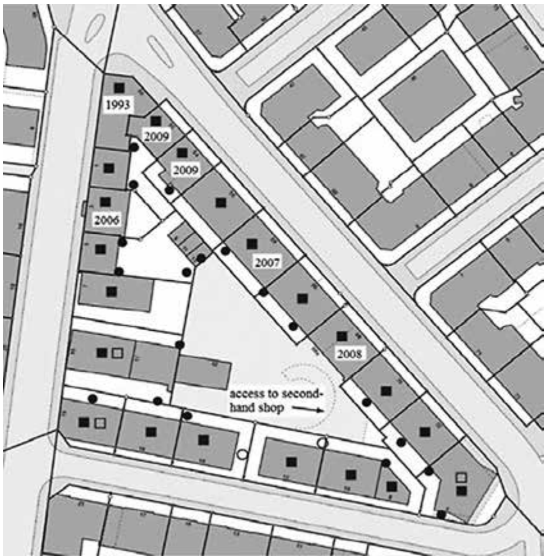

Figure 1. Tenement block I. Monitoring of B. orientalis in 2010. Years indicate when the UPAS received the first report of oriental cockroaches in the building. Traps: ■ = in basements; □ = in flats; ●= outdoor trap; ○ = not trapped.

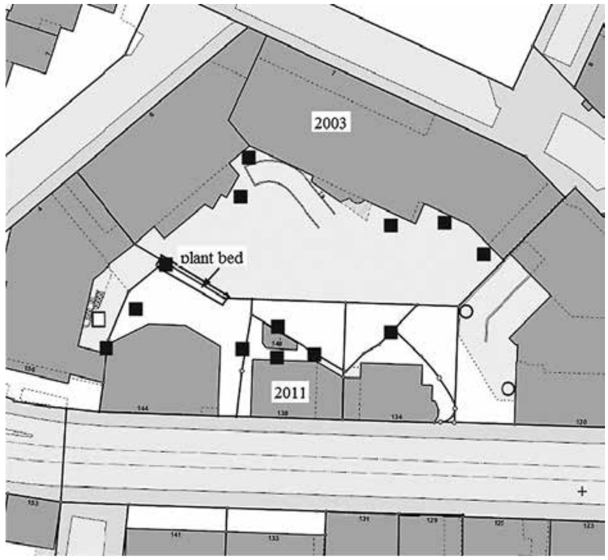

Figure 2. Tenement block II: Outdoor monitoring of B. orientalis in 2012. Block consists of 7 buildings, 3 grocery stores, 2 restaurants on ground floor. Traps: ■ = on trap; □ = not trapped; ○ = trap missing.

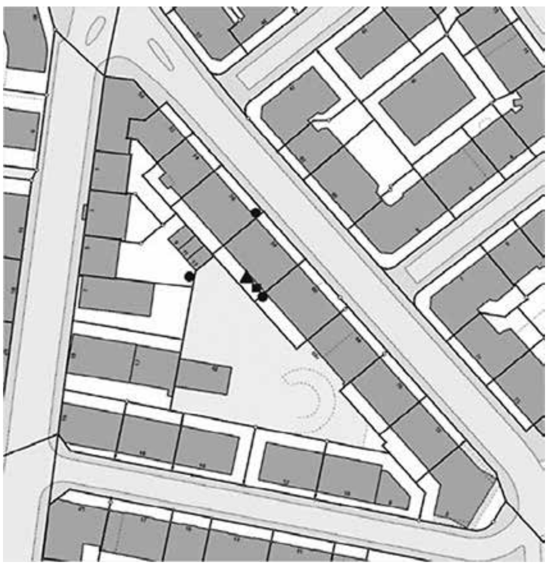

Figure 3. Tenement block I. Monitoring of B. orientalis in 2011- 2013. Traps: ● = 2011 trap catch; ■ = 2012 trap catch; ▲ = 2013 trap catch.

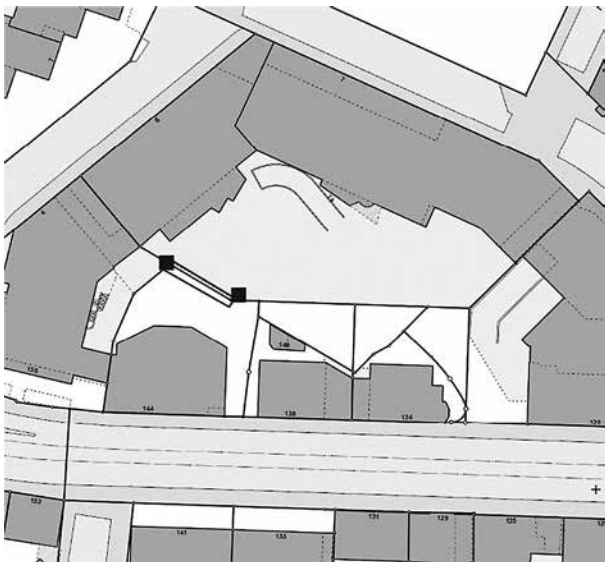

Figure 4. Tenement block II: Outdoor monitoring of B. orientalis in 2013. Traps: ■ = 2013 trap catch.

Damme (Germany) they were also present in the sewers and manholes were treated with insecticidal varnish (Freise, 2005). Therefore infestations of the sewer system should be considered in future cases. In both blocks a single source of cockroaches in unpaved ground could not be eradicated yet, so the monitoring and control measures will be carried on.

CONCLUSIONS

Altogether the strong reduction of the oriental cockroach in the backyards of both blocks has improved the situations substantially. In none of the buildings in block I cockroaches appeared after the first coordinated control action. Due to this success the UPAS started to have a closer look at other tenement blocks where oriental cockroaches have been seen outside in summer. At the moment we are involved in the control coordination of 4 different blocks of houses in Zurich.

ACKNOWLEDGMENTS

We thank Prof. Wolf Blanckenhorn for the reading and very useful comments on the manuscript.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

BEHAVIOR OF *RETICULITERMES FLAVIPES* (ISOPTERA: RHINOTERMITIDAE) DURING COLONY FUSION

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Abstract Limited data exist on foraging, agonistic and feeding behavior of subterranean termites of the same species but from different colonies. We conducted research to determine if subterranean termites, *Reticulitermes flavipes* (Kollar) from different colonies may express unusual foraging, agonistic and feeding behavior during colony fusion at various temperatures. Fifty *R. flavipes* workers from two different colonies (colony #1 undyed and colony #2 dyed with Nile blue-A)) were placed in separate feeding chambers and connected with polyethylene tubes to a common feeding chamber for foraging interaction at 15, 20, 23, 25 and 30°C. Each treatment had three replications. Data were recorded on foraging, agonistic/aggressive behavior and food consumption of termite workers at 1, 3, 24, 72 h and 7, 14 and 21 d intervals. Our data revealed that different colony members did not display agonistic behavior and fused together at 15-25°C with maximum food consumption at 20°C. Food consumption was not significantly different ($P \geq 0.05$) at 15-30°C and the 30°C was not the optimum temperature for termite survival.
Key words Subterranean termites, *R. flavipes,* agonistic behavior.

INTRODUCTION

Agonistic behavior refers to the social interaction of aggressive responses when new termite workers from the same species but different colonies are introduced (Haverty and Thorne, 1989). There are very few reports on Reticulitermes spp. of such interactions in North America, with the exception of Zootermopsis spp. (Haverty and Thorne, 1989; Thorne and Haverty, 1991), Heterotermes spp. (Binder, 1988; Jones, 1993), Coptotermes formosanus Shiraki (Su and Scheffrahn, 1988; Shelton, 1997a, b). The fundamental mechanisms associated with agonistic responses in subterranean termites are highly variable. When reproduction within the colony is controlled by a single king and queen, the colony is referred to as a simple family. After the death of one or both primary reproductives, the colony may undergo several cycles of inbreeding by the secondary neotenics. This is referred to as an extended family. Termite colonies have been also known to fuse together (DeHeer and Vargo, 2004, 2008; Johns et al., 2009). In certain cases, when workers within a colony express genetic ancestry to more than one colony, it is known as fused or mixed family. Colony fusion is difficult to detect when colonies have a complex genetic architecture, for example, when queen has multiple mating or when multiple queens reproduce offsprings (DeHeer and Vargo, 2004, 2008).

Haverty and Thorne (1989) reported that the termite colony members will act aggressively towards individuals from other nest-mates during nest-mate recognition phase. However, the level of inter-colony aggression within a species varies from one colony to another one. Studies on *C. formosanus* have demonstrated that inter-colony agonistic behavior does not always result from pairing colonies in the laboratory (Su and Haverty, 1991). Clement (1986) reported that colonies of

Reticulitermes spp. either accept or reject other colony members but it depends on the seasonal change (spring, summer or fall) during the termite collection. However, Grace (1996) did not see any agonistic behavior in *R. flavipes* colonies collected in the fall, whereas Clement (1986) suggested that European *Reticulitermes* colonies were rejecting other colony members.

Reticulitermes termite nestings were grouped into multiple-site nesters (Shellman-Reeve, 1997). When the wood as food source is depleted, the colony migrates to another suitable wood resource. *Reticulitermes* termite wood preference depends on wood species, stage of decay and moisture content (Waller and LaFage, 1987). Moreover, preferred wood might have already been occupied by other inhabitants. If the migratory colony is not able to find new preferred wood resource, it is most likely to invade another colony to access new food resource. Consequently, the host colony will either fight or fuse with the invading colony (Shellman-Reeve, 1997).

In this study, we conducted experiments to determine foraging, agonistic, feeding behavior and colony fusion correlation while pairing workers from two distinct colonies of *R. flavipes* at five different temperatures.

MATERIAL AND METHODS

Source of Termites. Wood logs infested with subterranean termites were collected from two different locations ≈500 m apart and returned to the laboratory at the University of Nebraska-Lincoln. The termites were extracted from each log from two different colonies and were maintained in separate polyethylene hard-plastic containers with lids and covered with black plastic for creating the subterranean environment. Termites were allowed to acclimate by maintaining all containers at room temperature (23 ± 2°C) for 30 d.

Temperature Setting. Percival growth chambers (Percival Scientific Inc., Perry, IA) were preset at 15, 20, 23, 25 and 30°C. Twenty termite workers plus one soldier were placed in a plastic petri dish (100 x 15 mm) with moist sand and corrugated cardboard. Later, these petri dishes with termites were placed in growth chambers at preset temperatures to ascertain that termites could survive for 30 d.

Feeding Arenas. Feeding arenas were similar to those used by Binder (1988) and Grace (1996). Each arena consisted a set of three polyethylene petri dishes/feeding chambers (100 mm diameter x 15 mm height, BD Falcon company, Franklin Lakes, NJ) and connected with 2 cm long of 6.35 mm diameter polyethylene tubing to accommodate approximately 1,000 termites (Figure 1). Each arena consisted of ~5 g sterile sand. The pinewood pieces (1.0 cm length x 1.0 cm width x 2.5 cm height) were oven dried overnight (80°C), cooled and weighed before their use. Each pair of pinewood pieces weighed ~1.5 g. One of the termite colony groups was stained with 0.1% (wt/wt) Nile Blue A by a no choice feeding of stained filter paper (Whatman No. 1, 9.0 cm in diameter) for 5-7 d (Abdul Hafiz et al., 2007).

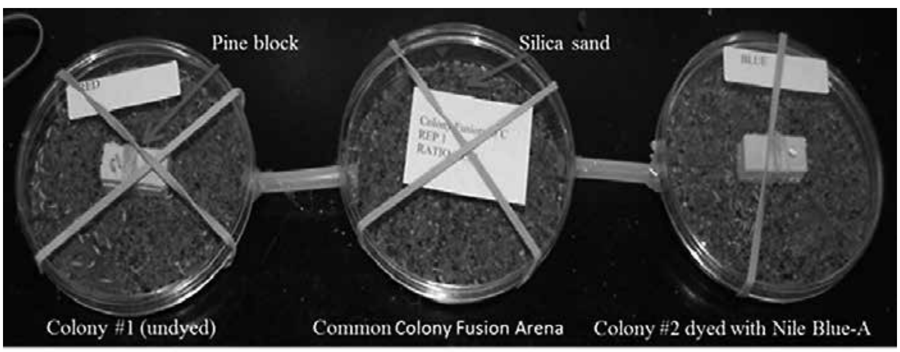

Figure 1. Feeding arena. Subterranean termites: Colony 1, undyed, Colony 2, dyed. Were placed in separate feeding chamber which were connected to a common arena and with food source in center

Feeding and Food Consumption. In the first experiment, 50 undyed termite workers from colony #1 were placed in the petri dish (feeding chamber) proximal of feeding arena and 50 Nile blue-A dyed termite workers from a colony #2 were placed in the petri dish (feeding chamber) distal to the feeding arena, leaving the middle petri dish (feeding chamber) unoccupied (Figure 1). Termite foraging activities were observed at intervals of 1, 3, 24, and 72 h and 7, 14 and 21 d. Food consumption was recorded at the end of 3 wk after dismantling the feeding arenas. The pair of pinewood pieces at each arena were cleaned, oven dried (80°C), cooled and weighted. The experiment design was the completely randomized block and each treatment had three replications. Analysis of variance (ANOVA) and *t*-tests (LSD) were conducted using SAS to test for significant differences in food consumption ($P \geq 0.05$).

Colony Merger/Fusion. In the second experiment, 50 undyed termite workers from colony #1 were placed in the petri dish (feeding chamber) proximal of feeding arena and 50 Nile blue-A dyed termite workers from a colony #2 were placed in the petri dish (feeding chamber) distal to the feeding arena, leaving the middle petri dish unoccupied (Fig. 1). The arenas were left on the laboratory bench for 30 min for acclimation. Each petri dish in the feeding arena was labeled reflecting appropriate treatment and the replication. The arenas were placed in 60.96 x 41.99 x 14.94 cm hard plastic boxes (Bella, Leominster, MA) covered with aluminum foil. Finally, the plastic boxes were placed in growth chambers that were programed for 15, 20, 23, 25 and 30°C. The sand in each petri dish was moistened weekly or as needed. Inter-colony pairings were monitored at intervals of 1, 3, 24, 72 h and 7, 14 and 21 d. After 3 wk of incubation, each feeding arena was dismantled and the number of undyed and blue dyed termites in each of the three petri dishes were recorded. For each test arena, connecting tubes were considered as part of the respective petri dish (feeding chamber). All colony pairing were categorized according to Fisher et al. (2004) as (a) sharing both nesting space food resources, (b) sharing food resources but maintaining separate nesting material or (c) maintaining separate nesting space not sharing food resources. Colony fusion was defined as the sharing of both nest sites and food resources.

Termite Mortality. After 3 wk of incubation, the feeding arenas were carefully dismantled. The number of blue dyed and undyed termites were recorded in each of three petri dishes (feeding chambers) for the pairing arenas (connecting tubes were considered part of the respective feeding chambers). Termite mortality was compared within each pairing for all five temperature settings using *t*-test (LSD). The data on termite mortality at respective temperature were subjected to analysis of variance (ANOVA) at the significance level of $P \leq 0.05$ using SAS (SAS Institute Inc. 2000).

Agonistic Behavior. The above described feeding arenas were used for observing agonistic/aggressive behavior at intervals of 1, 3, 24, 72 h, and 7, 14 and 21 d for all five temperature settings as mentioned earlier. Agonistic/aggressive behavior is defined as offensive and defensive responses between competing termite workers from two different colonies (King, 1973). The termites from pairing colonies were observed for 10 min at each time interval and at each temperature.

RESULTS

Feeding and Food Consumption

Termites fed actively at 15-25°C and the highest feeding was at 20°C with mean wood consumption of 0.34 g and the lowest feeding consumption at 15°C. The 25°C was considered the upper limit with mean wood consumption of 0.29 g (Table 1). However, the *t*-test (LSD) indicated no significant differences in wood consumption amongst all temperatures (15, 20, 23, 25 and 30°C) ($P \geq 0.05$) (Table 1).

Colony Merger/Fusion

All fifteen termite colony-pairings (100%) shared both nesting areas and food resources at all temperatures until 21 d. In all colony pairings during the first hour at all temperature settings, the termites were still foraging within the original feeding chamber and searching for suitable nesting site and food source. At 25°C, all the pairings shared both nesting space and food resources. At 15, 20 and 23°C, only 33.3% of colony pairings shared both nesting space and food resources, while 66.7% termites shared food resources but maintaining separate nesting material. After 3 h, all the colony pairings were sharing both nesting spaces and food resources at 15-25°C. However, at 30°C only 33.3% of termites shared both nesting and food resources, while 66.7% termites shared food resources but maintained separate nesting materials (Table 2). After 24 h, all the colony pairings at all temperatures shared both nesting and food resources, and the termites were very active in all feeding chambers. After 14 d, all the pairings still shared both nesting space and food resources except at 30°C. In addition, at 30°C the termites were not actively moving in colony pairing. After 21 d, the termites in colony pairings shared both nesting areas and food resources at all temperatures. Furthermore, termites in colony pairings were sluggish at 30°C after 21 d. Termites in colonies that fused/merged were observed grooming, participating in trophallaxis and foraging with unrelated nest mates.

Termite Mortality

At 30°C, pairing-termites had 65-75% mortality (Table 3). The optimum temperature for the pairing-termites was 25°C with only 15-41% mortality. In addition, at 15, 20 and 23°C termites had <50% mortality. Based on *t*- test analysis, termite mortality did not differ significantly at 15 to 25°C except for 30°C ($P \leq 0.05$) (Table 3). Temperature setting from 15 to 25°C also can be ideal temperatures for the termite pairings and colony fusion. Overall, temperatures have a significant effect on termite mortality ($P \leq 0.05$).

Agonistic/Aggressive Behavior

Our data indicated that termites show agonistic behavior for the first 3 h. This behavior was observed with head banging against each other. However, no agonistic/aggressive behavior was observed in termite colony-pairings after 24 h until 21 d.

Table 1. Mean food consumption by *Reticulitermes flavipes* after 21 days. Means with the same letter are not significantly different, $P \leq 0.05$.

Temperature (°C)	No. replicates	Mean* food consumed (g) ± SD
15	3	0.29 ± 0.04^{a}
20	3	0.34 ± 0.02^{a}
23	3	0.30 ± 0.04^{a}
25	3	0.29 ± 0.03^{a}
30	3	0.33 ± 0.03^{a}

Table 2. Colony fusion of *Reticulitermes flavipes* observed after 1, 3, 24, 72 hours, and 7, 14 and 21 days. A: Sharing both nesting space food resources; B: Sharing food resources but maintaining separate nesting material; C: Maintaining separate nesting space not sharing food resources

Temp.	Reps.	Time Interval							
		1h	3h	24h	48h	72h	7d	14d	21d
15^0C	Rep 1	A	A	A	A	A	A	A	A
15^0C	Rep 2	B	A	A	A	A	A	A	A
15^0C	Rep 3	B	A	A	A	A	A	A	A
20^0C	Rep 1	B	A	A	A	A	A	A	A
20^0C	Rep 2	A	A	A	A	A	A	A	A
20^0C	Rep 3	B	A	A	A	A	A	A	A
23^0C	Rep 1	A	A	A	A	A	A	A	A
23^0C	Rep 2	B	A	A	A	A	A	A	A
23^0C	Rep 3	B	A	A	A	A	A	A	A
25^0C	Rep 1	A	A	A	A	A	A	A	A
25^0C	Rep 2	A	A	A	A	A	A	A	A
25^0C	Rep 3	A	A	A	A	A	A	A	A
30^0C	Rep 1	C	A	B	A	A	A	A	A
30^0C	Rep 2	B	B	A	A	A	A	A	A
30^0C	Rep 3	B	B	A	A	A	A	A	A

Table 3. Mean individual *Reticulitermes flavipes* mortality of (colony #1 plus colony #2) for each pairing colonies after 21 days. Means followed by same letter not significantly different $P \leq 0.05$.

Temperature (^{o} C)	Replicates	Total percent termite mortality in individual feeding chamber (Col.1 + Col. 2) $\pm$ SD	Total percent mortality after pairing, Colony 1 + Colony 2
15	3	22.83 $\pm$ 8.30 b	<50%
20	3	18.67 $\pm$ 10.51 b	<50%
23	3	22.00 $\pm$ 8.34 b	<50%
25	3	18.67 $\pm$ 6.73 b	15% - 41%
30	3	33.67 $\pm$ 13.66 a	65% - 75%

DISCUSSION

Our data suggest that optimum temperature range and suitable food resources are inter-related for foraging and colony fusion. According to our data, the field collected subterranean termite colonies from different locations can merge/fuse together in the laboratory conditions at 15-25^{o}C. However, the temperature >30^{o}C may delay the termite colony-fusion process. According to Grace (1996) less agonistic behavior in introduced populations of termites in Toronto, Canada can lead to colony merging. A laboratory study conducted by Fisher et al. (2004) indicated that fused colonies can develop new functional replacement reproductives originating from only one colony. Fisher et al. (2004) further reported that there was no evidence of interbreeding by the secondary reproductives originating from two different colonies. Matsuura and Nishida (2001) suggested merging increases the colony size and it may have adaptive significance under certain environment conditions. In addition, Matsuura and Nishida (2001) found that colony fusion might be related to the nymph ratio in a colony. These authors denoted that the host colony showed agonistic behavior if the intruder colony had a higher nymph ratio than the host colony. But, if the host colony had a higher nymph ratio the intruder colony will merge.

In our study, agonistic behavior was only observed during first 3 h for termite colony-pairing at all temperature settings. Several studies on ants and termites indicated low genetic differences in nearby termite colonies which may be associated with low aggression (Beye et al., 1997; Husseneder et al., 1998; Tsutsui et al., 2000). Colony fusion due to recognition errors is one mechanism that can allow the formation of complex termite colonies reminiscent of ant super colonies (DeHeer et al., 2005). Most *Nasutitermes corniger* (Motschulsky) colonies are simple families, with a single queen and king (Atkinson and Adams, 1997); however, natural populations also include colonies with multiple unrelated queens and up to 50 nests. According to Pedersen et al. (2006) *N. corniger* from different nests within the same complex colony are mutually tolerant, but are highly aggressive toward other colony members of the same species. In addition, genetic bottlenecks have been hypothesized to explain loss of aggression among social groups, allowing the formation of complex colonies (Tsutsui et al., 2000). When colony recognition cues are heritable, loss of genetic diversity can result in greater similarity among colonies, reducing the ability of insects to discriminate against non-nestmates.

In addition, individual agonistic behavior varies. In a study using workers of *R. flavipes* and *R. virginicus,* only 4.5% of arenas with intraspecific colony pairs displayed agonistic behavior (Polizzi and Forschler, 1999). When agonistic behavior of individual workers of *R. flavipes* and *R. virginicus* was examined, 89% of previously aggressive workers displayed aggression during a second test, while 88% of previously passive workers were passive during a second test (Polizzi and Forschler, 1999). Therefore, there could be a division of labor among termite workers regarding the display of aggressive behavior (Polizzi and Forschler, 1999). Furthermore, it has been suggested that the mechanism of kin recognition involves multiple stimuli, including chemical, a behavioral and digestive cues (Thorne and Haverty,1991). Cuticular hydrocarbon composition differences on the epicuticle of *C. formosanus* were not correlated with inter-colony agonistic behavior (Su and Haverty, 1991). Agonistic behavior of *C. formosanus* colonies from Hawaii and Florida was not correlated with geographic distance (Su and Haverty, 1991; Shelton and Grace, 1997a). In addition, aggressive behavior between *C. formosanus* colonies in Hawaii did not correlate with genetic similarities between colonies (Husseneder and Grace, 2001). In our study, no agonistic behavior was observed at lower or higher temperatures after 24 h. Low temperature conditioning reduced inter-colony agonistic behavior presumably due to the elimination or suppression of kin recognition cues (Shelton and Grace, 1997b). Colony pairs of laboratory-reared *C. formosanus* did not display any agonistic behavior although these same colony pairs did display agonistic interactions when field collected termites were used (Shelton and Grace, 1997a).

In our study, temperatures around 15-25^{o}C are considered to be optimal for the termites to be actively interacting with each other. Again, no clear agonistic display was observed, but at 30^{o}C the termite activity seemed to be slower after two weeks of pairing. The field collected *Reticulitermes spp.* studied by Getty et al. (2000) showed an agonistic behavior in laboratory conditions for over 18 m. Fisher et al. (2004) witnessed that intruding termites performed maintenance behavior like grooming and trophallaxis. Similar observations were recorded in our study where most of the workers were grooming each other including the intruder workers at 15-25^{o}C. At 30^{o}C the termites seems to groom actively for first 72 h but grooming declines after one wk.

CONCLUSIONS

Colony merger/fusion can occur in the laboratory at 15, 20, 23, 25 and 30°C. Termite feeding activities were prolific at 15-25°C. All termites in colony pairings forage actively the first 72 h but least activity was observed at 30°C after 2 wk. Termite mortality was proportionately increased at 30°C after 3 wk.

ACKNOWLEDGEMENTS

We greatly appreciate the support and guidance from Urban Entomology Lab. members, Tim Husen and Ralph Narain. Special thanks to Ibrahim Salah, Department of Plant Science, University of Nebraska, Lincoln, NE for statistical analysis support.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

THE ECONOMICS OF TERMITE BAITING IN A SOUTH EAST ASIAN SCENARIO

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Abstract Termite baiting has evolved in recent times as a sustainable method for managing termites. Reports that baiting is not cost efficient ar frequent. This case study performs an analysis on the economics of running termite baiting in the city of Manila, Philippines. The article finally shows that inherent characteristics of the local market help make termite baiting not only a stand-alone and successful technique but also sustainable in generating profit for the practicing practitioners.
Key words Termite baiting, Southeast Asia**.**

INTRODUCTION

Termite management is the largest urban pest control activity in South East Asia. The gross damage termite cause in this region has never been estimated, but figures for the amount spent on controls could be in millions of US dollars. Though in general termite control remains an industry formalised work, where majority continue to treat soil for remedy, alternatives methods of treatments have appeared in recent times. Termite baiting is one such method, which was introduced to the Asian market in the early part of 2000. Since then it has evolved as a sustainable method for managing termites. However reports that baiting is not cost efficient and expensive is frequent. On the contrary this author estimates in the Asian cities termite baiting has taken over 25% of post construction termite control job based on gross revenue.

Termite baiting has proved to be popular and also sustainable and profitable among new generation practitioners. Their successes are due to a number of characteristics unique to Asian cities and are as follows: High prevalence of termites; higher density of buildings and structures; absence of mandatory termite proofing of structures during construction; presence of large number of commercial structures with common partition wall/fire wall separating buildings; lack of slab-on ground type of floor construction; frequent use of non-resistant timber in construction; commonness of above ground infestation; presence of *Coptotermes* as the major termite pest species; large number of new generation pest control operators (less than 10 years old companies); and favourable cost benefit ratio.

This paper reviews and examines each of the above characteristics in an Asian city such as Manila, in order to show controlling termites by baiting is sustainable and profitable method for practitioners.

MATERIALS AND METHODS

The data and information were collected by literature surveys and conducting interviews with responsible persons such as builders and pest control practitioners. Where ever needed surveys and experiments were conducted.

High Prevalence of Termite in Existing Buildings

To determine the prevalence of termite in existing structures, two trained personal were asked to walk through a 500 meters of a popular avenue in the central business district of Makati City, Manila. A total of 80 buildings were covered in this survey. Both personnel were specifically asked to meet/contact the responsible person of each building such as owner/caretaker or housekeeper/maintenance staff and determine the following: presence of active termites in any part of the building; previous record of termite infestation; whether termite control action was undertaken.

High Prevalence of Termite in Soil

To determine the prevalence of active termites in soil, a second survey was conducted using a single pest control company. Exterra in ground baiting stations provided by Ensystex Philippines Inc. were used for this purpose. Five hundred (500) in-ground stations containing wooden interceptors were installed and monitored for termite activity. The stations were all installed between the months of February to May 2012 covering 28 properties. The stations were monitored and the data presented here accounted for the first 3 months of activity.

Determining Termite Species Infesting Structures

A single pest control operator took part in this work over a period of 4 years between 2005 and 2009 to determine the dominant species infesting structures in Philippines. A total of 450 infested structures formed part of this survey. Data were collected during the survey and inspection of the infested structures. During inspection samples of live termites were collected for identification. Once identified the information was tabulated in 2 categories, namely belonging to higher and lower group of termite. Higher termite species included, *Microcerotermes losbanosensis, Macrotermes gilvus,* and *Nasutitermes luzonicus* and lower included *Coptotermes gestroi*.

Commonness of Above Ground Infestation.

In the above survey information on the method of entry into the structure was also investigated and noted. Entries were categorised as above ground when evidence pointed to clear termite entry from an above ground source such as a planter box, roof gardens, adjacent tree touching the building, mound along the building wall or from a temporary constructions touching the main structure. Rest of the entry were all marked as below ground.

Determining Cost / Benefit Ratio

A single pest control operator making use of termite baiting as his principle method agreed to take part in a survey to determine the economics of termite baiting system. Data was gathered on a number of parameters to check the cost benefit ratio of running a termite baiting system.

RESULTS AND DISCUSSION

High Prevalence of Termite

The survey covering 80 individual buildings in the central business district of Manila revealed 42.5 % buildings had some type of termite infestation either in the past or having an existing infestation. Seventy two (72) buildings out of the 80 reported that they have undertaken termite control action both in the past and in the present, to contain termite infestation.

The results of the experiment to determine the prevalence of termite activity in soil is summarised in Table 1. The data presented show 20% of the installed in ground baiting station intercepted termite colonies in 3 months. The major species in the soil was *Macrotermes gilvus* followed by *Microcerotermes losbanosensis* and *Coptotermes gestroi*.

Table 1. Interception of termite species by using In-ground Bait stations (IGS).

Number of IGS	**No interception**	***Coptotermes gestroi***	***Microcerotermes losbanosensis***	***Macrotermes gilvus***	***Nasutitermes luzonicus***
500	80%	1%	7%	11.8%	0.2%

The survey conducted on 450 infested structures showed *Coptotermes gestroi* as the major termite species. The number was significantly higher than other species. Also this species was significantly more common in urban areas compared to suburban areas, where there is more number of infestations from higher group of termite species (Table 2). Analysis also show structures located in suburban areas have significantly higher number of above ground entry compared to urban areas. Consequently structures in urban areas have significantly higher underground entry compared to suburban areas.

Cost / Benefit Ratio of Termite Baiting

The various parameters determinant to run a termite baiting system is shown in Table 3. The total business of the company using baiting for 2012 was recorded to be PHP 6 million. Cost of labour was computed to be 12.5%. After deducting all expenses the net profit from this venture is calculated to 43.75 %.

Table 2. Termite species, location and entry method for 450 infested structures.

Parameters	**Urban**	**Suburban**	**p value**
Species			<0.0001
Higher species	67	95	
Lower species	226	62	
Method of Entry			<0.0001
Above Ground	39	123	
Below Ground	254	34	

Table 3. Cost of termite baiting for a single pest control practitioner for 2012.

Itemized incomes	**Itemized costs**	**Itemized Amount collected (in Peso)**
New Linear Meters (LM) of business sold in 2012		4,800,000
Linear Meters Renewed from previous years		1,200,000
Gross Business from Termite Baiting for 2012		***6,000,000***
	Cost of Products	2,200,000
	Cost of Labour (2 teams with 2 technicians, working 8 hours a day, 5 days a week)	750,000
	Annual cost of Fuel/Car maintenance (covering an average of 100 km a day for the 2 teams)	325,000
	Others Expenses	100,000
	Total Expenditure	***3,375,000***

Since the removal of organochlorine compounds, long term termite control of structures relied on continuous maintenance and monitoring. It became imperative that termite management program need sustainable methods which will combine cost, termite elimination and environmental concerns together. Termite baiting soon found its position in the market as it combined all three concerns. But soon reports surfaced that baiting is not cost efficient and less profitable, in addition to it being slow acting and the method of treatment does not leave residual effect on the treated zone. Most of these concerns were expressed in North American termite control market. However termite baiting found favour and became popular in Asian cities such as Manila due a number of inherent characteristics of the market. Manila a metropolis with its dense population of 43,079 inhabitants per km2 presents a large concentrated termite market for practitioners. To keep up the growing population, buildings are regularly constructed. As an example the National Statistical Office (NSO, 2013) reported 13,378 approved building permits for residential and non-residential structures for the year 2011 in the national capital region of Manila. This number is 11.9 % of the total number of approved building permits for the country. The number also constitutes an estimated 6.3 million square meters of floor area which represents 29.5% of the total floor area for the country. Added to this total would be another estimated 2 million square meter of approved building permits for commercial and industrial types (NSO, 2013). Consequently Manila has become a focal place to run a termite control business due to favourable economics. Surveys have shown buildings are being built without specific building codes necessary for preventing termite infestations, in spite of high prevalence (Table 1). Studies have shown that a quality concrete slab can work as barrier against termite and in its absence structures are vulnerable to infestation (Schafer and Guirguis, 2003). Surveys by this author has found absence of quality concrete slab or using underspecified concrete slabs, thus increasing the probability of termite entry into buildings through future cracks. Also lesser use of a slab-on-ground floor types and preference for sectional slabs for residences adds up the chances for gaps and termite entries. Use of non-resistant timber in structures and decorations such as baseboards and wall cabinets are also a reason for initiating infestations.

It is noticeable that in Asian scenario customers call for termite control when a live infestation is detected by them. Very less interest is shown by the customer for prevention or using a termite management in the absence of termite infestation. Presence of active colony helps the process of baiting as the practitioners instantaneously install an above ground baiting station and drastically reduces the time for colony elimination by ensuring quick dispersal of active ingredient into the colony. Baiting is an attractive form of treatment for the existing colony and favour practitioners by saving time.

Prevalence of *Coptotermes* as a major urban species infesting buildings in urban Manila also favour the use of termite baits (Table 2). The species presents a unique advantage as they feed easily on processed cellulosic bait and can be suppressed and eliminated quickly from a given structure. Amount of bait consumed by the species is also significantly less compared to other species, thus making the work of a practitioner economical. Studies by the author have shown that structural activity of *Coptotermes gestroi,* the dominant urban termite in the Philippines, can be suppressed and eliminated in 12 weeks. Further evidence of this observation is supported by studies conducted by Garcia et al. (2007). The time could be further reduced by replenishing the bait more frequently, compared to the conventional method of once in a month which is generally practiced by practitioners. Colonies of higher termites may however take more time (Dhang, 2011).

It is true that the process of baiting does not leave a residual effect in the soil as compared to soil treatment. This is a significant disadvantage. To overcome this gap a monthly monitoring of the structure is undertaken by the pest control operators in running a baiting program which provide the much needed replacement for residual effect needed in termite management. Realistically it is accepted in the local industry that it is near impossible to create a full proof chemical barrier around a structure knowing termites could use a number of strategies to penetrate. However a monthly monitoring performed under termite baiting program based on inspecting of all in ground stations, collecting client feedbacks and check on alteration to the structure provides a better option to keep a check on the termite activities and longer protection.

As the on-going structural protection depends upon diligent monitoring for new evidence of termites in the future, it is often thought such program would be expensive to run. The cost benefit ratio as presented in Table 3 clearly shows that practitioners with large number of customers could easily restrict the cost of labour to 12.5% in running a baiting program. Similarly practitioners with limited customers have made the baiting profitable by focusing their business in a specific zones or areas of the city. This significantly cuts down cost of labour and time.

New generation practitioners have realised that treating termite colonies is the best method to eliminate the pest from structures. This realization has helped popularity of termite baits and non-repellent chemicals. However baits allow quick and sure dispersal of active ingredient into the colony compared to non-repellent termiticides. Baits are specifically added in aggregation devices either installed over an active site or installed in the ground. This makes the process more precise and prevents overuse of chemicals in urban environment.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

SEWER BAITING FOR RATS IN THE UNITED KINGDOM – IS IT MONEY DOWN THE DRAIN?

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Abstract In November 2000, Water UK (a representative organisation which brings together all the water and waste water utilities) and the Local Government Association (A national voice for local government in the UK), published the National Sewer Baiting Protocol which listed a number of principles that Water and Sewerage Companies (WASC) and local authorities (LAs) were expected to follow when undertaking sewer baiting programmes. The protocol was intended to provide LAs and WASC with a basis for closer working relationships, with the aim of streamlining spending whilst tacking the issue of rats in sewer networks; preventing ineffective spending and inefficient treatments. The National Pest Advisory Panel (NPAP) (a panel of experts offering advice and guidance to the Chartered Institute of Environmental Health (CIEH)) strongly support the suggestion that Control of rats in sewers and drains is an essential part of any rat treatment on the surface. NPAP sent a detailed survey to all LAs in England, Wales and Northern Ireland in 2002 (n=406) and again in 2012 (n=368), exploring their approaches to sewer baiting pest management in order to monitor the LAs and WASC sewer baiting activities to assess how these may have changed over time, and highlighting if baiting activities are effective and efficient.
Key words Commensal rodents, National Sewer Baiting Protocol, public health.

INTRODUCTION

The control of rats in urban environments involves more than just dealing with above ground rodent populations. Sewers provide a protective and stable environment for rats with an underground habitat free from predators where they have the freedom to live and breed (Brooks 1962). The brown rat, *Rattus norvegicus,* or sewer rat is thought to prefer living underground but when above ground will look to inhabit areas that provide protection and shelter (Brooks, 1962 and Illinois Department of public health)

Sewers are historically considered to be the main reservoir for rats (Brooks 1962, CIEH, 2003) with signs of rats above ground being an indication of possible increased population numbers below ground. Research carried out by Battersby et al. (2002) and Bonnefoy et al. (2008) reports that over half of surface rodent infestations in the urban environment are connected to defects in sewers. Staff within the local authorities (LAs) and Water and Sewerage Companies (WASC) have a detailed knowledge and understanding of rodent behaviour and the impact sewer rat populations have on society and public health (Battersby, 2004). Sewer rats are cunning and will use defects in a sewer as a way of finding an escape into the above ground environment putting public health at greater risk. Controlling rat populations and maintaining a high standard of maintenance of the sewerage networks can greatly reduce this risk to public health. (Musa and Cheong, 2004, International Pest Control, 2013)

Successful sewer baiting activity relies on effective integrated pest management and monitoring of the environment. Proactive monitoring, maintenance of the sewer systems and baiting could minimise the public's need for rat treatment requests (CIEH/NPAP, 2013). In the past, LAs utilised proactive baiting to control the rat populations living below ground in sewers. The objective of the 2000 National Sewer Baiting Protocol was to facilitate improved co-operation and communication between LAs and WASC. Seven key points were made covering areas of information sharing regarding new baiting activities; facilitating success of rodent controls in sewers and jointly reviewing the LAs and WASC operations and sharing information regarding costs. Reports to CIEH/NPAP from numerous LAs pest liaison groups (voluntary groups whose members comprise of pest control department operatives, supervisors and managers from local authorities across the UK) noted that the implementation of the 2000 protocol appeared to be patchy and in some areas, was alleged to have been ignored completely. This feedback strongly advocated the need for refinements to the protocol and for the introduction of an operational guidance document on sewer baiting treatments which should include training and qualifications of pest control technicians; health and safety aspects; treatment methodology. This document is available at www.urbanpestsbook.com (CIEH/NPAP, 2013; Murphy and Oldbury, 2002).

METHODS

The National Pest Advisory Panel, established in 2001 sent out a comprehensive survey to all local authorities in UK in 2002 (n=406), with a response rate of 64% (n=263). The survey explored LAs and WASC sewer baiting activities in relation to the 2000 protocol, in order to provide a baseline of sewer baiting services provided. This survey was repeated in 2012 (n=368) with a response rate of 41% (n=151), in order to gauge a 10 year picture of the LAs and WASC communications and workings in relation to the 2000 protocol, the change to legislation in October 2011 now making WASCs responsible for lateral drains and private sewers, and how austerity measures of 2010 with an impact of 28% cuts in public funding within local authorities, may have impacted on the provision of sewer baiting and rodent control measures by LAs and WASCs.

RESULTS AND DISCUSSION

In 1989 the 10 publicly owned water and sewerage authorities in the UK were privatised and became Water and Sewerage Companies (WASC), now individually and independently managed. Following privatisation the responsibility for undertaking treatments to control rat in sewers and funding of sewer baiting activities became unclear. However, the 2000 National Sewer Baiting Protocol states, where possible sewer baiting and baiting to combat rat infestation should be undertaken in a complementary manner by agreement between the Water UK member and the local authority (OFWAT). The recent austerity measures and financial pressures placed on LAs, and the expectations of joint financing for sewer baiting activities may be adding unnecessary pressure to LAs in achieving their legal responsibility of rodents control in their districts. (PDPA, 1949)

Respondents provided figures with regards to their LAs and WASC spent on sewer baiting per annum. Table 1 details the average spent for 2002 and 2012 by each contributing party. Between 2002 and 2012 there has been a £2,566 reduction in spending for sewer baiting on behalf of the LAs per annum with an increase of £3,796 spent on sewer baiting from the WASC per annum.

Table 1. Average spent on sewer baiting by local authorities and Water and Sewerage Companies in 2002, 2012.

	2002	2012	Difference spend between the years:
LAs average Spent per annum	£10,295	£7,729	- £2,566
WASC average Spent per annum	£7,447	£11,243	+ £3,796

Proactively baiting in sewers helps to actively monitor and control sewer rat populations, preventing them from becoming an above ground problem using integrated pest management (Murphy and Oldbury, 2002). Many debates have surrounded the effectiveness of proactive baiting in sewers compared with reactive baiting in sewers. It is believed WASCs opt for reactive baiting of sewers in the belief that it is more cost-effective (CIEH, 2003), Murphy and Oldbury (2002) state, reactive sewer baiting did little to actually manage rat populations or solve localised problems. The CIEH believes that proactive sewer baiting is the most cost-effective method for the control of rats in sewers, and in the past local authorities have utilised proactive baiting activities to control these sewer rat populations. Since privatisation, the WASC are believed to have taken sewer baiting back under their control in many geographical locations, and with this, are thought to have an increasing tendency for only reactive baiting to be carried out by the LAs (CIEH/NPAP, 2013). When asked which baiting technique best describes the one used by their LA; the data highlighted a 4% drop in the number of LAs in 2012 (21.5%) who carry out proactive sewer baiting techniques compared with 2002 (25.5%). The results showed there had been an increase of 3% in the number of LAs who stated they provide only reactive baiting treatments in 2012 (42%), compared with that of the 2002 (39%) data. Some authorities provided both reactive and proactive baiting of the sewers, between the 2002 (25.5%) and 2012 (36.5%) there had been a 1% increase in the number of respondents who now conduct their sewer baiting service this way (the percentage split between proactive and reactive for this option was not questioned.).

Table 2. Is your liaison with the Water and Sewerage Companies on sewer baiting?

	2002	2012	Percentage differences
Regular: (Planned or when necessary)	35%	45%	+10%
Irregular: (Irregular or never)	65%	55%	-10%

The 2000 Water UK protocol was designed with the aim that communication and co-ordination between LAs and WASC would become more streamlined. Table 2 identifies the LAs respondent's liaison activities with their WASC. One of the protocol points states, Water UK members and local authorities should jointly review on a regular basis their operation of this protocol with a view to improvement. In 2002 65% of LAs respondents reported they met on an irregular basis with some advising never liaising with their WASC, this fell by 10% in 2012 (55%). Between 2002 (35%) and 2012 (45%), Regular planned meetings had seen a 10% increase in the number of LA respondents who liaise with their WASCs whether it be on a planned or when necessary basis. OFWAT (The water service regulation authority in the UK) recommend regular operational meetings in order to maintain treatment efficiencies.

LAs have a responsibility to ensure the control of rat activity in their district (PDPA, 1949). Correlating rat activity at the manhole means LAs can communicate information back to the WASC and map hotspot areas for rodent activity above ground in order to gain control. The survey asked respondents if they record the infestations found at the manhole. Sixty eight percent of the LAs respondents in 2002 advised they do not record this data with this seeing a significant fall of 7% in 2012 (61%) (P=0.003), resulting in more LAs now recording this data. Keeping a record of infestations found at the manhole helps builds a local knowledge of rat population hotspots. Highlighting rat population hotspots at a manhole helps to build the knowledge of heavily infested areas, defects in sewers and aid effective management and control of rat populations above ground linked to sewerage networks below ground.

CONCLUSION

The history of sewer baiting has been one of good intention with the launch of the 2000 National Sewer Baiting Protocol. However, the information within the protocol seems to be limited when it looks at how its expectations are to be achieved by the intended parties. There is no specific funding programme in place for WASC, with individual WASC choosing what actions they will take in order to control rats in sewers, with limited knowledge this emphasises the need for LAs input to sewer baiting activities. The research highlights the lack of joint working, co-operation and communication between LAs and WASC which could greatly contribute to increased sewer rat populations reaching the surface as a result of reactive rather than proactive monitoring. The revised National Sewer Baiting Protocol, Best Practice and Guidance Document by NPAP 2013 published by Chartered Institute of Environmental Health, highlights the importance of adopting best practice, sound strategies and sewer baiting techniques in order to achieve effective rat control in the sewers. The only way to successfully control rat populations in sewers is the provision of effective maintenance of the sewerage network infrastructure together with proactive sewer baiting programmes. As with other initiatives, this will require adequate funding and communication between those responsible for the sewers and those with geographical knowledge and public health protection responsibilities. Controlling rat populations in the sewer networks across the UK will ultimately reduce the need for second generation anticoagulant rodenticides (SGAR) used to control some above ground rodent populations. Identifying defects in the sewer networks will have a profound impact in reducing the number of sewer rat populations reaching the surface. This in turn will reduce the need to use SGARs and the risk of secondary poisoning to non-target species; something which is constantly monitored by European Union.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

MUS MUSCULUS DOMESTICUS CARRYING VKORC1^{SPR} AND SUSCEPTIBILITY TO RODENTICIDES

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Abstract This paper describes the control of a mice infestation of a bakery in Lyon (France). Where other anticoagulant molecules used for the treatment of the situation failed, the use of difethialone grain showed a great efficacy in the control of this population. Live mice captured on the site where genetically tested and appeared to be *M. musculus domesticus* species with a *M. spretus Vkorc1* gene naturally introgressed. Susceptibility to AVKs of VKORC1^{spr} was *in vitro* evaluated by expressing recombinant enzymes in yeasts. The use of this *in vitro* yeast system is of great interest for monitoring the anticoagulant activity of VKORC1 in AVK resistant rodents and for the development and the evaluation of new anticoagulants molecules.
Key words Anticoagulant rodenticides, vitamin K.

INTRODUCTION

Rodent population management (rats and mice) should be based on different rules such as hygiene, natural behavior, environment structure… but also implies to use chemicals. Anti Vitamin K anticoagulants (AVKs) have been, for more than 50 years in Western Europe, the principal molecules used. Rodent populations adaptability and natural selection pressure involved animal's natural adaptation, and the development of resistance strategies, making these molecules less and less efficient. Among resistance mechanisms, the one called target resistance is the most frequent. The AVKs are inhibitors of the Vitamin K cycle by inhibiting the mechanism of recycling Vitamin K through their action on the Vitamin K epoxyde reductase.The gene responsible for this enzyme synthesis, *Vkorc1*, and the corresponding protein were described in 2004 (Rost et al.. 2004). AVKs are strong inhibitors of the enzyme activity of VKORC1 protein and as a consequence of the Vitamin K cycle. Inhibiting this cycle that ensure the production of clotting factors (IX, X, VII and II) involves lethal bleeding.

Mutations of this gene were found on resistant rodents (Pelz et al. 2005), but the *in vivo* proof of a *Vkorc1* mutation for the resistant phenotype was demonstrated by introgressing the Y139F mutation in wild strain of rodents (Grandemange et al 2009) which led to rodents with a resistant phenotype. Different mutations of this gene were reported on mice (Pelz et al.. 2012; Pelz et al., 2005; Rost et al., 2009). Three mutations or mutation groups seems to be particularly frequent: L128S, Y139C and a set of 3 or 4 mutations Arg12Trp / Ala26Ser / Ala48Thr and / or Arg61Leu. This set of 3 or 4 mutations seems to be the consequence of the adaptive introgression of *Vkorc1* gene from *Mus spretus* into the genome of *Mus musculus* (Song et al., 2011). An initial crossing between these two mice species might have occurred in their cohabitation area (North Africa or Spain). Descendants should have mated with *Mus musculus* and acquired the *Vkorc1* gene of the *Mus spretus* strain (*Vkorc1*^{spr}). These mice (*Mus* *musculus* with *Vkorc1*^{spr}) were found in Spain and Germany, but not in Italy or Greece. Concerning France that is half way between Spain and Germany, no information is available concerning the existence of this genotype.

This study describes the presence of such this type of mice in the Lyon (France) area. An efficacy field trial of difethialone on a site where only homozygote animals were found was set up. It was interesting to describe more in details the resistance induced by this genotype by expressing the recombinant VKORC1^{spr} in yeasts and analyzing this specific protein catalytic properties toward the action of AVKs used for mice pest control.

MATERIALS AND METHODS

Sequencing of *Vkorc1* and *Cyp b* Gene

Mice were trapped from various sites located in Lyon (France) or around Lyon. Genomic DNA was extracted from mice tails using Nucleospin Tissue kit (Macherey Nagel) according to the manufacturer's recommendations. *Vkorc1* gene was amplified by PCR using two specific primers sets: F1 $^{5'}$GATTCTTCCCTCCTGTCC$^{3'}$ and R1727 $^{5'}$AGACCCTGTCTCAAAACCTA$^{3'}$, and F1252 $^{5'}$GAAAGCAGAACACTTAGCAGG$^{3'}$ and R2512 $^{5'}$AACCAACAGCAGAATGCAGCC$^{3'}$.

PCR products were sequenced (Biofidal, Vaulx en Velin, France). *Cyt b* gene was amplified by PCR using specific forward $^{5'}$TCTCCATTTCTGGTTTACAAGAC$^{3'}$ and reverse $^{5'}$AACAATGACATGAAAAATCATC GTT$^{3'}$ primers.

In vitro Characterization of Resistance

Recombinant VKORC1 enzymes were expressed in *Pichia pastoris* as described previously by Hodroge et al. (2011, 2012). Microsomal fractions containing recombinant hVKORC1 proteins prepared as described previously (Hodroge et al., 2011, 2012) were used to determine the susceptibility of VKORC1 proteins to rodenticides. VKOR activity and reaction products analysis by liquid chromatography-mass spectrometry were performed as reported (Hodroge et al., 2011, 2012).

Figure 1. Live mice trapping localization in the neighborhood of Lyon

Field Trial

The field trial was performed in a bakery invaded by mice which is localized in the sixth district of Lyon. Thirty baiting boxes containing wheat (with difethialone 25 ppm) were distributed in the bakery. Consumption was evaluated every 3 days and dead mice were collected for genetic analysis. The baiting boxes were refilled every 3 days until the consumption was null. The field trial lasted 23 days.

RESULTS AND DISCUSSION

Trapping of Mice

Alive and dead mice were caught in Lyon and around (Fig. 1). In Lyon, 12 mice were captured in the "Parc de la Tête d'Or" and 18 mice were captured from the above mentioned bakery of the sixth Lyon's district. In the Lyon neighborhood (i.e. in a 50 km radius from downtown Lyon), 9 mice were trapped in Savigny (in 40 km on the West of Lyon); 5 mice were trapped in Saint-Cyr-le-Chatoux (in 50 km on the northwest of Lyon); 6 mice were trapped in Pontcharra-sur-Turdine (in 40 km on the northwest of Lyon).

Characterization of the Species

It's quite impossible to precisely determine the mice species on the basis of morphological analysis. Although the Algerian mouse *Mus spretus* known to be endemic in the South of France is considered as a small mouse with a short tail, it is very easy to confuse an Algerian mouse with a house mouse *M musculus domesticus*. In order to determine the species of the mice trapped in Lyon area, *Cyt b* gene was amplified by PCR from the mice tail-extracted genomic DNA and sequenced. *Cyt b* mitochondrial DNA gene is frequently used for phylogenetic analysis of organisms. The comparison of the sequences of the PCR products with databases allowed us to conclude that all the 49 mice trapped in and around Lyon were all mice belonging to the sub-specie *M. musculus domesticus*. While numerous pest control operators reported the presence in Lyon of small mice, the result obtained herein suggests that in spite of the global warming the Algerian mouse *Mus spretus* distribution remains limited to the South of France.

Sequencing of *Vkorc1* Gene

In order to detect a possible resistance to rodenticides due to VKORC1 mutations, the *Vkorc1* gene was amplified by PCR from the mice tail-extracted genomic DNA and sequenced. Mice captured in Pontcharra showed the previously described L128S and Y139C mutations (Pelz et al., 2005; Rost et al., 2009). The sequence analysis of all three coding exons from mice captured in Lyon ("Parc de la Tête d'Or" and Bakery), Savigny and Saint-Cyr-le-Chatoux, revealed the presence of mutations in five nucleotides positions as compared to the published *M. musculus domesticus Vkorc1* gene. Four mutations are located in exon 1 (C34T, G76T, A111G, G142A) and one is located in exon 2 (G182T). The A111G mutation did not result in amino acid substitution (silent mutation E37E); the 3 others led to amino acid substitutions. Arginine-12 was found to be substituted by tryptophane (Arg12Trp), alanine-26 by serine (Ala26Ser), alanine-48 by threonine (Ala48Thr) and arginine-61 by leucine (Arg61Leu). Out the 44 mice carrying these four mutations, 41 were homozygous for all these mutations and only three, from Saint-Cyr-le-Chatoux, were heterozygous for all these five mutations. These mutations were reported by Pelz et al. (2005) and Rost et al. (2009). Shortly after, Song et al. (2011) described that these four non-synonymous mutations were introduced into the *M. musculus domesticus* genome by an adaptive introgressed hybridization with *M. spretus* (*i.e*., the naturally occurring process including inter-specific hybridation followed by generations of backcrossing and selection of introgressed alleles). Indeed, *M. spretus* VKORC1 contained tryptophane, serine, threonine and leucine in amino acid positions 12, 26, 48 and 61 respectively. *M. musculus domesticus* carrying the complete or partial *Vkorc1* allele of *M. spretus* (*Vkorc1^{spr}*) were observed in Spain and Germany (Song et al., 2011).

Consequences of the Susceptibility to Rodenticides

To evaluate the consequences of the introduction of the four mutations Arg12Trp, Ala26Ser, Ala48Thr and Arg61leu into the *M. musculus domesticus Vkorc1* gene on the susceptibility to rodenticides, we expressed recombinant wild type VKORC1^{dom} and VKORC1^{spr} enzymes as a c-myc fused recombinant protein in *P. pastoris* and determined for both enzymes the inhibition constants (Ki) towards various rodenticide molecules. Whatever the enzyme we analyzed, all the molecules inhibited the VKOR activity in a non-competitive manner (data not shown). K_i values obtained for VKORC1^{dom} and VKORC1^{spr} enzymes are reported in Table 1. For VKORC1^{spr}, K_i values obtained towards the first generation rodenticides (i.e., warfarin, coumatetralyl, chlorophacinone) used in this study were dramatically increased (~80 to 140-fold) compared to those obtained for VKORC1^{dom}. Towards bromadiolone and the second generation molecules (i.e., difenacoum, difethialone), K_i obtained for VKORC1^{spr} were also increased compared to VKORC1^{dom}. Nevertheless this increase was more moderated compared to the first generation molecules. Taking together, these results suggest that mice carrying *Vkorc1^{spr}* gene must be more resistant to rodenticides than mice carrying *Vkorc1^{dom}*. The use of second generation molecules must be preferred in the presence of mice populations carrying *Vkorc1^{spr}*. This is coherent with the results obtained by Song et al. (2011). Indeed, they demonstrated that 80%, 91% and 20% of *M. musculus domesticus* carrying *Vkorc1^{spr}* survived coumatetralyl, bromadiolone and difenacoum trials, respectively.

Table 1: Ki values towards various rodenticides for VKORC1^{dom} and VKORC1^{spr} and resistance factor to rodenticides of VKORC1^{spr} comparatively to VKORC1^{dom}

	K*i* (µM) VKORC1^{dom}	**K*i* (µM) VKORC1^{spr}**	**Resistance factor**
Warfarin	1.1	97	88
Coumatetralyl	0.14	20	137
Chlorophacinone	0.16	12	73
Bromadiolone	0.15	1.4	7
Difenacoum	0.17	0.8	5
Difethialone	0.05	0.2	5

Field Trial

In order to assess the efficiency of second generation molecules, especially difethialone for the control of mice populations, we performed a field trial on the mice infested bakery located in Lyon.

Reocurring problems of mice infestation were observed in this bakery for several years, with numerous unsuccessful attempts to control them. Infestation signs where important at the beginning of the treatment (droppings, stains, urine smell, dead bodies found…). Control attempts where there, in particular baiting box and old baits contained brodifacoum (blocks, wrapped soft baits, injected soft bait).

About 30 bait boxes with difethialon wheat tech (25 ppm) where positioned in the bakery and its basements. The total consumption rose until 885g / 5 day after only 5 days of baiting. Consumption started to decrease until nothing after 23 days (see Fig 2). All the dead mice found during this baiting period where collected to run analysis and sequencing of *Vkorc1*. All the mice found were homozygous for *Vkorc1^spr^*. These field results show that the second generation rodenticide Difethialone remains, inspite of resistance due to *Vkorc1^spr^*, efficient to control *Vkorc1^spr^* mice populations.

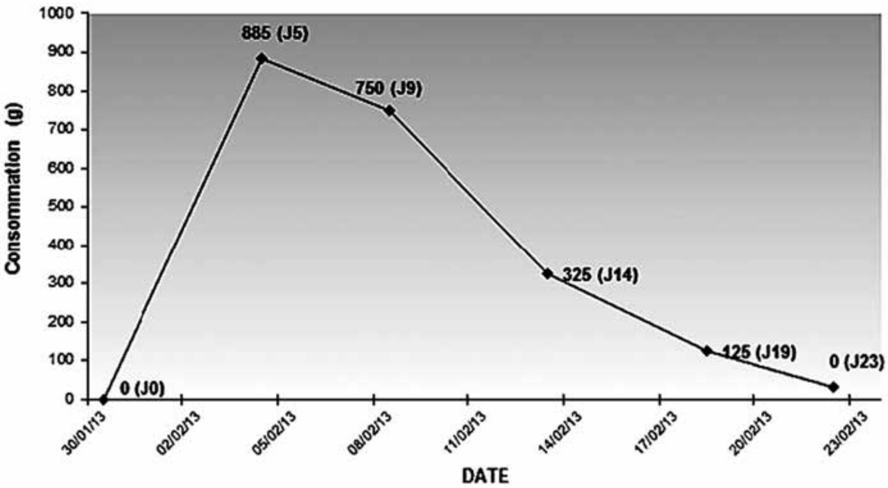

Figure 2. Daily Difethialon Wheat Tech consumption in the Lyon bakery

CONCLUSION

The present study highlighted that despite *Mus spretus* were not found in Lyon (France), evidence of natural gene introgression of *Vkorc1^spr^* into *Mus musculus* was found, due to natural cross breeding in natural conditions. The *in vitro* analysis of VKORC1^spr^ involves a more important resistance factor to Antivitamin K anticoagulants, especially for first generation anticoagulants, but also for second generation anticoagulants. Despite this resistance factor, field trials showed that the control is possible with difethialone, when correctly applied in the right conditions. The next step will be to confirm the *in vitro* results of K*i* values with live mice from the lab and *in vivo* results. That way all the anticoagulants will be screened and better control program will be issued.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

QUEENSLAND'S NEWEST INVASION: FERAL URBAN DEER

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Abstract Deer are not endemic to Australia and, until recent decades, were not a major component of the landscape. With the collapse of deer farming in the 1990s, subsequent deliberate release and translocation of deer from defunct farms, feral deer numbers are increasing dramatically. Historically, only urban centres associated with Royal National Park (New South Wales) have had to contend with urban feral populations. Recently deer have begun to encroach into other urban areas, including South Eastern Queensland. We interviewed stakeholders associated with urban deer management and concluded that awareness was poor there was a need to strategically deal with this emerging issue.
Key words Stakeholder knowledge, introduced deer.

INTRODUCTION

Deer are not endemic to Australia. They were first introduced by acclimatisation societies in the 18th century (Groves and Bishop, 1989) for game hunting and aesthetics. The practice of release continued into 20th century (Firth, 1973). Of the 18 species introduced, six (*Axis axis chital, Axis porcinus,* hog, *Cervus elaphus, red, Cervus timoriensis,* rusa, *Rusa unicolor,* sambar, and *Dama dama,* fellow) formed feral populations (Strahan, 1995). Four (fellow, red, rusa, chital) have established in Queensland (DAFF, 2012). Moriarty (2004a) reported that of the 200,000 feral deer in Australia, 14 herds (two in Queensland), some with in excess of 10,000 deer emerged from these releases. They represent 87% of the Australia's feral deer.

Deer have been farmed in Australia since 1803 but until recent decades all feral deer herds originated from acclimatisation society releases. The deer industry, based on captive animals, was modest until the 1970s/1980s when its popularity exploded with a 'massive' increase in farmed stock. However, the boom was short-lived. The industry collapsed in the 1990s due largely to low commodity prices (MacDonald, 1995; Moriarty, 2004 a, b). Although always a trickle of escapes, the collapse of deer farming resulted in the release of many deer on site. These releases have added 10 herds in Queensland, typically of < 500 individuals.

Although illegal throughout Australia, deer from defunct farms are translocated into bushland for game hunting. Many of these joined existing herds (descendants of earlier releases) or formed the basis of new ones (Low, 1999; Moriarty, 2004a). This has added an additional 20 feral herds in Queensland, typically of < 500 individuals.

Feral deer have formed 96 herds in New South Wales (NSW), compared to 32 in Queensland, and are a recognised issue in urban areas abutting Royal National Park (e.g., Southern Sydney, Moriarty, 2004a; Wollongong, WCC, 2013). In contrast, urban feral deer is just becoming an urban issue. This is because there are expanding herds throughout much of the State (Moriarty, 2004a), many of which have the potential to encroach into urban centres (Moriarity, 2004a). For example, recently thousands of feral deer have invaded Brisbane suburbs (McCathy, 2013). In parallel, with the expansion of herds (numbers and distribution), the human urban population continues to increase and expand its footprint (ABS, 2013). These parallel expansions will inevitably increasingly overlap.

Unlike the United States of America (Conover et al., 1995; Cornicelli et al., 1996), urban deer are not on the 'radar' as an issue in Queensland. However, based on the current indications (McCathy, 2013) and the known pattern of increase of deer (Jesser, 2005) and humans (ABS, 2013), we consider that strategically the development of policy/legislative instruments is required before the agenda is driven by necessity. A first step is to assess current awareness of urban feral deer. This is the aim of our paper. We report here on interviews with the most influential Queensland stakeholders, and compare the results with NSW counterparts, the only Australian State with an acknowledged urban feral deer problem.

MATERIALS AND METHODS

Those interviewed to determine awareness of urban feral deer were not chosen randomly because even among our academic environmental colleagues the issue had not surfaced. We therefore deliberately focused on individuals most likely to be appointed to a government advisory group to develop policy. Omission of any stakeholder group was because there was no representative identifiable. Telephone interviews were conducted in late March/early April, 2013. Respondents encompassed State and local governments, the community (environmental activists) and deer researchers.

RESULTS AND DISCUSSION

Themes explored, and the major points distilled from the respondents are listed in Table 1. Respondents considered community views of deer were polarised. Estimates of 20–50% were considered to view deer positively, the rest of the population considered negative. One researcher only commented on deer as an 'important hunting resource'. The only previous Queensland survey focused on rural producers - 50% had a positive attitude to deer on their land, 64% a saw them as a hunting resource, 39% a pest (Finch and Baxter, 2007). These data supported the view of polarisation.

Table 1. Question themes and response of representatives from governments (State (1) and local (2)), community (3) and researchers (4) from the Sydney (New South Wales) and Brisbane (Queensland), the areas of largest human population in areas of expanding deer populations

	General attitude *(Brisbane)*	General attitude *(Sydney)*
1.	Polarised (negative/'Bambi factor', unaffected – ambivalent)	Polarised (80% negative/20% positive)
2.	Polarised (50/50 - love/hate)	Not aware of any issue
3.	Asthetically attractive animal, not a pest, not feral but native	Polarised (Majority negative vs minority positive)
4.	Polarized (50/50 - love/hate), hate due to vehicle collisions	Hunting resource, large populations in Royal National Park

	Population changes *(Brisbane)*	Population changes *(Sydney)*
1.	Increasing	Increasing (Northern NSW)
2.	Increasing (estimation problems)	'No idea'
3.	No awareness, rare in urban areas, seldom in media	Increasing in numbers
4.	Increasing (especially number of males)	Increasing in numbers and species
	Deer impacts *(Brisbane)*	
1.	Damages to gardens, lawns, fruit trees, farm property damages, grazing turf, sorghum	
2.	Overgrazing, damage native vegetation, transmit diseases browse ornamentals, cause soil erosion, water pollution, vehicle collisions	
3.	No general knowledge of deer in urban areas, but damage private properties and vehicle collisions (need warning signs on roads)	
4.	Vehicle collisions (30 in 3 years), grazing/browsing, preventing spread of native species	
	Deer impacts *(Sydney)*	
1.	Vehicle collisions (2 lethal), disturbance in urban areas, damage in Royal National Park	
2.	No awareness of damage on private properties in urban areas	
3.	Agricultural and native vegetation damages, vehicle collisions, rare private property damage	
4.	Major issue is deer-vehicle collisions	

Most considered deer numbers were increasing, although a minority had 'no idea'. The latter comment was justified with 'deer are rarely observed in urban areas and seldom in the media'. Several of the Queensland-based respondents spoke of issues in Northern NSW but not South Eastern Queensland, implying they did not consider deer an issue in their jurisdiction. With one exception deer-vehicle collision was identified as an issue, although whether perceived as an urban issue was unclear because, despite the interviewer's focus on urban deer, respondents tended to concentrate on non-urban issues, such as competition with cattle, grazing on native vegetation, damage to national parks, preventing spread of native species, although damage to urban gardens was discussed. Another indication that respondents often failed to focus on urban deer, were the offering of views on hunting, landholders on large properties, although others also acknowledged that shooting was inappropriate or difficult in urban areas. There was, comment that local governments carried out control; for example, by trapping in urban areas. Several demonstrated a lack of knowledge of urban deer: not aware of any issue, no knowledge of urban deer. However, despite a claim that deer were seldom a media topic, one respondent reported that, in Queensland, culls after major fires (2001) failed due to a media campaign. (*Table 2.)*

The answers to questions directed at urban deer management were also confused with management in rural areas: landholders of large properties, need for different treatment of long-established and farm escapes; difficulties of finding sufficient recreational shooters. One respondent had no idea how to manage urban populations, another considered the solution was trapping, which occurs on request in much of urban SE Queensland, although it was stated that trapping and relocation had no effect on the overall population's size. Indeed, most respondents that explicitly referenced urban deer management included (or implied) the need for increased shooting/trapping to reduce pest numbers. One respondent identified the need to identify strategies to reduce urban populations. To deter deer from urban gardens, repellents applied to plants preferred by deer was suggested. Other suggestions included householders keeping gardens garbage free, and although acknowledged as costly, exclusion fences were suggested as was use of ‘urban bow hunting’ instead of guns.

Table 2. (Continuation) Question themes and response of representatives from governments (State (1) and local (2)), community (3) and researchers (4) from the Sydney (New South Wales) and Brisbane (Queensland), the areas of largest human population in areas of expanding deer populations

	Management *(Brisbane)*
1.	Declared pest species (landholders of large properties control deer population densities), in urban areas difficult to manage (local governments carry out control – trapping)
2.	Joint planning/managing of urban/suburban landscapes. Who is responsible for deer management? Lethal control: trapping, shooting (trapping is successful in Gold Coast). Relocation: no effect on population size (animal welfare), exclosures: fence building too expensive
3.	No understanding on how to manage urban populations, and it is costly to recruit hunters
4.	Shooting (impossible in urban areas), trapping (in Brisbane City/Moreton Bay local governments trap deer on request)
	Management *(Sydney)*
1.	Long-established herds and farm escapes perceive differently (impact monitoring). Population reduction strategies in urban areas, residents must keep areas garbage free, fencing in high density urban areas. Increase hunter numbers. Urban bow hunting?
2.	Interest conflict - community positive attitude, state government desire removal of deer, local government not aware of current situation. Deer much lower priority than other feral animals
3.	Conflict among stakeholders (e.g., after 2001 fire, attempts to reduce deer numbers failed due to media campaign against reduction. Drivers should be aware they are in deer territory. Difficult to find enough recreational shooters.
4.	Trapping/shooting, recreational hunting licenses increase, use repellents (e.g., chilli on prefered forage in urban gardens)

It was acknowledged that urban deer management was problematic. Development of joint planning/urban management instruments were suggested although it was acknowledged that it would be a challenge to name a single stakeholder responsible since common decisions were required multiple stakeholders. A Queensland-based respondent suggested that due to the difficulty of managing urban deer, local government currently 'carried out control'. Presumably this comment referred to the trapping on request by local governments including Brisbane City, Moreton Bay and Gold Coast, (much of SE Queensland urban area). Respondents also identified the need to raise awareness, for example, educating licensed drivers with warning signs in deer-prone areas. To achieve, just this one outcome, requires jurisdiction beyond local government, and thus supports the suggestion that urban deer management individual local governments do not have the resources/responsibilities to deal with issues of encroachment into urban areas.

CONCLUSIONS

We have identified that there is general acceptance that deer are increasingly in numbers. However, even though we deliberately sought stakeholders who definitely had deer encroachment into their urban jurisdictions, we considered that the level of awareness of the issues of urban deer was poor. For example, there was constant confusion between urban and rural issues. Apart from response to complaints, and the subsequent trapping and removal, there appears to be no clear management strategies in place. One respondent admitted that the issues were considered less important than other feral species problems. However, there is no doubt that deer are increasingly encroaching on urban areas (e.g., Melbourne, Webb, 2013; Wollongong, WCC, 2013; Brisbane, McCarthy, 2013), and with 94% of Queensland herds having developed in the last 20 years, it is inevitable that the issues of urban deer will increase. As Warren (1997) predicted for the US, we suggest that management of urban deer will become one of the 'greatest challenges' in Queensland, and 'undeniably' the most complicated due to the polarisation of views associated with deer.

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INVADING SPECIES: A CHALLENGE FOR PEST MANAGEMENT

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Abstract Global warming together with global trade and tourism gives foreign organisms a lot of opportunities to enter Europe and to find suitable habitats to establish. Many of these species already have a status as pests in their native countries and now start to be of major concern in Europe. A survey is presented on the species which were sent for identification from 2010 to 2014. Of these 128 species 4.0% arrived from the Mediterranean area and 23.4% have their origin on other continents. Invasive species readily accept the conditions of their new habitat. Examples are ants like *Technomyrmex vitiensis* and *Lasius neglectus* but also different cockroach species. The strategies of some species, to find ecological niches where they survive or even expand and the challenge for pest control are discussed. Most alien arthropods have a different way of life than native species and require special control strategies. Detailed descriptions of economically important species are often found in their countries of origin or in the American and Australian literature. Modern pest management requires global knowledge of pest control strategies and pest control literature. Some control advice may also be found in the internet, when the respective alien species has been identified.
Key words Aliens, exotic Formicidae, climate change

INTRODUCTION

Pest organisms accompany man, since he began to settle down (Robinson 1996). In the past centuries a lot of pests arrived from tropical and subtropical areas in Central European harbours with commercial trade of foreign goods. However, since the middle of last century, the pest fauna has changed dramatically in Central Europe, which is mainly caused by the increasing introduction of goods from overseas without having a sufficient pest management or quarantine of goods in the harbours. Sensitive foods are transported by aircraft and reach Central Europe within 24 hours from their overseas origins. Large livestock farms, industrial food processing and modern heating systems, provide constant high temperature conditions even in the winter months and allow tropical insect species to establish in Central Europe (Pospischil, 2001).

This development is accompanied by an increasing number of organisms which arrive in Europe (Klotz et al., 2008). South European species benefit from the rising temperatures caused by climate change and move north to Central Europe (Rabitsch and Essl 2010; Nentwig, 2011). These changes in the abundance of pest organisms are shown by the specimens sent by pest management professionals for identification.

MATERIAL AND METHODS

Several 100 specimens were submitted for identification since 2010 from many places in Germany and from neighboring countries belonging to 128 species from 14 Orders and 56 Arthropod families. Mostly adult arthropods were sent, but egg-batches, larvae, pupae, faecal pellets and gnawing particles and pieces of wood with signs of attack arrived also. In urgent cases or when larger specimens were found, pictures were sent per e-mail. The samples came from pest management professionals, museums, scientific institutes, botanical and zoological gardens etc. Development stages which could not be identified were cultured until hatching of adults. Identified specimens (particularly Siphonaptera, Coleoptera, Formicidae, and Heteroptera) were pinned or embedded on a slide after identification and included in the collection. Photos were taken of the samples (specimens, signs of infestation) to save the entries for further studies.

This survey refers to specimens which were sent by pest management professionals, institutes, veterinarians, physicians or private clients. Only species which arrived in Europe after 1492 are identified as exotic species or aliens in this survey.

RESULTS AND DISCUSSION

Pests were introduced into Europe with trade since hundreds of years. However, due to quarantine regulations and pest control during unloading in the harbours exotic pests could hardly spread further. With the use of containers and short loading and unloading times often without inspections infested goods came directly into the domestic areas. The pest fauna has changed dramatically in Central Europe since the middle of last century, and the increasing standard of living and world trade also allowed tropical species to establish (Pospischil, 2001). Climate change has an additional effect on the naturalization of exotic species (Rabitsch and Essl, 2010; Nentwig, 2011).

One hundred twenty eight species were identified, of which 72.6% are native Central European inhabitants. 4.0% come from the Mediterranean region and 23.4% are exotic species (Table 1). Most submitted specimens were Coleoptera with 43 species and 17 families (Table 2). 74.4% are part of the Central European fauna, and 4.0% of the Mediterranean fauna, respectively. Exotic species are present in the Families Dermestidae, Bostrychidae, Anobiidae, Coccinellidae, Silvanidae, and Bruchidae. Some specimens sent as pictures are protected species: the leather beetle *Carabus coriaceus* (Carabidae), and the musk beetle *Aromia moschata* (Cerambycidae).

Table 1. Number of submitted species of different orders and areas of origin

Area of origin	**Central Europe**		**Mediterranean**		**Other continents**		**Total submitted**
Order	**No. of species**	**%**	**No. of species**	**%**	**No. of species**	**%**	
Coleoptera	32	74.4	2	4,7	9	20.9	43
Hymenoptera*	13	52.0	1	4.0	11	44.0	25
Diptera	10	90.9	1	9.1	0	0	11
Blattaria	2	25.0	1	12.5	5	62.5	8
Heteroptera	4	80.0	0	0	1	20.0	5
Siphonaptera	5	100	0	0	0	0	5
Acari	6	100	0	0	0	0	6
Araneae	5	62.5	0	0	3	37.5	8
Others	16	92.9	0	0	1	7.1	17
Total	93	72.6	5	4.0	30	23.4	128

Table 2. Families of the Order Coleoptera and areas of origin.*[1] Number of Central European species;*[2] Names of introduced species in brackets

Area of origin	Family (Species)	No. of species	%
Central Europe*[1]	Carabidae (4 species), Staphylinidae (2 Species), Histeridae (1), Dermestidae (3) Anobiidae (5 species), Ptinidae (3 species), Tenebrionidae (2 species), Lamellicornia (1) Lathridiidae (2), Throscidae (1), Malachiidae (1), Cerambycidae (5), Bruchidae (1), Curculionidae (1)	32	52
Mediterranean area*[2]	Bostrychidae (*Amphicerus bimaculatus),* Anobiidae (*Nicobium castaneum*)	2	4
Other continents*[2]	Dermestidae (*Trogoderma angustum, Attagenus smirnovi, Anthrenocerus australis, Dermestes peruvianus),* Bostrychidae (*Lyctus brunneus*, *Sinoxylon anale*), Coccinellidae (*Harmonia axyridis*), Silvanidae (*Oryzaephilus surinamensis*), Bruchidae (1)	9	44
Total		43	100

Ants

In 1999, 147 species of ants have been recorded living in non native habitats (McGlynn, 1999). As social insects ants have developed an impressive diversity of species and occupy many ecological niches. Most species are beneficial in natural ecosystems, a few are detrimental in urban areas (Holway et al., 2002; Klotz et al. 2008). Surveys on exotic ant species in Central European tropical houses of botanical and zoological gardens were published by Boer and Vierbergen (2008) and Pospischil (2011). 25 ant species were identified in this survey belonging to the subfamilies Formicinae (11 species), Dolichoderinae (1 species), Myrmicinae (12 species) and Ponerinae (1 species) (Table 3). 44% are from overseas and one (*Crematogaster scutellaris*) comes from the Mediterranean coast (caravan from Croatia with insulation material from Italy). *C. scutellaris* colonies survive several years in unheated structures under the Central European climate and allates were found outside a building in February at 10°C.

Technomyrmex vitiensis, *Plagiolepis* sp., *Tetramorium insolens*, *T. bicarinatum* and *Solenopsis molesta* were submitted from tropical green houses. An established colony of *S. molesta* was additionally found in a private house in North Rhine-Westphalia 2012. *Technomyrmex vitiensis*, which was submitted 8 fold during the last 2 years, and *Plagiolepis* sp. are now the most common species in Central European tropical buildings and can survive indoors in private homes at least for a month. In a restaurant attached to a greenhouse, workers of *T. vitiensis* were found in 2013. The colonies are decentralized over large territories with regular exchange of workers. Inseminated queens are only found in new colonies and then replaced by intercastes which can hardly be differentiated from workers. The transfer of nutrients occurs through trophic eggs rather than trophallaxis (Yamauchi et al., 1991).

The small exotic species *Cardiocondyla obscurior* which is already known from some tropical greenhouses was sent from Munich, where a nest was found in cavities of a potted plant.

These ants are night active and their colonies are hidden in small cavities of plants and can easily be transported (Heinze et al., 2006; Pospischil, 2011). Several workers of the red imported fire ant were identified on a sticky trap which was submitted from an international German airport 2012. The establishment of this species in urban areas of Central Europe seems to be possible according to a small colony which was kept under Central European indoor temperature conditions for one year in 2000.

Lasius brunneus was submitted 15 fold during the last 2 years. *L. brunneus* establishes new colonies often in buildings with a moisture damage which has not been repaired correctly. This species is therefore an important indicator of structural damage. *Lasius neglectus* was sent twice: one from a location in Baden Wurttemberg in 2011, and a second from a market garden near Ludwigshafen in 2012. Both places are in the distribution area of *L. neglectus* which is already described by Ugelvig et al. (2008).

Cockroaches

The cockroach samples consist of two native Central European species (*Ectobius lapponicus* and *E. sylvestris*), one species from the Mediterranean region (*Ectobius vittiventris*) and 5 exotic species from overseas which are already established in Central Europe (*Blattella germanica*, *Supella longipalpa*, *Periplaneta americana*, *Pycnoscelus surinamensis* and *Blaptica dubia*). Many cockroach species have the typical attributes of invasive species. They live in human environment and have a broad food spectrum and a hidden mode of life. It is therefore not surprising that most of the submitted cockroaches are exotic species (Pospischil 2010). The migration of the South European *Ectobius vittiventris* from Italy to Switzerland is well documented (Landau et al., 2000). The species followed in the past 6 years the Rhine valley north to the lower Rhine district and was submitted 2013 twice from Bonn and Dusseldorf, respectively.

Heteroptera

The submitted Heteroptera (5 species) are native European species with the exception of *Leptoglossus occidentalis*. This species was first found 1999 in northern Italy and spread rapidly over most of Europe (Rabitsch 2010). The species reached the South of Germany 2006 and is now widespread along the Rhine valley north to the lower Rhine district. Specimens are frequently found in homes when they invade structures to hibernate. The specimens which were included in this study, were submitted from Bochum and Wuppertal (North Rhine-Westphalia).

Flies

The order Diptera is presented with 11 species. 10 species belong to the Central European fauna and one species has its original distribution in the Mediterranean region (*Clogmia albipunctata*, Psychodidae) (Table 1). Other orders are Isopoda, Collembola, Dermaptera, Saltatoria, Psocoptera and Thysanoptera which are represented with only a few Central European species in this study.

Other Arthropods

8 species of spiders were collected from fruit display in supermarkets (mainly bananas) or plant departments and submitted for identification (Table 1). A Brazilian wandering spider (*Phoneutria* sp.) was found in one banana box. Central European house spiders (*Tegenaria atrica*) were submitted from another four supermarkets und one Wolf spider (*Trochosa terricola)*. The tropical species *Achaearanea tepidariorum* was found in a fruit display. The feather-legged lace weaver *Uloborus plumipes* was introduced about 20 years ago to Europe with plant and is now present in most garden centers. The 6 mite species belong to the Central European fauna (Table 1), and 2 of them are parasitic (*Dermanyssus gallinae* and *Ornithonyssus bacoti*)

Table 3. Ant species sent for identification from 2010 to 2013 and area of origin.

Area of origin	Species	No. of species	%
Central Europe	*Lasius brunneus , L. emarginatus , L. flavus , L. niger , L. fuliginosus , L. alienus, Camponotus ligniperdus , C. herculeanus , Messor structor, Tetramorium cf caespitum Temnothorax unifasciatus, Myrmica rubra, Hypoponera punctatissima*	13	52
Mediterranean area	*Crematogaster scutellaris*	1	4
Other continents	*Lasius neglectus, Camponotus maculatus, Plagiolepis.* sp*., Technomyrmex vitiensis, Solenopsis invicta, S. molesta, Monomorium pharaonis, Cardiocondyla obscurior, Pheidole* sp. *, Tetramorium insolens, T. bicarinatum,*	11	44
Total		25	100

It is conspicuous that not only exotic species started in the last years to be a concern for pest management in Central Germany but also native species which were not mentioned in pest management before. Examples are *Anthocomus fasciatus* (Malachiidae, Coleoptera) and *Eilema complana* (Arctiidae, Lepidoptera). *E. complana* started 2 years ago to be a concern for pest management professionals. The larvae feed on algae, lichens and mosses on the roof of large buildings and enter the interior of the building to pupate.

CONCLUSIONS

The introduction of exotic species including pathogens which is favoured by the global trade and short transport times is strongly increasing. Urbanization proceeds and the mean temperatures in larger cities offer tropical species adequate life conditions.

Climate change has an influence on the further spread and establishment of these exotic species outside buildings. The prerequisites for the pest management to master these challenges are a profound knowledge of the species and their ecological competence. The pest management will further need interdisciplinary cooperation with international teams of specialists with different emphasis including veterinary and human medicine, natural conservation organizations, transportation, agriculture and food production.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

RECENT RANGE EXPANSION OF BROWN MARMORATED STINK BUG IN EUROPE

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Abstract A public survey to determine the current distribution of the invasive brown marmortaed stink bug, *Halyomorpha halys* in Switzerland showed that by the end of 2013*,* it was present in 11 Cantons, including six newly invaded Cantons (Basel-Landschaft, Aargau, Genève, Solothurn, Ticino, Thurgau, and Bern). In total, *H. halys* was found in 76 locations in 38 cities across Switzerland with large breading populations being present in the cities Zurich, Basel, Bern, and Lugano. There is now evidence that *H. halys* was already present in 2004, three years before its first official detection in 2007. Although *H. halys* has been present in Switzerland for nearly 10 years, its natural spread can be considered slow. Large distribution gaps between Swiss cities Zurich, Basel and Bern indicate that founder populations from Zurich arrived in these locations through human activity and movement of goods along the main motorways connecting the cities. The new list of host plants in Europe contains 51 host plants in 32 families, including many exotic and native plants. High densities of nymphs and adults were observed on *Catalpa bignonioides*, *Sorbus aucuparia*, *Cornus sanguinea*, *Fraxinus excelsior*, and *Parthenocissus quinquefolia*. With growing populations in Switzerland, France and Italy *H. halys* may further extend its distribution and become a nuisance pest in many European cities.
Key words *Halyomorpha halys,* nuisance pest, host plants, European distribution.

INTRODUCTION

One of the most harmful invasive insect pests is the brown marmorated stink bug, *Halyomorpha halys* Stål (Heteroptera: Pentatomidae), which is native to eastern China, Japan, Korea and Taiwan (Zhu et al., 2012). In North America it was first reported in the mid-1990s (Hoebeke and Carter, 2003), whereas in Europe it was first recognized in Zurich, Switzerland, in 2007 (Wermelinger et al., 2008). Until recently, the distribution of *H. halys* in Europe was restricted to four cantons of Switzerland, but it is now also present in France and Italy (Wyniger and Kment, 2010; Callot and Brua, 2013; Pansa et al., 2013, EPPO 2013). In the north-eastern United States, *H. halys* developed into a severe agricultural and horticultural pest, but due to its behaviour to enter human houses in large numbers for overwintering, it is also considered a nuisance pest in 21 states (Inkley, 2012; Northeastern IPM Center, 2013a). Adults entering the houses are a nuisance mainly because of the unpleasant odor they emit when disturbed, the remains of their frass, and their abundance, which can exceed 25,000 individuals in a single house in a single year (Inkley, 2012). In Switzerland *H. halys* is not considered an agricultural pest, but Mueller et al. (2011) showed that reports of *H. halys* in private residences in Zurich have increased exponentially between 2007 and 2010. Due to this increased number of reports in private homes in Zurich and other Swiss cities, the objectives of our study were to re-investigate the distribution of *H. halys* and their associated host plants to elucidate the current development of this nuisance pest in Switzerland.

MATERIALS AND METHODS

To determine the current distribution in Switzerland a public survey was conducted in 2012/13. Several articles were published in Swiss newspapers, public talks were given, and a webpage (www.halyomorphahalys.com) was launched, asking private homeowners to report findings of *H. halys* and infested host plants. Since *H. halys* adults are often confused with the native *Raphigster nebulosa* Poda (Wyniger and Kment, 2010), only those records that were validated by the authors based on the submission of dead individuals or digital images were considered as positive finds. Areas where *H. halys* was never reported before were visited by the authors whenever possible in person to confirm the presence of the bug. In addition, highly infested cities in Switzerland (Zurich, Basel, Bern, Lugano) and France (Strasbourg, Schiltigheim) were visited several times to compile a new list of host plants of *H. halys* in Europe.

RESULTS AND DISCUSSION

In total, the public survey resulted in 154 validated submissions. 51.9% of the submitted records were *H. halys*, followed by the native *R. nebulosa* (29.2%), and the invasive western conifer seedbug, *Leptoglossus occidentalis* Heidemann (16.2%). Other species reported were the native *Dolycoris baccarum* L. (1.3%) and the invasive *Nezara viridula* L. (1.3%). The most remarkable submission was a digital photograph taken May 5th, 2004 in Zurich-Seefeld by R. Burtscher, showing undoubtedly an adult *H. halys*. This proves that *H. halys* was already present in Switzerland long before the first official records in 2007 (Wermelinger et al. 2008). Wyniger and Kment (2010) reported that besides large populations in the Canton Zurich, single individuals had also occurred in the Cantons Basel-Stadt, Schaffhausen, and St. Gallen. The present survey showed that by the end of 2013 the bug had extended its range to 11 Cantons, including the newly invaded Cantons Basel-Landschaft, Aargau, Genève, Solothurn, Ticino, Thurgau, and Bern. In total, *H. halys* was found in 76 locations in 38 cities across Switzerland with large breading populations being present in the cities Zurich, Basel, Bern, and Lugano (Figure 1).

Figure 1. Distribution of H. halys in Europe according 2012/13 surveys and published records.

Although *H. halys* probably arrived in Zurich in the early 2000s, the majority of records were still submitted from an area of approximately 40 km around Zurich, suggesting that its natural spread was relatively slow. Large distribution gaps between populations in Zurich and other cities such as Basel and Bern indicate that founder populations from Zurich arrived in these locations through human activity and movement of goods along the main motorways connecting the cities, which was recently confirmed by Gariepy et al. (2013). The accidental movement of Swiss populations is likely also responsible for individuals discovered in Liechtenstein (Arnold, 2009), Germany (Heckmann, 2012), France (Callot and Brua, 2013), and Italy (EPPO, 2013; Pansa et al., 2013) (Figure 1).

Previously, the highly polyphagous *H. halys* was reported from eight plants in Switzerland (Wermelinger et al. 2008; Wyniger and Kment 2010), but with the present survey the list of host plants was extended to a total of 51 host plants in 32 families, including many exotic and native plants (Table. 1). In comparison, in the United States it was recorded from 166 plant species (Northeastern IPM Center 2013b), and in Asia a total of 106 host plants have been reported (Lee et al., 2013). High densities of sometimes more than 100 nymphs and adults per tree were particularly observed on *Catalpa bignonioides* Walter, *Sorbus aucuparia* L., *Cornus sanguinea* L., *Fraxinus excelsior* L., and *Parthenocissus quinquefolia* (L.) Planchon. Plants with large numbers of unripe fruits, buds and pods tended to host more individuals than plants without these parts, a phenomenon which was also observed in the US (Northeastern IPM Center, 2013b).

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CONCLUSIONS

The present survey demonstrated that *H. halys* is already far more widespread in Switzerland than previously assumed. With growing populations in Switzerland, France and Italy an expansion of the current distribution can be expected, and in the future *H. halys* may become a nuisance problem in many European cities. Areas of high risk are those cities that are connected by motorways with cities where large *H. halys* populations already exist. Remarkably, no breeding populations have been reported from Germany yet, but it is highly likely that *H. halys* will soon establish in German cities in the near vicinity of infested areas, e.g. Kehl, Weil am Rhein, and Konstanz. The reasons why *H. halys* populations seem to do best in larger cities remain unclear. Since *H. halys* develops better at warmer temperatures (Nielsen et al., 2008), one explanation could be the phenomenon of urban heat islands (UHI), which are metropolitan areas that are significantly warmer that their surrounding rural areas due to human activities (Oke, 1967).

Table 1. Host plants of *Halyomorpha halys* in Europe; n = native to Europe; e = exotic ([a]Wermelinger et al., 2008; [b]Wyniger and Kment, 2010).

Family	Common name	Scientific name	origin	High densities observed
Apocynaceae	oleander	Nerium oleander L.	n	
Aquifoliaceae	European holly	Ilex aquifolius L.	n	+
Araliaceae	Japanese angelica tree	Aralia elata (Miq.) Seem.[a]	e	+
	common ivy	Hedera helix L.	n	
Arecaceae	Chinese windmill palm	Trachycarpus fortune	e	
Asparagaceae	sicklethorn	Asparagus falcatus (L.) Druce	e	
Asteraceae	sunflower	Helianthus annuus L.	e	
Betulaceae	hornbeam	Carpinus betulus L.	n	+
	common hazel	Corylus avellana L.	n	
Bignoniaceae	Chinese trumpet vine	Campsis grandiflora (Thunb.) K.Schum.[b]	e	+
	cigar tree	Catalpa bignonioides Walter	e	+
Convolvulaceae	morning glory	Ipomoea sp.	(?)	
Cornaceae	common dogwood	Cornus sanguinea L.	n	+
Fabaceae	-	Caragana brevispina Royle ex Benth.	e	
	Judas tree	Cercis siliquastrum L.	n	
	lupine	Lupinus sp.	(?)	
	runner bean	Phaseolus coccineus L.	e	
Fagaceae	holm oak	Quercus ilex L.	n	
	English oak	Quercus robur L.	n	
Hydrangeaceae	hortensia	Hydrangea sp.	e	
Lamiaceae	harlequin glorybower	Clerodendrum trichotomum Thunb.	e	
Lauraceae	bay laurel	Laurus nobilis L.	n	
Lardizabalaceae	dead man's fingers	Decaisnea fargesii Franch. J. Bot. (Morot)[a]	e	+
Malvaceae	Hibiscus	Hibiscus sp.	e	
Magnoliaceae	Magnolia	Magnolia sp.	e	
Meliaceae	Chinese Toon	Toona sinensis (A.Juss.) M.Roem.	e	
Moraceae	common fig	Ficus carica L.	e	
	white mulberry	Morus alba L.	e	
Oleaceae	European ash	Fraxinus excelsior L.	n	+
Plantaginaceae	figwort	Asarina scandens (Cav.) Pennell	n	
Rosaceae	juneberry	Amelanchier lamarckii F.G.Schroed.[a]	e	
	black chokeberry	Aronia melanocarpa (Michx.) Ell.	e	
	cotoneaster	Cotoneaster cochleatus (Franch.) G.Klotz	e	
	common hawthorn	Crataegus monogyna Jacq.	n	
	apricot	Prunus armeniaca L.	e	
	sweet cherry	Prunus avium L.	n	
	almond	Prunus dulcis Batsch	n	
	peach	Prunus persica (L.) Stokes	e	
	nectarine	Prunus persica var. nucipersica	e	
	blackthorn	Prunus spinosa L.	n	
	firethorn	Pyracantha coccinea M.Roem.	n	

	garden roses	Rosa spp.	e	
	blackberry	Rubus fructicosus L.	n	+
	raspberry	Rubus ideaeus L.	n	+
	mountain-ash	Sorbus aucuparia L.	n	+
Sapindaceae	box elder	Acer negundo L.	e	
	Japanese maple	Acer palmatum Thunb.	e	
	Norway maple	Acer platanoides L.	n	
	sycamore maple	Acer pseudoplatanus L.[a]	n	
	bottlebrush buckeye	Aesculus parviflora Walter	e	
Scrophulariaceae	summer lilac	Buddleja davidii Franch.[a]	e	
	mullein	Verbascum sp.	(?)	
Sequoioideae	giant redwood	Sequoia sempervirens (D. Don) Endl.	e	
Simaroubaceae	tree of heaven	Ailanthus altissima (Mill.) Swingle	e	
Solanaceae	sweet pepper	Capsicum annuum L.	e	
	eggplant	Solanum melongena L.	e	
Theaceae	Japanese Stewartia	Stewartia pseudocamellia Maxim.[a]	e	+
Tropaeolaceae	Indian cress	Tropaeolum majus L.[a]	e	+
Ulmaceae	elm	Ulmus sp.	(?)	
Vitaceae	Virginia creeper	Parthenocissus quinquefolia (L.) Planch.	e	+
	common grape vine	Vitis vinifera L.	n	

The list of host plants for *H. halys* will undoubtedly grow as the pest spreads to new areas in Europe. The wide variety of exotic and native plants planted in parks and private gardens in cities provides a nearly unlimited food source for *H. halys* throughout the season and could be another reason why *H. halys* is mainly a nuisance problem in urban environments and less in the countryside. To avoid an increase of *H. halys* populations within cities, municipal nurseries and and private home owners should consider less suitable host plants when planting streets, public parks or private gardens.

ACKNOWLEDGEMENTS

The authors would like to thank all Swiss citizens that participated in the public survey. We are particularly grateful to R. Burtscher for providing the oldest record of *H. halys* in Switzerland.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

SCIENTIFIC EXPERIENCES FROM PEST ADVISORY IN VORARLBERG, AUSTRIA

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Abstract The biological advisory service (BAS) of the "inatura-Erlebnis Naturschau Dornbirn" is a free service for the general public, authorities and professionals in the state of Vorarlberg (Austria). Pests and annoying intruders in houses and gardens appear as a main topic for the advisors. They are regularly confronted with new pests, mass occurrences and invasive alien species. The preferred migration corridors of selected species are explained. Some of the invaders enlarge their range naturally are forced by climate changes. Others have been introduced by worldwide trading activities.
Key Words *Argiope bruennichi*, *Cheiracanthium mildei*, *Cylindroiulus caeruleocinctus*, *Ectobius vittiventris, Harmonia axyridis*, invasive species, *Leptoglossus occidentalis*, mass occurrence, *Sceliphron curvatum*, *Scutigera coleoptrata*

INTRODUCTION

The "inatura – Erlebnis Naturschau Dornbirn" is a museum for natural history in Vorarlberg (Austria). It was founded in 1960 by the state government and by the city of Dornbirn. In 2003 it was transformed into a limited liability company. Besides the classical challenges of a museum like exhibition, conservation and research, inatura also offers a biological advisory service (BAS). This service works on enquiries of the general public, mass media, authorities and professionals (e.g. pest controllers or physicians). A wide range of questions concerning animals, plants and fungi have to be handled. An increasing part of the consultants' work is pest advisory in a broad sense, including troublemakers and intruders. The contract to fulfill the BAS is written down in the nature conservation law of Vorarlberg. This also includes the enforcement of public relations. The advisors constantly publish explanatory leaflets, articles and press releases. At present the advising team consists of three part-time biologists.

Vorarlberg is the most western state of Austria. It has an extension of 2.601 square kilometers and more than 374.000 inhabitants. The Alpine Rhine Valley and the Walgau are the zones with the highest population density. With more than 2/3 of the whole population they show a distinctive urban sprawl. The biggest city is Dornbirn with more than 46.000 inhabitants. In the east and south of Vorarlberg high mountain ranges form natural boundaries to the other states of Austria and to Switzerland. In the west the Alpine Rhine River marks the border to Switzerland and Liechtenstein, Lake Constance the region where Switzerland, Austria and Germany meet. In the north the soft foothills of the Alps form the border to Germany.

MATERIALS AND METHODS

Enquiries to the BAS can be made personally, via telephone and e-mail. In many cases clients deliver specimen or samples for identification or they mail digital photographs. Many questions can be answered spontaneously or after consulting specialized literature or external partners. Sometimes outdoor activities are necessary, especially to survey mass occurrences or extraordinary phenomena.

Since 2003 all enquiries have been registered in annual advisory tables (MS Excel). Ideally these records contain the species or genus of the specimen, the purpose of the question, the location and circumstances of discovery and contact information of the client. In cases of particular incidents the complete e-mail correspondence is stored in digital files. Digital pictures, videos or sound samples complete the documentation. Selected specimen are preserved in alcohol or dried as further references.

RESULTS AND DISCUSSION

Annual Statistics

In the past ten years the amount of enquiries has increased to approximately 2.500 a year, especially the ones made by authorities and mass media. More than 100 interviews are given each year. The annual statistics show the number of enquiries classified according to systematic units, client cohorts and other characteristic values. They form an important reference for the authorities and a basis for the publication in inatura´s quarterly magazine inatura aktuell. Many of the advisory records are directly forwarded to external scientists to support all-Austrian projects about single species (raccoons) or animal groups (exotic marsh turtles).

Pests and Annoying Intruders

More than one third of the questions concern unwanted animals or pests in houses and gardens. Some of these enquiries regard vertebrates, particularly mammals like mice, martens, foxes or raccoons but also crows, snakes and others. However, there are many more bothersome intruders and pests among the arthropods. There are occurrences that do not need any measures, others can be treated easily by the clients themselves with the guidance of the BAS. Only some dangerous pests need to be combated by professional pest controllers. In Vorarlberg pests like cockroaches, bedbugs or pharaoh ants are not notifiable, therefore no universal statistics about their occurrences exist.

Mass Occurrences

Since 2004 a mass occurrence of the millipede *Cylindroiulus caeruleocinctus* (Wood) in the small village of Röns (Vorarlberg) has been observed by Zimmermann (2013). During almost ten years the residents of three houses have been confronted with periodical appearances of thousands of individuals. The cause of this phenomenon is unknown (Passig and Scholz, 2007). Various methods were tested to protect the affected persons and to force back this pest. Strangely enough, this mass occurrence signifies the first recorded appearance of this species in Vorarlberg. *C. caeruleocinctus* is indigenous in Central Europe, but there are several gaps in the stocks. This species might have been accidentally imported with soil or agricultural products. Meanwhile ten further mass occurrences of *C. caeruleocinctus* in Vorarlberg have been documented by the BAS.

Examples of Introduced Alien Species

The thermophilic Mud Dauber *Sceliphron curvatum* (F. Smith) is indigenous in Central Asia, its first European occurrence was in 1979 in southeastern Austria (Gepp 1995). From there it extended to all directions. Appearances were documented in 1994 by Aistleitner (2000) in Bregenz (Vorarlberg) and in 1998 by Schmid-Egger (2005) in Basel (Switzerland). This species has been regularly registered in Vorarlberg by the BAS from 2003 onwards (Table 1). The Harlequin Ladybird, *Harmonia axyridis*

(Pallas), was imported from Asia to control aphids in the glasshouses of the Netherlands and Belgium. The first European outdoor occurrence was observed in 1999 in Germany. In Vorarlberg *H. axyridis* was documented for the first time in 2006 by Rabitsch and Schuh (2006) in Götzis. In the same year the BAS registered three mass occurrences in Dornbirn, Feldkirch and Hittisau (Table 1).

The Western Conifer Seed Bug, *Leptoglossus occidentalis* Heidemann, originates from Northern America. Most probably it was accidentally imported to Europe with timber. In 1999 it was reported in northern Italy, six years later it reached Austria (Rabitsch and Heiss, 2005). In Vorarlberg *L. occidentalis* has been recorded by the BAS from 2009 onwards (Table 1). This bug is believed to have spread over Central Europe by its own efforts. But accidental transportations with garden products are possible as well.

Table 1. Numbers of individuals (*S. curvatum*, *L. occidentalis*) or mass occurrences (*H. axyridis*) of introduced alien species registered by the BAS since 2003.

	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
S. curvatum	2	5	5	12	14	13	5	16	17	7	9
H. axyridis				3	8	85	17	7	41	23	7
L. occidentalis							4	4	6	6	17

Table 2. Number of individuals of invasive Mediterranean species registered by the BAS since 2003.

	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
A. bruennichi	7	4	5	36	8	30	11	12	9	13	4
E. vittiventris							8	7	11	11	20
Ch. mildei											1
S. coleoptrata											1

Examples of Invasive Mediterranean Species

The Wasp Spider *Argiope bruennichi* (Scopoli) appeared in western and central Europe already 80 years ago (Auer, et al., 1989). According to the records of the BAS (Table 2) stocks in Vorarlberg have been increasing for more than 20 years. The field-dwelling cockroach *E. vittiventris* (Costa) has been registered in northern Switzerland from 1985 (Baur, et al., 2004).The first occurrence in Vorarlberg was in 2009 (Table 2). Three years earlier local pest controllers had probably been confronted with *E. vittiventris*.

The Northern Yellow Sac Spider, *Cheiracanthium mildei* L. Koch, appeared in southern Germany already 30 years ago (Muster, et al., 2008). First indications for its presence in Vorarlberg come from 2006. In 2013 the first proven specimen was registered by the BAS in Dornbirn (Table 2). The first appearance of the House Centipede, *Scutigera coleoptrata* (Linnaeus), in Vorarlberg was documented by the BAS in Gaissau (A) in 2013 (Table 2). Observations from Switzerland and Germany show that this species occurs in many places along the High Rhine Valley and around Lake Constance.

CONCLUSIONS

Being the first information facility for enquiries about all kinds of pests, mass occurrences and invasive species in Vorarlberg, the BAS offers the possibility to create awareness for new critical occurrences in the general public. Various thermophilic species spread their range from the Upper Rhine Valley eastwards along the High Rhine Valley and Lake Constance to Vorarlberg and neighbouring regions. These migration trends might be a reaction to local climate changes and have to be observed accurately.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

INSECTICIDE RESISTANCE MANAGEMENT IN URBAN PESTS

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Abstract Whilst the reports of insecticide resistance are numerous, and many mechanisms of resistance have been studied, there has been less emphasis placed on developing and promoting Insecticide Resistance Management (IRM) programmes to address this issue. It is argued that only through the implementation of an IRM programme, can the effective control of insect pests be maintained in the long-term. The Insecticide Resistance Action Committee's insecticide mode of action classification is a key part of any IRM programme. It enables the insecticide user to identify insecticides in the same class, and hence those that will provide selection pressure for resistance mechanisms which may affect all insecticides from that class. A model is also presented that identifies the path of activities and events that follows when a decision is taken to control an insect pest, and how they impact, and are impacted by, insecticide resistance development. The IRAC mode of action classification and the model of insect pest control can be used to develop effective and integrated IRM programmes.
Key words Integrated pest management.

INTRODUCTION

There are many reports and papers published describing insecticide resistance in urban insect pests. However, few offer practical advice on how to minimise the development of insecticide resistance, or how to manage the pest population when reduced susceptibility to a given insecticide has been identified. Insecticide Resistance Management (IRM) is a practical approach to managing an insect pest population in such a way that the effectiveness of the control interventions are maintained in the long run.

The Insecticide Resistance Action Committee (IRAC) was formed in 1984 and is a specialist technical group of the agrochemical industry association CropLife International. IRAC was created to provide a coordinated industry response to the development of resistance in insect and mite pests, and has the aim of promoting resistance management for sustainable agriculture and improved public health (McCaffery and Nauen, 2006). IRAC defines insecticide resistance as, "a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species" (IRAC, 2011).

DISCUSSION

Synthetic insecticides have been extensively used since the 1940s to control urban and public health insect pests. However, insecticide resistance rapidly developed, with house flies resistant to DDT identified only a few years after its introduction in 1949 (Keiding and Van Deurs, 1949). By 2012, the

Michigan State University Arthropod Resistance Database contained 10357 reports of resistance in 574 species of arthropod to 338 pesticides (Whalon et al., 2012).

The loss of susceptibility to an insecticide in an insect pest population has a number of undesirable consequences. The pest controller will have a smaller choice of insecticides to use when controlling that pest population. Insecticides with less desirable environmental properties may need to be used. Higher insecticide application rates may be used in an attempt to control the resistant population, resulting in increased burden on the environment. Pest numbers may increase as the population becomes harder to control; this is of particular concern if the pest is of public health importance.

Resistance develops due to selection pressure on an insect population where a subset of the population is able to survive and reproduce after exposure to an insecticide application. The mechanisms by which insect pests resist insecticides has been widely reviewed elsewhere (Hemingway and Ranson, 2000; Nauen, 2007). If a heritable trait allows the individuals to survive the insecticide exposure, then the proportion of the population carrying that trait will increase post exposure. Subsequent exposure of that population to the same insecticide, or one with the same mode of action, will result in a still greater proportion surviving, potentially leading to control failure. Depending on the mechanism of resistance, insects with reduced susceptibility to one insecticide are likely to also have reduced susceptibility, or cross resistance, to other insecticides with the same mode of action. IRAC has produced a mode of action classification to identify which insecticides share the same mode of action (Elbert et al., 2008). Regularly updated, this mode of action classification can be accessed online (http://www.irac-online.org/).

Behavioural resistance is an expression of insecticide resistance that is independent of mode of action class. It occurs when an insect population is still susceptible to an insecticide, but individuals have altered their behavior, such that they don't come into contact with the application. Bait aversion is the classic example of behavioural resistance. Silverman and Ross (1994) reported that a number of field populations of German cockroach, *Blattella germanica*, displayed avoidance behaviour to a cockroach bait formulation. These populations, therefore, did not pick up a lethal dose of insecticide and were not controlled. It was found that they were averse to glucose, a constituent of the bait matrix. Replacement of glucose with fructose significantly improved bait acceptance and hence efficacy.

The key to preventing the development of insecticide resistance in a pest population is to minimise the selection of the genes that confer the ability to survive the insecticide application. Insecticide resistance is often conferred by a number of mechanisms, which work together to resist a full label dose of the insecticide. Individually, they may only confer "resistance" to a sub-label dose, or incomplete application. Insect pests that have only developed partial, or low level resistance to a given insecticide class, may still be controlled by exposure to an application at the recommended label rate. As only individuals that survive the insecticide application can pass on their genes for reduced susceptibility, actions that ensure these individuals are controlled will minimise the further selection of insecticide resistance. These actions include non-insecticide based activities that reduce or exclude the pest population and can be summarised as best practice integrated pest management (IPM). A number of authors outline effective IPM strategies for urban pests and highlight the benefits from taking this approach (Lacey, 2002). Further information on IPM and IRM strategies is also given by IRAC (2011).

Figure 1 shows the activities and events that occur when an insecticidal intervention is used to control a pest population. The first step is to design the control programme and identify whether an insecticidal intervention is required, or whether the pest population can be reduced to an acceptable level through exclusion, and removal of conducive conditions. If insecticides are required, to minimise the selection pressure for resistance development, only insecticides to which the target pest is known to be susceptible, should be used. Insecticides from the same mode of action class should not be continuously used at a given location; instead insecticides with different modes of action should be rotated through time. If a large area is to be treated, within which the target pest population can freely move, it is beneficial to employ a matrix approach, where insecticides with different modes of action are used in different locations within the treated area. Once the most appropriate insecticide class is chosen, the choice of insecticide product should be based upon fitness for purpose. "Fit for purpose" should include overall efficacy of the product, and suitability for its use under the conditions where it will be deployed. The insecticide product label should be followed using correctly calibrated and serviced application equipment. The applicator should be trained in the effective and safe use of the product, and have sufficient knowledge to correctly identify the target pest and the biology pertinent to its control. Under-dosing, incorrect placement, the use of substandard products and poorly timed applications, will increase the probability of the target pest being exposed to a sub-label dose, increasing the likelihood that individuals with reduced susceptibility will survive.

Figure 1 covers the four steps in the journey the insecticide needs to make to control the pest population, highlighting potential losses *en route*. Reduced susceptibility at each step, manifested as a reduction in the available insecticide capable of binding at the target site, can limit the control of the pest. By effectively delivering the recommended label dose of the insecticide to the target pest population, the impact of the potential loss mechanisms are reduced, and a greater proportion of the target pest population will be controlled. This increases the effectiveness of the control intervention, and minimizing the probability of reduced susceptibility developing.

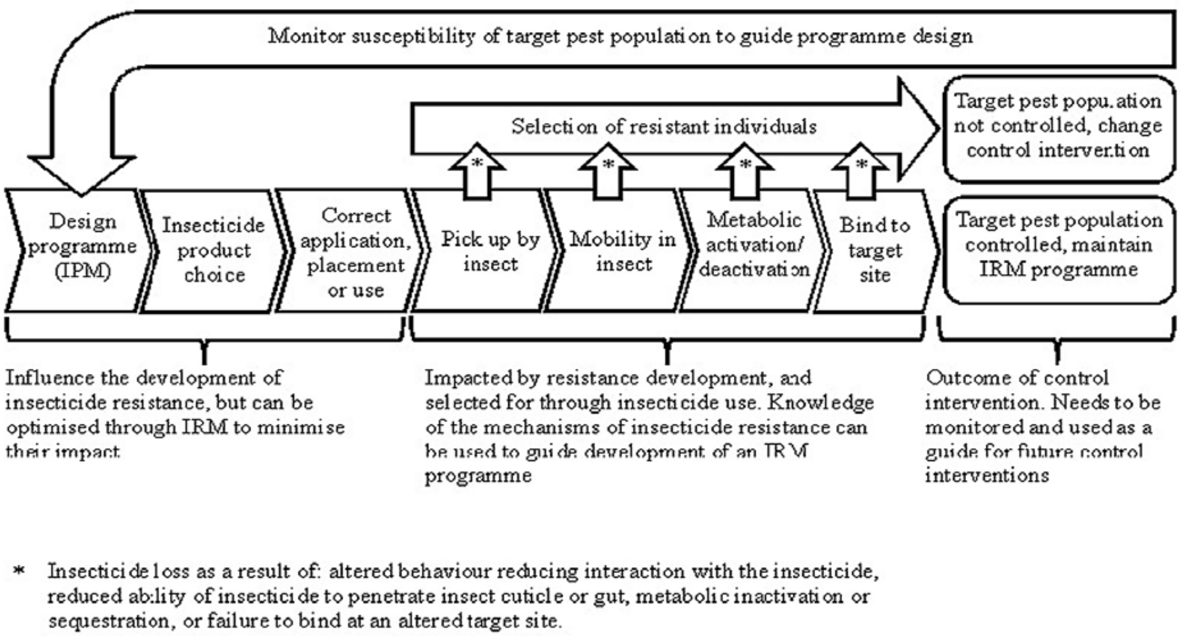

Figure 1. Model of the activities and events that occur when an insecticidal intervention is used to control a pest population

CONCLUSIONS

Insecticide susceptibility in a pest population is a valuable asset which needs to be maintained. Actions which minimise the selection pressure for resistance development, before an insecticide resistance problem is identified in the target pest, should therefore be encouraged. IRM should be considered as part of a wider IPM programme. Rotations and mosaics of insecticides from different mode of action classes form the basis of an IRM strategy. The IRAC mode of action classification scheme is a valuable tool to support the informed selection of insecticides for such rotations and mosaics. An appreciation of the activities and events that occur when an insecticidal intervention is used to control a pest population, exemplified in the model presented in Figure 1, help to identify those that can be optimised in the development of an effective IRM programme.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

TREATMENT OF WOOD-BORING BEETLES IN OXYGEN-FREE ATMOSPHERES

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Abstract The investigation showed lethal effects to development stades of three different wood boring insects commonly found in storages and exhibition rooms of museums. Hermetically closed chambers without floor sealing contained atmospheres < 1 vol.-%, the results of 100 % mortality could only be determined by examining test insects.
Key words Controlled atmospheres, museum pests, wood-boring, powderpost beetles.

INTRODUCTION

Caused by loans from all over the world, new acquisitions and changing exhibitions, a pest infestation occurred in the storage of the MMK Museum of Modern Art Frankfurt Main. All kinds of material and aspects concerning the collection are handled by the conservator of the MMK. Due to the active acquisition policy and ongoing projects, the storage is in constant motion. Beyond paintings and sculpture, artists may use any material or method in their artwork or installation and each has different parameters for preservation and conservation. Deterioration is determined by the art material's stability and its interaction with the environment. An accumulation of minor, often unnoticed events is usually only found when examining the entire collection over a long period of time. Changes in temperature or relative humidity may create varying stress levels in components or layers. We try to create an environment for the artworks that possibly does no harm. That includes light, temperature, moisture but also any gas that could lead to an uncontrolled chemical reaction. Acidic or other gases and vapours readily attack cellulose and other materials, and cause irreversible, cumulative damage to the appearance and supporting structure of works of art.

After previously finding beetles in the storage, they were examined by the Senckenberg Institute in Frankfurt to see whether they could survive or even breed in the acclimatized storage. Aware of the risk, we had implemented a constant monitoring. An early warning system with adhesive films, UV light traps and pheromone was installed. Still, a massive infestation was found in early 2011 in one spot probably brought in with a crate. A task force was founded with external specialists to find the most effective way to get rid of this pest. Thorough investigations showed that the beetles had already spread throughout. We learned that the powder-post beetle (*Lyctus brunneus*), and its life cycle is still part of scientific research.

It became clear that there was only one way to get rid of the pest: We had to remove everything from storage and redo the entire sapwood floor. For conservation reasons, we decided to use a sapwood-free oak floor. The Museum has a 20th Century collection of more than 4500 artworks comprised of more that 15.000 single pieces: they range from large convolutes of graphics and b/w photographs, colour prints, paintings, objects, installations, organic materials and technical equipment. We then tried to separate the conifer from the hardwood to reduce the costs for the anoxia treatment. But it was a bigger effort to separate all endangered materials than tenting a whole heavy-duty shelf. All crates and packing had to be opened, bubble wrap foil had to be removed to allow oxygen to escape as quickly as possible. Because of special packing and the fragility of the objects, they could only be moved by trained art handlers.

Recommendations had called for oxygen levels in controlled atmospheres in museums at less than 1 Vol.-% (Gilberg, 1989). A lethal effect is more easily achieved on adult insects than on their eggs. (Gilberg, 1991) tested eggs of *Lasioderma serricorne*, *Stegobium paniceum*, *Anthrenus vorax* and *Tineola bisselliella* with a gas mixture of 0.4 % oxygen over a period of three weeks at 30°C. The eggs of all species showed 100% mortality after exposure. These results are not feasible for treatment of modern art in controlled atmospheres. Conservators only allow maximum temperatures of 20 to 22°C.

Few past studies have shown mortality results in eggs from wood borers at standard room temperatures of 20°C and applied oxygen levels at less than 1.0 Vol.-%. Frank (1991) tested inert gases to control powder-post beetles at 20 °C for more than 3 weeks treatment for 100% mortality. This paper will show the feasibility to eradicate different stages of wood boring species in controlled atmospheres under feasible conditions and at oxygen < 1vol.-%.

MATERIAL AND METHODS

To investigate the efficiency in oxygen-free atmospheres, a XXL-tent with a volume of 600 cbm was applied to eradicate a powder-post beetle infestation. A total package with floor-foil was impossible because all infested objects in heavy boxes were stored on industrial storage shelves and wrapped in Vacupac made up of HDPE, aluminium and PET. For reversible fixation, the aluminium foil was stuck together with gas-tight aluminium tape and fixed to the industrial storage floor.

For humidity control, a humidifier and a dehumidifier were placed inside the tent and connected via an electronic control unit (INVAN4) outside. The unit records oxygen, humidity and temperature levels and regulates the humidity inside the tent. To reduce oxygen below 1 Vol.-%, nitrogen was flushed first and a nitrogen generator used to maintain the oxygen concentration inside the tent. Figure 1 shows the exposure data for humidity, temperature and oxygen levels.

To evaluate the efficacy in terms of mortality, house longhorn beetle (*Hylotrupes bajulus*), furniture beetle (*Anobium punctatum*), and powder-post beetle (*Lyctus brunneus)* contained in 20 pieces of softwood, were placed into the tent. Control organisms of the same batch were stored beside the tent and samples of the same batch were also tested at the laboratories of Materialprüfungsamt Eberswalde. The different stages of test organisms used are described below.

Exposure of Larvae

Six living larvae of the powder-post beetle *Lyctus brunneus* were applied to a suitable specimen that could fit seamlessly into the sampling block. For comparison, six larvae of the common furniture beetle *Anobium punctatum* and three larvae of the house longhorn beetle *Hylotrupes bajulus* were introduced into the specimens, and these subsequently inserted into sampling blocks. The silicon-sealed sampling blocks were then placed into the fumigation tent. Post-fumigation, the sampling blocks were removed to study the larvae. Identically fitted sampling blocks were placed simultaneously outside of the tent and not subjected to the treatment with oxygen low atmosphere.

Exposure of Eggs

Insect species used: 2–9 days old eggs of the common furniture beetle *Anobium punctatum* and 1 – 4 days old eggs of the powder post beetle *Lyctus brunneus* from the MPA lab. Material for the egg deposit: Incubation medium, predominantly consisting of oak formed into a cube of approx. 1 cubic meter. See Table 1 and 2. The beetle pair was placed on a semi-synthetic diet of compressed nutrient as a breeding ground. The female deposited eggs within two to three days for *Anobium* and 24 hours for *Lyctus*. It was not possible to determine the number of eggs as some were deposited deeply into the nutrient media. Each of these nutrient media was then placed inside a glass vessel, tightly closed off with air-permeable gauze and taken to the fumigation tent. The nutrient media were removed after fumigation and checked daily for larvae hatching. After finding the first hatching the investigation ended. Larvae that hatched later were not taken into consideration. Egg deposits for the control study, were prepared and transported in the same way as the fumigated samples but kept outside of the fumigation tent. Egg deposits post-fumigation were stored at the breeding room at 20 °C and 80% RH for *Anobium* and breeding room at 26°C and 70% RH for *Lyctus* at MPA Eberswalde.

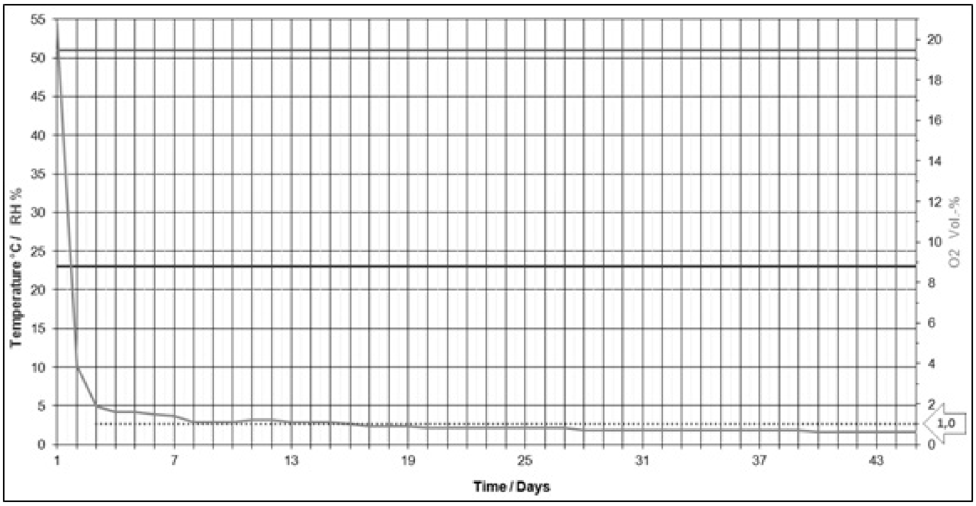

Figure 1. Graph showing RH, temperature and oxygen levels of the treatment.

Determining Mortality

Mortality was determined by examining the insects microscopically for any perceptible movement. Larvae and eggs were held for subsequent development. Unexposed controls. On the day of exposure, the same species and stadia were set up as unexposed control samples. These were placed beside the tent in identical basins and at identical RH and temperature as the insects being exposed.

Table 1. Eggs of *Anobium punctatum* post-exposure.

Type of Eggs	Sample No.	Maximum age of eggs at exposure (days)	Period of exposure (days)	Hatching of larvae after exposure (days)
Treated	1	9	12	-
	2	6	12	-
	3	5	12	-
Control	8	6	-	22
Treated	4	6	19	-
	5	5	19	-
Control	9	6	-	22
Treated	6	5	26	-
	7	2	26	-
Control	10	5	-	

RESULTS AND DISCUSSION

Oxygen-free atmospheres were achieved in an in-situ chamber with gas-tight sealing and purging nitrogen from a generator. Figure 1 shows the data for oxygen level and climate conditions with RH and temperature inside the chamber.

The efficacy of oxygen-free atmospheres for art and cultural heritage has been investigated for many decades. In this investigation we found that hermetically closed mobile ´chambers´ without floor seal could be used to control major wood boring beetles. Exposure time for eradication of three species of wood boring insects in different stages represented a range for practical application. Controlled atmosphere of < 1 Vol.-% O_2 (Figure 1) resulted in 100 % mortality of larvae of *Hylotrupes bajulus*, *Lyctus brunneus* and *Anobium punctatum* after 43 days. 100 % mortality of eggs from *Anobium punctatum* and *Lyctus brunneus* were achieved in a controlled atmosphere of < 1 vol.-% O_2 at 22°C temperature after an inspection of 12 days (*Anobium*) and 11 days (*Lyctus*).

The usual recommended oxygen levels of 0.1 to 0.3 % could be higher, as in cases with XXL-tenting, but should always be controlled by using test insects of the target species. More scientific investigations and technical standards are needed to have safe data for different parameters of higher oxygen levels (1.0 to 1.5 %), exposure time, temperature and different insect species.

Table 2. Eggs of *Lyctus brunneus* post-exposure.

Type of Eggs	Sample No.	Maximum age of eggs at exposure (days)	Period of exposure (days)	Hatching of larvae after exposure (days)
Treated	5	2	6	4
	6	2	6	6
	9	1	6	4
	10	1	6	4
Control	15	2	-	12
	17	1	-	17
Treated	1	4	11	-
	7	2	11	-
Control	16	2	-	10
Treated	3	3	18	-
	11	1	18	-
Control	18	1	-	20
Treated	2	4	25	-
	8	2	25	-
Control	13	4	-	8

After inspecting every single piece, we were able to confirm that the *Lyctus brunneus* only got as far as the stretchers and into some crates. All measures are now implemented as we have to guarantee to the insurance carrier that everything is being done to avoid future pests. We built a vast double door system within the outer storage, connected to an anoxia chamber. Every artwork or crate that is not guaranteed to be free of hardwood will have to go through our anoxia chamber before entering a storage area. Knowing that the *Lyctus brunneus* is gaining ground wherever factory-made wood is in use, we allow only conifer to be used for construction and in crates, bases, temporary walls.

ACKNOWLEDGMENTS

We thank Jochen Wiessner, wood pests consultant, for assistance and support. We also thank Eva Fennert of Materialprüfungsamt Eberswalde for her assistance in maintaining test insects and working out the results of the mortality rate.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

CURRENT STATUS AND TREATMENTS FOR *ANOBIUM PUNCTATUM*

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Abstract This paper outlines the current status in Europe of *Anobium punctatum* and reviews its biology, environmental requirements and food sources. It will also review historic insecticidal treatment methods, their efficacy and the long term problems their residues may cause. It evaluates the efficacy of current treatments used both commercially and domestically for the control of outbreaks and the developing methods of monitoring, using insect traps and attractants, to detect the early presence of an infestation. It reviews the affect of the recent EU Biocides Regulations on chemical and non-chemical treatment options.
Key words Biocides Directive, fumigation, anoxia.

HISTORICAL DISTRIBUTION

Woodworm, or common furniture beetle, *Anobium punctatum*, is considered to be one of the most destructive pests of timber in the United Kingdom and Northern Europe. Wood-boring beetles closely related to *A. punctatum* have been found in amber from the Eocene period. They lived in dead trees and fallen logs long before man came on the scene. One of the oldest recorded infestations in the UK of *A. punctatum* comes from archaeological excavations of Roman material in York (Buckland, 1976). The species was first described from Swedish specimens by Degeer in 1774 as *Ptinus punctatus*. It seems strange that the beetle does not seem to have been known to Linnaeus. The name *Anobium punctatum* was the first adopted by Petro Rossi in 1794.

The first published reference in the UK was by Stephens in 1839 who stated that it was abundant in old houses throughout the country. It was also a relatively common insect out of doors where it attacked dead parts of trees, fallen timber and logs. The beetle is found throughout Europe. Hedges (2013) mapped the distribution of *A. punctatum* in Europe from the evidence of damage to wooden printing blocks. He has shown a clear pattern of distribution from 14th century to the 19th century of *A. punctatum* north of a line about 46 degrees. South of that line the damage to the wood blocks has been caused by another species of wood borer, *Oligomerus ptilinoides* which is the predominant wood borer in South Europe. *A. punctatum* has spread to Australia, New Zealand, North Africa, South Africa, the Eastern Seaboard of North America and Asia.

BIOLOGY AND PEST STATUS IN THE PAST

The biology and ecology of *A. punctatum* is covered by Hickin (1975) and Ridout (2000, 2012). The life cycle in the UK can be summarised as follows: Adults emerge from April to July and will fly under certain conditions of temperature and light and will live for 20-30 days. The females lay eggs in rough wood, end grain, cracks or crevices and sometimes in old flight holes. The eggs hatch within 15-25 days and the larvae tunnel into the wood for a period of 2-5 years depending on temperature, wood moisture content and nutritional value of the wood. The larval tunnels are packed with excreta (frass) which has a characteristic gritty feel and barley-grain shape when seen under a microscope. The number of larval instars is thought to be six. When they are fully grown, the larvae tunnel towards the surface of the wood and make an enlarged pupal chamber. Adults emerge from the pupae after 2-3 weeks and the adult chews through the remaining wood leaving a characteristic circular exit hole1.5-2 mm diameter.

The incidence of *A. punctatum* infestation in buildings was well documented in the past because of surveys carried out by Government agencies and companies doing remedial wood treatment. The incidence of *A. punctatum* in buildings increased in the years immediately before 1940, and then dramatically over the next 15 years. This was probably due to the large amount of unseasoned timber used in house repairs and construction after World War II. Infestation in buildings probably reached its peak in the late 1950s and early 1960s. Although damage can be caused by the tunneling of larvae, the importance of this pest as a causative agent of structural damage has probably been over-emphasised. Evidence from museum collections shows that damage to furniture and other wooden objects has been severe in the past, particularly when objects have been kept in unheated buildings or in damp basements and attics.

The beetle will breed in a variety of coniferous and hardwood timber (Hickin, 1975), including; fir, pine, maple, poplar, beech, ash, elm and oak. It is frequently stated that *A. punctatum* has a preference for wood which has been cut at least 20 years. This seems to be contradicted by evidence which shows that relatively fresh sap-wood is the most suitable for rapid development. Plywood made with animal glue adhesive is susceptible to attack and can be destroyed very rapidly because of its increased nutritional value.

A survey by Berry et al. (1993) showed that there had been a marked decline in the importance of *A. punctatum* as a structural pest in domestic properties in the UK over the latter years of the 20th Century. There appeared to be very little or no incidence of infestation in houses built after 1960 and there was also 50% drop in infestation levels in property built before that date compared with a previous survey (Tack, 1966). Berry (1995) suggested that the reduction in infestation in newer houses may partly be due to the increased levels of heating and ventilation now found in most houses. Larvae cannot establish themselves and complete development below about 65% relative humidity (equivalent to 15% timber moisture content). The moisture content of timber is not likely to exceed this value in an efficiently heated and ventilated building.

The effect of lower humidity and moisture content on the restriction of infestation of historic collections is also convincing. The only active infestation seen in museum collections in the last 30 years have been from recently introduced objects which have been kept in unheated outbuildings. None of the major museums in the UK known to the authors have active infestation of *A. punctatum* in their buildings or recent collections, other than open air museums with vernacular buildings.

Although the incidence of *A. punctatum* has clearly dropped to very low levels in buildings with good air circulation and/or climate control, there does seem to have been a recent increase in buildings which have been designed to be more energy efficient. Better insulation, reduction in ventilation and air movement all help to create micro-climates with higher levels of humidity which allows *A. punctatum* to develop. Couple this with an increase in higher starch content sustainable-grown wood and you may have increased damage by woodworm.

DETECTION AND MONITORING OF INFESTATIONS

Several detection methods have proved partially successful, including measuring respiratory carbon dioxide and the use of high frequency acoustic detectors (Vaiedelich and Le Conte, 2013) and ultra wide band radar sensors (Herrmann et al., 2013). Detection can often be difficult as the larvae do not eat and move continuously, especially at lower temperatures. Detection of active infestations is usually by observation of fresh exit holes and the accompanying faecal material (frass). The mobility of the adult beetles is defined by the surrounding temperature; they will not normally fly at temperatures below 20^{o}C. They have been caught on sticky fly papers (Kigawa, 2013) and in standard sticky traps (Child, 1993). The sex pheromone attractant for *Anobium punctatum* is known but is difficult to isolate from its stereoisomers (White and Birch, 1988).

Differentiating between active infestations and inactive ones can be difficult. Owing to the long larval development, emergence holes may appear over a number of years. Recording the holes and noting new ones in successive years has proved successful in long-term monitoring (Creffield, 1991). Adhering a patch of tissue paper with a water soluble glue over affected areas and observing any new emergence holes through it in the following years has proved successful.

TREATMENTS

Treatment options against furniture beetle infestations have changed radically in the last 20 years. The former use of toxic insecticides and fumigants has now largely been superseded by environmental manipulation and new generation insecticides.

ENVIRONMENTAL CONTROL

Experimental data confirms that *Anobium* larval growth is halted at timber moisture contents below 12% (Ridout, 2000). This relates to an ambient relative humidity of 65-70%. Temperatures below 15^{o}C inhibit movement, mating and eating and lower temperatures are increasingly fatal to all stages. Low temperature control limits state that infested material kept at -18^{o}C for 2 weeks will kill all insect stages. At -30^{o}C the treatment time is reduced to 1 week. The affected material needs to be sealed in a plastic bag with minimum air content to provide a micro-climate and prevent damaging condensation. Temperatures 52^{o}C and above will kill all stages in 1 hour (Strang, 1992 and 1995). Raising air temperatures lowers ambient relative humidity so care must be taken to humidify the heated air.

Anoxia. Reducing oxygen levels to below 0.5% will kill by a combination of suffocation and desiccation (Biebl and Lang, 2013). Objects are placed in gas-tight containers and the air replaced with inert gases such as nitrogen, or oxygen absorbers are used to reduce the oxygen content to below 0.3%. The treatment is only effective at temperatures above 20^{o}C and for extended periods of time (several weeks).

Mechanical Treatments. Non-chemical treatments to kill eggs and deep seated larvae include the use of microwaves and gamma radiation (Steinbach 2013).

Fumigation. Fumigation treatments involved highly toxic gases such as hydrogen cyanide and methyl bromide. Fumigant gases currently allowed in the European Union include phosphine. Sulfluryl fluoride (Drinkall, 1996) is currently registered as an effective fumigant in many countries, and carbon dioxide as a concentration of 60% has been used as a fumigant at high temperatures. In the European Union, sulfluryl fluoride, carbon dioxide and nitrogen are registered under the Biocides Directive 528/2012 to specific companies.

Insecticides. The Biocides Directive 528/2012 which came into force on September 2013 is designed to harmonise the use of biocides. These are active substances intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means. The affect of the legislation has been the demise of many currently used insecticides, as they no longer conform to the new regulations or are un-economic to register them.

Treatments against *Anobium* using insecticides require them to be effective for several years as they are surface treatments which kill eggs and emerging adults. They have the perceived problem of pesticide residues that may persist for many years. Some manufacturers are replacing their active ingredients with alternatives such as insect growth regulators. Insecticidal treatments against *Anobium* traditionally used insecticides such as lindane (□-hexa-chlorohexane), DDT (dichlorodiphenyltrichloroethane), dissolved in an organic solvent. Because of the toxicity and flammability of the solvent modern formulations are now micro-emulsions of the insecticide in water. Application onto affected material is by spraying or by fogging. Treatments using pyrotechnic insecticides have been successfully used against wood-boring insects (*Anobium* and *Xestobium rufovillosum*) in difficult to reach structural timbers (Ridout, 2000). Releasing the pyrotechnic smokes over successive years is intended to kill emerging adults and eggs.

NEW DEVELOPMENTS

Increasingly the treatment of structural and decorative timber against wood-boring infestations is continuing to moving away from the use of toxic insecticides. As a result of the new EU Biocides Directive, companies are concentrating more on the use of environmental solutions and the use of non-chemical treatments.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

BRACONID WASPS: A BIOLOGICAL CONTROL METHOD FOR THE COMMON FURNITURE BEETLE (COLEOPTERA: ANOBIIDAE)

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Abstract The aim of our research was to develop and test a biological control method for *A. punctatum*. After mass rearing of the host-specific braconid wasp species *Spathius exarator* (L.), laboratory control tests of the parasitism rate were conducted. Praxis tests of the efficiency of the braconid wasps were performed in six churches for the duration of two years. Monitoring of success was based on exit holes from wasps and *A. punctatum*. Laboratory control tests with defined numbers of living *A. punctatum* larvae in small lumbers showed 79% parasitism by braconid wasps after three months and 98% parasitism after ten months. The results of the praxis tests revealed a steady increase of emerging braconid wasps in all churches; up to 80% less new exit holes of *A. puncatum* were counted in 2013 compared to 2012. The results demonstrate the biological control of *A. punctatum* with the braconid wasps.
Key words *Anobium punctatum*, *Spathius exarator*, church, museum pests.

INTRODUCTION

A. punctatum belongs to the family of Anobiidae, derives originally from Europe and has spread worldwide (Pinniger and Child, 1996). It causes huge economic damage by the destruction of wood, especially in attics, stairs and in antiques of churches and museums. The eclosion of *A. punctatum* occurs between April and August, preferentially in May and June (Vite, 1952) and can be identified by a circular hole on the wood surface with a diameter of about 1 -2 mm (Becker, 1983). The beetles lay 20-60 eggs primarily in soft- and sapwood with wood moisture over 10% (Becker, 1940; Hickin, 1963; Cymorek, 1982). The *A. punctatum* larvae eat their way through the wood and thereby produce dust heaps typically found under active infested wood. The development of the larvae takes between two and five years (Becker, 1940; Hickin, 1963).

The methods of control range from local insecticide or thermal treatment to fumigation with carbon dioxide, nitrogen and sulfuryldifluoride. Especially in extensive infected buildings, such as churches or museums, these treatments often are impossible or unwanted. A new approach lies in the biological pest control with natural enemies. Several antagonists of *A. punctatum* are known, but remained unstudied for about 50 years (Becker, 1954). Thus, the aim of our work was to rear and test the host-specific parasitic wasps *S. exarator*, which are described as one of the most common natural enemies of *A. punctatum* (Becker, 1942). The development of a single wasp proceeds on *A. punctatum* larvae of different larval stages and the eclosion of the adult wasps happens through an own gnawed exit hole on the wood surface with an average diameter of 0.5 mm (Lyngnes, 1956).

MATERIAL AND METHODS

Laboratory Tests

After successful mass rearing of the braconid wasps, the parasitism rate was tested on twelve small lumbers (10 cm x 5 cm x 3 cm) with the same batch of living *A. punctatum* larvae, prepared by the Federal Institute for Materials Research and Testing (Berlin, Germany). At given times, parasitic wasps were added to half of the twelve lumbers (test samples). Other six lumbers without the addition of wasps served as a control (control samples). Table 1 shows the detailed overview of dates and numbers of added wasps. From December to February no further parasitic wasps had to be added, because the reproduction occurred internally. From March to July wasps were added again. About ten months after the first addition of wasps the experiment was finished.

Table 1. Overview of the addition of parasitic wasps to the test sample.

Date	Parasitic wasps
7.11.12	10 ♀, 5 ♂
26.11.12	10 ♀, 5 ♂
18.3.13	10 ♀, 5 ♂
30.5.13	10 ♀, 5 ♂
6.7.13	10 ♀, 5 ♂

The conditions in the laboratory were adjusted to 18h light, 6 h darkness, 21°C room temperature and 75% relative humidity. Once a month all wood dust heaps per lumber were counted and removed. In addition all new *S. exarator* exit holes per lumber, which match the number of parasitized *A. punctatum* larvae, were documented. At the end of the experiment, the wood moisture was measured with a wood moisture meter GMH 3810 (Greisinger Electronic). To determine the exact number of living and dead larvae, the lumbers were disintegrated with a pry bar.

The number of exit holes of wasps in addition to the number of living *A. punctatum* larvae at the end of the experiment result in the total number of vital larvae at the onset of the experiment. With this number, the parasitism rate per month was calculated in percent.

Praxis Tests

To test the ability for parasitism outdoor, six different infested churches were treated with the braconid wasps six to eight times a year within duration of two years. Monitoring of success was based on the intensity of the infestation of *A. punctatum* in contrast to the effectiveness of the parasitic wasps. Heavily infested wood surfaces were bond with commercial wrapping paper and 10% tylose glue (Noldt, 2007). After each treatment, the new exit holes of *A. punctatum* and braconid wasps, distinguished by the diameter, were marked with colored pens and counted.

DATA ANALYSIS

Statistical analysis was done with Microsoft Excel. The correlation between the parasitism rate and the number of wood dust heaps was calculated with Pearsons´correlation and the significance of the correlation (one-tailed) was determined with the software of Soper (2014). The difference of exit holes from A. *puncatum* between 2012 and 2013 was calculated using a one-tailed two-sample-t-test for dependent samples. P-values of $p \leq 0.05$ were considered significant and marked with *.

RESULTS AND DISCUSSION

Laboratory Tests

The measurement of wood moisture of the lumbers gave an average value of 13.7 ± 0.77 % relative humidity. This moisture provides optimal conditions for the development of *A. punctatum* larvae.
To evaluate the parasitism rate, the exact number of living *A. punctatum* larvae at the onset of the experiment was determined and is listed in Table 2. This value is an addition of the number of exit holes of wasps and the number of living *A. punctatum* larvae at the end of the experiment.

Table 2. Number of living larvae and exit holes in the lumbers.

	Number of exit holes of wasps	**Living *A. punctatum* larvae at the end**	**Living *A. punctatum* larvae at the beginning**
Control sample	0	38 (+1 adult beetle)	39
Test sample	39	1(+2 pupae of parasitic wasps)	42

The control samples with its 38 living *A. puncatum* larvae and one emerged adult beetle at the end of the experiment proved optimal life conditions for *A. punctatum*. In the test samples 42 living *A. punctatum* larvae were present in the lumbers at the onset of the experiment. At the end, only one *A. punctatum* larva survived and two partial developed wasp pupae rested in the lumbers. Moreover, 39 exit holes of the wasps were detected, corresponding to the number of parasitized larvae. The increase of parasitized *A. punctatum* larvae per month is shown in Figure 1, together with the number of monthly counted wood dust heaps.

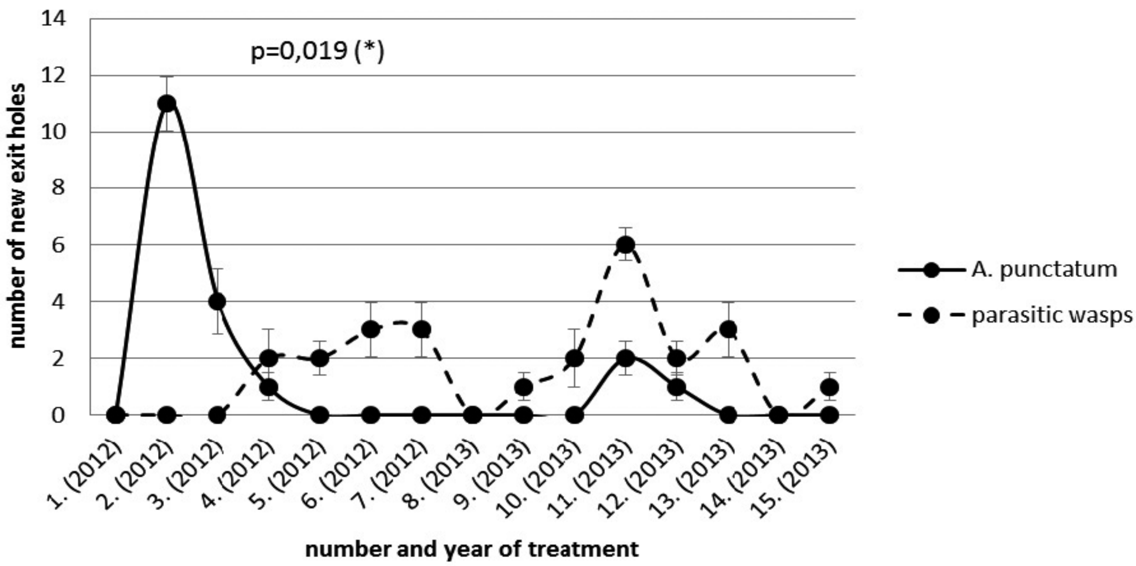

Figure 1. Parasitized *A. punctatum* larvae (round dots) and numbers of wood dust heaps (triangular dots) in the test samples. Standard derivation results from differences between the six lumbers. The correlation coefficient after Pearson was -0.85 with a significant correlation between the two curves (p=0,016). The arrow indicates the date of the initial addition of the parasitic wasps at 7.11.12.

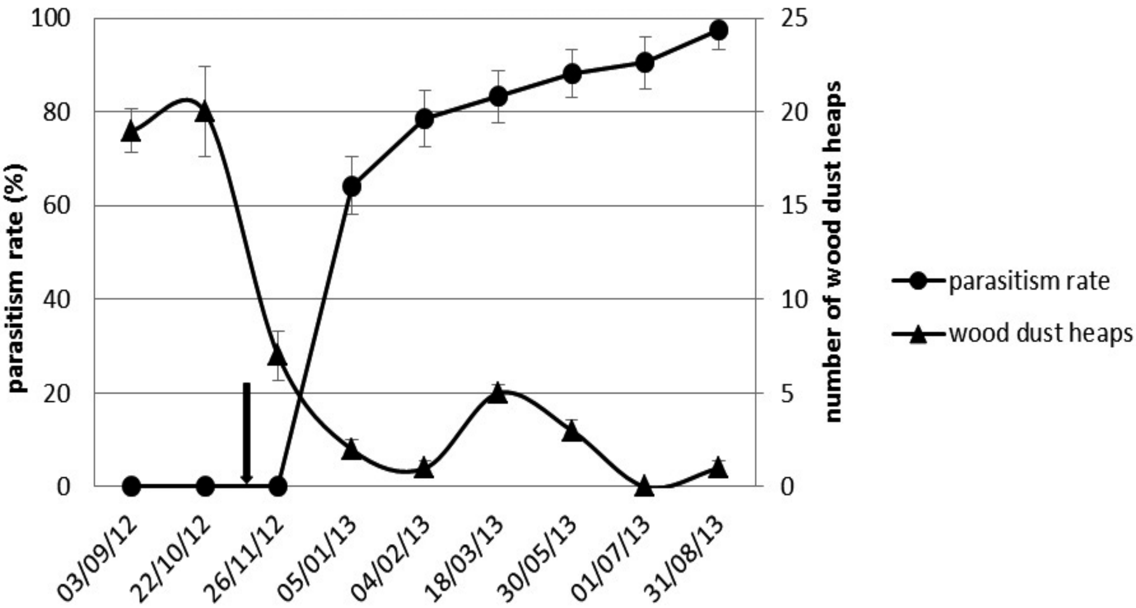

Figure 2a. Church N. Number of exit holes of *A. punctatum* (continuous line) and of parasitic wasps (dotted line) of pews in the church N. (a) and S. (b), masked with wrapping paper. Values are given as total amount of exit holes from four parts of the pew (0.7 m^2) (a) and from two different parts of the pews (together approximately 0.15 m^2) (b) with the mean standard derivation. Statistical analysis between the numbers of exit holes of *A. punctatum* from 2012 and 2013 was carried out with a one-tailed two-sample t-test for dependent samples.

Three months after the first addition of *S. exarator*, 79% of the *A. punctatum* larvae were parasitized. At the end of the experiment, an almost complete parasitism was achieved with 98% killed *A. punctatum* larvae. The increase of parasitized larvae showed a high negative correlation with the decrease of wood dust heaps with a correlation index of r=-0.85. The statistical analysis revealed this value as significant (p=0,016).

Praxis Tests

In 2012 and 2013 six different churches were treated with parasitic wasps against *A. puncatum*. Monitoring of success was performed by counting new exit holes from braconid wasps and *A. punctatum* as described above. Representative results from two different churches were shown in Figure 2a and b.

Figure 2a shows the summarized number of new exit holes of *A. punctatum* and parasitic wasps on four infested parts of the pews in the church N., masked with wrapping paper. Two hatching phases of *A. punctatum* can be identified. The first happened between the first and fourth treatment (from June to August 2012), the second hatching phase was between the tenth and twelveth treatment (June to August 2013). The number of emerged *A. punctatum* decreased from 16 beetles in 2012 to 3 beetles in 2013, according a significant reduction of 81% (p=0,019). During the entire treatment period 25 *A. punctatum* larvae were parasitized in the monitored area, as identified by the exit holes of the wasps.

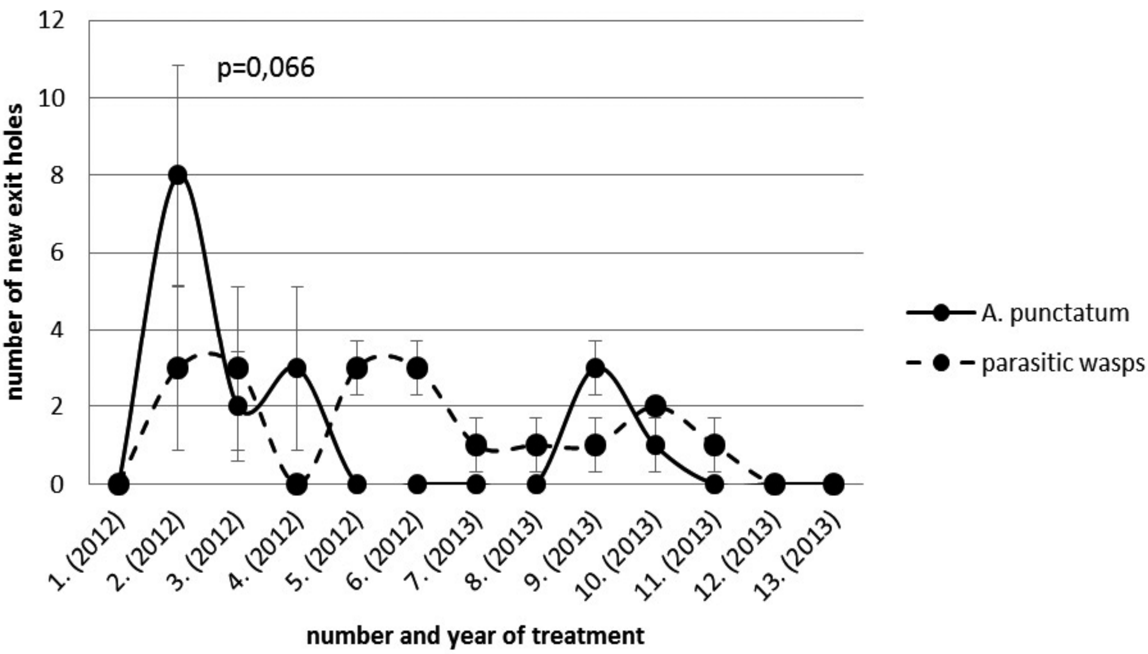

Figure 2b shows the number of summarized exit holes on two parts of the pewage in the church S. In 2012, a total number of 13 hatched *A. punctatum* were counted, with a peak between the first and third treatment. In 2013, four *A. punctatum* hatched, corresponding to a 70 % reduction (p=0,066). This reduction was caused most likely by the parasitism of the braconid wasps, which killed 17 *A. punctatum* larvae in different stages of development in the monitored area.

CONCLUSIONS

The results of the laboratory tests show a fast, effective parasitism of *A. punctatum* by the host-specific *S. exarator*. Moreover, the praxis tests in several churches proved successful parasitism of *A. punctatum*. This tests lead to a reduction of up to 80% hatched *A. punctatum* beetles within strong infested areas in the second year of treatment. Therefore it can be reasoned that the biological treatment with suited braconid wasps is an effective, easy-to-apply method for large-area *A. punctatum* infested objects. However, the further development of infestation by *A. punctatum* and the need for additional, selective treatments remains to be investigated in the following years.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

ERADICATING THE ASIAN LONGHORNED BEETLE IN WINTERTHUR, SWITZERLAND

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Abstract In 2012, an infestation of Asian Longhorned Beetle (ALB) was detected in Winterthur (Canton ZH, Switzerland). With more than 140 adult beetles and several hundred eggs and larvae, the infestation was by far the most extensive in Switzerland. Based on experiences in Austria where the first ALB infestation in Europe occurred, the beetle was fought with extensive actions: removal of all infested trees, preventive removal of potential host trees and installation of a focus- and a buffer zone. Transport of plant material out of these zones was banned and a close monitoring regime including specialised dogs put in place. Tere were no adult beetles and only a few living larvae detected 2013. 700‘000 Swiss francs were spent in 2012, not including hundreds of working hours spent by employees of the Canton Zurich. The programme is estimated to cost another 2.8 million Swiss francs, if no more living beetles are detected. The experiences from Winterthur show that elimination of a small ALB infestation is possible. The costs demonstrate the need for strong boarder controls or alternative packaging materials for imports from infested regions.
Key words Infestation zone, monitoring, pest elimination.

INTRODUCTION

Asian Longhorned Beetle (ALB) is one of the most dangerous plant pests worldwide, causing millions of dollars of damage and the reason for thousands of trees cut in urban space. ALB was introduced into the United States in the early or mid nineties, and has since then become a major economical and ecological problem in the US, Canada and Europe, where it was introduced first in Braunau (A) in 2001. Its broad host spectrum, its ability to attack completely vital trees and its larvae's capability of final development even in dead timber enhance ALBs spread and the damage it causes after infestation.

In summer 2012, an infestion of ALB was discovered in Winterthur, a city of some 100‘000 inhabitants near Zurich, Switzerland. Compared to earlier infestations in the US, Canada or Europe, the infestation was discovered at an early stage (likely in its third generation), and was therefore smaller in extent. Nevertheless, it was by far the most extensive infestation in Switzerland: more than 140 living beetles and several hundred eggs were discovered. The infestation occured in an industrial area, where a new street had been constructed. It was surrounded by industrial property and waste land, with no private housing in the immediate proximity of the infestation. From the beginning, authorities in Switzerland were cooperating closely with partners from Braunau, and decisions were taken based upon experiences in the Austrian town, where ALB could be declared extinct in

2013. Consequently, ALB was fought with rigorous actions immediately after discovery, and this active phase was followed by an intense monitoring programme, that also included organisational measures with respect to transport of plant material. At the moment, this monitoring programme is in its second year, and as there were new larvae found in 2013, will go on at least until 2017.

MATERIALS AND METHODS

A number of different short-term and long-term measures were taken in order to erase ALB in Winterthur, based on three different zones defined:
Infested area (living beetles or trees with larvae, eggs or exit holes detected),
Focus zone, 200-500 m around infested area, depending on density of broadleaf trees,
Buffer zone, up to 2 km around infested area.

Measures in infested area: Immediate removal of all infested trees. Trees were shredded on-site and all material was disposed of at a waste incineration plant; Removal of potential host trees in a radius of 100m around infested trees.

Measures in focus zone: Mapping of all potential host trees; Intense control of broadleaf trees with specialised sniffer dogs and tree climbers; Trees indicated suspicious by sniffer dogs have to be controlled with tree climbers, as well as trees that are not easily controllable from the ground; Preventive removal of many potential host trees ; Information of the public by personal contact.

Measures in buffer zone: Sampling of potential host trees with dogs and from the ground, including controls in forest stands; Information of public by flyers; Control and information of companies at risk (importers of stone-material).

Zones were marked with signs. Transport of any plant material out of these zones was banned, a central collecting point was installed inside the infested area. Stem wood as well as firewood could only be moved out of the zones with cantonal permission or had to be shredded at place. From the beginning on, there was a close cooperation between municipal, cantonal and federal authorities, as well as with research groups and partners abroad. Monitoring as well as control measures have to be continued for four years without any living beetle, larvae, egg or any new exit hole to be found for the infestation to be declared as erased. In Winterthur, this will be in 2017 at the earliest.

Legal Background

Combating ALB was based legally on the enactment of plant protection, where ALB ist listed as one of the most dangerous plant pests that has to be reported and fought. The Federal Department of the Environment ordered the Canton of Zurich to implement the measures described above. Generally, based on the enactment of plant protection, the canton has to pay for the monitoring, while the community with an infestation on its territory has to pay for eradication measures.

Import Control

As a direct reaction on the large ALB infestation in Winterthur, the federal office for plant protection strongly increased border controls. All wooden packing material imported from different asian countrys has to be declared in advance and is controlled visually or with sniffer dogs upon arrival. Suspicious batches have to be aerated. In parallel, political efforts have been made to increase heat treatment in the exporting countrys. The stone industry, the main importer of suspicious wooden packing materials, took measures in order to decrease the risk for ALB introduction: new packaging methods, inspections on production sites as well as efforts to increase heat treatment frequency and quality by the stone suppliers from Asia.

RESULTS AND DISCUSSION

So far, the measures taken have proved effective. After removal of all infested trees and the intense search for ALB, no more beetles have been found. In 2013, only some living larvae were found, but ALB was not expected to fly in that year. Thus, a very close monitoring has to take place in the warm season of 2014. During 2013, detection dogs kept on finding larvae and a few infested trees, and there were a number of trees under suspicion. Most of them were removed precautionarily. In addition, border controls found only a few shipments contaminated with ALB, which shows an increased awareness within the stone industry. However, it will only be after the 2014 flying season that a more consolidated statement about the eradication measures in Winterthur can be made.

Financing

Switzerlands first big ALB infestation occured in Winterthur, a city with sufficient personal, machinery and financial capability to deal with the infestation. The direct costs for 2012 had to be borne by the city, and they rocketed to 700‘000 Swiss francs (770‘000$) within a few months. Not included in these numbers are hundreds of working hours spent by employees of the Canton Zurich, by members of the Swiss Federal Institute of Forest, Snow and Landscape Research WSL and by employees of the Federal Office for the Environment (BAFU). The 4-year monitoring programme is estimated to cost another 2.8 million Swiss francs (3.08 million $), which will, based on a special enactment of the local governement, be payed for by the canton.

It is clear, however, that Winterthur itself was lucky in two respects: the infestation was in its third generation and thus shortly before becoming very hard to control, as several forest stands are nearby. And secondly, because Winterthur was able to deal with the infestation initially. Another big plus was the fact, that a mainly industrial area was in the focus, not private property with gardens full of beloved trees and bushes. This certainly made rigorous actions easier and it prevented the question of compensation from being raised. In fact, the legal basis for such a compensation is not really clear, as ALB is considered a forest pest, but the infestation took place on city grounds. Legally, there are big differences between forests, agriculture and public or private green, with different administrative units in charge.

One consequence of this ALB infestation is the need for a clearer legal basis with respect to compensations that might be or might have to be provided after eradication measures have taken place. Secondly, there must be a concept for smaller communities that a) do not have enough personnel and machinery to deal with an infestation, and b) do not have the financial resources to have this work done by external services. At the moment, there are discussions about a cantonal action force equipped with enough capacities to intervene whenever necessary. However, as there are not that many ALB infestations, this action force should be integrated into other cantonal duties. Last but not least, clarity about the costs of such an infestation is needed. Specifically, it has to be determined within a legal frame, which cost are to be borne by the community, the cantonal or the federal administrations, respectively. On the practical side, knowledge transfer has to be guaranteed. This refers to both eradication measures and monitoring (detection dogs, tree climbers).

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

LIKELIHOOD OF INFESTATIONS BY *TINEOLA BISSELLIELLA* (LEPIDOPTERA: TINEIDAE) FROM NATURAL RESERVOIRS

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Abstract The webbing clothes moth *Tineola bisselliella* is economically the most import pest on wool, fur, and feathers. The larvae cause damage in stores, museums and households. Infestation can be through import of infested material or by new infestations out of natural reservoirs. Natural reservoirs are believed to be bird, rodent or insect nests. Rearing experiments with collected different nesting materials have shown that *T. bisselliella* is capable to breed in animal nests as well as on stored seeds. Outdoor trapping with pheromones for catching males or with attractive food for the larvae to catch females indicate that the presence of *T. bisselliella* outside buildings is very limited. Although the webbing clothes moth has the potential to survive in non-synanthropic environments, it does not frequently occur there. Intra-guild competition with other tineid moths prevents *T. bisselliella* from establishing sustainable outdoor population. New infestations from the field are unlikely and preventive control must focus on quarantine to avoid the pest's introduction via contaminated material.
Key words clothes moth, invasion, animal nests, quarantine, out-door trapping.

INTRODUCTION

The common or webbing clothes moth *Tineola bisselliella* (Hummel) is one of the most destructive pest insect world-wide of wool, hair, feathers, furs, or articles manufactured from these materials (Kemper, 1935; Flint and McCauley, 1937; Turner and Walden, 1937; Back, 1940; Hinton, 1956; Becker, 1983; Rajendran and Parveen, 2005). In homes, frass by clothes moth larvae leads to quantitative and qualitative damage in woolen fabrics, clothing, and upholstered furniture (Parker, 1990). In drapery shops and rug stores, clothes moth infestations can cause economic losses (Hammers, 1987). *Tineola bisselliella* is also considered a severe museum pest because it infests and destroys ethnological and natural history exhibits which usually contain unique and precious artefacts (Pinniger, 1994).

Control measures to hinder larvae from feeding or to kill intruding flying adult moths of *T. bisselliella* are manifold and include use of insecticides, fumigants, extreme temperature, traps and the release of parasitoids (Bry and Simonaitis, 1975; Florian, 1987; Parker, 1990; Trematerra and Fontana, 1996; Plarre et al., 1999; Pinniger, 2010). However, prevention of new infestations from the beginning would be the key strategy and is an essential part of any IPM program. Although several observations and monitoring studies had been carried out (Child and Pinniger, 1993; Brand and Wudtke, 1997) the pathways of new infestation for this pest remain obscure. For clothes moths two major scenarios for new outbreaks can be thought of: import of infested material or invasion out of reservoirs from the outside. The later requires sustainable moth populations in the natural environment and an excellent mobility by the adults. We have tested the likelihood of *T. bisselliella* occurring outside buildings away from direct synanthropic conditions which are usually found in homes, stores or museums. We have set up pheromone-traps and artificial nest-traps out-doors to catch migrating male and female adult moths (Robinson, 1988a). We have collected empty nest material from birds and insects and inoculated these with eggs of *T. bisselliella* to check for their developmental potential on these substrates respectively diets. We critically analyzed published faunistic records of clothes moth findings in bird nets.

MATERIAL AND METHODS

Pheromone traps. Outdoor pheromone trapping was carried out in two consecutive years from August 2008 to July 2010. Several trapping locations, 23 in the first and 13 in the second year, were set up within and outside the city limits of Berlin (Germany). The sticky traps were placed outside buildings but in sheltered areas. Traps contained a sex-pheromone lure for *T. bisselliella* provided by Insects Limited (Indianapolis, USA). Trap catches were checked and lures were changed on a biweekly basis.
Artificial nest-traps. Artificial nests were made of goose quills which had been soaked in 10 % nutritional yeast/water solution and oven dried. This material has successfully been used for rearing *T. bisselliella* in the laboratory at BAM. Quills were loosely packed into a plastic bucket (12 cm in diameter, 12 cm in height) which was placed into a modified wooden bird box (18 cm x 18 cm base area, 22 cm in height) with an entry slit (Figure 1). Similar to the pheromone traps, artificial nests were placed in sheltered areas outside houses in and near Berlin at 9 locations (Table 1). Prior to that, tests at ca. 25°C and 65% RH in a laboratory flight room (5 m x 2 m x 2 m) revealed the principal suitability of the trap design to catch webbing moth females (Plarre, 2011). Traps were checked and replaced by new material on a quarter-yearly basis for two year (August 2011 to July 2013). After initial visual trap inspection for insects the artificial nest-material was incubated at rearing conditions of 27 ± 1°C and 75 ± 5 % RH. in the laboratory to allow development of any instars with a final check after 6 weeks.

Table 1. Qualitative evaluation of trapped *Tineola bisselliella* in artificial nest traps at different locations in and around Berlin, Germany for two years.

trapping location	**short description of location** (number in brackets corresponds with trapping location of figure 2)	***T. bisselliella* trapped** (yes or no)
city center	urban-city, multifamily house, balcony 1^{st} floor (-)	no
	urban-city, multifamily house, balcony 2^{nd} floor (14)	yes
	suburban, multifamily house, balcony 5^{th} floor (12)	no
	suburban, single family house, backyard (10)	no
	suburban, single family house, backyard (8)	no
	suburban, single family house, backyard (9)	no
	urban fringe, single family house, backyard (-)	no
	urban fringe, single family house, backyard (-)	no
country side	landscape area, single family house, backyard (20)	no

Table 2. Development of *Tineola bisselliella* from egg to adult on different breeding materials.

Material tested for development of *T. bisselliella*	Development from egg to adult
blue tit *Cyanistes caeruleus* nest foundation, plant material only	2 replications with no successful development
blue tit *Cyanistes caeruleus* breeding nest with dead mummified chicks	1 replication with 1 successful development
empty wasp nest *Vespula vulgaris* with insect debris	1 replication with 1 successful development
insect corpses of adult European house longhorn beetle *Hylotrupes bajulus*	2 replications with 2 successful developments
insect corpses of adult webbing clothes moths *Tineola bisselliella*	3 replications with 1 successful development
insect corpses of adult case bearing clothes moths *Tinea pellionella*	3 replications with 2 successful developments
wheat kernels *Triticum* spec.	1 replication with 1 successful development
wheat kernels *Triticum spec.* with insect corpses of adult grain weevils *Sitophilus granarius*	2 replications with 1 successful development
insect corpses of alate dry wood termites *Incisitermes marginipennis*	2 replications with 2 successful developments
insect corpses of alate dry wood termites *Kolotermes flavicollis*	1 replications with 1 successful developments

Breeding experiments. A number of different substrates were tested whether *T. bisselliella* was capable for a successful development from egg to adult. The materials were collected by chance or were byproducts of the BAM multi insect rearing facility. They are not considered exhaustive nor exceptional representative. All material may be encountered in natural reservoirs (Table 2).

Figure 1. Artificial nest trap in modified wooden bird box to attract females of Tineola bisselliella. The plastic cup contained yeast soaked and oven dried goose quills, a substrate suitable for larval development and thus attractive to egg-laying females.

RESULTS AND DISCUSSION

Pheromone traps. Figure 2 (2A for 2008/2009 and 2B for 2009/2010) shows cumulated catch data of trapped male moths per year and per trapping location arranged from city center (left) to country side (right). The further away from the city the fewer was the number of *T. bisselliella* captures up to total absence of this species in the country side. Interestingly, besides negligible by-catches one other tineid moth, the brown-dotted clothes moth (*Niditinea fuscella* L.), was caught in also high amounts. It is known that males of this species are attracted to alcohol compounds (Hwang *et al.*, 1978), and most likely they were lured into the trap by the webbing clothes moth pheromone´s solvent. The brown-dotted clothes moth is a typical species from bird nests in Central Europe and trapped with alcohol based lures (Trematerra and Fiorilli, 1999). Trap catches of *N. fuscella*, were reciprocal to those of *T. bisselliella* with highest numbers in the country side.

Artificial nest-traps. Table 1 shows presence or absence of clothes moths in nest traps after initial visual inspection and after incubation. Trapping locations are arranged from city center (top) to country side (bottom). Clothes moths were caught in only one single trap located in the city center. All other traps lagged the presence of this species. Because the principal suitability of the trap design to catch flying webbing clothes moth females had been demonstrated in the laboratory, it is believed that in this survey female moths were either not present in the respective out-door environment or the overall abiotoc seasonal conditions regarding temperature and humidity prevented extent flight behavior (Titschack, 1925; 1927; Griswold, 1944). Regardless of the reason, female *T. bisselliella* appear not to extensively commute out-doors.

Breeding experiments. *Tineola bisselliella* was capable of successful development from egg to adult on any of the tested material of animal origin (Table 2). Keratin, a fibrous structural protein, was believed to be essential and obligatory for clothes moth development (Robinson, 1988b). Our rearing experiments on insect corpses, however, suggest that chitin can serve equally well. Thus accumulation of insect debris which may occur in seasoned insect nests (bees, wasps, ants, termites) must also be considered as excellent breeding reservoirs for *T. bisselliella*. Even starch dominated material of purely plant origin like wheat seeds seem to allow complete development of this moth species (Table 2). Findings by Ishii and Kawahara (1966), Becker (1980), Sellenschlo (1990) and Stejskal and Horak (1999) support the oligophagous potential of *T. bisselliella,* which can be regarded as a pre-adaption to successfully invade the synanthropic environment. In our experiments only the base-nest of the blue tit which was made of small wooden sticks and moss was not suitable for clothes moth development.

Review of faunistic records. Reports in the literature regarding the presence of *T. bisselliella* in natural habitats away from human housings are rare and confusing. For example, in his summary of bibliographies on nidicolous insects Hicks (1959) lists 15 references to *T. bisselliella* findings in bird nests. Thus, in secondary and tertiary literature, it is often listed as a common species in bird nests (Niethammer, 1937; Uhlmann, 1937/1938; Hinton, 1956, Petersen, 1969; Hannemann, 1977; Klausnitzer, 1988; Pinniger, 2001; Cox and Pinniger, 2007). Our own studies of the listed references, however, do not allow for this general conclusion. First one notices duplications of citations as well as generalized faunistic reports from secondary literature without specified data. This limits the number of original works reporting the presence of webbing clothes moths in bird nests to only six references (Boyd, 1936; Herfs, 1936; Kemper, 1938; Büttiger, 1944; Woodroffe and Southgate, 1951/1952; Woodroffe, 1953), The abundances of webbing clothes moth individuals in these finding are all very small (Weidner, 1961).

Indirect negative reports on *T. bisselliella* exist: Nordberg (1936) did not find any webbing clothes moths in a total of 422 nests of various bird species, while he did find large numbers of other moths including *N. fuscella*. Hinton (1956) never found webbing clothes moth in bird nests. Similarly, the webbing clothes moth is not listed in the results of the faunistic investigations by Green (1980) and Krall (1981).

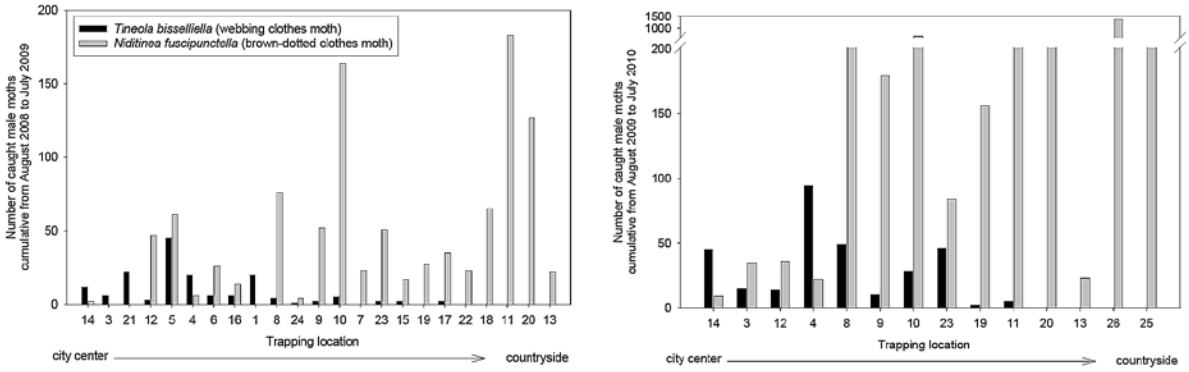

Figure 2. Pheromone trap catches of *Tineola bisselliella* (black bars) and *Niditinea fuscella* (grey bars) at trapping locations in and around Berlin, Germany, from city center to countryside. Numbers of trapped male moths are cumulated for each location and year (1st year left and 2nd year right).

CONCLUSION

It is generally accepted that the natural habitats of most pest insects can be found outside the synanthropic environment in layers of leaf litter, under bark, as well as in rodent or bird nests. Indeed, most of the common pests have been reported as being facultative nidicolous (Linsley, 1944). Therefore infestation of commodities by pest insects out of these reservoirs is one considerable possibility. However, the likelihood of a pest´s occurrence and survival out-doors largely depends on its ecological potential and competitiveness against other species of the same ecological guild. Some pest species are rarely found in wild habitats, especially in those regions where they are not native and where they have been introduced by man.

The fabric pest *T. bisselliella* serves as a good example. Most likely originating in Central or Southern Africa this insect was introduced into Europe probably not earlier than the late 18th century (Weidner, 1970; Plarre and Krueger-Carstensen, 2011). Being more tolerant to dry environments than other fabric pests its economical importance increased during the 20th century when in-door climates changed because of central heating systems. *Tineola bisselliella* should be regarded as an eusynanthropic species. Its occurrence in out-door natural habitats must be regarded as accidental. Reported founds of webbing clothes moth larvae in bird nests have been largely overstated in the literature. Although theoretical possible, *T. bisselliella* must be considered to be an exception and a seldom to very seldom occurrence in animal nests of paleoarctic biocoenoses. The females' very poor flight ability at moderate temperatures (Hinton, 1956), as well as the strong competition from other species in natural habitats may be the causes. Other tineid moth, especially of the *Tinea*-group and several dermestid beetle species of *Anthrenus* and *Attagenus* are the most common keratinophagous nidicolous insects found in mild climate zones (Hicks, 1959; Petersen, 1963). Economically relevant new infestations by *T. bisselliella* occur through the displacement and receipt of infested materials. Therefore good quarantine measures are key requirements to prevent damage.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

DISTRIBUTION OF *CTENOLEPISMA LONGICAUDATUM* (ZYGENTOMA: LEPISMATIDAE) IN THE NETHERLANDS

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Abstract In The Netherlands, until 2002 it was thought that next to *Lepisma saccharina, Thermobia domestica* was present in residences as well. It appeared *T. domestica* had been identified incorrectly and was *Ctenolepisma longicaudatum*. It is hypothesized that the latter species is better adapted to the indoor environment than the other two species. *Thermobia domestica* has not been found in the last years. *Lepisma saccharina* is present far less than *C. longicaudatum*, whereas the latter is spread throughout The Netherlands and found within modern residences. Significantly more individuals of *C. longicaudatum* than *L. saccharina* are sent in by the public as have been found visually during house searches.
Key words Gray silverfish, common silverfish, firebrat.

INTRODUCTION

From the order of Zygentoma, over 400 species are known worldwide (Mendes, 2002). Only a few species are considered to be pests. *Lepisma saccharina* (the common silverfish) and *Thermobia domestica* (the firebrat) are relatively well studied indoor pest species and are (probably) cosmopolitan. Their pest status is based on their presence indoors and damaging of materials, such as old book binding, clothing and wallpaper glue (Robinson, 2005). *L. saccharina* occurs in warm and damp locations. *Thermobia domestica* is thermophilic with an optimum temperature of 37 to 39^{o}C (Ebeling, 1975). Both *L. saccharina* and *T. domestica* have been described to be present in The Netherlands early in the 20th century (Oudemans, 1900). Oudemans (1900) described *T. domestica* to be found at or near bakery ovens, although Sweetman (1938) described this species to be a common household pest. Until 2002, it was thought to be common in The Netherlands, until Beine Nierop and Hakbijl (2002) discovered *T. domestica* had been incorrectly identified and were actually *Ctenolepisma longicaudatum* (gray silverfish).
Ctenolepisma longicaudatum differs from *L. saccharina* and *T. domestica* in that they are not as limited to temperature and moisture conditions (Ebeling, 1975).

Modern residences are built with increasing insulation values. In combination with the increased use of central heating, this provides a stable dry and warm environment. It is therefore hypothesized that *C. longicaudatum* is the dominant species within the modern household environment in The Netherlands.

In this study both the distribution of *C. longicaudatum* and the abundance of this species as a pest in the indoor environment as compared to the other species of Lepismatidae in The Netherlands are presented.

MATERIALS AND METHODS

Data for this study are acquired from three sources: archival data from identifications performed in 2007 until 2012, a survey in which people were requested to send in silverfish, and from visual inspections and manual capture within residences.

Archival Data

Data from samples that have been sent for identifications and advise on pest management that were sent to the Dutch Expertise Centre for Urban Pests (Kenniscentrum Dierplagen) in the period of 2007-2012 have been used to determine both the distribution as abundance of Zygentoma species within the household environment. Houses (terraced, semidetached, detached), apartments, flats, care shelters and attached sheds and garages, were considered as the household environment.

Coordinates of the addresses where the samples were found, were used for the map. In case the address was not known, the coordinates of the centre of the city, town or village were used. The coordinates were transformed to fit a grid with squares of 5x5 kilometres. Multiple samples within a 5x5 kilometre square are shown as a single square. The map was created using ArcGIS version 9.3.1 (Esri Nederland B.V., Rotterdam). Samples of Zygentoma species were identified using Beine Nierop and Hakbijl (2002), Gorham (1991), Weidner (1993) and Weidner and Sellenschlo (2010).

Survey

In October and November 2013 a survey was conducted in which people were contacted and encouraged to send in as many indoor Zygentoma as possible. The request was placed on the website (www.kad.nl), was sent by online newsletter and picked up by several media (including www.natuurbericht.nl and www.vroegevogels.nl). Respondents were asked to answer a few questions regarding the date of construction and type of building, and nuisance and control measures taken.

Residential Inspections

A total of 17 single-family houses within the municipality of Houten, The Netherlands were visited in May 2013 and visually inspected for the presence of Zygentoma species. From the nineteen seventies, the population of Houten increased from approximately 4,000 to approximately 45,000 in 2006. Within this period several residential districts were built in different decades. The inspected residences were selected on period of construction, which ranged from 1964 until 2007 with the exception of one house dating from early 19th century, and were located throughout town. Residents were contacted in person and were asked for permission to inspect their premises. Standard inspection locations were the crawling space, broom closet, meter cupboard, attic, storage rooms, sanitary areas, laundry, and central heating boiler. Based on the information provided by the residents, other areas were included as well. Residences were inspected by two persons. Zygentoma species. were hand collected and stored in plastic vials for further identification according to Weidner and Sellenschlo (2010). The surface temperature and relative humidity of locations where Zygentoma species. were found, and similar locations if they were not found, were measured using an infrared thermometer (Optris MS, Optris GmbH, Berlin, Germany) and a thermo-hygrometer (Testo 625, Testo BV, Almere, The Netherlands), respectively.

Data Analysis

Differences between number of individuals from different species are tested using the Mann Whitney U test. Numbers of individuals found with the residential inspects in houses built in different periods of construction were tested using the Mann Whitney U test. The observed number of *C. longicaudatum* from two different periods of construction were tested against the expected number of *C. longicaudatum*, based on the housing stock from these periods of construction, by a one way chi square test.

Data was analysed using IBM SPSS Statistics 20 (IBM Corporation, Armonk, United States).

RESULTS AND DISCUSSION

There was a significant difference (p= 0.004, Mann Whitney U) between the number of *C. longicaudatum* and *L. saccharina* that were sent in for identification from the household environment. *Ctenolepisma longicaudatum* makes up, on average (± s.e.), 11.5 ± 1.0 percent of all samples that were sent in from the household environment for identifications, whereas this is only 0.7 ± 0.2 percent for *L. saccharina*. *Thermobia domestica* was not among the samples received. In the survey that was conducted in 2013, a total number of 35 samples was sent in, in which only one contained a single *L. saccharina*, whereas in total 416 *C. longicaudatum* (>99%) were sent in, with an average (± s.e.) of 13.4 ± 2.9 individuals per residence. The maximum number of *C. longicaudatum* received was 62, whereas some respondents sent in a single one. From the pest species of Zygentoma that occur within buildings, *C. longicaudatum* is by far the most abundant.

Furthermore, *C. longicaudatum* can be found spread over the entire country, from east to west and from north to south (figure 1). It is even found on Ameland, one of the islands in the Wadden Sea.

The results of the survey of 2013 show a high percentage of samples of *C. longicaudatum* from residents of relatively recent periods of construction (67% in the period of 1991-2010), whereas the percentage of samples from earlier periods of construction was lower (33% in the period before 1991). The housing stock, however, shows an opposite distribution based on the period of construction. Residences built within the period of 1991 until 2010 make up only 22% of the housing stock, however (den Otter, 2012). The observed numbers of *C. longicaudatum* are significantly different from what was expected based on the housing stock (p< 0.001, one way chi square). This means that relatively more of these insects were sent in by people living in residence of recent construction. Altered construction requirements, methods and materials account for an indoor climate change, making the household environment warmer and drier. It is therefore expected that the ratio in abundance between a species that prefers warm, but can colonize drier environments, such as *C. longicaudatum*, and a species that is bound to a more humid environment, such as *L. saccharina,* increases through the decades. When comparing the number of insect samples received in the period of 1972 until 1977, the percentage of *L. saccharina* (2.3% ± 0.3 on average ± s.e.) is indeed significantly higher (p= 0.004, Mann Whitney U) than of *T. domestica* (0.1% ± 0.0 on average ± s.e., data from Anonymous (1978)), another thermophilic species capable of colonizing drier environments, although it is to be expected these were *C. longicaudatum* as well.

There was a significant difference between the number of *C. longicaudatum* found within the nine residences (seven terraced, one semi-detached and one apartment) built after 1990 (9.1 ± 2.2 on average ± s.e.) and the number found within the eight residences (six terraced, one detached and a single maisonette) built before 1991 (1.2 ± 0.5 on average ± s.e., p= 0.002, Mann Whitney

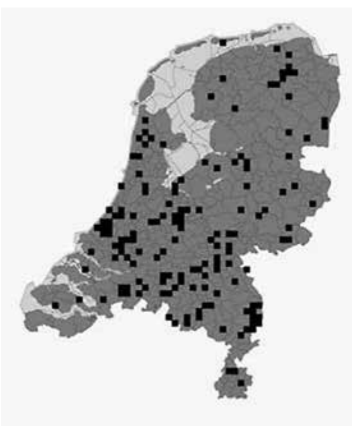

Figure 1. Distribution of Ctenolepisma longicaudatum in The Netherlands, based on data from 2007 – 2012 and the survey of 2013. Squares represent areas of 5 x 5 kilometres.

U). Although slightly higher, no difference was found in overall temperatures and relative humidity between locations where *C. longicaudatum* was found and locations without these insects, but these differences were not significant, perhaps due to the low number of houses visited.

There is a low level of tolerance for these insects, as ninety percent of the respondents considered them a nuisance, because of hygienic reasons (33%), damage (30%) or just their presence (36%), and nearly half of the respondents had taken control measures before: vacuuming, capturing by glue boards or by hand, using insecticide sprays, ventilating, reducing temperatures and cleaning up; see Figure 2.

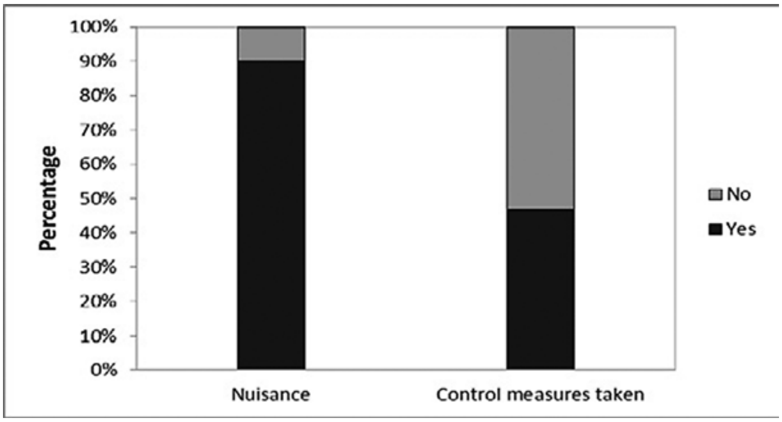

Figure 2. Percentage of respondents (N= 35) that consider Ctenolepisma longicaudatum as a nuisance and have taken control measures.

CONCLUSIONS

The majority of Zygentoma that are found within the household environment in The Netherlands are *C. longicaudatum*. *Lepisma saccharina* is found much less and *Thermobia domestica* not at all. *Ctenolepisma longicaudatum* is spread throughout the entire country and is mainly found within modern residences.

ACKNOWLEDGEMENTS

We would like to acknowledge Brian Dijkman, Yelmer Everink, Jos van Gemert and Corisjo Schroevers from HAS Den Bosch, for their effort in collecting gray silverfish in the residences of Houten. Furthermore, we would like to acknowledge Bart Merkus for his help in collecting and analysing data from the survey, Ton Brink for identification of the Zygentoma in the period of 2007 until 2010, and Prof. dr. Willem Takken for his suggestions on the manuscript.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

CONTROLLED ATMOSPHERE FOR PEST CONTROL IN CLIMATE CHAMBERS AND SILOS

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Abstract The controlled atmosphere (CA) principle is based on low-oxygen in combination with increased temperatures. CA treatments are commercially used world-wide and have gained acceptance from both governments and industries as the non-toxic fumigant technology for a variety of applications. As a pest control method CA has many advantages over traditional fumigants like products are free of residues and safe for people and the environment. The treatments are independent of atmospheric influences. CA can be used for quality preservation purposes for long term storage of food and feed commodities. It does not compromise the treated products in any way. CA treatments are applied in airtight climate controlled environments like climate chambers, barges or containers with the use of fixed or mobile installations. To apply CA in existing silos, a pressure tests needs to be conducted in order to establish the level of gas tightness of the silo.
Key words Fumigation, insects control, silo treatments, tobacco

INTRODUCTION

Low oxygen atmospheres have been used for many years to control stored product pests (De Lima, 1990). When O2 in the environment is reduced, insects lower their metabolic rate, and very low metabolism imposes stress on the insects (Mitcham et al., 2006). Studies have concluded that the oxygen level needs to be below 3% for this stress to be lethal; in most cases, it needs to be below 1% for rapid kill.

Controlled atmosphere treatments can be applied in airtight environments (like custom made climate chambers) ranging from 1 to 1000 cubic meters. The atmosphere is established by means of a converter that is able to create inside the airtight environments O_2 levels varying between 0% and 1.5%. The treatment eliminates the insects regardless their life stages. The lack of oxygen causes the eggs, larvae, pupae and adults to dry out and suffocate. Each treatment is fully monitored and controlled 24/7 via remote control. Each treatment has its own parameters based on the treated product and the type of insect(s) that need to be controlled. These parameters are based on a database of insect control data and knowledge of products. The treatment data are recorded using software programmes for full traceability

POST HARVEST TOBACCO 2004-2011

Stored and processed tobacco can be infested by the *Lasioderma serricorne* and *Ephestia elutella*. These insects need an O_2 level of 0.5% to be killed. To study the effectiveness and the duration time of the controlled atmosphere treatment on stored tobacco, several tests and trials where done over a period of 8 years. Besides testing the effectiveness in killing of the insects in all life stages and the most efficient duration time, these tests can also indicate if and if so what kind of (unwanted) side effects of the treatment might occur. Like discolouring or decolouring, taste deviations and loss of moisture in the tobacco.

Different varieties of tobacco were used for the tests: From tobacco seeds to raw tobacco in transport boxes to cigarettes and cigars packed in commercial packaging and packed in carton transport boxes. Some carton boxes were placed on pallets and wrapped in plastic transport foil. Holes were made in the foil so that the flow could go through the pallets more easily, (Figure 1). The tests where conducted in Vietnam, Indonesia, United Kingdom, Africa and the Netherlands. The products are placed in an airtight chamber or container. Various sensors are placed throughout the chamber or container at certain set points. These sensors measure the oxygen level and temperature. The moisture level of the cigarettes and cigars were measured separately before and after the tests were ended. Samples of *Lasioderma serricorne* and *Ephestia elutella* including eggs, larvae, pupae and adults were put in glass bottles, covered with a net on top, and placed inside the carton boxes.

The various tests indicate that within 9 hours, the required level of 0.5% O_2 was reached in the core of the products (Figures 2, 3). The commercial and transport packaging did not hinder the effectiveness or the duration of the controlled atmosphere treatment. There were no signs of living insects found. Regardless the life stages, all insects were killed. The quality of the products were in no way compromised; no decoloring or discoloring of the tobacco or tobacco products occurred, no taste deviation and the moisture level of the products stayed stable.

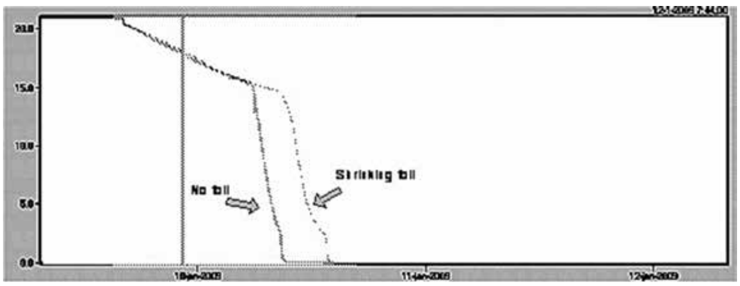

Figure 1. Influence shrinking foil on the flow air through pallets.

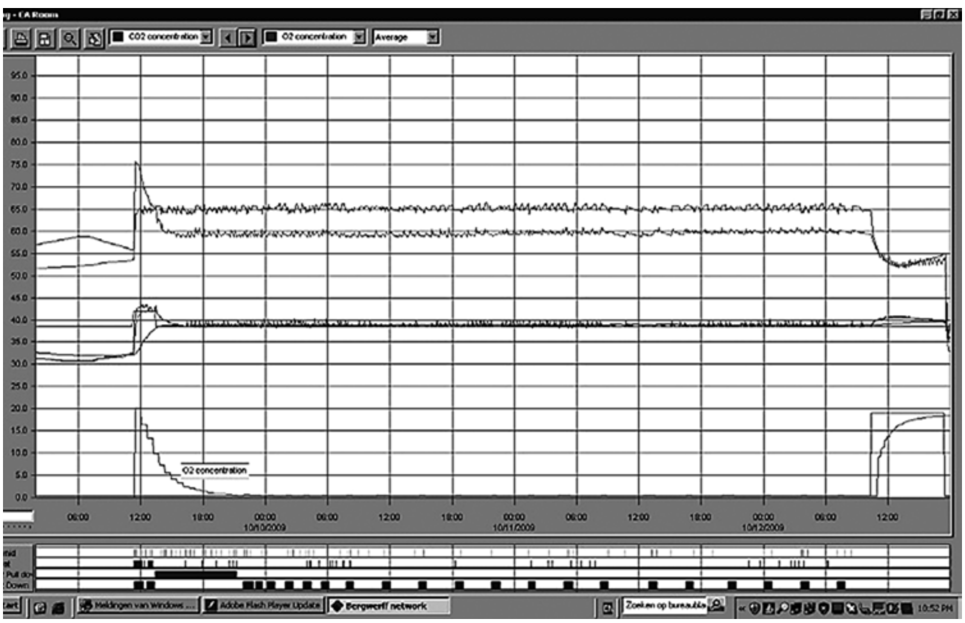

Figure 2. Test result reaching the 0.5% O_2 level within 9 hours.

CONTROLLED ATMOSPHERE IN SILOS

Since a very large portion of the storage of food and feed commodities is done in silos, there is a need for the application of controlled atmosphere in silos. Silos are constructed in various manners, different materials are used and the usage may differ. Some silos are only used for storage, others for storage and as a treatment facility. The most important aspect is the air tightness of the silo. The more air tight the silo is, the quicker the right low oxygen level will be reached within the silo. This can be established after conducting a pressure test.

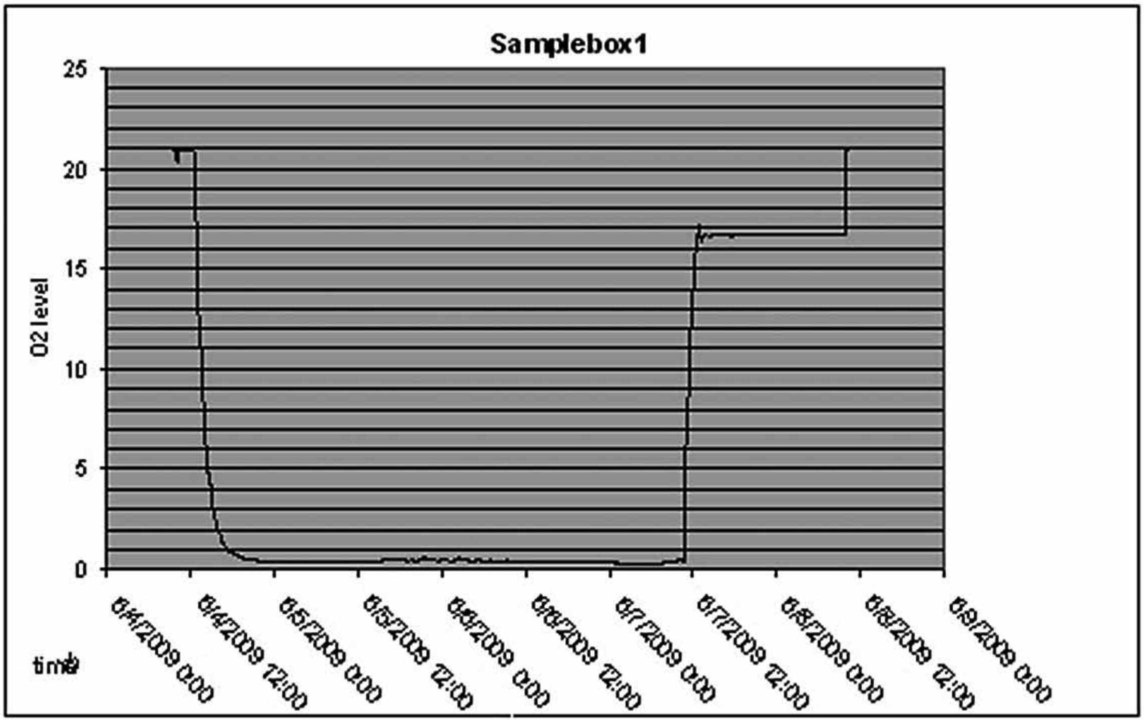

Figure 3. Test result reaching the 0.5% O_2 level within 9 hours.

SILOS TESTS

Several tests were conducted in silos in Switzerland with Desinfecta, CDL in Italy and together with Agrospecom in Greece. The various tests where done to establish the level of gas tightness of the silos, what time duration was needed to reach and keep an oxygen level of 0.5% . This O_2 level is required to kill of the *Sitophilis granarius*.

The tests silos were wheat filled corrugated silos with an open top. That means that in the roof of the silo air openings are present. To measure the pressure, the top of the silos needed to be closed. It was covered with thick plastic sheets. All seams were taped to prevent air leakage and dust. Kit was used to close any gaps around the valves, walls and floor. The parameter for this test was set for 0.5%, this to kill of the *Sitophilis granarius*. This test showed that it took 18 hours to reach 0.5% O_2 level within the silos. (Figures 4, 5, 6). The term for reaching the 0.5% O_2 level was set within 24 hours. The test showed that the existing silos reached the required level well within the set time frame. This level was held for the whole duration of the test, even though there were only minimum measures taken to make these corrugated silos more gas tight.

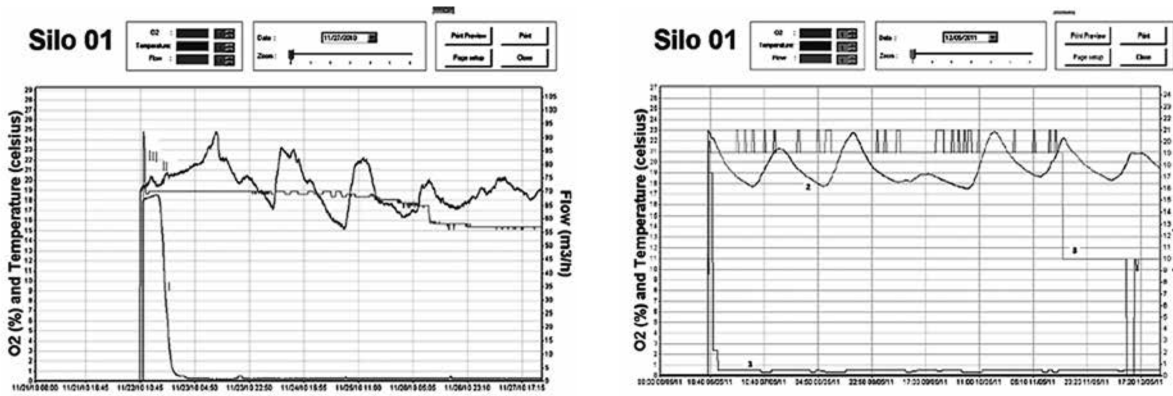

Figure 4, 5. The 18 hours to reach 0.5% O_2 level within the silos. I = O_2 level, II = Temperature, III = Flow; 1= O_2, 2 = Temperature, 3 = Flow.

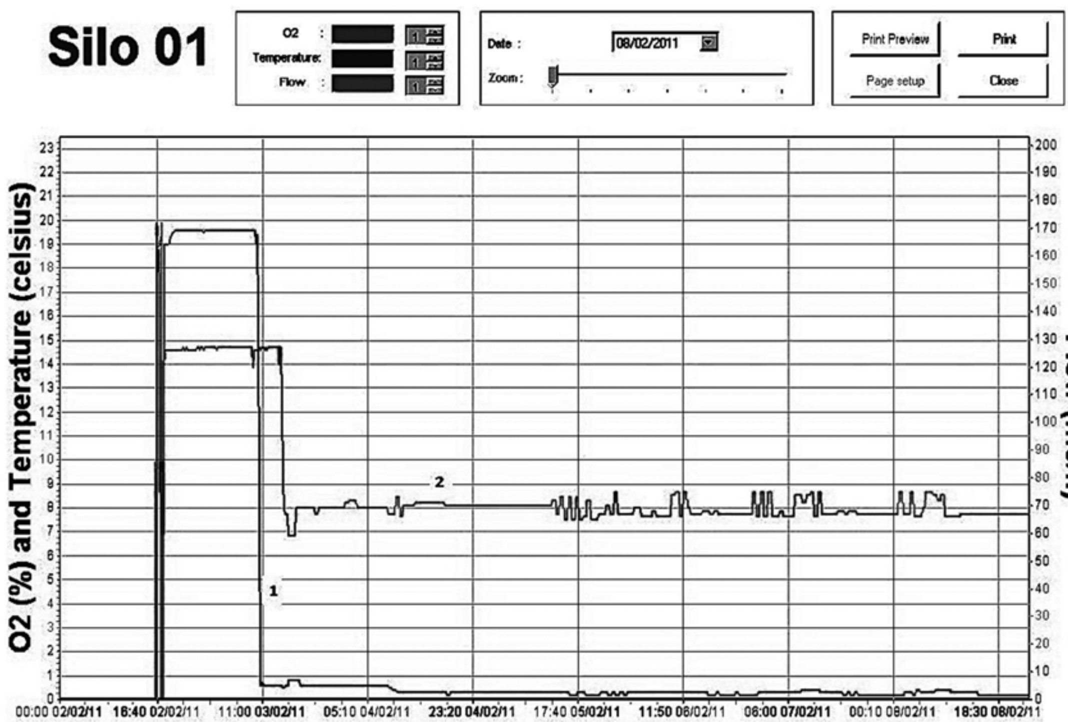

Figure 6. Test showed that it took 18 hours to reach 0.5% O_2 level within the silos. 1= O_2 level, 2= Flow

CONCLUSIONS

Controlled atmosphere treatment is an effective alternative for chemical fumigants. It holds it competiveness against the chemical fumigants on points as price, treatment time, availability and usability. Chemical fumigants like Phosphine face problems of causing resistance, leaving residues, long treatment duration, affecting the ozone and a negative image; they become overall less competitive in comparison to natural alternatives. The conventional food products market will choose for this alternative more often because of the growing trend in awareness of food safety. Controlled atmosphere treatments reduce the risk for working personnel and consumers. All systems are used without waiting for a fumigator.

The principle of the treatment is simple; without oxygen no insect can survive. Each insect stage of the insect species is controlled, taking into account that pupae and eggs are the most difficult ones. Each treatment is adjusted to the insect specie that needs to be controlled and the product that is treated regardless if it is applied in a treatment chamber or a silo.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

WARM AIR TREATMENT WITH RECIRCULATING AIR

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Abstract A warm air treatment is a non-toxic method to eradicate all stages of stored product insects or insects interfering with food hygiene and human health. The heating units reach temperatures of 50° to 60°C inside the buildings and rooms everywhere and are maintained during a sufficient period of time. This temperatures are never exceeded otherwise damage due to heat may occur to the building and the machinery. Our machines work only with warm air instead of heat, together with a constant air turnover and electronic regulation. The system was developed to be used in the flour milling industry and similar production facilities. In these environments the hazard caused by combustible dust has to be considered. The unit was constructed to satisfy the classification ex II 3 D according to the ATEX 100 which means it may be operated in the Zone 22 (areas containing conductive dusts). Treatments can be done in rooms as well as in whole buildings.
Key words Stored food pests, heat damage.

INTRODUCTION

Not only human beings but also a considerable number of insect species regard grain and cereal based products as an important basic food. The most common insects threatening this kind of stored products are the grain weevil (*Sitophilus granarius*), the flour moth (*Ephestia kuehniella*) and the rusty-red flour beetle (*Tribolium castaneum*). It is generally believed that the optimum temperature for these insects is somewhere between 15° and 35°C. At temperatures above 45°C both the insects and their eggs are killed within a few hours. As you all know insects cannot reduce their body temperature by perspiring or breathing. At this temperature they will die due to coagulation of their body proteins.

REQUIREMENTS

Warm air treatment is a convenient non-toxic method to eradicate all stages of stored product insects or insects interfering with food hygiene and human health. Several major prerequisites must be fulfilled to carry out the advanced method successfully. At the one hand a temperature of 50 to 60°C must be reached everywhere and be maintained during a sufficient period of time. At the other hand this temperature should never be exceeded, because otherwise damage due to heat to the building and the machinery may occur.

Furthermore inside the building should be optimal warming of walls, floors, machines and constructions in order to reach lethal temperature all over. The project time at a building has to be at least 48 hours and for single room min. 24 hours to guarantee a moderate warming. Also, the system must be flexible, modular and designed for different types of areas and uses energy utilization in an optimal way. One of the most important facts is that the method is able to handle certain plant conditions like explosive atmospheres generated by combustible dust.

METHOD

The procedure described here is based on the principle that only the air from inside the building has to be heated and so high air temperatures are not necessary. The heater causes a circulating airflow inside the building and results in a requested very low energy consumption.

During warm air treatment the temperature is controlled directly. Each heater automatically monitors and controls the temperature of its airflow and consequently that of the floor/area/sector it serves so that a treatment can work with lower temperatures and lower energy consumption. This is essential in factories with sensitive electrical components or electronic control boxes Two heaters can easily be installed in such a way that they produce a heat build-up. This is very helpful if you have to treat for example, flour storage bins that are several floors high. Owing to the construction of the heater and ground fans, the air movement and the heat transfer are encouraged to be at floor level because insects will emerge from machines, flanges and cable conduits and fall onto the floor but are not able to escape to cooler areas.

EQUIPMENT

The heater is equipped with an axial fan of 0.75 kW, two heating registers each of 9 kW, two control thermostats, a safety device to prevent overheating and a switch panel containing the electrical equipment. The three-phase power supply (380-400 Volt) is provided by a 15 meter long cable fitted with a CEE plug. The CEE plug is equipped with a switch to swop two of the phases if the fan direction needs to be changed. The dimensions of the heater are 430 by 610 mm by 1,040 mm high. The energy consumption is 9.75/ 18.75 kW (9/18 kW for heating, 0.75 kW for the fan). The weight of the heater including the 15 meter cable is 75 kg. An axial fan sucks in air at the floor level and blows it over the heating elements. The heated air leaves the top of the heater horizontally.

The temperature of the circulated air is controlled by the integral thermostats. When the room temperature reaches about 50°C the thermostat switches off the heating elements. As soon as the temperature falls the heating element is reactivated to maintain the lethal temperature in the room at between 50°-55°C. In case of a malfunction - when the air temperature in the heater exceeds 140°C the STB (safety-temperature-switch) automatically switches off the main energy supply. The heater is fitted with two wheels and a handle which makes it easy to reposition it during the heating period. The heater was primarily developed to be used in the flour milling industry and similar production facilities to replace methyl bromide and other toxic agents used for pest control. In these situations the hazard caused by combustible dust has to be considered.

In Europe the EU-regulation 94/9EG (also known as ATEX 100 a) concerning equipment used in explosive atmosphere has to be complied with. The heater was constructed to satisfy the classification ex II 3 D according to the 94/9/EG which means it may be operated in the Zone 22. The heaters are connected to existing CEE sockets. If the customer's plant does not have enough CEE sockets there is the possibility to provide one or more power distributors panels with up to fifteen 32A sockets. This distribution panel can be connected directly to the main energy supply.

For a better air distribution and to work against the thermal effect, ground fans are used in addition. They suck on the upper warm air on top of the machine and bring it out via an integrated pyramid in all four directions straight over the floor. The used fans have the same diameters like the heaters only without heating elements and 16A CEE plugs. The inserted amount of ground fans depends on the construction of the treated building. For a fewer needed number of CEE sockets there is the possibility to connect up to five ground fans with the help of a small distribution board on one 32A CEE socket. Adapters, deflectors and extension cables complete the equipment.

APPLICATION FIELDS

Within the last 15 years the system has been used successfully in different branches and industries: Food: processing industry, mill, bakery, pasta, baby food, catering; pet-food/feed: pet food plant, premix plant, feed-plant; non-food: clothes, tobacco industry, wooden subjects, IPPC-standard (ISPM 15), room drying, climate improvements. Bed bugs: hotel, motel, accommodation, living rooms, train, and plane. The maximum building volume that can be treated economically is between 50,000 cubic meters and 70,000 cubic meters. Larger buildings should be divided into sections which are treated one after another.

COSTS VS. BENEFITS

To carry out a treatment has with rental for equipment, performance man power, transportation charges and energy consumption four different parts of expenses for the customer. Opposing it isn't only a pest control procedure because it has further fundamental benefits. During the whole treatment it's possible to stay inside the treated rooms and especially during the warming up to see how insects leave their hidings. This fact helps the customer's employees for future hygiene management procedures. They are able to do intelligent cleanings or structural improvements. They learn to see their plants with different eyes. After the treatment the whole building inclusive inside machinery is dried out. All caking inside silos or inside tube systems which may contain mold are now easy to remove. Also the germ number is reduced compared with the former situation.

COMPARISON

Using the system brings a lot of different advantages compared with other applied methods. Warm air treatment vs. fumigation (SF2, PH3, MB, mixtures, etc.):
Non-toxic for person and no residues in products, no development of insect resistances, no pressure test of the building, no official authorization, rooms always accessible, insects are visible, lethal for all insect stages of development. Warm air treatment vs. heat treatment (oil or gas burning units, >80°C). No heat damages, more flexible, adaptable and modular, user-friendly, easier distribution of warm air, lower energy consumption, closed machines and facilities because of permanent warm circulating air. Heat treatments are independent from the outside temperature and can be performed all the year round. Also it's only an internal procedure without outside standing machines or any poison-warning sign outside of the building.

EXAMPLES OF USE

Use in a Mill

For an effective building treatment six basic rules have to be observed: 1) Shut down the entire production equipment, 2) Clean all the rooms carefully, remove dust deposits, wrapping paper, bags, packaged product and other removable inflammable objects from the rooms which are to be heated, 3) Remove gas containers (like spray cans) from the area to be heated, 4) Switch off compressors and ventilate the pressure tanks, 5) Remove combustible fluids, 6) Switch off all electronic components.

The heating period in production buildings with several floors could extend to 48 h. A single-room heat treatment, e.g. laboratory, sample room, normally takes 24 h.

During the heating period, inspect the heated areas at regular intervals, to check that the heat distribution is uniform and to identify cool areas. Normally, at least 2 (but often more) heaters WEO 9/18 and ground fans are used on each floor. It depends on the total base area and volume of the building. They are positioned in such a way that they produce a uniform heat distribution. In a single-room heat treatment one heater should be enough. The heaters are connected to existing CEE sockets or in most cases via power distributors. A heating power of 18 kW needs a single 32A fuse. If the power socket is only fitted with a 16A fuse the heater can be used at half power (9 kW) as well.

Secluded Areas

Apart from treating whole buildings or areas this system is a great tool to treat separated areas within a building or any kind of premises. There are already treated fully successfully: Separated areas inside a plant (for example parts of a plant, production areas in the same areas as warehouse areas); production lines inside a plant; separated rooms inside plants (just one or a few floors of a building, production areas that are just by technical rooms); only parts of a production line (treating just fermentation chambers of a production line); just part of equipment such as trays or cups or similar items; hotel or Hospital rooms (mainly bed bugs).

It is important when you have to treat secluded areas inside buildings to previously plan how we are going to be able to treat them. It's fundamental to make sure that everything is taken into account so we don't face problems when we have to start the treatment or during the same treatment.

Things we should always think of when planning a job: How are we going to construct the separation between the area that has to be treated from the rest of the plant or building (use of a wooden structure, just use of frames covered with carton or plastic, plastic covers, etc.); when constructing the separation, we have to think that there's going to be heavy air moving inside the secluded area (the construction has to resist the heavy air pressure); minimization of the heat loss (in order to save energy and ensure the efficacy of the treatment). Make sure that pipe lines, the structure itself, etc., are being taken care of; how are we going to be able to enter and get out from the secluded area during treatment; during treatments is necessary to check for cold areas and act accordingly; how we will detect and control insects trying to escape from the treated zones.

NETWORK

The warm air treatment is being practiced in Europe and in several Asian countries and has been receiving a steadily growing appreciation. Partner companies are located in the main countries which have own equipment to be next to customer's requirements. Outside Europe there are plans to build up two new partner companies to meet increasing worldwide demand. For a quality assurance this system is distributed only through certifed operators to follow up the efficient practice and the safe use of heat treatment in food industry and other applications. The company headquarter in Germany provides partners, customers and smaller national partners with equipments, single machines, services, training and consulting.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

THERMAL TREATMENTS FOR BED BUGS

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Abstract Thermal control is one of the possible control methods for bedbug infestations. It is however often assessed controversially. This assessment is not always based on objective arguments and rarely on own, practical experiences.
On the basis of practical trials and experiences from over 450 heat treatments in different room types we show how and where hidden hollow spaces can be opened and treated, how heat damage can be prevented or largely reduced and how the escape of the bedbugs from the treated rooms can be suppressed. We show the technical and commercial feasibility and the advantages of thermal bedbug treatment on the basis of the number of recalls (additional treatments).
Key words Pyrethroid resistance, lethal temperature, heat related damage, silica dust

INTRODUCTION

The control of bed bugs with insecticides became more and more difficult after 2005, because the EU has systematically eliminated elder active ingredients from the official registration list. Mainly synthetic pyrethroids remain, against which bed bugs have increasingly developed resistance (Boase et al., 2006, Pinto et al., 2007a; Boase, 2011; Romero, 2011; Pfeiffer, 2013; Zu et al. 2013; Miller, 2013). An increased effort for insecticide treatments of bed bugs and increasingly dissatisfactory results convinced the pest control company (PCC) Ratex to change to thermal treatments with Thermonox® heating devices in October 2011. For Swiss conditions Ratex is a middle sized family company with four pest control technicians and eight additional roofers to do marten and pigeon control installations. At present thermal treatment of bed bugs makes up around 15 percent of the company's turnover.

Heat is an excellent bed bug killer, is non toxic and only heat sensitive items have to be removed from the room (Miller, 2013). The company ranked these advantages higher than the disadvantages like high costs and no residual activity.

The head of operations has an education as electrician. This is an advantage, because the heat devices need high voltage current, which is not always available in many buildings. He can install a temporary connection immediately. Overall the PCC has made good experiences with this method. It is very important that all measures are taken to prevent heat damage of the infrastructure, equipment and furniture. Damage normally starts at temperatures above 60°C (140°F). All possible refuges of bed bugs and parts of buildings that they could disperse to have to be investigated und made accessible for the heat (Hasenböhler and Kassel, 2011; Hasenböhler 2012). Therefore the Pest control technician (PCT) inspects the rooms to be treated together with the customer and discusses necessary structural measures and restoration (Hasenböhler, 2012).

MATERIALS AND METHODS

The company Ratex controls bed bugs with heating devices of the brand Thermonox® WEO 4.5 / 9 (Hasenböhler, 2006 / 2012; Hasenböhler and Kassel, 2011) and depending on the room size and architecture with one or more additional fans.

Before the thermal control the PCTs inspect the infested rooms just like before an insecticide control. At the same time they undertake all necessary measures to prevent heat damage and bed bugs from escaping. They also check the concerned structures for spaces that can't be reached by the heat (Pinto et al., 2007b, Hasenböhler and Kassel 2011). These measures can easily take up two and a half to three hours of work time.

The warm air current of the heating device is directed towards the highest heat demand and a fan is installed in the shadow of the heater depending on room size (Hasenböhler and Kassel, 2011). A maintaining period with a constant temperature between 50°C (122°F) and 60°C (140°F) for 24 hours follows a heating-up period of 8 to 15 hours. About all 8 hours supervising measurements are performed in the room (three for the whole heating period) to ensure that the needed temperature is maintained and to relocate the heating device or the fan if necessary (Hasenböhler and Kassel, 2011; Hasenböhler 2012).

The different materials of the building structure and the fixtures and fittings differ in their time to heat up because of their different heat conductivity and heat capacity. This monitoring during the maintaining period is necessary to make sure that the killing temperature interacts long enough in all parts of the treated room. Then a cooling phase of 12 hours follows. The thermal treatment process cannot be shortened, because of the risk of the killing temperature not reaching the eggs in deep cracks or other hiding places and of cold spots not heating up enough to kill the bed bugs or their eggs. This would prevent the necessary eradication (Hasenböhler and Kassel, 2011; Hasenböhler 2012: Hammond, 2012). After the cooling phase 1.5 to 2 hours are spent to reset the room. This includes remounting the light switches, sockets and lamp coverings, removing all the masking tape or plastic coverings and reinstalling the baseboards and other fittings. While reinstalling the fittings, silica dust (diatomaceous earth) is applied as a residue. The complete heat treatment costs add up to CHF 1500 (~USD 1660) per room. If three rooms have to be done at the same time the costs drop to CHF. 1200 (~USD 1330) and from five rooms up the costs drop to CHF 1000 (~USD 1110) per room. The house owner, the caretaker and the adjacent neighbors have to be informed about the heat treatment because especially in buildings with precast concrete slabs the ceiling or the floor will heat up to 30 °C (86 °F) or more (Hasenböhler and Kassel, 2011). Uninformed neighbors might call the fire department. Such a false alarm can amount up to CHF 1400 (~USD 1550).

RESULTS

Between October 2011 and February 2014 the pest control company Ratex has heat treated overall 452 rooms with only 8 recalls that include a second guarantee treatment (Figure 1). The recalls concerned rooms of 4 different private apartments, one residential home, one backpacker hostel, one three star hotel and one emergency accommodation for the homeless.

In three cases temporary sealing with tape was not performed or not done careful enough. The first concerned an old window, from which the bed bugs reinvaded the room after the treatment. In the second the opening to the roller shutter casing gave the bed bugs a refuge and a return possibility to the treated room. In the third case the cracks of the hangers of a radiator, which lead to an insulated hollow space, were sealed, but not thoroughly enough. This space was finally opened and heated up as well.

In two cases the wirings were not sealed or only sealed on one side, while in the latter case the bed bugs came out of the cable duct in the adjoining room. This then had to be treated as well after sealing the other side of the opening. In the other case the cable duct that was not sealed belonged to a fire detector on the ceiling. To seal it, the detector had to be deactivated and removed from the ceiling. Two further cases concerned the ceiling. In one case the suspended ceiling was temporarily sealed off with a thin foil to prevent the bed bugs from spreading into this insulated area. The foil reacted to the heat with fissures and the bed bugs could wander off into the cooler zone behind. In the second case the owner had already sprayed the bed bugs unsuccessfully for four months without telling the company and a temporary sealing of the ceiling was in vain. Before the second treatment this ceiling containing sound reducing material was sealed off completely thus enclosing the bedbugs within. In the last case concerning an emergency accommodation, the power line was timed to turn off between 22 hours until 5 o'clock in the morning. The company however was not informed about this. The bed bugs were killed but after 2 weeks larva appeared, because the necessary temperature had not been held long enough to penetrate and to kill the eggs in the deeper cracks.

DISCUSSION

Infested private homes make up for over 60% of the treatments, while the other three categories hotels, public buildings and public welfare accommodations each are treated at a low percentage of 11.5 to 14 % (Figure 1). These numbers are not representative and can change from one company to the next. There are over twenty PCC operating in Zürich and surroundings. Swiss people are very critical towards insecticidal contamination of their own home. Therefore a high proportion of people might prefer the heat treatment to the insecticide treatment in spite of the higher costs. This is also the case for public buildings and for most hotels. These additionally have their employees trained to get rid of infestations immediately and therefore avoiding customer complaints and lawsuits. This is in accordance to Robinson and Boase (2011) where most infestations are found in residential premises and hotels. However in contrary to the above mentioned research, in Switzerland there is no knowledge of infestations in premises visited or used by a broad range of residents such as shops, theatres, offices and administrative buildings. There are reports of bed bugs in train coaches with sleeping compartments. Heat treating of railway wagons is expensive and the risk of heat damage to the interior with all the different plastic materials is high (personal communication C. Zehnder). Therefore Ratex is not interested in treating railway wagons.

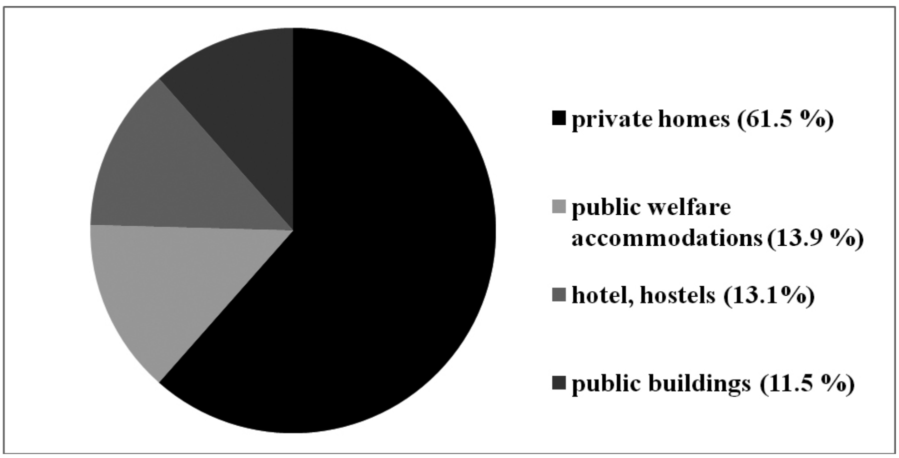

Figure 1. Percentage of bed bug heat treatments in accomodation groups.
Public welfare accommodations = accommodations paid by public welfare, asylum- and supervised accommodations. Public buildings = hospitals, old people's homes, school camp houses.

Our experiences from 452 heat treatments and the eight recalls showed that the most important points to be checked before treatments are as follows.

Avoid Heat Damage to Furniture, Equipment, and Fixtures

If fire alarms are installed, they have to be deactivated, to prevent unnecessary alarms. The duct of the electric cable to the alarm has to be sealed off. If there are fire extinguishers in the treatment area they have to be removed.

All heat sensitive objects have to be removed by the residents. These are for example oil paintings, candles, records, perfumes, sprays (aerosol cans), medicine, plants and all food. This is selectively checked by the pest control technician. The residents are however responsible for this task. All other items stay in the room to be treated and to prevent the displacement of bed bugs into other rooms (Hasenböhler, 2006; Hasenböhler and Kassel, 2011). All electric devices like the television, refrigerator, stereo equipment or computer have to be turned off and plugged out. They normally endure these temperatures without any problem. A backup on an external hard disk is strongly recommended. Furniture with edge-jointing adhesive are fixed with masking tape so that they cannot slip or peel off when the adhesive (on the base of thermoplast) becomes soft during the heat treatment.

Prevent Bed Bugs From Escaping and Dispersing

All plug sockets, switch boxes and emergence points of electric cables of ceiling lamps in the room are treated with silica dust. Then they are sealed with heat resistant acrylic silicone. Each electric line out of the room to be treated is followed to the next cable exit and sealed here as well.

Baseboards are removed and checked for bed bugs. If there are traces or live bed bugs, all cracks and crevices in this area are treated with silica dust and again sealed with acrylic silicone. Wooden structures in the ceiling area, e.g. beams, old, untight windows, shutter covers, cracks in the wall at heater pipes or hangers for the radiator, ventilation shafts and the room door have to be temporarily sealed with tape to prevent the bed bugs from escaping.

The thick electric cable for the heat device has to be inserted through the door opening. This prevents the door from closing tightly and sealing of the door is made more difficult. Nowadays there are flat cable joints fitting under the door and allowing the door to be closed and sealed off correctly. Cable ducts are sealed off with fire protection material. Some of the panels of suspended ceilings are removed to heat up the space above. If the bed and the kitchen are in the same room, the kitchen can be sealed off with a lock of plastic sheet. Inaccessible areas are checked with an endoscope and if necessary treated with silica dust and sealed.

Open Thermally Protected or Insulated Spaces

All furniture close to the wall has to be moved away so far that it can be heated up sufficiently. Fittings like beds that are fixed in the wall or built in closets have to be dismantled because the area behind cannot be heated up enough and will otherwise be used as retreat by the bed bugs.

Multiple floor layers have to be removed completely or the top layer has to be sealed completely because the heat will not penetrate through these layers. Floors can be heated from both sides, from the bottom and from the top, but that is more costly. Bay windows and window boards have to be closely examined. If there are cracks they have to be picked out or sealed with acrylic silicone. Temporary sealing with tape is necessary for cracks in parquet floors, the shaft with the setting valves of floor heating systems and air ducts.

Following these rules will result in a successful heat treatment. If one escape possibility or one hiding place is missed or the chosen length of heat impact is to short like in the eight recalls, not all the bed bugs are killed and therefore complaints and expensive secondary treatments on the costs of the company will follow. Heat treatments in combination with insecticides (Boase, 2011; Hammond, 2012; Miller, 2013) are not necessary except in cases with inaccessible cold spots for the bed bugs to find refuge. In all cases the company applies silica dusts in hollow spaces, behind fittings and baseboards, into electrical fittings and ducts and any obvious cracks and crevices. Lots of the latter are sealed permanently and if the rooms are reinfested with bed bugs, the preparation time and the treatment price is lower. Silica dust alone can rarely eradicate a bedbug population, but it's presence before an infestation might stop it or at least slow down the population growth (Potter et al., 2013).

ACKNOWLEDGMENTS

We thank Dr. Gabi Müller for the reading and very useful comments on the manuscript.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

HEAT TREATMENT FOR INSECTS

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Abstract Heat treatment has been used to control stored food pests, bed bugs, cockroaches, and other household pests, and structural timber and wood pallets. The energy requirements for treating different materials, spaces, and timber require careful attention to maintaining a heat regimen and training of application personnel.
Key words Insect control, store food pests, structural timber, bed bugs.

INTRODUCTION

The use of heat for insect control in flourmills is not a new idea, it has been used since the early 1900s (Menon et al., 2000). It was shown to be effective in controlling a variety of pests (Sheppard, 1984). However, the use of heat declined after the advent of the gas fumigants. There is renewed interest in using heat to control insects in mills because of phase out of methyl bromide. Typically, the method involves raising ambient temperature to 50° C or above for 24-36 hours to kill insects within the mill, including within machinery. Heat has been used in a controlled environment in the treatment of museum objects and building preservation (Nicholson and Von Rotberg, 1996). Heat treatment is effective for controlling cockroaches (Zeichner et al., 1996), bed bugs (Kells, 2006; Pereira and Koehler, 2011; Hasenbohler and Kassel, 2011; Schrader and Schmolz, 2011), and stored food pests (Fields, 1992; Tang et al., 2007)

Annual space fumigations of flour mills and other food facilities were effective for a few months. However, the months leading up to the next fumigation, insect levels were at dangerous levels in terms of product contamination risk. Fogging only hid adult insects from customer audits and drove real infestation levels deeper into machinery and closer to product. What was needed was an ongoing treatment technique that gave year round control of stored product insects. A system was developed of detailed ongoing inspections of machinery during mill shutdown periods. Control was achieved by a combination of cleaning, insecticidal sprays and dusts to non-food contact surfaces and spot fumigation with a 3:1:1 mixture of carbon tetrachloride, ethylene dibromide and ethylene dichloride to kill eggs, larvae and pupae inside machinery. However, these gases were identified as carcinogens and banned.

HEAT TREATMENTS

Practical heat treatment can be divided into five main types: space treatments,
spot treatments, chambers and kilns, silos and bins, and temporary structures. Heat has been used in the following situations: food industry including confectionary, mills and bakeries, breweries, hotels and hostels, textile pests, fleas and bed bugs, shops, cars, lorries, trains and buses, aircraft, ISPM15 (International Standards for Phytosanitary Measures), timber pests, drying, and combination treatments with insecticides or gas.

The broad concept of heat treatment is to use the following strategy to control all stages of the insect pest life-cycle: detailed inspection by contractor or client biologists or hygiene staff; agree on a treatment strategy with client, including any engineering concerns regarding heat treatment of sensitive machinery, a fire detection and suppression system, and cleaning requirements before and after treatment; and install heating system, ducting and temperature logging sensors.

Heat treatment for bed bug control in hotels is successful using bubble type heating systems and the 56° C for 30 minutes core temperature heat. The same heating requirements have led to the use of heat treatment in timber pest control. This temperature (56° C) has been set to take into account reduced treatment times during pallet manufacturing, or not measuring the cold spots or centre of the thickest piece of timber. Timber beams in houses can be treated to control wood boring insects. The target temperatures of 52° C for 1 hour must be measured in the coldest locations and in the thickest beams. This requires drilling and then filling to locate temperature sensors.

DISCUSSION

That Europe has clearly survived the last 10 years without methyl bromide is testament to the wisdom of the decision to ban it under the Montreal Protocol. Heat treatment has proved more successful in many situations and is safer. Rapid 24 hour commodity treatments remain difficult, and the food supply industry has learned to factor in the 5 days or more needed for phosphine fumigation Training of is crucial to successful treatment without damage. There is commercial focus on different types of machinery, more efficient or more powerful heaters. The success is in how it is applied and the understanding the operator has of how the heat is behaving in the target treatment area.

CONCLUSIONS

For the future, heat treatment of some commodities is possible. However because of the time and energy required for heat to penetrate packaging, treatments will more likely be in fixed in – line installations, or in adapted heat treatment containers. The previous methods of covering commodity with a sheet and treating; heating shipping containers is not feasible due to lack of internal air circulation. Phosphine and CO_2 are still available for commodities and both work well in conjunction with heat. In Europe, applicators allow 5 days instead of 24 hours for fumigations. Heat and phosphine combinations may bring this time down when using phosphine.

The future for heat treatment is good, and its benefits are for all industry not just the organic market. However, heat treatment is not a panacea but should be used in conjunction with a proper integrated pest management system. Good cleaning in conjunction with other pest control techniques should be used to target eggs and larvae hidden in areas inaccessible to conventional pest control measures.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

A EUROPEAN STANDARD FOR PEST MANAGEMENT SERVICES

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Abstract The Confederation of European Pest Management Associations (CEPA) and its members are sponsoring the development of a European standard for the pest management industry. This would be in collaboration with the European Standards Institute and the National Standards Institutes of the 28 member states of the European Union as well as Norway, Iceland and Switzerland. This European standard would specify the requirements, recommendations and basic competences under which pest management servicing companies must operate to meet the needs of their customers. This would include private companies, public authorities or the general public. All European national associations who are members of CEPA would require their member organisations to uphold this standard once it is in place. The objectives of the presentation are to review the need for professional standards, what these standards would cover in terms of service, and the steps necessary to initiate them.
Key words Pest management service, industry standards, pest managers.

INTRODUCTION

The Confederation of European Pest Management Associations (CEPA) and its members are sponsoring the development of a European standard for the pest management industry. This would be in collaboration with the European Standards Institute and the National Standards Institutes of the 28 member states of the European Union as well as Norway, Iceland and Switzerland. This European standard would specify the requirements, recommendations and basic competences under which pest management servicing companies must operate to meet the needs of their customers. This would include private companies, public authorities or the general public. All European national associations who are members of CEPA would require their member organisations to uphold this standard once it is in place. For pest managers it will provide a benchmark for a level of professionalism to strive towards, or to maintain or improve upon. For legislators it provides reassurance regarding the responsible attitude of our industry and a better definition of what professional pest management service stands for.

The concept of global quality standards for professional pest management services was discussed by Duggal and Siddiqi (2008). They provided a historical review of the guidelines for structural pest control services in the United States, and reviewed the guidelines for professional service for the food processing industry, wood packaging and fumigation, and general standards for the pest control industry. The objectives of the presentation are to review the need for pan-European standards for professional pest management services, and to propose what these standards would cover in terms of service and the steps to initiate them.

CONFEDERATION OF EUROPEAN PEST MANAGEMENT ASSOCIATIONS

The Confederation of European Pest Management Associations (CEPA) is a not-for-profit organization base in Brussels, Belgium. It represents, at the European level, 23 national industry associations and an industry with a turnover of € 2200 million and the interests of 8000 companies with over 38,000 employees. In addition to the national associations the CEPA membership also includes 23 partners from industry, including manufacturers, distributors and service companies in Europe. CEPA was set up in 1974 by the Belgian, British, Dutch, French and Spanish national associations. These organizations had the vision to see that national associations could not, by themselves, defend the interests of their members at a European level.

EUROPEAN STANDARDS

A European standard is a document established by consensus and approved by the European Standards Institute (CEN). It contains a series of specification and/or recommendations in relation to products, systems, processes or services that can be adopted by the industry across all states of the European Union. A European Standard can also be used to establish a common terminology within a specific sector. Once ratified by CEN, a European standard has to be implemented by CEN members as an identical national standard and any conflicting national standards will be withdrawn. A European standard potentially substitutes 31 (28+3) different national standards. Although European standards are voluntary, once they are adopted, it is obligatory that they replace existing national standards, but not national legislation. Once the European process has started, no further work is permitted on National Standards.

EUROPEAN STANDARD FOR PEST MANAGEMENT SERVICES

As sponsors of the initiative for establishing standards for the European pest management industry, CEPA wishes to engage the industry in this project to ensure that the industry is recognized for responsibly protecting European citizens and the environment in which they live against public health risks. Other important values resulting from the pest management industry's engagement in this project have been identified. The most significant ones are: 1) The project will create awareness for the value to society that the pest management industry represents through the maintenance of health and hygiene; 2) The initiative will contribute to controlling unprofessional use and provide support for products that protect public health but are under threat of restriction or deregistration measures; and 3) The introduction of a European standard will enhance CEPA's interaction with the European institutions and all its other European stakeholders.

Certification can be awarded only to organisations (service providers) not to individuals. A certificate shall be valid for no more than three years. Organisations that wish to join the CEPA scheme shall be audited by a certification body approved by CEPA. Once they have demonstrated to the certification body that they conform to the requirements of The Standard, they can be certificated. The Protocol guiding the Certification Scheme sets out the requirements for the audit and certification processes. It is based on the Principles of Auditing set out in the International Standard ISO 19011:2011.

CEPA believes certification is crucial as it will enable the users of pest management services to identify those providers that offer an effective, safe and legal service. It will provide pest management companies across Europe with the opportunity to benchmark themselves against their peers. It will demonstrate the professionalism of the pest management sector, and offer a strong platform for the establishment of a global standard for service providers. CEPA and its European Standard for Pest Management Services expect publication of the final draft in the last quarter of 2014.

CEN STANDARD AND USE OF ANTI-COAGULANT RODENTICIDES

CEPA recognizes the public health consequences if the means to adequately control rodent populations in Europe were to be curtailed by restrictions on rodenticides. As today no significant and effective alternative to anti-coagulant rodenticides is readily available and that a major infestation would have serious consequences for the public health. The use of rodenticides based on anti-coagulant active ingredients remains essential in order to manage pest populations successfully.

Based on concerns for public health, CEPA recommends that anti-coagulants should remain accessible for trained pest management professionals as long as no viable alternative is available to manage rodent pests. Furthermore, through the publication of the CEPA/CEN standard and the promotion of certification to this standard, CEPA will contribute to identify trained pest management professionals.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

A POLYMER ENHANCED FORMULATION TO PROLONG THE EFFECTIVENESS OF SURFACE SPRAYS

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Abstract A new long-lasting, polymer-enhanced formulation has been developed for suspension concentrates of synthetic pyrethroids which addresses and remedies the above mentioned problems. This polymer technology, called PolyZone™ technology, prolongs the effectiveness of deltamethrin suspension concentrates on different kind of surfaces. This technology has been integrated into a new deltamethrin-based product called Deltamethrin SC-PE, which extends residuality on concrete and other materials. The World Health Organization has recently recognised this formulation as having a residual lifespan of 6 months, and it has been shown to greatly extend persistence in outdoor applications due to enhanced rain-fastness as shown in outdoor weathering trials.
Key words Residual spray, vector control, Malaria, Malaria control.

INTRODUCTION

Modern vector control relies on a multitude of tools to combat malaria transmitting mosquito vectors to limit the spread a disease that still causes several hundred thousand deaths per year, predominantly amongst young children. Although insecticide-treated nets (ITNs) and more recently long lasting insecticidal nets (LNs) are highly effective for preventing malaria, it is also recognised that by themselves LNs and ITNs are unable to deliver the reductions in malaria burden necessary to eliminate malaria in Africa and that both indoor residual spraying (IRS) and LNs should be implemented (Kleinschmidt, 2009; Hamel, 2011; Fullman, 2013).

The residual lifespan of IRS is a key element to its suitability as a control tool. Pyrethroids play an essential role in malaria vector control as roughly 50% of the WHO recommended insecticides for IRS against malaria vectors belong to this class of chemistry. Synthetic pyrethroids are commonly very stable on wood, tiles and other inert surfaces, providing good residual efficacy. On more aggressive and alkaline surfaces such as mud and concrete however, the duration of activity is often reduced (Camilleri, 1984; Demoute, 1989).

To provide an innovative deltamethrin-based IRS-solution with improved residuality also on these difficult surfaces, different formulation concepts were investigated and screened using an accelerated stress test on laboratory scale. Results of one particular concept, called polymer-enhanced formulations, in semi-field, village-scale field trials will be presented and discussed. Further studies revealed that the same technology that protects the active substance on surfaces from chemical degradation also enables the active ingredient to withstand wash-off induced by rain and irrigation. This feature is beneficial to the performance in outdoor pest control applications. Outdoor perimeter treatment is a use pattern where the perimeter of a house is sprayed to prevent the invasion of outdoor pests into buildings. Under these weathering conditions, the main destructive factor is moisture not alkalinity (Jorgenson, 2010; Jorgenson, 2012).

MATERIALS AND METHODS

Laboratory Residual Testing

Laboratory testing of Deltamethrin SC-PE and prior development candidate formulations was done according to standardised Phase 1 testing procedures adapted from WHO guidelines on concrete and plywood materials (WHO, 2006). Blocks of substrates were sprayed using a potter tower or an automated spraying robot with correctly diluted insecticide. Surfaces were allowed to dry to completion before further handling. For bio-assays, *Anopheles gambiae* were exposed for 30 minutes before being moved into clean containers. Insecticide-induced knock-down was recorded after 1 h and 2 h, and mortality 24 h post exposure. Tests were done 1 day after spraying and subsequently every 4 weeks. 3 replicates of 10 mosquitoes were tested per treatment. In between bio assays surfaces were aged at 30°C; 80% RH without the influence of direct daylight.

Artificial Hut Residual Testing

Artificial, uninhabited simple huts were erected in Tanzania/ Lower Moshi district. These huts, measuring 3 m wide, 3 m long and with walls 2 m high were brick built structures with an iron roof and eave gaps between roof and walls. Walls were covered with different local materials such as concrete plaster (concrete/sand mix), mud, plywood and palm thatch. Walls were sprayed with a compression sprayer with diluted Deltamethrin SC-PE to achieve a surface loading of 25 mg active ingredient/m². Surface loading for the standard DDT was 2000 mg active ingredient/m². Laboratory reared mosquitoes (local susceptible *Anopheles arabinesis*) were brought into the huts every four weeks and tested in a standard cone bioassay to determine residual efficacy of the treated surfaces.

Outdoor Weathering Trials

These trials were run as variation of standard residual surface trials as described above. Instead of being aged in climate chambers, treated surfaces were moved to an outside location where surfaces were exposed to 50% shaded sun light and naturally occurring precipitation. Surfaces were then assessed in laboratory bio-efficacy trials against *Blattella germanica* as test insect on a weekly basis.

RESULTS AND DISCUSSION

Selection of Residuality Enhancing Formulations

The formulation finding process explored a new in-situ encapsulation technology by using aqueous polymer dispersions (latices) which are suitable to form water-insoluble polymer films upon spraying and drying. Since incorporation of these latices into solid formulations is not possible, we have been focusing on the development of liquid formulations. Bioefficacy screening of various polymer adjuvants on laboratory scale resulted in the identification of only one class of polymer adjuvants of interest. This polymer class is characterised by a set of unique polymer and physical-chemical properties, being responsible for the improved residuality on porous, alkaline surfaces.

We propose a mode of action where the polymer particles act like a protecting primer and only partially encapsulate the active microcrystals on the surface, ensuring bioavailability and protection against surface alkalinity. The electron micrograph shown in Figure 1 of active microcrystals with polymer on a surface seems to suggest this.

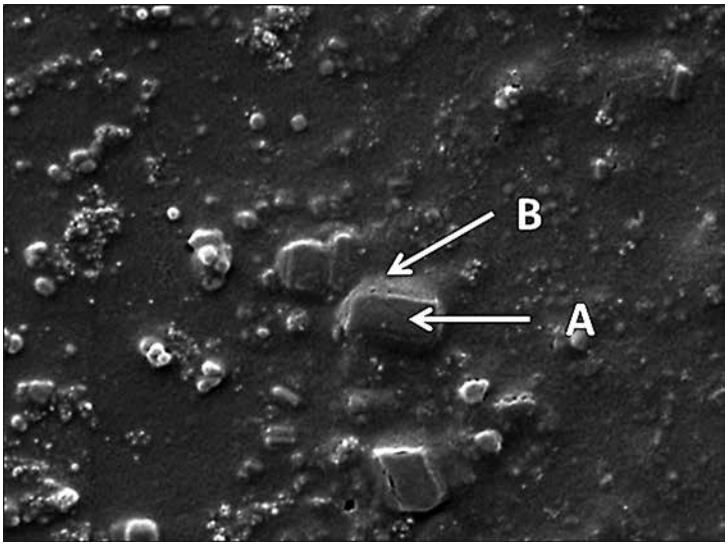

Figure 1. Transmission electron microscopy picture of partially embedded Deltamethrin crystals in a polymer film on a non-porous surface. A: Deltamethrin microcrystal ; B: polymer deposit.

Performance on Two Surfaces in the Laboratory

The formulation was tested on two surfaces against K-Othrine® WG 250 (Deltamethrin WG 250), an existing deltamethrin-based vector control product (Figure 2). On concrete, Deltamethrin WG 250 at a rate of 25 mg active ingredient/m² showed reduced performance from week 12 onward. Final mortality levels occasionally exceed 80% after 24 hours, but mortality values fluctuated greatly which was an indication of insecticide levels being at breaking point. Deltamethrin SC-PE applied at the same rate had residuality levels well above 80% for at least 52 weeks. On an inert wooden surface, Deltamethrin WG 250 and Deltamethrin SC-PE showed excellent performance (data not shown). Throughout the trial duration of 80 weeks, none of the three treatments fell below the threshold of 80% mortality after 24 hours. The surfaces were stored under stress conditions of 30°C and 80% RH between trials.

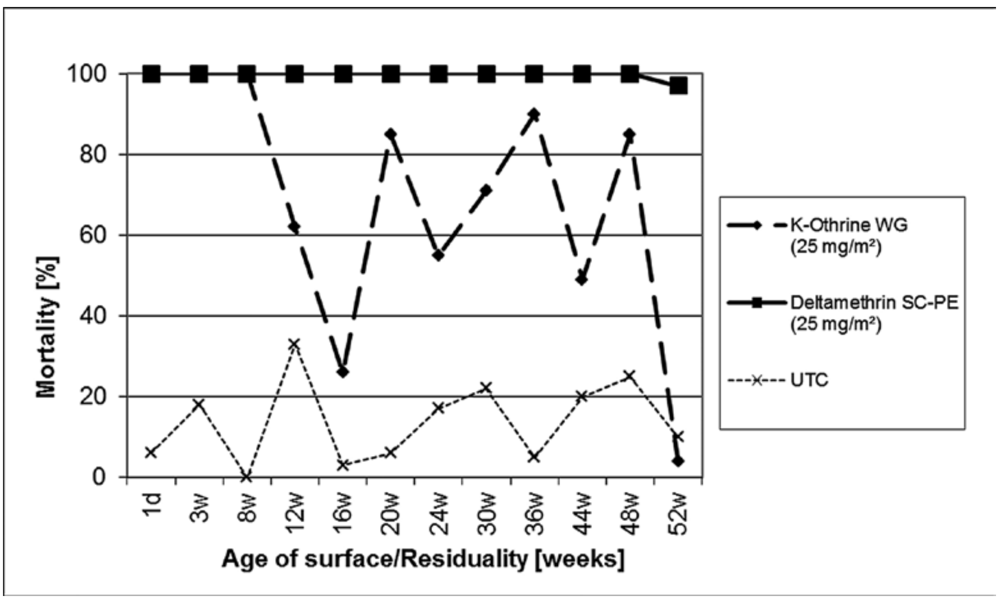

Figure 2. Final (24 h) mortality of *Anopheles gambiae* after 30 minute exposure to sprayed concrete surfaces.

Performance n Artificial Huts in Tanzania

For confirmation of the suitability of dose and residuality tests were carried out under natural condition in Africa by spraying locally-used housing materials and testing the residual effect by means of cone bioassays. In huts, mud, concrete plaster, plywood and palm thatch walls were treated and periodically bio-assayed. The treatments compared were Deltamethrin SC-PE sprayed at 25 mg DLT/m^2 and a DDT based product at 2000 mg DDT/m^2. In bioassay results depicted in Figure 3, Deltamethrin SC-PE showed better initial kill and longer residual activity than DDT on concrete by killing a greater proportion of *A. arabiensis* for a longer period. On mud and wood performance of Deltamethrin SC-PE and DDT were similar (data not shown).

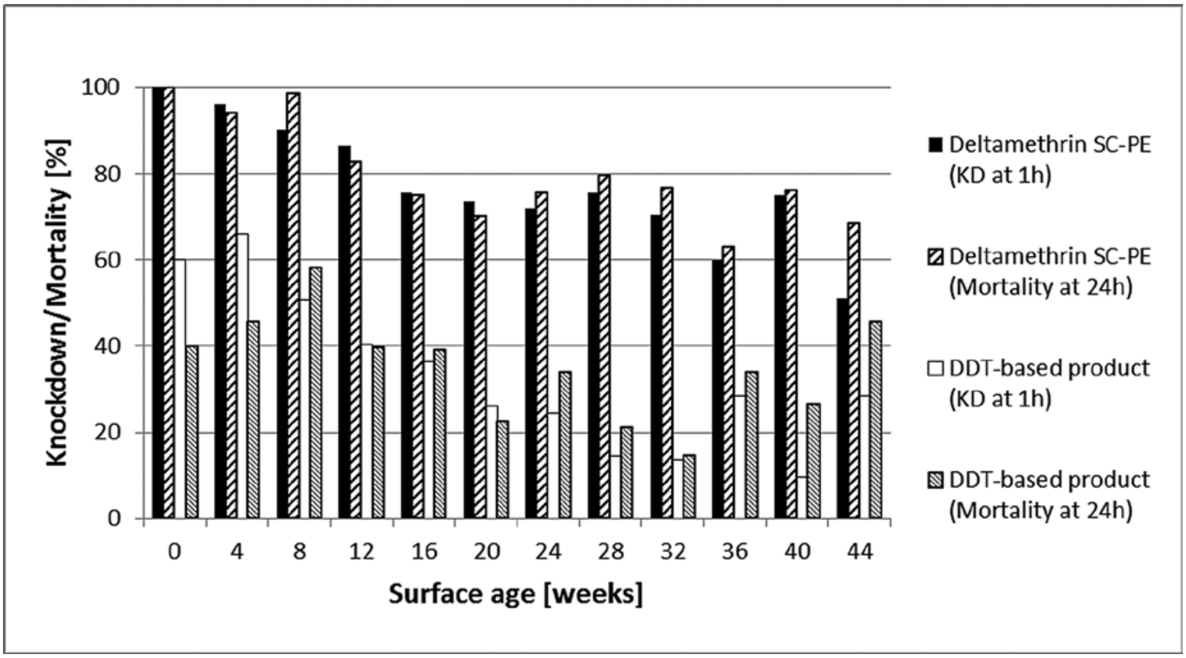

Figure 3. Knockdown and mortality of *A. arabiensis* on concrete walls aged in huts in Tanzania. Deltamethrin SC-PE used at 25 mg/m² and the DDT-based product was applied with 2000 mg/m².

WHOPES Evaluation and WHO Recommendation

Deltamethrin SC-PE was evaluated in the WHO certification process run by World Health Organization Pesticide Evaluation Scheme (WHOPES). Following this 3 phase process, a recommendation was issued and reported by the annual WHOPES working group. Using trial data of Deltamethrin SC-PE from laboratory scale, small scale field trials and village scale field trials by the WHO in Vietnam, India and Mexico and supportive data submitted by industry, the following recommendation was issued in July 2013: The use of Deltamethrin SC-PE for indoor residual spraying against malaria vectors at a target does of 20-25 mg active ingredient/m² will result in residual efficacy of 6 months (WHOPES, 2013). All other pyrethroids, including other formulations of Deltamethrin, have a recognised residual efficacy of 3 to 6 months; the fact that there was no lower limit range for Deltamethrin SC-PE indicates that it is recognised as being the longest lasting residual pyrethroid product available for malaria control.

Outdoor Weathering Trials

Evaluations were conducted to determine whether the PolyZone™ technology has an impact on outdoor, weather-induced destruction of insecticidal layers. Trials assessed the resistance of Deltamethrin SC-PC compared against various commercial insecticide formulations to exposure to UV radiation in sunlight with daily temperature fluctuations and exposure to rainfall or irrigation (Jorgenson, 2010; Jorgenson, 2012).

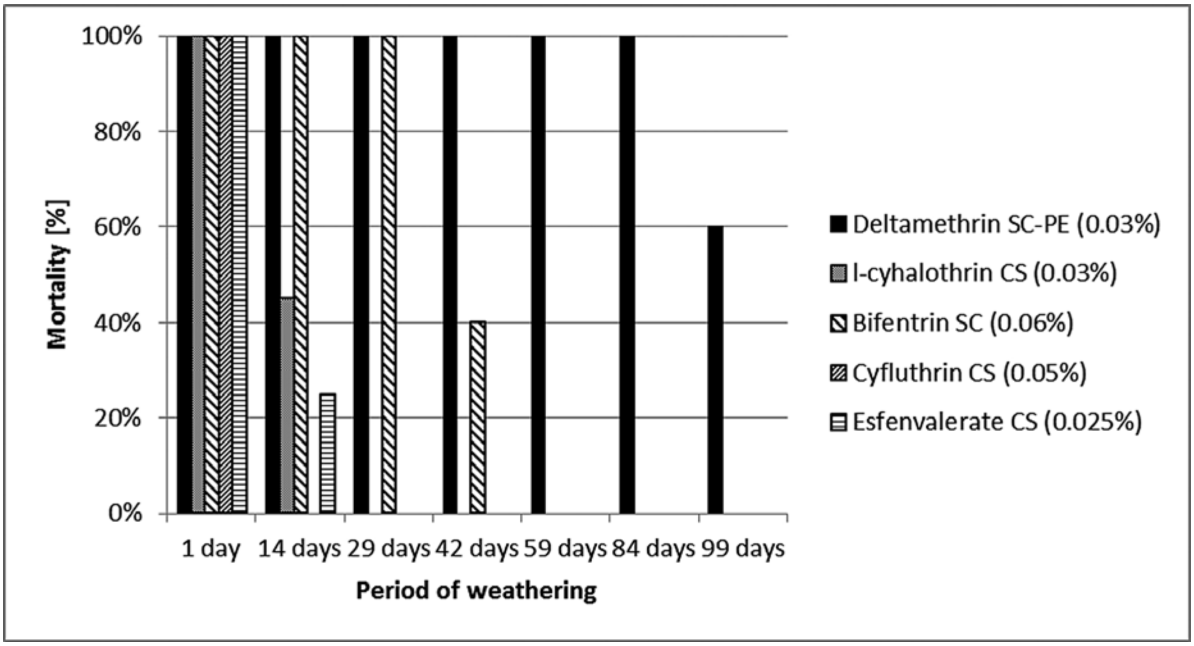

Figure 4. Mortality of *B. germanica* exposed to surfaces treated with the given insecticidal products at the indicated rate. Total rainfall in the test period was 177 liter/m².

Insecticidal formulations were sprayed onto surfaces at label rate. Treated surfaces were exposed to naturally occurring weathering conditions and periodically assayed for bio efficacy as described above. Deltamethrin SC-PE showed an extended period of full effectiveness. Figure 4 shows that Cyfluthrin CS was ineffective and l-cyhalothrin and Esfenvalerate CS were ineffective after 14 days of exposure to outdoor aging. The suspension concentrate of bifentrin caused substantial mortality for 1 month. Deltamethrin residues protected in the PolyZone™ formulation remained 100% effective through 3 months of weathering.

CONCLUSION

Data presented in this paper demonstrate that a novel polymer enhanced technology provides a longer residuality for key use pattern. Deltamethrin SC-PE extends effective insect control both in indoor applications on difficult aggressive surfaces, as well as in outdoor conditions under the influence of precipitation. Longer residuality decreases the frequency of insecticide re-application which reduces the output of chemicals into the environment and improves the operational efficiency of anti-Malaria spray teams or pest control operators.

ACKNOWLEDGEMENTS

Parts of this work, namely hut trials in Tanzania, were supported by the Innovative Vector Control Consortium (IVCC), Liverpool, UK. Their knowledge and expertise was greatly appreciated.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

CYANTRANILIPROLE: A NOVEL INSECTICIDE FOR CONTROL OF URBAN PESTS

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Abstract. Cyantraniliprole is a novel insecticide that belongs to the anthranilic diamide class of insecticides. The novel mode of action of cyantraniliprole depletes calcium from insect muscle, affecting muscle contraction, causing paralysis and eventually death. We report the results of testing of two formulations of cyantraniliprole, including a sprayable formulation for control of crawling insects and a fly bait formulation for house fly control. Cyantraniliprole was effective as a direct spray (contact activity) and as a residual surface application for control of German cockroaches, crickets and house fly. A proprietary bait comprised of a unique matrix and 0.5% cyantraniliprole, was shown to be highly effective against house fly. Due to the novel mode of action, cyantraniliprole can be a valuable rotational product towards integrated pest management and insecticide resistance management programs.
Key words Cyantraniliprole, house fly, ants, cockroaches, insecticide.

INTRODUCTION

Cyantraniliprole (CYNT) is an anthranilic diamide insecticide discovered by DuPont Crop Protection and more recently, is being developed by Syngenta for uses against selected urban pests. Cyantraniliprole is classified by IRAC as a ryanodine receptor modulator [Group 28] (IRAC, 2012). Cyantraniliprole binds to ryanodine receptors, causing depletion of calcium from muscle cells, leading to muscle contraction, paralysis, and death of the insect (Cordova et al., 2006; Satelle et al., 2008). Cyantraniliprole was categorized as a reduced-risk insecticide (EPA approval granted 2011) with low toxicity to vertebrates. The compound is systemic in plants and has been the subject of development for controlling pests important to agricultural crop production. In the area of urban entomology, Mao et al. (2011) reported results from topical applications and treatment of sand substrates with CYNT against *Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* Shiraki. Mao's results showed cyantraniliprole to have LC_{50} and LD_{50} values similar to those reported for chlorantraniliprole (Altriset), another anthranilic diamide, for both termite species. In recent years, an effort to profile CYNT for activity against certain urban pests was undertaken. This paper reports on the activity of CYNT against important pests in urban settings.

MATERIALS AND METHODS

Laboratory bioassays were conducted beginning in 2009 through 2013 to evaluate the efficacy of cyantraniliprole on urban pests. Tests were conducted at the DuPont Stine-Haskell facility in Newark, DE, the Syngenta-Vero Beach Research Center, Vero Beach, FL, and the Syngenta Stein, CH, facility, as well as by a contract researcher. Tests of sprayable formulations of CYNT were either by direct spray of target insects or by exposure to residues on treated surfaces. For direct-spray trials, insects were sprayed directly using a spray chamber designed to simulate a contact spray under field conditions, i.e. the spray chamber floor was made of wire mesh so that treated insects would not be subject to spray accumulation. After treatment, insects were transferred to clean containers, provided with food and water and observed for mortality for 2 days. Data were subjected to ANOVA and means were separated with LSD tests. For tests with residues on treated surfaces, non-porous, glazed tiles were sprayed with each treatment with 1 gallon per 1000 ft^2 volume, and allowed to dry before exposing insects. Insects were exposed to the treated tiles for 30 minutes, transferred to an untreated container with food and water and monitored for mortality for 72 hours. Data were subjected to ANOVA and means were separated with LSD tests.

Trial #PR096161a. Insecticide-susceptible laboratory-reared cockroaches (J Wax strain) were used for testing, with10 adult males per replication, and 4 replications. A suspension concentrate (SC) formulation of CYNT and indoxacarb 20WG (Arilon®) were used with dilutions of 0.01%, 0.05% and 0.1%.

Trial #USVT0I0472013. Insecticide-susceptible laboratory-reared cockroaches (Orlando strain) were used for testing, 10 adult males per replication, with 4 replications. A 20% SC formulation of CYNT was used at dilutions of 0.05% and 0.1%, and Demand® CS (lambda cyhalothrin) at 0.03% was tested.

Trial #2013.2750.1. Insecticide-susceptible, laboratory-reared cockroaches were used for testing, with 10 adult males per replication, and 4 replications. A 20% SC formulation of CYNT was used at dilutions of 0.05% and 0.1%, and lambda cyhalothrin at 0.03% was used for comparison. Treated tiles were stored up to 60 days and the test repeated on aged tile residues.

Trial USVT0I0482013. House crickets (1 week old, sourced through Carolina Pet Supply) were used for testing, >30 per replication, with 4 replications. The 20% SC formulation of CYNT was used at dilutions of 0.05% and 0.1%, and lambda cyhalothrin at 0.03% was used for comparison.

Trial # PR096162a. Insecticide-susceptible, laboratory-reared house flies (Benzon strain) were used for testing, 10 adult per replication with 4 replications. A 20% SC formulation of CYNT was used with dilutions of 0.01%, 0.05% and 0.1%. Indoxacarb 20WG was tested at the same dilutions. After application, flies were transferred to clean containers, provided with food and water and observed for mortality and knockdown at 4 hours through 7 days.

Trial# PR109105. Laboratory colonies of insecticide-susceptible house flies (Entomology Consultants, LLC, Chaparrel, NM) were used in the trial. Choice tests were arranged in cages under laboratory conditions, using one hundred 3-day old adult flies per replicate. Flies were fed a 50:50 mixture of dry instant milk + granulated sugar, along with water, *ad libitum* throughout the study as an alternative choice to each test bait. Pre-weighed quantities of each bait product (based on recommended label rates) were provided in individual cages (along with milk/sugar bait and water) as a series of paired choice tests. Assessments of knock-down (inability of a fly to right itself) and mortality were recorded in each cage/replicate at 5, 15, 30, 60 minutes and 3, 7, and 24 hours. Analysis of variance was performed.

RESULTS AND DISCUSSION

Contact Activity and Residual Spray Tests

For direct sprays of German cockroaches, cyantraniliprole 20 SC provided greater than 97.5% control at dilutions ranging from 0.01 to 0.1%, comparable to levels of mortality observed with indoxacarb (Table 1). Mortality was more rapid with cyantraniliprole compared with indoxacarb, although this observation is expected since indoxacarb is a pro-insecticide, requiring metabolism by insects to be activated. Cyantraniliprole 20SC provided high levels of mortality with German cockroaches as a residual spray application (Table 2). In the two trials, >90% mortality was observed from exposure to treated tiles, although in trial 1, the progression of mortality moved slowly, requiring 72 hours for maximum mortality. The slower action contrasts with the rapid activity observed with lambda cyhalothrin. Although CYNT appears not to be as fast acting as a pyrethroid, this insecticide shows potential when applied as a contact or residual spray for German cockroach control. When surface residues of CYNT were aged indoors to 32 and 60 days, mortality was high with both rates of CYNT, suggesting good residual under indoor conditions. Because of the mode of action, CYNT could be a valuable for managing resistance in German cockroach populations.

Table 1. Percentage mortality and morbidity of *Blatella germanica* sprayed with cyantraniliprole dilutions, DuPont –Stine/Haskell RC, 2009 [PR096161a]. Means with same letter not significantly different (p<0.05).

	Mean[1] % Dead and Moribund		
Treatment and Rate	**1 HAT**	**4 HAT**	**24 HAT**
Cyantraniliprole 20SC 0.01%	25a	95ab	100a
Cyantraniliprole 20SC 0.05%	32.5a	90abc	100a
Cyantraniliprole 20SC 0.1%	27.5a	87.5abc	97.5ab
Arilon 20WG 0.01%	0b	0d	57.3c
Arilon 20WG 0.05%	0b	2.5d	100a
Arilon 20WG 0.1%	0b	5d	100a
Untreated	0b	0d	0d

The effectiveness of cyantraniliprole of residual sprays on house crickets was determined (Table 3). At rates of 0.05% and 0.1%, mortality of house crickets was 96.5 and 100% at 72 hours exposure. As with German cockroaches, the onset of mortality was slower than with lambda cyhalothrin.

Cyantraniliprole 20SC was also effective against house fly as a direct spray (Table 4). At dilutions as low as 0.01%, >90% of treated flies were dead or moribund within 1 hour and all flies were affected within 24 hours. Efficacy was also achieved with sprays of indoxacarb 20WG at all rates tested but flies were affected more slowly than with CYNT.

Table 2. Mean percentage mortality of German cockroaches (*Blatella germanica*) exposed to glazed tile surfaces treated with cyantraniliprole sprays, Syngenta –VBRC [USVT0I0472013] and Stein, Switzerland [2013.2750.1], 2013.

	Mean % Mortality							
	Trial 1 (VBRC)[1]							**Trial 2 (Stein)**[2]
Residue Age	**1 Day**			**32 Days**		**60 Days**		**7 Days**
Treatment	24	48	72	48	72	48		24
Cyantraniliprole SC 0.05%	36.5b	60.8b	86.5a	80.0b	95.0a	97.5a		93.0 (±0.6)
Cyantraniliprole SC 0.1%	23.5bc	76.8b	87.3a	66.3b	95.5a	97.3a		93.0 (±0.6)
lambda cyhalothrin 0.03%	100a	100a	100a	100a	100a	100a		93.0 (±0.6)
Untreated	4.8c	4.8c	4.8b	0.0c	25.0b	10.3b		93.0 (±0.6)

[1]On tiles aged 1 day in laboratory; means with same letter not significantly different, LSD p=0.05).[2]On tiles aged 7 days in laboratory; numbers in parentheses are standard deviations.

Cyantraniliprole Bait for House Fly Control

In a trial using a milk/sugar fly bait with 0.5% CYNT (PR106405b-Harrison & Kudile), >95% mortality was observed within 24 hours, after a 6-hour exposure to the bait, compared with <3% mortality with a blank bait. Based on these results and other rate studies with CYNT, development of a proprietary bait product for house fly control was undertaken. The bait matrix was incorporated with 0.5% CYNT for testing as a house fly bait. In choice tests, the CYNT fly bait, (Zyrox® Fly Granular Bait) was effective in controlling house fly, providing 100% mortality within 24 hours (Table 5).

Table 3. Percentage mortality of house crickets (*Acheta domestica*), exposed to glazed tile surfaces treated with cyantraniliprole sprays, Syngenta VBRC [USVT0I0482013].

	Mean % Mortality[1]		
Treatment and Rate	**Hours after exposure**		
	24	**48**	**72**
Cyantraniliprole 20SC 0.05%	53.8b	95a	96.5a
Cyantraniliprole 20SC 0.1%	42.5b	89.3a	100a
Demand CS 0.03% lambda cyhalothrin	100a	100a	100a
Untreated	0c	0b	0b

[1]On tiles aged 1 day in laboratory; means with same letter not significantly different, p<0.05).

Efficacy of CYNT was not significantly different to that observed with Golden Malrin® (1.1% methomyl bait) and Quikstrike® (0.5% dinotefuran) at 7 and 24 hours, although mortality occurred somewhat slower. High levels of knockdown with cyantraniliprole fly bait was observed in 5 min with nearly 100% of flies affected within 30 min. While the mode of action of CYNT would be expected to exhibit delayed action, the activity on house fly was relatively rapid. Compared with Maxforce® fly bait (0.5% imidacloprid), cyantraniliprole bait provided significantly better control. Cyantraniliprole fly bait is a highly attractive novel fly bait product and should be an effective tool in managing house fly populations, particularly with pyrethroid- and imidacloprid-resistant populations.

Table 4. Percentage mortality and morbidity of *Musca domestica* sprayed with cyantraniliprole SC dilutions, DuPont –Stine/Haskell RC, 2009 [PR096162a].

	Mean[1] % Dead and Moribund		
Treatment and Rate	**1 HAT**	**4 HAT**	**24 HAT**
Cyantraniliprole 0.01%	95.0a	100.0a	100.0a
Cyantraniliprole 0.05%	92.2a	92.2a	100.0a
Cyantraniliprole 0.1%	95.0a	100.0a	100.0a
Arilon 20WG 0.01%	2.5b	12.5c	90.0a
Arilon 20WG 0.05%	2.5b	25.0bc	100.0a
Arilon 20WG 0.1%	0.0b	42.5b	100.0a
Untreated	0.0b	0.0c	7.5b
[1]On tiles aged 1 day in laboratory; means with same letter not significantly different, $p<0.05$).			

Table 5. Mean number of dead *Musca domestica* per 100 total after feeding on various bait products; laboratory choice study, Lee (2011) [PR109105].

Treatment	**Average number of dead house flies (of 100)**			
	1 HAT	**3 HAT**	**7 HAT**	**24 HAT**
Cyantraniliprole Fly Bait	67.8ab	88.5b	97.5a	100.0a
Golden Malrin®	92.5a	100a	100.0a	100.0a
QuikStrike®	79.5a	99a	100.0a	100.0a
Maxforce® Granular Fly Bait	49.3b	76.8c	90.5b	93.0b
Untreated Check	0.0c	0d	0.0c	0.5c
[1]On tiles aged 1 day in laboratory; means with same letter are not significantly different, $p<0.01$).				

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

REDUCING PESTICIDE RUNOFF IN URBAN WATERWAYS

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Abstract Treatment strategies focused on reducing the amount of fipronil applied and the amount fipronil and its metabolites detected in the water runoff. Directed applications around the perimeter of the structure, narrow pin-stream applications, and no spray zones reduced the amount of fipronil detected. Alternative formulations such as foam may also have potential in reducing the amounts of fipronil in the water runoff. A standard treatment that avoided the driveway and garage provided satisfactory control and the lowest levels of fipronil in the water runoff.
Key words Fipronil, pyrethroids, Argentine ant.

INTRODUCTION

Ants are a major pest in the urban environment in California and represent 60-85% of pest problems on residential accounts for Pest Management Professionals (PMP). Argentine ants, Linepithema humile (Mayr), accounted for up to 85% of the ants encountered by PMPs (Field et al., 2007). A summary of research conducted from 2003 to 2012 (CASQVA, 2013) reported that the pyrethroid bifenthrin was found in 69% of the sediment samples and 64% of the water samples in California. Fipronil was detected in 39% of the water samples and 19% of the sediment samples. Maximum observed levels were higher than the reported toxicities of fipronil and its degradates. The conventional use of these sprays by PMPs clearly poses a problem and challenge.

Runoff from concrete treated surfaces contained bifenthrin and permethrin even 3 to 7 months after treatment. Water events (irrigation, washing, and rain) to treated concrete surfaces should be avoided since concrete surfaces may act as a reservoir for pesticides (Jiang et al., 2012). A consortium of university, industry and governmental agencies organized the Ant Pest Management Alliance (Ant PMA) and developed procedures that reduced the amount of pyrethroids applied by 75% (CDPR 2010). The lower risk IPM routes provided excellent ant control at costs slightly less than conventional treatments. However, single applications of fipronil were applied in most cases instead of pyrethroids.

The objective of this study was to expand upon the continued the efforts of the Ant PMA and reduce the amount of fipronil applied to control ants. Several different formulations of fipronil and application techniques were applied to residential homes and the amount of fipronil and its metabolites in the water runoff and the efficacy of the treatments were determined.

MATERIALS AND METHODS

Collection of Water Runoff

A technique reported by Greenberg et al. (2010) was used to collect the water runoff at the curb. A custom-made 'U'-shaped Styrofoam dam was enclosed in a thin plastic liner and placed firmly against the sides of the street curb. Once water began to fill the cutout, samples were continuously taken from the center of the pooled water. The samples were transported to the laboratory in ice boxes and stored at 4°C until analysis.

Pesticide Analysis

Pesticides were identified using a procedure outlined by Greenberg et al. (2010). Water samples (1000 ml) were extracted with 40 ml methylene chloride three consecutive times using glass separatory funnels. For analysis of fipronil and its metabolites, the residue was recovered in petroleum ether + acetone (70 + 30 by volume; 1.0 ml) and subjected to a further cleanup. The extract (1.0 ml) was then passed through the conditioned cartridge and eluted with petroleum ether + acetone (70+30 by volume; 10ml) at a flow rate of 0.5 ml min^{-1}. The concentrations of target compounds in the final extracts were analyzed using an Agilent 6890 series GC equipped with a Ni63 microelectron capture detector (ECD; Agilent Technologies, Wilmington, DE). An HP-5MS column (30 m×0.25mm×0.25 µm; Agilent Technologies) was employed for separation. The typical retention times for desulfinyl fipronil, fipronil sulfide, fipronil, and fipronil sulfone under these conditions were 10.7, 12.9, 13.1, 15.2 and 17.8 min, respectively. A preliminary experiment showed that the method detection limits for the analytes were 0.001 µg l^{-1}. The recoveries of spiked analytes were higher than 85% using the above extraction and analysis steps.

Field Treatments

All treatments were applied during July 2010. The standard 0.06% fipronil preparation (Termidor SC, BASF Corp., Grenesboro, NC) was applied using a 19 L backpack sprayer (Birchmeier, Stetten, Switzerland) that had a 1.5 mm Duro mist nozzle. The fan spray was applied around the entire house foundation, approximately 30 cm up and 30 cm out from the house. At the driveway, it was applied to the garage door/driveway interface. Approximately 3.7 liters of spray were applied around each of 5 homes.

In the no spray zone treatment, the 0.06% fipronil was applied similar to the standard treatment, except that the driveway and hardscape areas near the driveway were not treated. About 2.8 liters of spray were applied to each of 5 homes.

The pin stream application of 0.06% fipronil was applied with the same equipment as the standard spray, except the spray was applied in a narrow pin stream. The pin stream was applied about 5 cm up and 5 cm out from the house. About 3.8 liters of spray were applied to each of 5 homes.

An application of dry foam using an aerosol can consisting of 0.65% fipronil (Whitmire Research Labs, St. Louis, MO) was applied to the entire house foundation approximately 5 cm up and 5 cm out from the house. At the driveway it was applied to the garage door/driveway interface. The amount used depended on the size of each house, ranging from 285 to 693 g (an average of 441 g).

An application of wet foam was applied using a Jack Plus Compressed Air Sprayer (NPD Products Ltd., Midhurst, Ontario, Canada). The 3.8 liters of fipronil was mixed with 40 ml of Pro Foam Platinum (Nisus Corp., Rockford, TN). The band of foam was applied 30 cm up and 30 cm out from the house. About 3.8 liters of fipronil plus foam were applied at each of 5 houses.

Monitoring Treatment Efficacy

The methodology used for monitoring Argentine ants and determining the efficacy of treatments has been reported in detail (Klotz et al., 2009). Ten vials of 15 ml 25% sucrose were placed around the house's foundation. Consumption of the sucrose/water over 24 hours was measured converted into the number of ant visits.

RESULTS AND DISCUSSION

Pesticide Runoff

At day 1, the standard spray and the crack and crevice treatment resulted in concentrations of fipronil lethal to mysid shrimp (Figure 1). At day 7 only the crack and crevice treatment provided a concentration in excess 140 PPT. At day 21, all the samples were below 140 PPT. The wash of the driveway with 151 liters of water to simulate a rain event resulted in fipronil concentrations high enough to kill *Ceriodaphnia*. The driveway flush at 56 days showed that the treatment that excluded the driveway had significantly lower runoff than any of the other treatments (Tukey's HSD test, $P < 0.05$ for each pairwise comparison).

Treatment Efficacy

The standard perimeter spray of fipronil provided up to 85% reductions in the number of ants visiting monitoring stations (Table 1). Initially the no spray zone treatment provided outstanding reductions, but the control declined by week 8. The pin stream application around the foundation and across the crack in the driveway in front of the garage door failed to satisfactory control. The wet foam provided about 55% reductions for the entire test period. The dry foam aerosol application initially provided satisfactory control, but the number of ants increased at week 4.

Table 1. The field performance of fipronil treatments against Argentine ants.

		Avg. ant visits per vial (% reduction) at week after treatment			
Treatment	Avg. Pre-count	1	2	4	8
Standard	11,595	3,188 (72.5)	2,883 (75.1)	5,023 (56.7)	2,022 (85.7)
Std. - driveway	16,388	3,621 (77.9)	1,215 (92.6)	4,783 (70.8)	5,924 (63.9)
Pin Stream	7,571	5,456 (27.9)	3,666 (51.6)	801 (89.4)	6,969 (9.3)
Wet Foam	13,152	5,348 (59.3)	6,009 (54.3)	5,534 (57.9)	6,046 (54.0)
Dry Foam	12,432	2,935 (76.4)	3,737 (69.9)	6,308 (49.3)	8,029 (35.4)

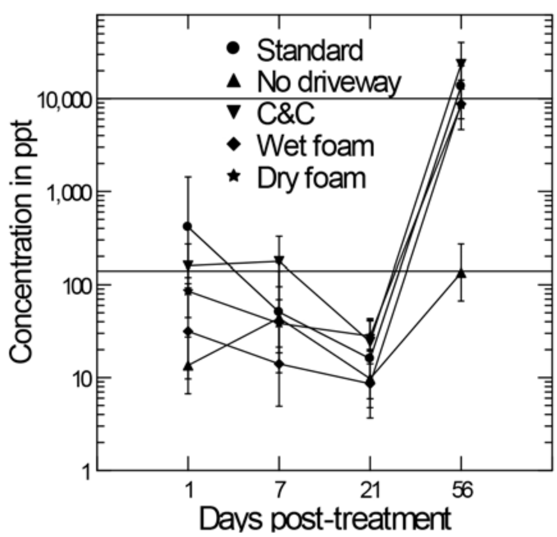

Figure 1. Fipronil runoff. Upper dotted line is Ceriodaphnia EC50 (10,000 PPT); lower dotted line is Mysid shrimp EC50 (140 PPT). Day 56 is the result from a 151 L wash of driveway and garage door.

CONCLUSIONS

The levels of fipronil in all treatments, except the no spray zone, produced levels toxic enough to kill 50% of the *Ceriodaphnia* and well above those necessary to kill all the Mysid shrimp.

The level of ant control decreased with those strategies that avoided the driveway. In the future, alternative treatment strategies that permit the treatment of the driveway and do not contribute to pesticide runoff and water quality issues need to be investigated.

ACKNOWLEDGMENTS

The research has been in part supported by grants from the California Department of Pesticide Regulation and the United States Environmental Protection Agency.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

INDOXACARB RESISTANCE IN THE GERMAN COCKROACH AFTER BAIT SELECTION

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Abstract In this study, we examined the resistance status of six field-collected strains from Singapore to eight insecticides, as well as the resistance of the parental through F_5 generations after they were selected with indoxacarb bait. All strains showed no resistance to acetamiprid (0.7–1.9x), imidacloprid (1.6–2.5x), chlorantraniliprole (2.3–4.0x), and bendiocarb (2.1–2.7x); low levels of resistance to indoxacarb (2.5–9.9x) and chlorpyrifos (2.6–8.7x); and moderate levels of resistance to DDT (>2.6x) and deltamethrin (5.8–55.6x). Very low resistance levels were detected in the indoxacarb bait assay test. Cross-resistance between deltamethrin and indoxacarb was found where LD_{50} and RR_{50} values for deltamethrin and indoxacarb were positively correlated ($P < 0.01$). Subsequent selection with indoxacarb bait on three strains significantly ($P < 0.05$) increased indoxacarb resistance (up to >30x), but it reduced deltamethrin resistance.
Key words Cross-resistance, *Blattella germanica*, pyrethroid.

INTRODUCTION

Heavy reliance on insecticides and high frequency of application have led to the development of insecticide resistance in the German cockroach, *Blattella germanica*. Field populations of *B. germanica* have developed multiple insecticide resistance mechanisms such as reduced cuticular penetration, increased detoxification rate and target site insensitivity. The German cockroach has developed resistance to at least 47 different chemicals, including to novel insecticides such as fipronil (Kristensen et al., 2005; Ang et al., 2013) and indoxacarb (Chai and Lee, 2010).

Indoxacarb is the first commercialized pyrazoline-like insecticide that acts by blocking the independent site of the sodium channel (McCann et al., 2001). It is readily bioactivated by esterase/amidase type enzymes to yield the potent insecticidally active bio-product *N*-decarbomethoxylated metabolite (DCJW), which results in pseudoparalysis and subsequent death of poisoned insects (Wing et al. 1998; Wing et al. 2000; Wing et al., 2005). The mode of action of indoxacarb and its metabolite are novel and entirely distinct from that of pyrethroid.

Chai and Lee (2010) reported that several field-collected German cockroach populations from Singapore were resistant to indoxacarb, even though these cockroach populations were never exposed to this insecticide. We suspect that this phenomenon was due to cross-resistance from pyrethroid. Several insect pests have shown cross-resistance between pyrethroid and indoxacarb due to elevated monooxgenase and esterase (Ahmad and Hollingworth, 2004; Shono et al., 2004;

Ahmad and Arif, 2009; Nehare et al., 2010). However, information about the indoxacarb resistance mechanism and the potential for cross-resistance between pyrethroid and indoxacarb in *B. germanica* are limited.

This study reports the resistance status of six field-collected strains of the German cockroach from Singapore to indoxacarb and other commonly used insecticides. In addition, three strains were selected and subjected to insecticide selection using indoxacarb bait.

MATERIALS AND METHODS

Six field-collected strains of German cockroach were used in this study: B1 Tampines Central, Beach Road, Boat Quay, Victoria Street, Cavenagh Road, and Ghimmoh Road. A laboratory insecticide-susceptible reference strain that originated at the Environmental Health Institute (EHI), Singapore was used for comparison. Technical grade deltamethrin, chlorpyrifos, imidacloprid, DDT and bendiocarb, indoxacarb, chlorantraniliprole, and acetamiprid diluted in analytical grade acetone were used.

Ten adult males aged 1–3 weeks were immobilized with CO_2 prior to topical application. One µl of insecticide solution was placed on the first segment of the cockroach abdominal sternites using a microapplicator (Burkard Scientific Ltd., Middlesex, UK). Treated cockroaches were transferred into a clean Petri dish supplied with dog food and a wet cotton ball. Mortality of the cockroaches was scored 48-h after treatment. A total of 3–6 doses resulting in > 0% to <100% cockroach mortality, were evaluated for each insecticide and the experiment was replicated 3 – 5 times.

Advion® (0.6% indoxacarb) cockroach gel bait was used for the bait assay against the susceptible and field-collected strains. Ten adult males were released into the test arena that was provisioned with harborage and water and acclimatized for 24-h before the bait placement. Dead or unhealthy individuals were replaced immediately before the bait assay. Dog food was provided ad libitum and placed at one corner of the arena, while 0.1 g gel bait was placed at the other corner. Mortality of the cockroaches was scored every 1–3 h until all cockroaches were dead. Each bait assay was replicated four times.

Based on the topical bioassay results, three strains (Cavenagh Road, Ghimmoh Road, and Boat Quay) were chosen and subjected to bait selection. Approximately 3 g of indoxacarb bait were introduced to the cockroaches in the rearing tank without an alternative food source. After 24–48 h of exposure when approximately 80% of the test cockroaches were dead, the dead and moribund cockroaches, and the bait were removed from the rearing tank. The remaining survivors were provided with dry dog food and reared under laboratory conditions. Adult male offspring (aged 1–3 weeks) of selected generations of the three strains were evaluated with topical and bait assays to determine the effect of bait selection on the susceptibility of the cockroach populations to indoxacarb.

All mortality data were subjected to probit analysis. Resistance ratio (RR) was calculated by dividing the LD of field-collected strains by the corresponding LD of the susceptible strain. The RR was classified into five categories according to Lee and Lee (2004) and Chai and Lee (2010): <1, absence of resistance; 1 to 5, low resistance; □5 to 10, moderate resistance; 10 to 50, high resistance; and >50, very high resistance. The synergism ratio (SR) was calculated by dividing the LD of deltamethrin alone by the LD of deltamethrin + synergist for the same strain. Comparisons of LD and LT values among cockroach strains were based on the overlap of their respective 95% fiducial limits (FLs). Relationships between all tested insecticides were determined using pairwise correlation analysis of LD and RR values.

RESULTS

Based on the toxicity results of the EHI strain, deltamethrin was the most toxic insecticide, whereas acetamiprid was the least toxic. The Cavenagh Road strain showed the highest resistance level among the field-collected strains. The results showed that all strains were susceptible to acetamiprid, imidacloprid and chlorantraniliprole. Deltamethrin resistance levels ranged from 5.8 to 55.6-fold, and DDT resistance levels were >2.6-fold. Low to moderate indoxacarb resistance was found in several strains. The Boat Quay, Cavenagh Road, and Ghimmoh Road strains showed low to moderate chlorpyrifos resistance. In contrast, no chlorpyrifos resistance was detected in the B1 Tampines Central, Beach Road, and Victoria Street strains. Low resistance to bendiocarb was also recorded in the studied strains. Out of the six field-collected strains tested on indoxacarb bait, only two strains exhibited no significant difference in LT_{50} values compared to that of EHI susceptible strain. The Cavenagh Road and Ghimmoh Road strains showed significantly higher LT_{50} values than the EHI susceptible strain.

Pairwise correlations of LD_{50} and RR_{50} values among field-collected strains for all insecticides tested revealed no correlation between all insecticides and the cockroach strains, except for a positive significant correlation ($P < 0.01$, $R = 0.973$ for LD_{50}, $R = 0.972$ for RR_{50}) between deltamethrin and indoxacarb.

Boat Quay, Cavenagh Road, and Ghimmoh Road exhibited increased indoxacarb resistance after selection. These strains showed reduced resistance against three other conventional insecticides (deltamethrin, bendiocarb, and chlorpyrifos). The Boat Quay strain exhibited the highest LT_{50} at F_5 generation. This value was significantly different ($P < 0.05$) from that of the parental generation. The F_5 generations of the Cavenagh Road and Ghimmoh Road strains showed significantly higher ($P < 0.05$) LT_{50} values compared to that of the EHI susceptible strain (Figure 1).

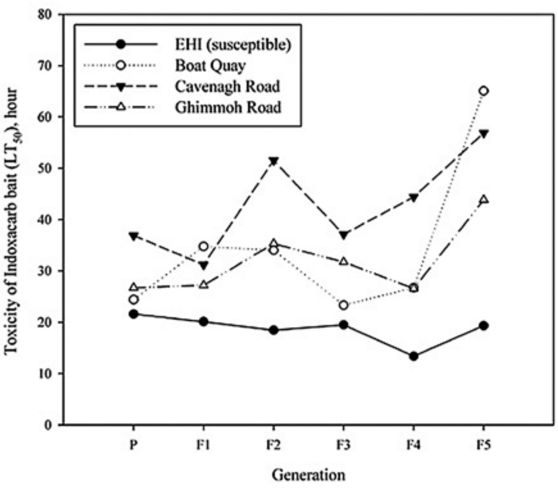

Figure 1. LT_{50} of all generations (from parental to F_5) of three indoxacarb-selected cockroach strains determined using bait assay.

DISCUSSION

The topical bioassay revealed a low level of indoxacarb resistance in the cockroach strains. Indoxacarb resistance was possibly caused through cross-resistance from deltamethrin and was shown in the correlation analysis. Indoxacarb in bait formulation, was never used against these populations of the German cockroaches in the field. The bait assay revealed that our cockroach strains had a significantly lower resistance to indoxacarb (i.e., oral ingestion) compared to topical application (Gondhalekar et al.

2011; Gondhalekar et al. 2013). This likely is because the midgut cells of cockroaches contain a high concentration of esterase/amidase-like enzymes, which are responsible for indoxacarb bioactivation (Wing et al., 1998; Wing et al., 2005). Reierson (1995) reported that a single adult German cockroach can consume at least 1 mg of food in a single meal. The indoxacarb bait formulation that we used contains 0.6% of active ingredient; thus, a cockroach will ingest ~6 µg of indoxacarb in 1 mg of bait. In addition, this compound exhibits poor cuticular penetration (Yu and McCord, 2007). The cross-resistance between members of pyrethroid class and indoxacarb, had been reported in other pest insects, such as the spotted bollworm *Earias vittella* (F) (Ahmad and Arif, 2009), obliquebanded leafroller *Choristoneura rosaceana* (Harris) (Ahmad and Hollingworth, 2004), and housefly *Musca domestica* (L) (Shono et al., 2004).

Deltamethrin and indoxacarb share a similar target site (i.e., the voltage-gated sodium channel) (Soderlund 2005). However, the differences in mode of action (Lapied et al., 2001; Narahashi, 2002; Yu, 2008) and binding sites in the channel (Wing et al., 2005) might explain the different resistance levels of deltamethrin (5.8–55.6x) and indoxacarb (2.5–9.9x).

After 5 generations of selection, no correlation was found between the susceptibility of the cockroaches to indoxacarb and those of deltamethrin. The deltamethrin resistance in the indoxacarb-selected populations was significantly reduced from that of the parental generations. The selection process using indoxacarb bait might have changed the resistance mechanisms in these strains that they became indoxacarb-specific. Deltamethrin may select for low indoxacarb resistance, however it is not possible vice-versa. Indoxacarb gel is still considered effective against these indoxacarb-selected cockroaches as all cockroaches died within 2 weeks after treatment.

In conclusion, cross-resistance is an important issue to consider, especially when a new insecticide with a similar target site is being introduced. This problem should not be underestimated because of its potential in shortening the longevity of the effectiveness of an insecticide. An understanding of the resistance mechanism and how resistance develops are essential to develop an effective resistance pest management strategy against urban insect pests.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

PIRIMIPHOS-METHYL 300CS, THE REINVENTION OF AN EFFECTIVE MOSQUITO ADULTICIDE

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Abstract Control of the Anopheline vectors is the most effective approach to malaria control. Reductions in mortality and morbidity from malaria have been achieved through the use of pyrethroid treated bed nets, pyrethroid resistant mosquitoes threaten the effectiveness of this valuable intervention. Insecticide resistance management (IRM) dictates that the target insect should not be continually exposed to the same class of insecticide to break the selection pressure for a resistance mechanism. Currently this cannot be achieved with the use of World Health Organisation's Pesticide Evaluation Scheme (WHOPES) approved Long Lasting Insecticide Treated bed Nets (LN). One approach to break the cycle of continual pyrethroid exposure is to employ indoor residual wall spraying (IRS) with a non-pyrethorid insecticide. IRS has been shown to be an effective method of controlling the Anopheline vectors of malaria. Pirimiphos-methyl is a WHOPES approved organophosphate insecticide which has been used as an emulsifiable concentrate formulation for IRS, and has been shown to control pyrethroid resistant mosquitoes. On some wall surfaces season long mosquito control may not be delivered. Supported by the Integrated Vector Control Consortium Syngenta developed a microencapsulated formulation of pirimiphos-methyl which provides control of *Anopheles* mosquitoes on typical wall surfaces. This paper discusses the biological behaviour of the microencapsulated formulation of the organophosphate insecticide, pirimiphos-methyl, developed specifically for use in mosquito vector control.
Key words Insecticide resistance management, bed nets, malaria

INTRODUCTION

It has been estimated that between 2001 and 2012 3.3 million deaths due to malaria were averted WHO (2013). The significant increase in vector control interventions has played a major role in this success, particularly the increased use of long lasting insecticide treated bed nets (LNs). However, during this period, the only LNs approved by WHOPES employed pyrethroid insecticides. Together with the continued use of pyrethroid insecticides for IRS, it is not surprising that pyrethroid resistance has developed in the Anopheline vectors of malaria, and is rapidly spreading throughout Africa (Ranson et al., 2011). As insecticide resistance continues to develop and spread, there is a real danger that these valuable vector control tools will be lost.

One approach to break the cycle of continual pyrethroid exposure is to employ IRS with a non-pyrethorid insecticide. IRS has been shown to be an effective method of controlling the Anopheline vectors of malaria (Mabaso et al., 2004) and to have an epidemiological impact on malaria (Pluess et al., 2010). One such non-pyrethroid, WHOPES approved, insecticide is the organophosphate pirimiphos-methyl, marketed by Syngenta under the trade name Actellic©.

Field studies have shown pirimiphos-methyl EC to have excellent residual insecticidal activity against Anopheline mosquitoes. Fuseini et al. (2011) found that pirimiphos-methyl EC effectively controlled *Anopheles gambiae* (Giles), identified as being resistant to pyrethroids, carbamates, dieldrin and DDT, when applied to concrete rendered walls at the end of the 15 week study period, in a trial in Ghana. Other studies, however, have found the pirimiphos-methyl EC formulation to be less robust in some circumstances. Okumu et al. (2012) found that when sprayed on mud walls or palm ceilings, the insecticidal activity of these surfaces rapidly decayed over two months.

Particulate insecticide formulations are known provide greater residual activity than EC formulations against mosquitoes when applied in IRS (Hadaway et al., 1970). Much research has gone into the development of particulate formulations specifically for the residual control of insects of public health importance, as exemplified by the development of a microencapsulated formulation of lambda-cyhalothrin, described by Wege et al. (1999). Given that pirimiphos-methyl has WHOPES approval, and shows promising insecticidal activity against pyrethroid resistant *Anopheles* species, a project was undertaken, in partnership with the IVCC, to develop a particulate formulation based on capsule suspension technology, the results of which are reported in this paper.

MATERIALS AND METHODS

Experimental substrates were treated using a track-sprayer fitted with a TeeJet 8003 nozzle to apply the formulations, diluted in de-ionised water, with an application volume of 40 ml/m^2. Treated substrates were stored at 26°C under low light conditions until used.

Bioassays were undertaken with three day old non-blood fed adult female mosquitoes, either *Aedes aegypti* (L.), *Anopheles stephensi* (Liston) or *An. aconitus* (Dönitz) Mosquitoes were exposed to the treated surface for one hour using a 9 cm Petri dish exposure chamber, they were then removed from the treated surface and placed in a recovery cup with access to a 10% sucrose solution. Assessment of mortality was made 24 hours post exposure. Given the non-excito-repellent nature of pirimiphos-methyl, a one hour exposure period was considered representative of likely field exposure.

RESULTS AND DISCUSSION

Laboratory Bioassays

Microencapsulation produces a particulate formulation that facilitates tarsal pickup, and hence bioavailability, of the insecticide by the mosquitoes from treated surfaces. The study presented in Table 1 demonstrated that pirimiphos-methyl 300CS remained bioavailable on porous, unglazed ceramic tile for at least 24 weeks, and controlled both *Ae. aegypti* and *An. stephensi* adult females exposed to the treated surfaces. The study presented in Table 2 supports these findings, highlighting the benefit of a particulate formulation on highly porous cement and mud, compared with an EC formulation.

Microencapsulation also physically separates the insecticide from the external environment, offering a degree of protection from aggressive surfaces, such as cement and mud, as highlighted by the study presented in Table 3. The residual insecticidal activity of pirimiphos-methyl 300CS was degraded to a much less extent on cement, than a bendiocarb wettable powder formulation, remaining effective on both surfaces for a minimum of 24 weeks. The study presented in Table 4 supports these findings, demonstrating the robust activity of pirimiphos-methyl 300CS on highly porous mud blocks, originating from Cote d'Ivoire, providing at least four months residual mosquito control.

Table 1. Mortality of female *An. stephensi* and *Ae. agypti* exposed to pirimiphos-methyl CS 1 g/m^2 residual deposits on unglazed ceramic tile. WAT = weeks after treatment.

	24 h % mortality at WAT				
Formulation	**1**	**7**	**12**	**20**	**24**
An. stephensi P-methyl 300CS	100	100	100	100	97
Ae. aegypti P-methyl 300CS	100	100	90	100	100
An. stephensi Control	3	3	0	7	7
Ae. aegypti Control	3	0	7	3	0

Table 2. Mortality of female mosquitoes exposed to residual insecticides. Trial stopped at 24 weeks.

		Weeks of >80% mortality 24 h post exposure	
Species	**Surface**	Pirimiphos-methyl 500 EC	Pirimiphos-methyl 300CS
Ae. aegypti	Cement	8	24*
Ae. aegypti	Mud	12	24*
An. aconitus	Cement	12	24*
An. aconitus	Mud	8	24*

Field Data

These laboratory results are confirmed by field trials. Chanda et al. (2013) report a study that treated cement and mud walls in houses in Zambia with pirimiphos-methyl 300CS at a rate of 1g/m^2. WHO cone bioassays were undertaken at intervals with laboratory reared *An. gambiae*. Complete mortality of exposed mosquitoes was recorded on both cement and mud walls five months after application, with eight months of greater than 90% control recorded on cement. The authors concluded that pirimiphos-methyl 300CS could be recommended for intra-domiciliary spraying for malaria control, as part of an IRM strategy. In a study undertaken by Rowland et al. (2013), it was found that in experimental huts with cement lined walls, pirimiphos-methyl 300CS at 1g/m^2 provided greater than 80% control of free flying pyrethroid resistant *An. gambiae* for nine months. The authors also concluded that pirimiphos-methyl 300CS applied at 1g/m^2 showed great promise for providing prolonged control of pyrethroid resistant *An. gambiae*.

Table 3. Mortality of adult female *Ae. aegypti* exposed to residual insecticide formulations applied to cement or unglazed ceramic tile (UGT).

			24 hour % mortality at WAT						
Formulation	**g ai/m^2**	**Surface**	**1**	**2**	**4**	**8**	**12**	**16**	**24**
P-methyl 300CS	1	UGT	100	100	100	100	100	97	93
P-methyl 300CS	1	Cement	100	100	93	90	90	87	83
Bendiocarb 80WP	0.4	UGT	100	100	83	17	17	20	13
Bendiocarb 80WP	0.4	Cement	20	7	0	0	0	17	13
Control		UGT	0	0	3	0	0	0	0
Control		Cement	3	0	3	0	0	0	0

Table 4. Mortality of adult female *Ae. aegypti* exposed to residual insecticide formulations applied to mud blocks originating in Cote d'Ivoire.

		24 hour % mortality at WAT					
Formulation	**g ai/m^2**	**1**	**2**	**4**	**8**	**12**	**16**
Pirimiphos-methyl 300CS	1	100.0	90.0	93.3	100.0	100.0	96.7
Labmda-cyhalothrin 10CS	0.025	100.0	100.0	100.0	93.3	93.3	53.3
Control		0.0	0.0	0.0	3.3	6.7	6.7

Taken together, these findings demonstrate that the rational reformulation of an established insecticide can rejuvenate its utility for IRS. Sufficient residual insecticidal activity has been demonstrated by pirimiphos-methyl 300CS to warrant its inclusion in IRS programmes, and highlights its utility in IRM programmes, particularly where pyrethroid resistance is present in the target mosquito population.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

EFFICACY TRIALS ON MOSQUITOES WITH NEW MONOMOLECULAR FILM

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Abstract Trials have been conducted with Aquatain AMF, a silicone based self-spreading liquid for mosquito control, in many locations and environments around the world. The trials included *Anopheles*, *Aedes*, and *Culex* mosquitoes. The product has been shown to be effective in very difficult conditions where other mosquito control products have not been successful. It is classified as a larvicide, but the trials demonstrated that it is effective on pupae. The film discourages females from laying eggs on the surface.
Key words Silicone film; mosquito control.

INTRODUCTION

The product is a silicone based liquid for mosquito control (Bukhari and Knols 2009; Bukhari et al., 2011). It spreads across the surface of standing water, forming a very thin film. As the silicone polymer called polydimethylsiloxane or PDMS has a low surface tension, larvae and pupae cannot attach to the surface to breathe, causing them to drown. The product has an entirely physical action, and does not contain any toxic chemicals.

Silicones are an ideal polymer for this application, as they impart a strong spreading action which enables the film to spread around dense vegetation. Silicones are also resistant to ultraviolet light and oxidation, resulting in an extended period on the surface beyond the petroleum based larvicide oils. Finally, they do not present any danger to humans or the environment, degrading into harmless silicates. The trials have been undertaken in 14 countries, covering almost all continents and a wide variety of conditions. All of the trials have been independent, and many of them have been peer-reviewed.

MATERIALS AND METHODS

Due to its strong spreading action, most of the trials have been conducted by simple pouring the product on to the water surface at the recommended dosage (1 litre per 1000 square metres of surface area). For very difficult areas in which the water surface is discontinuous, spray equipment has been required. In most cases, larvae have been counted using standard dippers.

RESULTS AND DISCUSSION

Sri Lanka Medical Research Institute

Trials were conducted on a heavily polluted canal in central Colombo. The predominant species was *Culex*. All of the larvae in the treated zone died within one day and there was still a 98% reduction two weeks after application. After four weeks, a 91% reduction was still observed, compared with the control area in which larval numbers had increased by 60% over the period.

Uganda Ministry Of Health

Trials were conducted at several sites in a swamp in Uganda. There was a 100% reduction in larvae after 12 hours at all sites, and no mortality in the control group. The trials were only conducted for a short period, so they couldn't draw any conclusions about longer term efficacy in this case.

Kenya Medical Research Institute and Wageningen University

Trial was conducted in a rice paddy in Kenya, following successful laboratory trials at Wageningen University in The Netherlands. The conditions were extreme, with heavy vegetation, slurries in part of the paddy, and workers coming in and out to weed the paddy field. Emergence of anopheline mosquitoes was reduced by 93% during the trial, and the authors concluded that the product is an effective agent for the control of mosquitoes in irrigated rice paddies. They also concluded that the product had no negative impact on non-target organisms, or on rice yields.

Cuba Directorate of Surveillance, Sancto Spiritus

Trials were conducted at five sites in the city of Sancti Spiritus in central Cuba. The sites were selected because other larvicides had been unsuccessful in reducing the population of larvae. They were either heavily vegetated, heavily polluted, or both – which explains why other larvicides didn't work. The number of larvae declined dramatically within 24 hours of application at all sites, to almost zero. After 15 days, they remained at around zero, although they had started to increase slightly at one of the sites.

Greece Directorate of Plant Produce Protection

Finally, these trials were conducted on a 1.2 hectare rice paddy in Greece. The paddy is divided into three sections, which are connected by channels. The rice plants were almost fully matured, creating dense vegetation and a challenge for the product to spread around all of the plants to cover the surface. It was applied at one side of the paddy only, in order to create the greatest challenge.

The larval mortality in section 1 - closest to where the product was applied – was 100% after three days, and was still at 75% on day 25 after application. The results weren't so impressive in sections 2 and 3, where larval mortality peaked at 80% and 40% respectively. However, the researchers noted that if the product had been applied at more locations, the results are likely to have been improved. They concluded that the product can provide larval control in habitats where other means of control are not effective.

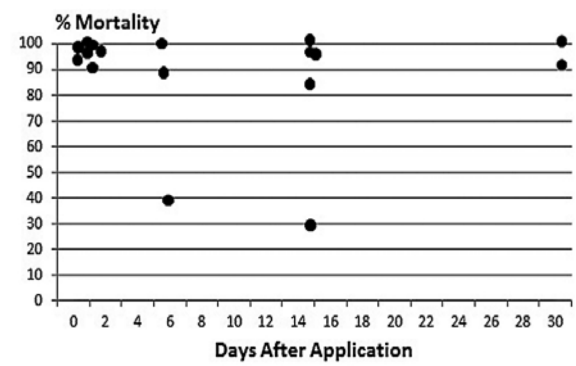

Figure 1. Mortality of Culex larvae.

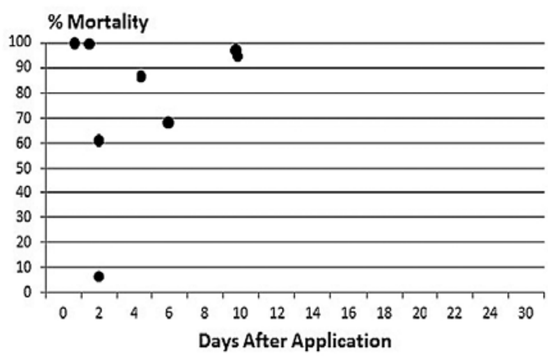

Figure 2. Mortality of Anopheles larvae.

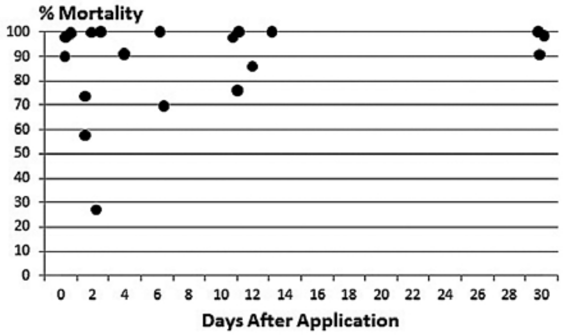

Figure 3. Mortality of Aedes larvae.

Figures 1, 2 and 3 show the results of all of the trials on C*ulex*, A*nopheles* and A*edes* larvae respectively. The product is very fast-acting and highly effective on *Culex* mosquitoes (Figure 1), with 90-100% mortality observed from Day 1 through to the duration. The two readings in the middle of the graph are those for the remote sections of the paddy field in Greece, where the product was deliberately applied at some distance away, in a heavily vegetated paddy, to gauge the limit of its spreading action.
For *Anopheles* mosquitoes (Figure 2) but the speed of action is slower. The pattern seems to be that the larvae die gradually over a period of days rather than within a day or so, as in the case of *Culex* larvae. Anopheles larvae may not come to the surface as frequently, and are therefore less affected by the film. However, after 10 days, or so, the result is the same. For *Aedes* mosquitoes – which transmit dengue fever – the results indicate (Figure 3) that the product is once again highly effective, but the timeframe for all of the larvae to die is around 10 days.

The impact on the pupae of all species is very rapid: within a few hours, all pupae are wiped out. No doubt this is because the pupae are at the surface at the final stage before emerging as adults, and they are immediately affected by the film. Also, no pupation has been observed in any of the trials.

The combined impact of the killing of all pupae and the absence of pupation is that there is no adult emergence after the film has been applied. Based on a limited number of trials: virtually no eggs are laid on the surface when the film is in place, but some females do drown while attempting to lay eggs.

CONCLUSIONS

In summary, the trials have shown that the product has multiple impacts on the mosquito lifecycle. Working through the various stages: the presence of the film discourages females from laying eggs; any larvae already in the water are wiped out, some of them within a day or so, and others gradually over a period of a few days; no pupation has been observed; and the pupae already in the water die very rapidly. With no pupae, there is no emergence of adults once the film is applied.

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Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

CULEX MOSQUITOES (DIPTERA: CULICIDAE) IN PRAGUE, CZECH REPUBLIC

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Abstract Breeding sites of *Culex pipiens molestus,* an autogenous and anthropophilic bioform of *Culex pipiens*, were frequently detected in flooded basements of buildings in Prague in the second half of the last century. The breeding sites gradually disappeared owing to improved maintenance of buildings after the political/ownership changes in 1989. The last report of a breeding place of *Cx. molestus* in Prague was made by the author in July 2010. According to our previous long-term studies, the anautogenous and mostly ornithophilic bioform of *Cx. pipiens* was always abundant in Prague and its surroundings. *Culex torrentium* was not detected in Prague before the turn of the 20th century. Its breeding sites were various water reservoirs or road puddles where the *Culex torrentium* larvae appeared either alone or along with those of *Cx. pipiens*. In June 2013, after heavy rains which caused disastrous floods in Prague, no larvae of flood mosquito species such as *Aedes vexans* appeared in the flooded zones. Later, *Culex* larvae (80% *Cx.pipiens*, 20% *Cx. torrentium* on average) appeared sporadically in flooded park meadows. The identification of Cx. *pipiens/torrentium* was based on the microscopic morphology of the hypopygia of the males reared in laboratory from larvae/pupae collected in the field. In flooded terrain depressions in Prague forests, *Cx. torrentium* (> 99%) larvae developed in densities often exceeding 100 individuals/dm^2 . Two dozen *Cx. modestus* larvae were caught on the edge of flooded meadows/fields in the southern outskirts of Prague. *Cx. territans* has disappeared from the Prague area as a result of progressive urbanization of land.

Proceedings of the Eighth International Conference on Urban Pests
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Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

POPULATIONAL STRUCTURIZATION OF *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE) POPULATIONS IN BRAZIL

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Abstract Microsatellites have been widely used in phylogenetic studies of various groups of insects. This study analyzed the genetic panorama of the urban species of mosquito *Culex quinquefasciatus* present in Brazil. Nine populations of *Cx. quinquefasciatus* mosquitoes from all regions of Brazil and one from Argentina were investigated by six microsatellite loci previously utilized successfully in *Culex pipiens*. A deviation from Hardy-Weinberg equilibrium was observed in 60% on the analyzed loci ($p<0.05$). The dendrogram of genetic distance among all populations showed two distinct clusters: one cluster from Rio Branco, Belem, Teresina and Pontes e Lacerda, located in the North and Midwest of Brazil, and a second cluster formed with the populations from Santa Vitória, Chapecó, Rio Pinheiros and PET located in the South and Southeast of Brazil. The population of La Plata showed distinct genetic patterns due to hybridization between *Culex quinquefasciatus/pipiens* and colony population has low heterozygosity for being more than 20 years in the laboratory, for these reasons they were segregated not grouping with any other population. The grouping of populations in clusters defined in the North-South axis, and the segregation of populations from La Plata and colony indicates significant genetic differences between populations of *Cx. quinquefasciatus* mosquitoes in Brazil. The segregation of La Plata population revealed significant genetic differences from those collected in Brazil. A better understanding of the genetic structure of mosquito populations may help anticipate distribution of diseases and play a decisive role in epidemiological interventions. FAPESP: 2008/57468-6 and 2012/19117-2.

Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

NEGATIVE CROSS-RESISTANCE AS A RESISTANCE MANAGEMENT TOOL FOR PYRETHROID RESISTANCE IN MALARIA VECTORS

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Abstract Malaria is a global health challenge. Vector control remains the fundamental, central and critical component of prevention and control of malaria. Insecticides remain the mainstay in vector control for malaria prevention. Pyrethroids are currently the insecticides for adult vector control intervention. The increasing and widespread development of pyrethroid resistance is a serious potential threat to malaria vector control programmes. Managing insecticide resistance is a key challenge for effective and affordable vector control. Spread of resistance to insecticides increase with prolonged use of the same or several insecticides exerting high selection pressure on the vector population. Hence, innovative strategies are essential to maintain and/or sustain vector control. Monooxygenases and esterases are the enzymes which are responsible for metabolism and detoxification of pyrethroid and also bio-activate chlorfenapyr and indoxacarb into an active toxic form, respectively. *In silico* enzyme and insecticide interaction activity studies also validated that there could be a possibility of chlorfenapyr and indoxacarb to exhibit negative cross-resistance (where detoxifying enzyme for one insecticide is responsible for bio-activation of the other insecticide) against pyrethroid resistance. It is suggested to use chlorfenapyr to manage resistance due to enhanced monooxygenases and indoxacarb with enhanced esterases. This novel approach will lead to improve the current vector-control approaches by introducing alternate insecticide, i.e., negative cross-resistance toxins to manage insecticide resistance employing alteration/ rotation. This innovative resistance management strategy can prolong the useful life of insecticides, while at the same time preserve or enhance the utility of existing insecticides in use.

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Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

IRRITANT EFFECT AND TOXICITY OF CHLORFENAPYR AND COMPARISON OF IRRITANT EFFECT TO DIFFERENT INSECTICIDES IN SUSCEPTIBLE AND MULTI-INSECTICIDE RESISTANT LABORATORY STRAINS OF *ANOPHELES STEPHENSI*

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Abstract Malaria vectors have acquired widespread resistance to different classes of insecticides including two synthetic pyrethroids owing to the continuous use in public health and sometimes in other sectors. Hence, there is need to develop alternative strategies including use of new insecticides with novel mode of action for effective management of insecticide resistance and vectors. For effectivity of an insecticide, its toxicity as well as the intrinsic chemical nature of the molecule that seldom cause irritability is important, because the net toxic effect depends on these two aspects. So far, among the insecticides classes used in disease vector control, the irritability and toxicity values are in the order pyrethroids>DDT>malathion. Among the new class of insecticides that readily can be used to manage insecticide resistance including pyrethroid resistance is chlorfenapyr, a pyrrole class insecticide. We have conducted tests of irritability and test of intrinsic toxicity on DDT, malathion, deltamethrin, permethrin and chlorfenapyr against *Anopheles stephensi* susceptible and resistant lab strains. Chlorfenapyr molecule has shown late acting nature and least irritant effect against susceptible and resistant strains among the insecticides tested. Hence, this study demonstrates and further supports that chlorfenapyr being the least irritable compound allows more landing time to the vector to pick up lethal dose of insecticide and thus could be an ideal candidate for IRS especially for the management of multiple insecticide resistant disease vectors including pyrethroid resistance.

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SPECIFIC AND POPULATION SENSITIVITY TO REPELLENTS OF BLOOD-SUCKING FLIES (INSECTA: DIPTERA)

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Abstract Studies were conducted in several climatic zones of the Western and Eastern Siberia. Blood-sucking Diptera in localities of these regions have rich species composition and reach high numbers. In addition to a considerable irritant effect on people, they are carriers of many infection diseases, and also cause allergic reactions and a dermatosis. The program of researches included studying of species composition of the mosquitoes, horse-flies and black flies, and also determination of the coefficient of repellency of repellents for the main species of blood-sucking insects. Effectiveness of 7 repellents from different chemical groups were studied with the help of olfactometers. An average coefficient of repellency in relation to separate species of mosquitoes, gadflies, black flies and level of reaction of these insects on repellents in various climatic zones was defined. Results of research showed that the sensitivity to repellents of different species of mosquitoes, horse and black flies, differed significantly. Besides, it was established that geographical populations of each species have different levels of sensitivity to repellents. As a rule, the species was most sensitive to repellents, in the climatic zone which was optimal for its ecological requirements. Its level of sensitivity usually correlated with level of specific abundance. Thus, the average level of reaction of blood-sucking Diptera to repellents in each natural zone depends on the one hand on the set of species and on the other hand on level of reactions to repellents of geographical populations of the blood sucking insects.

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Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

PLANT BASED MOSQUITO REPELLENTS – A SOLUTION FOR PEOPLE AVOIDING CHEMICAL REPELLENTS?

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Abstract There are several regions in Poland where mosquitoes emerge in huge numbers almost every year. Some of them are areas of protected nature - mainly wetlands, where the insects cannot be controlled in mass actions. Many inhabitants or tourists visiting these regions try to cope with the problem on their own - the solution is the use of repellents for personal protection. People more willingly use agents advertised as "based on naturally occurring plant oils" than biocides containing synthetic chemicals, especially for protection of children. The aim of the study was to evaluate efficiency of several products (biocides and cosmetic products) present on the Polish market containing plant oils, declared as giving protection against mosquitoes. The evaluated formulations were: tissues, bracelets, patches and sprays. The products were tested against adult *Aedes aegypti* female mosquitoes on human volunteers under laboratory conditions. Our results did show, that small cosmetic products, such as tissues and patches reduced mosquito bites up to 48% after 1 hour. Bracelets gave significant protection after warming up from the body: after the 1st hour 70% reduction was observed and up to 85% after 4 hours, respectively. Tested sprays based on plant oils protected against mosquito bites as well as biocidal products with 15% DEET.

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TICK ABUNDANCE IN THE CITY OF ZURICH, SWITZERLAND

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Abstract Ticks and their caused diseases lyme disease and TBE are an increasingly discussed topic in Switzerland. The habitat preferences of ticks have been studied frequently, but there exist almost no data about the abundance of ticks in urban areas. Due to that, the purpose of this study was to examine the tick density in urban parks in the city of Zurich. In addition there was analysed whether there is a relation between the abundance of rodents and ticks and whether the collected ticks were infected with the bacterium *Borrelia burgdorferi*. The ticks were collected by flagging low vegetation in 14 park areas in the city of Zurich. The study areas were classified in three categories. Category 1 includes areas with a high amount of forest, whereas category 3 contains cultivated, open parks and category 2 is situated in between. During the flagging, temperature and humidity were measured on each area. The results include the analysis of the climatic data and of the study areas, the comparison with the rodent data, the outcome of the borreliosis tests and the determination of the development stage. 15 ticks of the species *Ixodes ricinus* were collected during the study. On 4 out of these 14 study areas ticks were found. These 4 areas belong all to the category 1. The average humidity over all tick findings was 65% (SD= 13.4) and the temperature 16.95°C (SD= 2.21). There was no relation between the occurrence of ticks and rodents. All results of the borreliosis tests were negative. The collected data are insufficient to give a statement about the tick density in the city of Zurich. There is no final answer that explains why there were no ticks found on category 2 and 3 areas. A possible explanation could be the lacking of high vegetation and organic matter at these areas. These two factors are essential for the existence of a microclimate. To obtain more meaningful results, it is recommended to adapt the method and to perform the study again.

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TICK-BITE PREVENTATIVE BEHAVIOR OF PEOPLE ON RECREATIONAL AREAS IN WARSAW

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Abstract The occurrence of *Ixodes* and *Dermacentor* ticks is not limited to natural biotopes. These arthropods inhabit recreational parks and small forests situated in the city and in the suburban zone of Warsaw as well. In studies carried out in Warsaw recently the level of infected by *Borrelia burgdorferi* s.l. ticks was recorded between 10,7–20,0%. The aim of our study is to determine how people visiting recreational areas in Warsaw protect themselves against tick bites. A questionnaire about the use of anti-tick prophylaxis was elaborated. Respondents were people who visited 5 recreational areas: parks in the city and small forests in the suburbs, 11 – 80 years old. About 400 completed questionnaires were analyzed. The evaluation of given answers showed that approximately 50% respondents did not use chemical repellents. About 60% did not wear appropriate clothes: long sleeves, long trousers in light colors, etc. when visiting recreational areas known for the presence of ticks. About 56% persons examined their body carefully after the walk in the forest or park. However, about 92% respondents confirmed their knowledge about tick-borne diseases; 90% of dog owners declared that they protect their pets with chemical agents against ticks.

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PROLONGED EXPOSURE OF BED BUGS TO SUB-LETHAL TEMPERATURES; MORTALITY, STERILIZATION, FERTILITY RESTORATION AND OFFSPRING EFFECTS

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Abstract The bed bugs comeback as a pest has revitalized research on several aspects of its biology to improve management. Temperature can be manipulated to control bedbugs, but has so far been focused on temperature extremes with mortal outcome. The present study focuses on exposure of bedbugs to temperatures of a sub-lethal character, and point at its potential application in the struggle against this culprit. We exposed adult bed bugs for 34.0, 35.5, 37.0, 38.5 or 40 ºC for 3, 6 or 9 days. 40 and 38.5 ºC induced 100% mortality when exposed for extended time, whereas 34 ºC had no observable negative effect on bedbugs. The sub-lethal temperatures did however interact with time to induce intermediate or low levels of mortality and had distinct effects on fertility. The number of eggs produced, the hatching rate of the eggs and the following nymphal feeding and moulting success decreased by increased temperature and prolonged exposure. The fertility remained low for up to 40 days after heat exposure, and the time until fertility restoration was strongly connected to the time and temperature exposure. Offspring originating from the populations with restored fertility also suffered from reduced ability to moult. This may indicate involvement of the ovarial transferred bacterial symbiont, *Wolbachia,* in the mechanism behind the observed temporary sterilization and reduced offspring survival. Finally, eggs produced by untreated populations at 22 ºC were exposed for 34.0, 35.5 or 37.0 ºC for 3 or 6 days to investigate egg mortality at relevant temperatures. Our study shows that raising temperatures to between 35 and 40 ºC for different periods of time will influence bed bug population dynamics negatively. This may be utilized in professional pest management to worsen conditions for bed bugs to reduce infestation size and potential comeback after treatment. The role of this rather cheap measure ought to be further investigated both as a part of pest managers arsenal against bed bugs, but also as a potential tool in “do-it-yourself” eradication.

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USE AND EVALUATION OF ANT IDENTIFICATION WORKSHOPS FOR PEST MANAGEMENT PROFESSIONALS IN WASHINGTON, OREGON, AND BRITISH COLUMBIA

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Abstract Workshops on ant identification have been offered in Washington, Oregon, and British Columbia to pest management professionals since 1996 during with winter season. Number of workshops varies from 3 to 7 each year. Classes are nearly always filled to a capacity of 24 for each four-hour session. Each participant receives a preserved collection of approximately 20-25 ants in alcohol, keys for identification, a hand lens, plus information on biology, life cycles, and management on specific ants. Workshop sites are selected where each participant has access to a dissection microscope with lights, usually at universities or community colleges. Common local ants encountered in pest management are presented as well as exotic or tramp species that have the potential of becoming an invasive species. Common ants include: thatching ants, moisture ants, false honey ants, odorous house ants, Pharaoh ants, pavement ants, harvester ants, velvety tree ants, and carpenter ants. Several species of carpenter ants are presented with keys for identification. Exotic or tramp ant species that PMPs may encounter are presented: Argentine ants, ghost ants, red imported fire ants, little fire ants, European fire ants and rover ants. Participants appreciate having the actual ant in their collection to keep for review. Evaluations by participants indicate the information and guidance in the use of identification keys plus the availability of the take-home specimens have added to their professional development and has been as asset in working with clients.

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Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
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IMPACT OF INSECTICIDE SPRAY ON NON-TARGET TERRESTRIAL ARTHROPODS WHILE TREATING FOR *LINEPITHEMA HUMILE* (HYMENOPTERA: FORMICIDAE)

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Abstract As part of a study investigating experimental insecticide formulations for control of Argentine ants, *Linepithema humile* (Mayr), in outdoor locations, the imidacloprid-based product Premise 2 (Bayer Environmental Science) was used as a commercial standard. During the study, Premise 2 provided the most significant control of *L. humile* compared to the experimental products. The focus of this abstract is the resulting management of *L. humile* with an emphasis on the impact of the treatment on non-target terrestrial arthropods. Premise 2 was applied at the highest label rate of 0.1% to four trees with heavy trails of *L. humile* on the campus of Clemson University, Clemson, SC, USA, and another tree about 20 m away with similar ant trails was used as a control. Approximately 4 L of spray was applied 1.5 m up trunks and approximately 1.5 m around tree bases. The control tree was treated with water in a similar manner. Ant numbers were determined by counting the largest trail of *L. humile* passing a point on each tree in 30-sec. Numbers were recorded at 1 wk pre- and 1, 4, 8 and 12 wks post-treatment. One pitfall trap also was placed within 1 m of each tree. Thirty ml of antifreeze (Arctic Ban, Camco Mfr. Greensboro, NC, USA) was added to pitfall traps for one week before collection. Samples (0, 1, 4, 8 and 12 wks) were identified to order, family or genus. Pretreatment ant numbers averaged 27 on treated and 37 on the control tree. At 8- and 12-wks post-treatment, the mean percent decrease of *L. humile* was 100% on treated trees, the percent ant increase for the control tree was 35 and 41 at 8-and 12-wks post-treatment, respectively. Many arthropods were collected, but adult beetles (Coleoptera) and ants (Hymenoptera) were the most abundant groups captured in traps. Ten beetle families were collected in pitfall traps, with nine in treated sites and four in the control site. These included Carabidae, Curculionidae (Scolytinae), Histeridae, Montomidae, Nitidulidae, Scarabaeidae, Silvanidae, Staphylinidae, and Tenebrionidae in treated sites; and in the control, Chrysomelidae, Curculionidae (Scolytinae), Nitidulidae, and Staphylinidae. The Nitidulidae were the most common beetles captured; there were no other discernable patterns in beetle diversity or abundance during the study. Ant genera collected did show some patterns. At treatment and control sites, one week before treatments, *L. humile* was the most abundant ant species collected in pitfall traps. Ant numbers were low with 24 *L. humile* trapped at treatment sites and three at the control site. *Paratrechina* also were captured at pre-treatment Premise 2 sites with one *Hypoponera* sp. at the control site. Throughout post-treatment evaluations (1-12 wks), *L. humile* was the only ant species collected in

pitfall traps in the control site. In treatment sites, *L. humile* decreased to zero at 4-wks post-treatment with a corresponding increase in diversity and number of ant genera in the control. From 1- to 12-wks around treated trees, *Paratrechina, Pheidole, Solenopsis, Strumigenys,* and *Technomyrmex* ants were collected, the most abundant being *Solenopsis molesta*. It was difficult to discern patterns in arthropod diversity and abundance between treated and untreated sites, but *L. humile* was a keystone species in relation to other ants. After *L. humile* were reduced, diversity and abundance of other genera increased. This study indicates that other ant genera quickly fill a void created by reduction of an invasive species. In general after treatment, the original pest ant may rebound.

Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

MOLECULAR CHARACTERIZATION OF ANTS OF THE *CAMPONOTUS* GENUS COLLECTED IN URBAN PARKS OF THE CITY OF SAO PAULO, BRAZIL

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Abstract Alate ants are being collected in two urban green areas of the city of São Paulo, differing in their distances to natural Atlantic Forest remnants, in order to characterize the species diversity and the importance of urban parks in the maintenance of such diversity. Ants of the genus *Camponotus* are major components of the ant fauna in urban areas and are largely represented among the alates captured in the light and Malaise traps employed in the current study. Morphological identification is hindered by the lack of keys for alates in this genus. Therefore, molecular methods are being evaluated as an auxiliary tool for the identification and/or characterization of the genetic diversity of the *Camponotus* alates. After separation into morphospecies through external morphological characters, specimens are subjected to DNA extraction, PCR amplification of 28S rDNA and mitochondrial cytochrome oxidase I (COI) genes, and DNA sequencing. Our results show that 28S rDNA sequences confirm that a morphospecies belongs to the genus *Camponotus*, but species identification through COI sequences is limited by the scarcity of sequences of South American *Camponotus* species deposited in public databases. To diminish these problems, *Camponotus* workers are being collected actively or through pitfall traps in the surveyed areas, morphologically identified when possible, and sequenced. We expect that by this strategy, it will be possible to identify a significant portion of the *Camponotus* alates and to compare the efficacy of alates or workers capture to characterize the diversity of this genus in the surveyed areas. Preliminary results of these studies will be presented.

Proceedings of the Eighth International Conference on Urban Pests
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Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

EVALUATION OF EDUCATIONAL PROGRAMS IN AN INTEGRATED PEST MANAGEMENT APPROACH FOR COCKROACH INFESTATION IN HOUSING

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Abstract This study was conducted to evaluate effectiveness of educational programs on individual knowledge of occupants and sanitation rate. The study's locations comprised three buildings in the South West of Iran. The educational program was initiated by putting up posters, handing out pamphlets, face to face interaction and gave informative lectures to all students in the intervention section. To evaluate the effectiveness of the educational program, 53 residential units were divided randomly into intervention (education) and control groups. Sanitation before and after educational programs in study locations, was evaluated using sanitation rates. Before education, a set of questionnaires in two sections were distributed among all occupants in order to collect information on occupants' attitudes towards sanitation in the study's locations and knowledge of residents about cockroach infestation, prevention and integrated pest management (IPM) system. After being exposed to the educational program the respondents' knowledge about IPM improved by 46%. Evaluation of the obtained scores after educational programs showed a significant difference between scores for pre- and post-educational programs (Mean= 6.93 Vs 8.63). Data was further substantiated by control group (Mean=7.55 Vs 7.4). Therefore the educational program improved scores and subsequent knowledge of students on IPM and cockroach management. The sanitation rates for the intervention units at the girls' dormitory had significantly improved (from median 4 to 1) after several educational programs were conducted. This finding was further substantiated by the control group, which did not show significant change after a similar duration. In conclusion, the sanitation and the educational programs were important strategies contributing to the success of IPM method.

Proceedings of the Eighth International Conference on Urban Pests
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Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

USE OF CLOTHIANIDIN AS A NEW ACTIVE SUBSTANCE IN COCKROACH GEL BAITS

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Abstract This poster will report on development of a new cockroach gel bait formulation based on the active ingredient clothianidin. We will review research conducted to establish a minimum effective concentration for clothianidin in the formulation that is necessary to kill cockroaches. A series of trials will be summarized to document the efficacy of the final formulation against several cockroach species and compare efficacy to commercial gel bait formulations. A series of comparison studies will compare and contrast performance of this new gel bait to prominent commercial gel baits in susceptible strains of the German cockroach. Since our discovery of the bait aversion phenomenon in the early 1990s, Bayer has actively collected German cockroach strains that are suspected of displaying aversion to gel baits. Using several of these field-collected, bait-averse strains, we also carried out trials evaluating the effectiveness of this new gel bait and several commercial products in these “finicky” German cockroaches. Finally, we have completed several replicated field trials around the world against several species of cockroaches will also be presented. As more German cockroach populations are discovered that display an aversion to gel baits containing sugary phagostimulants, by offering a highly palatable bait matrix based on unique food ingredients and novel phagostimulants, this clothianidin gel bait will effectively control populations that have developed (or are developing) bait aversion characteristics. The bait gel market today is dominated by just two chemical classes, the phenylpyrazole and oxadiazine insecticides with modes of action as GABA gated Cl- channel agonists and voltage dependent Na+ channel blockers, respectively. This clothianidin gel bait has a distinctive mode-of-action, acting as an agonist at nicotinic acetylcholine receptors, which will be effective in rotation strategies to limit development of insecticide resistance. This new product has important attributes: the bait formulation is free of all common food allergens, the formulation is practically non-toxic to mammals, and is eligible for classification as a least toxic pesticide under the LEED Tier III classification scheme. These and other attributes were recognized by the US EPA when designating this new product was eligible for an expedited, reduced-risk registration decision.

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Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

COMPARISON OF EFFICACY AND ATTRACTIVENESS OF FIVE COMMERCIAL GEL FORMULATIONS TO CONTROL COCKROACHES IN LABORATORY TESTS

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Abstract We report the results achieved in laboratory tests conducted to compare the efficacy and the attractiveness of commercial gel formulations, containing abamectin, fipronil, indoxacarb, acetamiprid and imidacloprid, for the control of nymphs and adults of cockroaches. The tests were replicated 4 times and were carried out by in special arenas of 1 1 m^2 surface each. *Blattella germanica* (L.), *Periplaneta americana* (L.) and *Periplaneta australasiae* (F.) were the species considered in the study. In particular *Periplaneta australasiae* settled recently in several cities in Italy, probably due to the importation of food from the South-East Asia which is sold in specialty stores. At any assessment all the cockroaches placed in the Untreated Control arenas resulted alive and fully active. In terms of number of dead individuals, all the products showed a variable activity on the three species of cockroaches considered, but in two cases no mortality was recorded, while in other two cases full mortality was observed. On the base of the mortality percentage achieved the data statistically elaborated indicate that after 48 hours only the fipronil based gel showed a statistically significant difference against both stages of *B. germanica,* which was totally controlled. All the other treatments, including fipronil on *P. americana* and *P. australasiae*, recorded a mortality of cockroaches without significant statistical differences. The tests must be considered as preliminary study, which allow to affirm that the different gel formulations currently available in Italy for the control of cockroaches, show a low efficacy 48 hours after application with no statistically significant difference among them against *P. americana* and *P. australasiae* at any stage of development. However, gel containing fipronil 0.05% reached an efficacy statistically significant and higher than the other gel formulations against *B. germanica* at both stages of development, while on *P. americana* and *P. australasiae*, achieved an efficacy statistically similar to the other 4 gel formulations tested. In case of limited control by gels in the short term, traditional sprays are recommended, limiting the use of the gel only at points of infestation deemed not at serious risk of diffusion, such as electrical cabinets, closets, toilets.

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Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

EVALUATION OF BAIT GEL DOSAGE WITH FIPRONIL AND HYDRAMETHYLNONE FOR *PERIPLANETA AMERICANA* CONTROL

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Abstract The aim of this work was to evaluated to a minimal dose and minimal lethal time of Fipronil and Hydramethylnone bait gel formulation at a concentration of 0.05 and 2.15%. The insects were food deprived during 24 hours and after confined individually with the respective dose of the gel, water and food feline ground into test arena 17 cm long, 13 cm wide and 5 cm high. The dosages of 0.005, 0.007, 0.01, 0.03, 0.05, 0.07, 0.1, 0.2, 0.3, 0.4 and 0.5 g gel bait containing 20 repetitions per treatment were evaluated at periods of 1, 2, 3, 4, 24, 48, 72, 96, 120, 144, 168, 192, 216 and 240 hours after confinement of individuals. The active ingredient Fipronil showed 100% efficiency for all doses tested. The smallest dosage to achieve 100% efficiency of the active ingredient Hydramethylnone was 0.1 g. The minimum lethal time to reach 90% mortality of individuals with the active ingredient Fipronil was at a dose of 0.5 g gel bait in 24 <LT90 <48 hours. The active ingredient Hydramethylnone showed lethal time of at least 90% of individuals of $168 < LT_{90} < 192$ hours at a dose of 0.5 g gel bait.

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Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

EVALUATION OF THIAMETHOXAM AEROSOL APPLICATION AGAINST *BLATTELLA GERMANICA*

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Abstract The control of the German cockroach *Blattella germanica* is a major challenge for Brazilian pest control operators (PCOs) due to their great capacity for reproduction and dispersal. The study described aimed to evaluate the efficacy of the insecticide Thiamethoxam (Optigard LT® 25% – Wettable Granules) when applied directly in cracks and crevices infested with *Blattella germanica* under laboratory and field conditions, using an aerosol generator (Micronizer®). In the laboratory study cracks and crevices were simulated with piles of bricks and the insecticide was applied using Micronizer®. The dosages tested were 8g, 12g and 16g of commercial product per liter of water, applied in approximately 2.5 ml per crack/crevice. Results showed excellent control with all three concentrations tested, ranging from 99% (dose: 12g/L) to 100% (doses: 8g/L and 16g/L) 1 hour after application. The field study was undertaken in the cities of São Paulo and Itaguaí (Brazil) and consisted of the localized applications of the insecticide Thiamethoxam (Optigard LT doses: 8g/L and 16g/L) in cracks and crevices using Micronizer®, by local PCOs. Results showed an excellent rate of control even with 75 % lower application volume than conventional methods. The applications (dilution: 8 or 16g/L of water) were made weekly (São Paulo) or biweekly (Itaguaí) at the beginning of treatment, and monthly thereafter, until the cockroach infestations were controlled, which occurred after 2 – 5 applications. The control rates obtained ranged from 90% to 100% and were measured with cockroach sticky traps, aspirations of insects and through visual assessment.

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PATHOGENICITY OF *METARHIZIUM ANISOPLIAE*, *BEAUVERIA BASSIANA* AND *ASPERGILLUS* SP. TO *PERIPLANETA AMERICANA* (BLATTODEA: BLATTIDAE) FEMALES

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Abstract Entomopathogens have been used in many agricultural and veterinary programs of pest control. Studies with fungi have shown great potential for control of urban pests, such as cockroaches, but there are few studies on the subject. Thus, the aim of this study was to investigate the pathogenicity of entomopathogenic fungi to females of *Periplaneta americana*. The treatments were: T1 - no fungus application and T2 - solution of 0.1% Tween 80 (TW). For T3-T11 suspensions were made with TW: T3 - 3 x 10^8 conidia/ml of E9 strain of *Metarhizium anisopliae* (Ma), T4 - 3 x 10^7 conidia/ml of Ma, T5 - 3 x 10^6 conidia/ml of Ma, T6 - 3 x 10^8 conidia/ml of IBCB 35 strain of *Beauveria bassiana* (Bb), T7 - 3 x 10^7 conidia/ml of Bb, T8 - 3 x 10^6 conidia/ml of Bb, T9 - 3 x 10^8 conidia/ml of JAB 42 strain of *Aspergillus* sp. (Asp), T10 - 3 x 10^7 conidia/ml of Asp and T11- 3 x 10^6 conidia/ml of Asp. Females were sprayed with 100 µl of the suspensions and kept at 27 ± 0.5°C and 80% RH in the dark. Mortality and fungus extrusion rate were evaluated for 20 days. Data was analyzed using Scott Knott ($p \leq 0.05$) and transformed to $\sqrt{x}$ +1. The highest mortality and extrusion rate (48%) occurred with T3. There was no significant difference in time of death (6 to 10 days). All fungi were pathogenic to *Periplaneta americana* females. T3 could be a tool for integrated pest management.

Proceedings of the Eighth International Conference on Urban Pests
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Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

IDENTIFICATION OF FUNGI PATHOGENIC FOR *PERIPLANETA AMERICANA* (BLATTODEA: BLATTIDAE) BY SEQUENCING THE REGION ITS1-5.8S-ITS2 AND EVALUATION OF A SCATTERING FORMULATION OF *BEAUVERIA BASSIANA*

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Abstract *Periplaneta americana* is a vector of various diseases and its control is usually done with chemical pesticides. However, this insect can be controlled with the use of entomopathogenic fungal formulations. Therefore, it is necessary to identify these control agents and also characterize sprays used in different concentrations. The aim of this study was the identification of three isolates of pathogenic fungi to *Periplaneta americana* and to evaluate the kinetics of surface tension and contact angle of droplets formed from suspensions containing *Beauveria bassiana*. DNA sequences were obtained from isolates JAB 68, IBCB 35 and JAB 42 and isolated the ITS1-5.8S-ITS2 region. Treatments of the scattering analysis were: T1 - ultra pure water; T2 - solution 0.1% Tween 80 (TW), T3 - suspension 2 x 10^8 conidia/ml of IBCB 35 isolate of *Beauveria bassiana* (Bb), T4 - 2 x 10^7 con./ml of Bb, T5 - 3 x 10^6 con./ml of Bb. The sequencing of ITS1-5.8S-ITS2 region of JAB 68 and IBCB 35 isolates showed 100% similarity with *Metarhizium anisopliae* and *Beauveria bassiana* species, respectively. The third sequence, corresponding to JAB 42 isolate had 100% similarity with *Aspergillus westerdijkiae* and *Aspergillus ochraceus* species from GenBank database. Phenetic tree separated the isolates into three distinct groups, showing differences between species. The results of scattering analysis showed that lower concentrations of *Beauveria bassiana* conidia suspended with Tween 80 promote greater spreading of the formulation on the insect surface.

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Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

KINETICS OF SURFACE TENSION AND CONTACT ANGLE OF *METARHIZIUM ANISOPLIAE* AND *ASPERGILLUS* SP. SUSPENSIONS

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Abstract An important aspect to consider in biological pest control is the formulation of biopesticides, since it may interfere with the effectiveness of the control target. The aim of this study was to evaluate the kinetics of surface tension and contact angle of droplets formed in suspensions of *Metarhizium anisopliae and Aspergillus* sp. The treatments for *Aspergillus* sp. were: T1 - ultra pure water (UPW); T2 - Solution 0.1% Tween 80 (TW), T3 - suspension 2 x 10^8 conidia/ml of JAB 42 isolate of *Aspergillus* sp. (Asp), T4 - 2 x 10^7 con./ml Asp; T5 - 3 x 10^6 con./ml Asp and for *Metarhizium anisopliae*: T1 - UPW; T2 – TW, T3 – suspension 2 x 10^8 conidia/ml of JAB 68 *Metarhizium anisopliae* isolate (Ma), T4 – 2 x 10^7 con./ml of Ma, T5 – 3 x 10^6 con./ml of Ma. It was done an image analysis with a tensiometer and automatic software to obtain the kinetics of surface tension and contact angle of the formulations on wings of cockroaches. Data was analyzed using Scott-Knott test ($p \leq 0.05$). Conidial suspension of Asp-T3 had higher surface tension at 90s and 180s, indicating less spread when compared with other suspensions. Suspension of Ma-T3 treatment presented higher surface tension than suspension of T4 and T5 treatments at 5 seconds, which reveals a smaller scattering of the liquid. There was no difference between TW with conidial suspension of Ma in contact angle formed with the surface, which shows that the form and amount of inoculum does not interfere with the spreading of suspension.

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Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

INFESTATION OF NATURAL BUILDING INSULATION BY THE CLOTHES MOTH, *TINEOLA BISSELLIELLA*

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Abstract Inside a two year old family house, clothes moth adults appeared in all rooms in 2012. Inspection of clothes did not find any moths. The moth population was heavy, with at least 10 moths caught daily. Thermal fogging inside the house with deltamethrin reduced flying moths for no more than 14 days. The natural wall insulation made from sheep's wool was suspected as a breeding site of the moths. Samples of wool extracted from inside the walls contained moth larvae. According to the insulation provider the ecologically friendly product was treated with a 10% solution of Molantin SP (10% permethrin), but as the two year guarantee had expired, the manufacturer refused any responsibility for the moth infestation. The house owner was so desperate that he considered removing the plasterboard from the walls and to replace the infested wool insulation with a traditional mineral one. The cost of such work was however unrealistic. We proposed treating the insulation by injecting a combination of the synthetic pyrethroids transfluthrin with a high vapour pressure as a fumigant, and permethrin for residual effect. A pilot pressurised aerosol product was prepared, containing 1% of transfluthrin and 1% of permethrin, and a non- flammable propelling gas. The can was equipped with a special nozzle allowing treatment of the internal voids filled by the insulation (1m width x 3m high x 5 cm depth). The product was applied into the internal voids through holes drilled into the plasterboard. Altogether 8 aerosol containers (each 750 ml) were used. The total volume of treated wool insulation was approx. 10 m^3. Up to 3 months after the treatment no flying moths were observed inside the house. Then 1 -2 moths began to appear again daily. The probable reason for the re-infestation was the fact that the internal voids behind the sauna were not treated, as we wrongly anticipated that moth larvae would not be able to survive high temperatures. Repeated application is planned and results will be presented on the conference.

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Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

TRANSDERMAL CHOLECALCIFEROL RODENTICIDE

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Abstract A genetic resistance to anticoagulants, and bait shyness, have made baited approaches to rodent management less effective. Baiting approaches rely on the rodent to consume the anticoagulant; however, there is no guarantee that effective amounts of the rodenticide will be ingested. The consequence of this sub-lethal dosing has been the natural selection of resistant strains. There is thus a need for an alternative to anticoagulant rodenticides which differs in active ingredient and manner of delivery. Through the development of an in-vitro in-vivo model we present an optimized, cholecalciferol based, transdermal rodenticide. The liquid rodenticide is designed for application to the back of the rodent, via an aerosol, allowing the delivery of cholecalciferol through the rat's skin. A laboratory efficacy evaluation was performed with the new rodenticide, conducted inline with European and Mediterranean Plant Protection Organization (EPPO) guidelines (EPPO 1998), in which 100% efficacy was achieved. This is greater than the 90% efficacy (European Commission 2009) deemed 'sufficiently effective' by EU Regulation No 528/2012 (European Commission 2012). The results signify the first steps in the approval of an alternative to anticoagulant baiting which both negates resistance and bait shyness by delivering acute doses of cholecalciferol through the dermis.

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Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

EKOMILLE IN RODENT MANAGEMENT PROGRAMS: TWO CASES STUDIED IN CENTRAL ITALY

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Abstract Ekomille trap is an electromechanical and ecological system for the capture of mice and rats. The trap is baited with natural foods without any type of toxic bait and it is able to capture over 80 rodents. In the present work are reported results of field trials carried out to evaluate the Ekomille use in rodent management. We selected two houses, one in Benevento and one in Pescara both representatives of periurban environment of Central Italy. Initially in both cases, rodent control was done with chemical rodenticides. From the end of September 2011, 5 Ekomille traps baited with natural foods were positioned in critical points outside in Benevento. During the first 15 days the devices were maintained inactive, in order to reduce rodents neophobia, after that, we switched the Ekomille on and then checked it monthly. As results from the beginning of October 2011 until the end of December 2013, we caught 266 Norway rats (10 in 2011, 145 in 2012 and 111 in 2013). From early January 2009 6 baited Ekomille were allocated in Pescara house, along the outer perimeter of the property. Traps were checked every two months. In this case from the beginning of January 2009 to the end of December 2013 were captured 491 Norway rats (99 in 2009, 96 in 2010, 99 in 2011, 105 in 2012 and 92 in 2013). After Ekomille trap disposal, a drastic reduction of damage by rodents activity was observed. These positive results indicate that the use of Ekomille trap for the control of rodents is a valid component of an integrated pest management (IPM)-based control strategy. In IPM programs, the use of these traps can lead to drastic reductions of chemical treatments, resulting in economic benefits and improvements for environment safety and quality.

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Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

FIELD EVALUATION OF PALATABILITY OF EXTRUDED AND WAX BLOCK RODENTICIDES AGAINST *RATTUS NORVEGICUS*

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Abstract Control of *Rattus norvegicus* in Brazil is frequently made by applying wax blocks and, more recently, with extruded blocks containing a second generation anticoagulant a.i., where wax blocks have shown low attractiveness. We conducted two field studies to evaluate the attractiveness of a wax block (Klerat® Wax Block 20 g) and an extruded block (TalonXT® 20 g), both with the active ingredient Brodicafoum at 0.005% concentration. They were compared with three other rodenticides commercially available in Brazil. The four blocks of each type were placed adjacent to each other in bait stations inside a basement near grain storage. During the period of February to April 2013, the wax blocks were replaced 8 times. Nine evaluations showed that Klerat consumption was higher and represented 46.50% of all rodenticide consumption, while consumption of first alternative bait (Brodifacoum 0.005%) represented just 24.38%; the second alternative bait (Brodifacoum 0.005%) represented 18.77% and the third alternative bait (Difethialone 0.0025%) represented just 10.35%. During the period of August to October 2013, the extruded blocks were replaced 9 times. Ten evaluations showed that TalonXT consumption was higher and represented 80.20% of all rodenticide consumption, while consumption of first alternative bait (Brodifacoum 0.005%) represented just 11.25%. Consumption of the second alternative bait (Difethialone 0.0025%) represented just 8.17% and the consumption of the third alternative bait (Bromadiolone 0.005%) represented just 0.38%. Klerat® and Talon® showed better palatability and this information is valuable when designing an effective rodent control program.

Proceedings of the Eighth International Conference on Urban Pests
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Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

GEOGRAPHIC INFORMATION SYSTEM IN PEST CONTROL PROGRAMS, AN EXAMPLE WITH FERAL PIGEON (*COLUMBA LIVIA*) CONTROL PROGRAM IN MADRID CITY

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Abstract Health Madrid as the municipal health administration of the City Council of Madrid (Environmental Health) manages pest prevention and control operations. Feral pigeons have increased during the last decades as one of the most frequent pest problems in Madrid City. The pest control sector, as almost all sectors, has always used maps. The inclusion of GIS technology has incorporated a new perspective, allowing spatial data to perform a much more important role due to the speed of complex geographical analysis or processes. This has represented a great opportunity for Health Madrid's Vector Control Unit to accomplish GIS-based programs (methodologies) for the prediction, prevention and control of cockroaches, rats, pigeons and other pests in Madrid City. After five years since the Unit systematically implemented Geographic Information Systems (GIS) in its feral pigeon control program, we present the know-how and results obtained in this period. GIS are useful in different phases of pest control, beginning with a simple map of residents' sightings, following with population census map or more complex correlated variables maps. As well as analysing control methods, the system allows the vector risks or Program results to be evaluated. After visualizing and understanding the implications of GIS-based programs we are convinced, and believe that other professionals will be too, that GIS is an essential tool for local pest management and particularly for managing urban bird populations.

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COMPARATIVE EVALUATION OF THE EFFICIENCY OF AN INSECTICIDE SOLUTION IN DIFFERENT EQUIPMENT USED FOR CONTROL OF INSECTS AND OTHER ARTHROPODS

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Abstract Of the techniques used by pest control operators to control synanthropic cryptic insects and other arthropods such as German cockroaches, ants, bedbugs, and scorpions, the application of insecticides directly into cracks is one of the most commonly used methods. Three pesticide application equipments: manual compression sprayer, pressurised aerosol spray and electric micro-sprayer with two-fluid nozzle atomizer. The test models were standardized, made up of three pieces of pine plywood, to contain a hollow space with an inlet hole at one end and an output hole at another end. The equipments were filled with a colored solution and the pressurised aerosol spray was not colored. Before application the flow rate and pressure of application of each equipment were measured. After application the time was measured which the solution needed to pass through the outlet hole. The test models were then opened and the dispersion of the solution was measured in each model. The results were compiled and analyzed by the Tukey test ($p<0.01$) and indicated that under the conditions of this experiment the electric micro-sprayer was the most efficient, secure and economical (99% cheaper) choice for this type of treatment.

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Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

CLEAN ECOLOGICAL METHODS FOR CONTROL OF URBAN PESTS

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Abstract Increasing pest diversity in urban areas is a reality of modern time. Actual biological technologies are unable to prevent this process. Sometimes control leads to negative results, in particular the use of chemical pesticides. Use of clean ecological methods must be the strategy of the future. The basis of such a technology must be imitation of wild nature processes. There are 4 groups of such a technique. They are: ecological, genetic, chemical and ecological, physical and ecological. Ecological ones are based on natural intra species relations. They are: competition, host-parasites and predator-prey. The use of competition is the only way to completely replace a pest species by a less dangerous one. Genetic methods are based on selection and gene engineering. Chemical and ecological methods are based on the use of natural compounds with biological activity, or their analogues. Such compounds are: hormones, anti hormones, biological toxins, attractants and repellents. Ecological-and-physical methods are based on the use of acoustic (sound, ultrasound, infrasound) or electromagnetic energy. The composition and rotation of all these methods may become the basis for effective control of urban pest populations.

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PHYSICAL METHOD OF COMBATING INSECTS - NEW APPROACH TO INHIBITION OF RESISTANCE TO INSECTICIDES

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Abstract A unique mixture of polymeric compounds for effective control of various insect species was tested. When applied in a free form or mixed with insecticide formulation has provided quick and even spreading increasing the likelihood that the toxicant will be transferred by contact enhancing the bioavailability of the active ingredient to the insect. It also enhances the penetration through the cuticular waxes of the exoskeleton of insect modifying the exposure profile. When applied in free form or in mixture with insecticide works through 1)suffocation -when applied it provides complete penetration of the surface of insects' body and fills its spiracles 2) Immobilization-formulation is tightly covering all developmental stages of the pest insects immobilizing them. Thus it increases the dehydratative potential of the product resulting in uncontrollable water loss , whilst increases the exposure to insecticide 3) dehydration - effectively removes hydrocarbons from insect's exoskeleton cuticular wax that normally provides the insect with protection against water loss and is therefore critical to its survival. When the protective wax is disrupted, water loss becomes uncontrollable and irreversible, leading to dehydration and death. It enhances the penetration of detoxification inhibitors inactivating the possible recovery process prior to exposure to insecticide. Hence, it would be a perfect tool to deal with expanding problem of resistance to insecticides.

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BIOLOGICAL DISPERSAL STRATEGIES OF THE NEW INVASIVE *MEGACOPTA CRIBRARIA* AT A REGION LEVEL

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Abstract Parameters defining the dispersal strategies of phytophagous insects at a local scale are key components of the fitness of these insects and may thus be essential in the adaptation to new-geographic environments that are structured in space and time. The Kudzu bug, *Megacopta cribraria*, is a new invasive insect recently detected in USA. Its rapid spread and exponential population growth have elevated it to be a serious urban and agricultural pest. We examined its life history, behavioural and physiological traits, as well as the abundance and distribution of feeding resource and evaluated the impact of the region-scale biological and ecological dispersal strategies on its invasiveness. Here we report that traits defining its dispersal strategies may be a cornerstone of host-plant specialization due to the fact that the effect of dispersal parameters on its fitness depends strongly on plant characteristics. The abundance and widespread of reproductive and feeding host plants, structured in time and space, provide kudzu bug with a high-level resource. The life history, behavioural, physiological and reproductive traits have evolved in response to the new environment, allowing the rapid spread of the Kudzu bug.