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DETERMINING THE SOURCE OF HOUSE FLIES (*MUSCA DOMESTICA*) USING STABLE ISOTOPE ANALYSIS

KATHARINA HEINRICH, HOWARD BELL, AND ROBERT WEAVER
Fera Science Ltd (Fera), National Agri-Food Innovation Campus, Sand Hutton, York, YO41 1LZ, UK

Abstract Intensive livestock units frequently produce flies in large numbers that, on migration, cause nuisance to the occupants of neighbouring dwellings.The resolution of such problems is often reliant on the unequivocal identification of the origin of the flies, particularly when several potential sources exist. This study evaluated stable isotope analysis as a method for differentiating adult house flies (*Musca domestica* (L.)) on the basis of their dietary history so as to determine their likely source. Flies were reared in the laboratory on several substrates, including chicken and cattle manure, laboratory diet and household vegetable waste. Different fly parts (wings, heads, and legs) and whole flies were analysed immediately after eclosion and after 10 days. The $\delta^{13}C$ and $\delta^{15}N$ values for adults that had developed on each diet type were highly distinct. Stable isotope analysis, therefore, shows potential to be employed to determine the likely source of various nuisance insects, and to contribute to the abatement of such problems.

Key words Nuisance insects, stable isotopes, EA-IRMS, intensive livestock, dietary history

INTRODUCTION

House flies (M*usca. domestica*), and other muscids, are a perennial problem associated with intensive livestock rearing facilities throughout the world. Flies also emerge from a range of other sources, notably domestic waste, carcasses and landfill sites. When conditions are optimal, large livestock units are a significant source of flies, which can often migrate to nearby premises. These flies can constitute a nuisance to affected householders and also pose a significant health risk due to the carriage of pathogenic organisms such as *Campylobacter*, *Escherichia coli*, *Salmonella*, and others. Persuading the management of suspected source facilities to abate the problem can often prove difficult for enforcement bodies as it is challenging to unequivocally prove that the suspected source is actually the premises generating the insects causing the nuisance problem, especially when several potential sources are found in close proximity.

The analysis of tissues using Elemental Analyser - Isotope Ratio Mass Spectrometer (EA-IRMS) techniques has proven to be extremely effective as an ecological tool for the identification of animal migration patterns and feeding habits (Hood-Nowotny and Knols, 2007). Stable isotope analysis has also demonstrated to be an effective technique elucidating the dietary histories of several insect species (Patt et al., 2003).

The current project was designed to provide baseline data to examine the potential for the exploitation of IRMS as a method for determining the origin of nuisance insects such as house flies. This initial work is based entirely on laboratory-reared flies, but builds a platform for a full assessment of this method using flies derived from the field. The potential applications for this technique in the context of resolving nuisance insect issues in the UK and elsewhere, are discussed.

MATERIALS AND METHODS

Adult flies were derived from a laboratory strain (Cooper) held at 25°C, 16:8 hours light:dark photoperiod and 70% relative humidity. Larvae were reared on a medium of bran, grassmeal, yeast, malt and milk powder (40:20:10:3:3) made up to a paste with water. On emergence, adults were held in 30 x 30 x 30 cm screen cages and provided with powdered milk, sugar and water ad libitum. Eggs were collected though placement of milk-saturated cotton wool swabs into adult cages which were subsequently collected ca. 12-14 hours after the start of oviposition. New larval cultures were initiated through seeding approximately 1000 eggs into 250 g of diet, using the conditions described for adult flies.

A series of diets were used to raise flies of potentially different stable isotope ratios. These were (i) standard laboratory diet (as described above), (ii) standard laboratory diet to which corn oil had been added (25% w/w), (iii) chicken manure (from an intensive egg production unit), (iv) cattle manure (from cattle kept on deep litter straw and fed maize silage) and (v) meat-free household waste consisting of liquidised potato peelings, carrot peelings and onion skins (4:1:1 by weight).

Approximately 50% of the insects were culled within 24 hours of emergence (D0) and immediately frozen at -20°C whilst the remaining adults were held in screen cages and provided with sugar water only (10% w/v cane sugar) for a further 10 days (D10).

Prior to stable isotope ratio determination, insects were dissected to provide samples of wings, heads and legs. The various body parts and whole insect samples were dried overnight in a Savant Speed-Vac. Cohorts of whole insects (n = 10-15) were milled to a fine powder using a ball mill (Retsch MM400), whilst the other body parts were analysed without further preparation. Each sample was weighed into tin-foil capsules with a minimum of three replicates prepared for each diet and fly type. In the case of wings and legs, appendages from ca. 10-20 flies were required to provide sufficient tissue whilst 2-3 heads were used for each determination.

Isotope ratio mass spectrometry was used to determine the $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratio of fly body parts, by calibration against reference materials of known isotope ratios. The IRMS system comprised of a GV Instruments IRMS (Isoprime Ltd, Manchester, UK), fitted with an elemental analyser combustion interface (Fisons1108) and operated with MassLynx Inorganic 4.0i software. Stable isotope ratios are expressed in per mil [‰] units, according to the following formula:

$$\delta X‰ = \frac{\left(\frac{X}{Y}\right)_{sample} - \left(\frac{X}{Y}\right)_{standard}}{\left(\frac{X}{Y}\right)_{standard}} \times 1000$$

where X and Y represent the heavier and the lighter isotope, respectively. The international standards V-PDB (Vienna-Pee Dee Belemnite) for $\delta^{13}C$ and AIR for $\delta^{15}N$ are referred to; which are defined as 0 ‰ on the respective δ-scale. The uncertainty of measurements using IRMS is typically ± 0.2 ‰.

The trophic shift of $\delta^{y}X$ diet to insect body is calculated by using the following formulae:

$\delta^{y}X$ trophic shift: Δinsect−diet = $\delta^{y}X$ insect − $\delta^{y}X$ diet

where y is an isotope and X is an element.

Data was analysed using one way analysis of variance (ANOVA) to examine the $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios of the diets and the flies produced by the different feeding regimes for a given time point (D0 or D10). Means were subsequently separated by Tukey-Kramer post hoc tests. Analysis was conducted using GraphPad Prism 5.0.

RESULTS AND DISCUSSION

Significant differences in the $\delta^{13}C$ and $\delta^{15}N$ values for the different diets, and the D0 flies (whole, milled) that had developed on them were obtained (Table 1).

The δ-values for whole milled adult *M. domestica* immediately after emergence (D0) indicated that ^{13}C and ^{15}N were, with one exception, more abundant in the adult flies than in the diets that they had developed upon. Enrichment with the heavier isotope is commonly observed between trophic levels and an increase of 0.4 ‰ (+/- 1.3 ‰ SD) in $\delta^{13}C$ and 3.4 ‰ (+/- 1 ‰ SD) in $\delta^{15}N$ is estimated (Post, 2002), values that were reflected in the "producer" (manure) - consumer (fly larvae) relationship, observed in the present study.

Table 1. $\delta^{13}C$ and $\delta^{15}N$ values [‰] for diets and *Musca domestica* reared on these respective diets, and the enrichment of δ^{13} C and δ^{15} N from diet to insect. The determinations were made for each diet type from a pooled sample of 10-15 whole flies. The uncertainty of measurements is typically ±0.2‰. For each isotope, values followed by different letters within each column are significantly different (one-way ANOVA, P<0.05).

Isotope (relative to standards)	Diet	Diet isotope values [‰]	*M. domestica* (D0) isotope values [‰]	Trophic shift $\Delta_{insect\text{-}diet}$ [‰]
$\delta^{13}C$ (vs V-PDB)	SLD*	-26.9 ± 0.1a	-23.0 ± 0.1a	3.7
	Chicken manure	-26.5 ± 0.1a	-27.3 ± 0.1b	-0.8
	Corn-oil / SLD	-21.2 ± 0.2b	-19.9 ± 0.1c	1.7
	Cattle Manure	-22.5 ± 0.1c	-21.4 ±0.1d	1.5
	House-hold waste	-27.3 ± 0.1a	-25.7 ± 0.1e	1.6
	Cane sugar	-11.7 ± 0.1		
$\delta^{15}N$ (vs AIR)	SLD*	3.8 ± 0.1a	9.1 ± 0.1a	5.3
	Chicken manure	18.4 ± 0.2b	21.8 ± 0.1b	3.3
	Corn-oil / SLD	3.6 ± 0.2ac	6.6 ± 0.1c	3.0
	Cattle Manure	8.2 ± 0.2d	10.9 ± 0.1d	2.7
	House-hold waste	3.5 ± 0.2c	8.7 ± 0.2e	5.1

*SLD = Standard Laboratory Diet

A positive isotopic shift of 2.9 to 5.1‰ for $\delta^{13}C$, indicative of increasing ^{13}C abundance, was recorded in all flies that had been kept on a cane sugar diet for 10 days post emergence, regardless of larval diet upon which they had been reared. The diet switch affected the $\delta^{15}N$ values of whole flies differently compared to fly body parts and no general trend was observed. Scatterplots correlating the $\delta^{15}N$ and $\delta^{13}C$ values for whole flies, wings, legs and heads illustrate how the values change as flies age (Figures 1A-D). By correlating $\delta^{15}N$ and $\delta^{13}C$ values, for whole milled flies (n = 10 to 15), full differentiation of their origin was achieved (Figure 1A).

Whilst, in many cases, the source of insects may be very obvious and readily identifiable through visual inspections, in some situations several alternative locations could be serving as breeding sites for flies. Given that different livestock facilities vary widely in their standards of hygiene, animal husbandry and production methods, knowledge of where flies are actually coming from provides useful information for any enforcement agency tasked with resolving such issues.

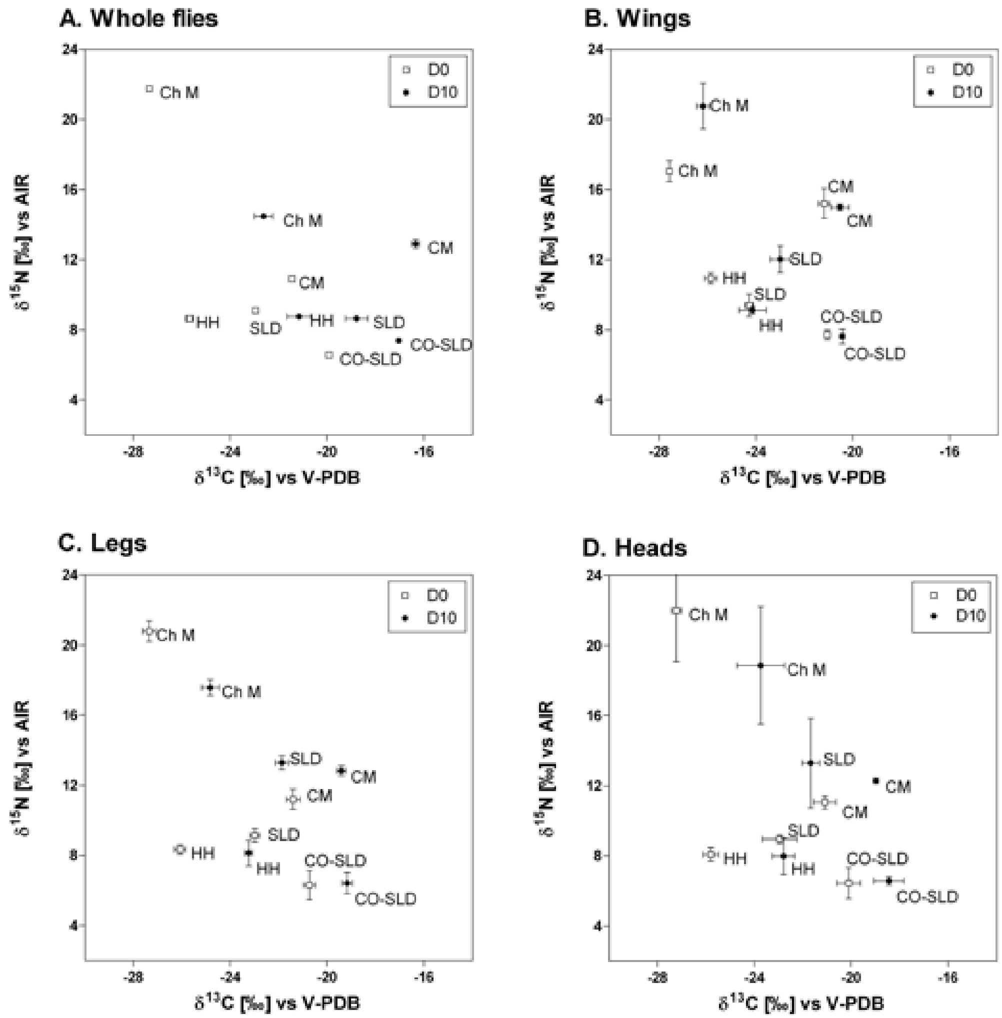

Figure 1. The correlation between the $\delta^{13}C$ values and $\delta^{15}N$ values for (A) whole flies, (B) wings, (C) legs and (D) heads. Error bars indicate two standard deviations of the mean values for a minimum of three determinations. Key to diets: SLD = standard laboratory diet, CO-SLD = Corn oil / standard laboratory diet, Ch M = chicken manure, CM = cattle manure, HH = household waste.

The observed changes in isotopic signatures as flies age would indicate that $\delta^{13}C$ and $\delta^{15}N$ signatures will be variable within a given *M. domestica* population, even when they have identical larval dietary histories, due to the variable ages of the insects present. However, the degree that flies will vary needs to be substantiated in scenarios that reflect the situations where *M. domestica* naturally develops. In these situations the adult fly will feed on a range of substrates, including the one that it had developed upon as a larva. The differences in the stable isotope ratios of insects that derive from certain substrates, such as chicken manure, were so different that, even after a prolonged period of feeding on a completely different adult diet, with no access to the larval substrate, the isotopic ratios still remained distinct.

Whilst the data presented here clearly indicates that the source of nuisance flies could be determined through correlating stable isotope ratios with potential developmental substrates, further experiments are required to cater for the broad range of diets that are exploited by house flies. It will be also necessary to monitor the isotope ratios of these substrates over time to identify possible fractionation, caused by decomposition or fermentation. In particular, a factor that must be considered is that animal manures tend to become isotopically heavier as, for example, volatilisation of ^{14}N ammonia is kinetically favoured over that of ^{15}N ammonia (Bateman and Kelly, 2007).

CONCLUSIONS

The results of this study are promising and demonstrate that flies can be readily differentiated on the basis of their larval diet. As such, stable isotope analysis could be employed to resolve the more intractable cases of insect nuisance, where the source of insects and, therefore, the responsible premises, are subject to a degree of doubt. Whilst *M. domestica* was used here as a model, the strength of this technique would allow to ascertain the dietary history of a range of other nuisance and peridomestic pests, such as mosquitoes, other dipteran species and cockroaches, and consequently determine where they have issued from.

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COMPARISON OF NUMBER OF *LIMNOPHYES NATALENSIS* (DIPTERA: CHIRONOMIDAE) COLLECTED WITH STICKY AND SUCTION LIGHT TRAPS IN FOOD FACTORY

[1]GORO KIMURA, [2]KIMIO HIRABAYASHI, AND [1]TSUTOMU TANIKAWA
[1]Technical Research Laboratory, Ikari Shodoku Co., Ltd., 579 Chibadera, Chuo-ku, Chiba, 260-0844 Japan
[2]Institute of Textile and Science, School of Science and Technology, Academic Assembly, Shinshu University, 3-15-1 Tokida, Ueda, Nagano 386-8567 Japan

Abstract Adult *Limnophyes natalensis* were collected using sticky and suction light traps during the course of a year in a Japanese food factory. Such physical control methods have been demonstrated effective for collecting adult midges. The numbers of adult *L. natalensis* collected with the two methods were compared by season. A total of 121 were collected over eight nights with the sticky light trap, and 194 were collected with the suction light trap, a significantly higher number. The numbers were not significantly different for winter. These results demonstrate that suction light traps are more effective for collecting adult midges in food factories.

Key words Chironomid midges, nuisance, physical control, sticky light trap, suction light trap

INTRODUCTION

Although Chironomidae are not listed as a household pest as are Diptera (Ito, 1982), adult chironomids can contaminate materials and final products in pharmaceutical and food processing factories (Tabaru et al., 1987). While they typically originate outdoors (e.g., Matsuzaki and Buei, 1993; Hattori and Moriya, 1996).

Colonization and massive numbers of *Limnophyes natalensis* (Kieffer) in Japanese food factories have been reported (Kimura et al., 2010, 2014). Genus *Limnophyes* is terrestrial and semi-terrestrial (Pinder, 1995), and *L. natalensis* have been reported to breed in rivers and streams (Sæther, 1990). An investigation at a certain food factory discovered *L. natalensis* larva in the organic residue from the drains and machines (Kimura, unpublished). Kimura et al. (2014) used a special light trap to investigate the abundance and flight activity of adult *L. natalensis* in a food factory and found that the thermal conditions affected both on a daily basis (Kimura et al., 2014).

While chemical insecticides are frequently used to control midge population, biological and physical control methods have also been used (Failla et al., 2015). Methods for controlling *L. natalensis* have not yet been established, because chemical control has been avoided in Japanese pharmaceutical and food factories, and biological control is not realistic in such environments. A more promising approach to controlling the adult *L. natalensis* population is to use light traps because this species exhibits phototactic behavior (Kimura et al., 2014).

In the study reported here, we compared the effectiveness of using sticky and suction light traps in a food factory as an ecological approach to the effective physical control of *L. natalensis*.

MATERIALS AND METHODS

Study Site

The food factory is in Chiba City, Chiba Prefecture, Japan and usually operates from 08:00 to 18:00. The lights are normally off from 18:00 to 08:00. Data on the seasonal abundance and flight behavior of *L. natalensis* in this factory were given by Kimura et al. (2014).

Collection of *L. natalensis*

The sticky light trap (Optclean VI, Ikari Shodoku Co., Ltd.) was equipped with a 20-W black fluorescent lamp (Figure 1), the suction light trap (Clean Eco Line GXmini, Ikari Shodoku Co., Ltd.) equipped with two 10-W black fluorescent lamps (Figure 2). Both were installed 1.0 m above the floor, with a distance of 5.0 m between them. They were operated from 17:00 to 8:00 over the course of a year (2013), and their locations were switched at the start of each sampling period: spring (March 19–20 and 21–22; vernal equinox on March 20), summer (June 20–21 and 21–22; summer solstice on June 21), autumn (September 21–22 and 22–23; autumnal equinox on September 23), and winter (December 21–22 and 22–23; winter solstice on December 22). The sticky sheet and insect net were replaced after each sampling period.

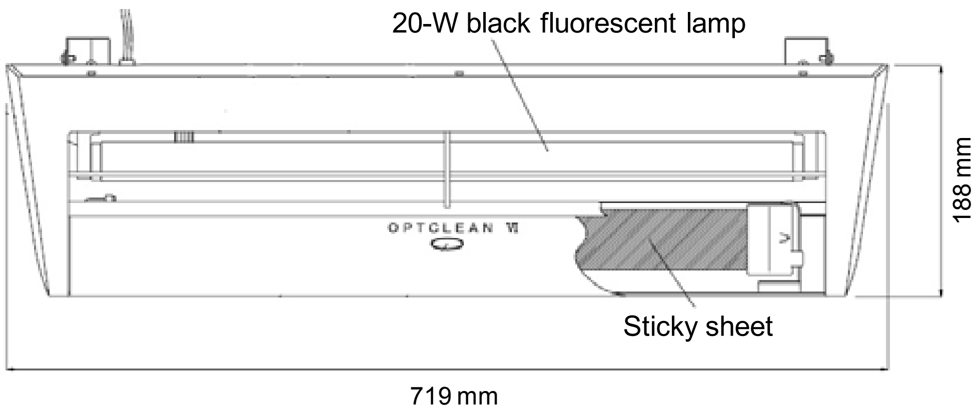

Figure 1. Structure of sticky light trap.

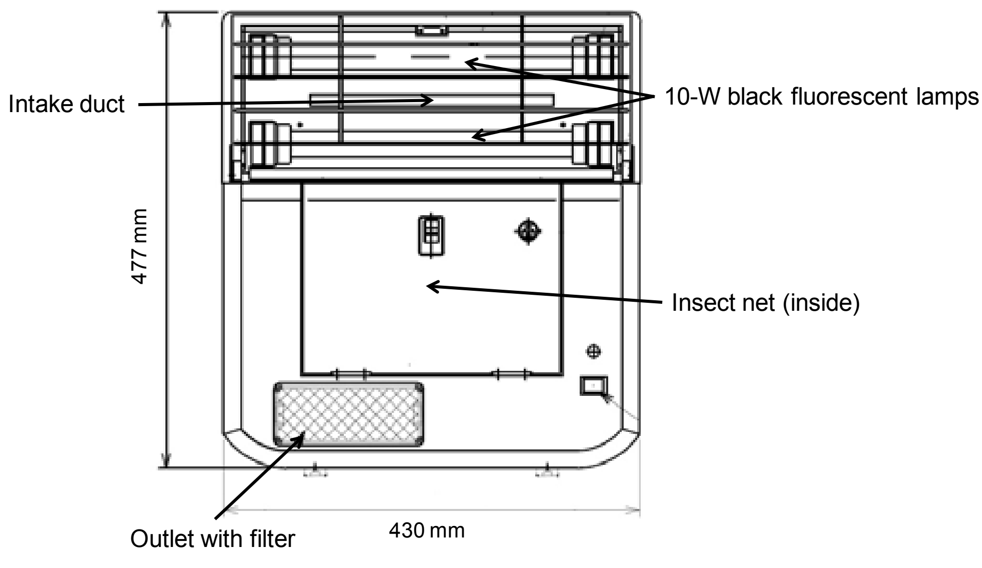

Figure 2. Structure of suction light trap.

Room air temperature was continuously measured with a temperature and humidity sensor (TH-001, Ikari Shodoku Co., Ltd.) each sampling period. The mean room air temperature ranged from 13.1±6.8°C (December 21–23) to 19.5°±0.7C (June 20–22), averaging 17.2 ± 4.6°C. The mean air temperature during the early morning hours (4:00–8:00) did not fall below 7.4°C at any time (Figure 3). The *L. natalensis* were counted using a binocular dissecting microscope.

Data analysis

All statistical tests were performed using SPSS version 11.5.1.J for Windows (SPSS Japan Inc.).

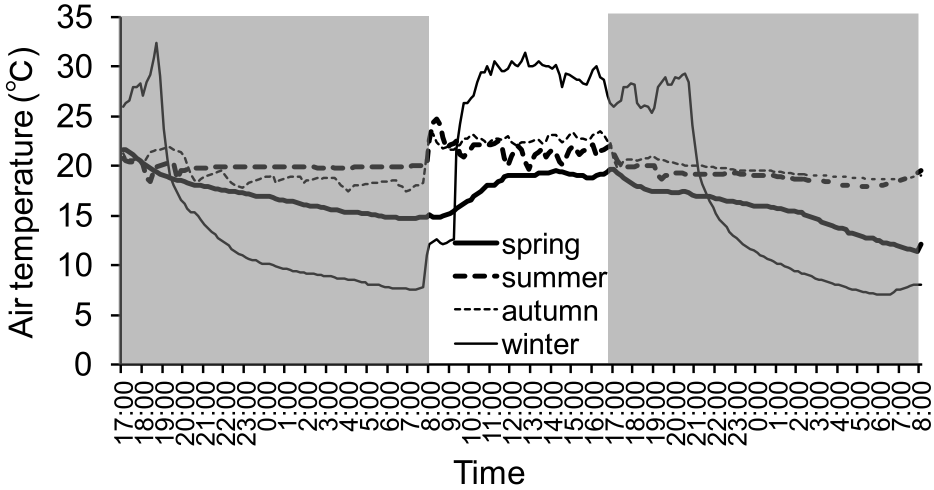

Figure 3. Air temperature in food factory by season. Shaded areas indicate times light traps were in operation.

RESULTS AND DISCUSSION

A total of 121 (male:female=78:43) adult *L. natalensis* were collected during the sampling periods with the sticky light trap, 194 (123:71) were collected with the suction light trap (Table 1). The sex ratio was biased towards male for each trap. The number collected with by suction light trap was significantly higher than the number collected with the sticky light trap (Binomial test, $p < 0.01$). The number was the highest for summer (sticky light trap: 68/collected over two nights; suction light trap: 95/collected over two nights), followed by autumn (34 and 54), spring (13 and 32), and winter (6 and 13). The number collected with the suction light trap was significantly higher for each season (Binomial test, spring: $p < 0.01$; summer and autumn: $p < 0.05$), except for winter (Table 1).

As mentioned above, the thermal conditions affect the abundance and flight activity of adult *L. natalensis* on a daily basis. Adult *L. natalensis* were collected in the morning, when the mean air temperature was higher than 7.4°C (Kimura et al., 2014). Therefore, the daily flight activity of *L. natalensis* showed two peaks (morning and evening) from spring to autumn, with only an evening peak in winter (Kimura et al., 2014), which agrees with the low number collect in winter.

These results demonstrate that suction light traps are more effective than sticky light traps for collecting adult midges in food factories. However, sticky light traps have been traditionally used in Japanese food factories because the sticky sheets prevent dispersal of insect debris and reduce insect contamination. The suction light trap used in this study has become widely used in Japanese food factories as a means of physical control because it is equipped with a filter to control the dispersal of insect debris (Figure 2).

Table 1. Number of adult *L. natalensis* collected using suction and sticky light traps.

Sampling period	Suction light trap			Sticky light trap		
(17:00–8:00)	M	F	Total	M	F	Total
March 19–20	8	1	32^{A}	8	1	13^{a}
March 20–21	20	3		3	1	
June 20–21	29	32	95^{B}	23	12	68^{b}
June 21–22	17	17		16	17	
September 21–22	8	1	54^{B}	16	7	34^{b}
September 22–23	29	16		8	3	
December 21–22	8	0	13	4	2	6
December 22–23	4	1		0	0	
Total	123	71	194^{A}	78	43	121^{a}

Aa, Bb: Values with different superscript in same row are significantly different in binomial test (Aa: $p < 0.01$; Bb: $p < 0.05$)

It has been shown that the attraction of chironomid species to a light source is directly proportional to light intensity (Ali et al., 1984, 1986; Hirabayashi et al., 1993). The suction light trap (Clean Eco Line GX, Ikari Shodoku Co., Ltd.) we used has a powerful light souse two 20-W black fluorescent lamps. This trap has been widely used in Japanese food factories as a means of physical control. The chironomid species responded more to the quantity of light than to the quality of light (Ali et al., 1984, 1986; Hirabayashi et al., 1993). There is little information about their attraction to the visible light of a LED. Kimura et al. (2014) demonstrated that chironomid midges are equality attracted to a white fluorescent lamp (emitting UV light) and a white LED lamp (not emitting UV light). Hirabayashi et al. (2016) reported that chironomid midges are attracted to several colors of LEDs, with a green LED being the most attractive, followed by a white fluorescent lamp, white LED, UV LED, red LED, and blue LED. Further research on the manipulation of wavelength should be undertaken to obtain a better understanding of how chironomid midges can be effectively controlled in food factories.

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ROLE OF LED LIGHTS IN THE DESIGN OF ULTRA-VIOLET LIGHT TRAPS FOR HOUSE FLY MONITORING AND CONTROL

MATTHEW GREEN
Rentokil-Initial PLC Foundry Court, Foundry Lane, Horsham, West Sussex. RH13 5PY

Abstract The light emitting diode (LED) lamp market is predicted to experience massive growth over the next decade. Outside the domestic lighting sector there are a number of specialist uses for LED technology that have warranted research into specific frequencies of light emission. Ultra-violet (UV-A waveband) LEDs are becoming powerful enough to offer a credible alternative light source to the fluorescent lamps currently used in ultra-violet light traps for the monitoring or control of flying insects in urban environments. Whilst there are significant energy savings to be made in employing this technology, other differences between LEDs and fluorescent lamps can be exploited to maximise their characteristics of light emission to catch flying insects. The hypothesis that an insect light trap using a UV-A LED luminaire can be as efficient at removing a known number flies from an environment as a light trap using fluorescent lamps is tested using common chasses with spectroscopy and replicated bioassays.

Key words House fly, *Musca domestica*, spectral sensitivity, visual response

INTRODUCTION

In 2015, the global price of LED bulbs was estimated to have fallen by 30-40% and by 2016 the global LED lighting market exceeded $30.5 billion US dollars (Anon, 2016). With the rise in domestic use of LED lighting manufacturers have sought to expand market share by leveraging technology into related niche markets including ultra violet (UV) and infrared (IR) applications. The largest market for UV LEDs is in ink curing, a rapid polymerisation process that fixes inks to surfaces that requires neither water nor solvents. The ink curing market is curiously fortuitous for the future of insect light traps in that the frequencies of UV light commonly used to fix ink (365nm wavelength) is close to a peak in spectral sensitivity in the visual apparatus of the house fly, *Musca domestica* (Goldsmith and Hernandez, 1968)

UV-A mercury phosphor lamps have been used in commercial insect light traps for over 40 years (Roberts, 1990) and such units are commonplace in commercial and industrial setting, particularly those handling foodstuffs. Mercury phosphor lamps have a non-negligible environmental impact, even in relatively niches uses as insect light traps and are subject to increasing regulation such as the European Union's recent Restriction on Hazardous Substances II (RoHS II) regulation, the implementation of which saw a shift from lamps emitting peak UV-A wavelength at 350 nm to those emitting at a peak of 365 nm due to the quantities of lead used to attenuate the output. There are important waste considerations for Pest Management Companies too; each lamp has a functional life of approximately one year, after which it degrades to a point where the peak wavelength has shifted and the intensity declined to a level that no longer draws flies to the trap having been saturated by ambient light.

House flies are an important urban disease vector (Davies, 2016) that are controlled or monitored with insect light traps by pest management companies that will respond to changes in change number or species by proofing to restrict the insects' access to the area in question or remove them from the environment where ingress is inevitable. Quantifiable measurements from these traps that purport to effect catch rate are often quoted as de facto figures for their efficacy, including total lamp power, UV-A output and lethal surface area. Whilst there is certainly some evidence to support the maximisation of these measurements (Pickens and Thimijan, 1986) previous studies and suggest that various design factors that may negate any advantages gained through such efforts (Hanley et al., 2009; Green, 2011; Jones et al., 2017).

Light emission spectrographic variation between the LED and fluorescent luminaires from previous (unpublished) studies shows that under known ambient light levels (400LUX) the shape of UV-A light cone produced by an LED luminaire differs from that of that of three 15 W UV-A fluorescent lamps mounted in an identical chassis. The 'forward facing' directional nature of LEDs throws light further away from the trap.

The aim of this work was to derive a relative efficacy rank from a consistent series of tests that enables direct comparison between LED and fluorescent luminaires operating on the same trap chasses. Removing flies from the environment as fast as possible is seen as the most important factor for the end user (Sargent, 2010) to reduce the risk to human health. This is the role of light traps when used to monitor areas where risk is low and hazard is high (usually traps with adhesive surfaces to aid identification for origin and cause/point of ingress); and when they are used as a control device in areas where risk is high and hazard is low (areas with direct access to the external environment where high voltage grids are employed in light traps). Catch rate in this study was quantified by estimating the best possible time to catch 50% of flies within a room.

A significant amount of prior investigation into catch rates for *Mucsa domestica* has centred on UV light wavelength attraction (Smallegange, 2003; Roberts et al., 1992; Syms, 1988). Black light bulbs sold for the fly killer market radiate light in the 330-385 nm ultra-violet range, within which there does not seem to be a consensus for the wavelength that is most attractive *M. domestica* in practice, despite electrophysiological studies (Smallegange, 2003).

MATERIALS AND METHODS

Two light trap chasses (Figures 1-4) were tested with two luminaires in identical controlled environment rooms (4 m^2 with a volume of 9 m^3), maintained at 25° C $\pm$ 2° C and 50% $\pm$ 10% RH, illuminated daily on a 12- hour cycle. The rooms were subject to 10 air changes per hour and sealed to prevent flies escaping.

Figure 1. Chassis A with fluorescent lamp (left) and LED (right) luminaires.

Figure 2. Chassis B with fluorescent lamp (left) and LED (right) luminaires. Role Of LED Lights In The Design Of Ultra-Violet Light Traps

UV lamps used in the traps were on for a minimum of 100 hours prior to testing. LED luminaires were on for 2 hours to ensure the light output was stable (assessed with a handheld UV spectrometer). Traps tested were mounted 1.8 m from the floor.

Bioassay protocol followed Green (2001): 100 unsexed adult *Musca domestica* were released at floor level from the centre of the room. The number of flies captured in the unit was counted at intervals of: 15, 30, 60, 90, 120, 240 minutes, 5 hours, 7 hours and 24 hours. After 24 hours all live flies in the room, dead flies on the floor or within the unit (not on the glue) and observed escapees were accounted for. Six replicates of each test were conducted with fresh glue surfaces for each test and luminaires emitting the same level of UV-A light.

RESULTS

An average time for half the number of available flies to be caught (C_{50}) was calculated by averaging the counts from six days of testing. The C_{50} score is the minimum possible time that it would take each unit to catch 50% of the available flies (given maximal performance of the unit based on the average recorded catch for each of the eight time intervals), using the following equation:

$$C_{50} = \frac{t}{\log_2 (N_0/N_t)}$$

where C_{50} is the fastest average catch time, t is the time elapsed, N_0 is the initial percentage of flies (100) and N_t is the percentage remaining after t.

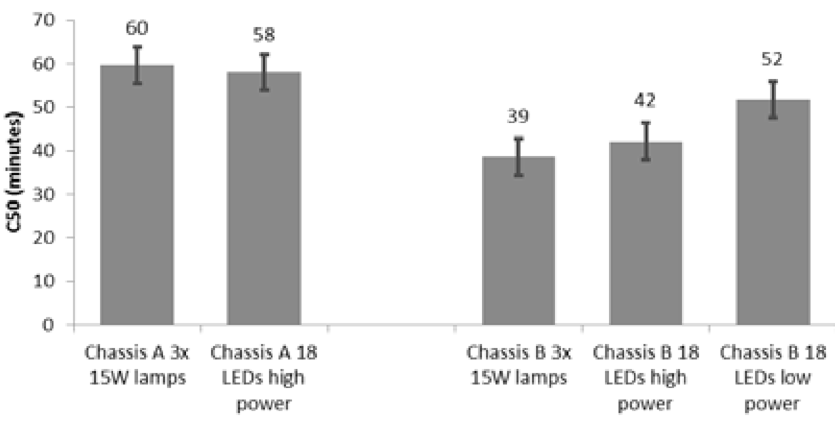

Figure 3. Catch rate variation with luminaire and power.

The results show chassis B to be more efficient than chassis A (although there is some notable cover colour and therefore contrast variation). At a high power setting the units with LED luminaires consume approximately 60% of the power of the units with fluorescent lamps, falling to below 50% at the low power setting over the test period. No significant difference (p=0.05) was found between luminaires for either chassis or power level.

DISCUSSION

Directional LED luminaires offer a number of interesting design choices for UV-A insect light traps. The use pattern and power dimming capabilities of LEDs make them a particularly good fit for use in insect light traps, the limiting factor currently being power output that will gradually be overcome as technological hurdles of cooling and reliability are addressed. Variable power supply to LEDs would initially seem to be of nominal use for light traps given some test methods currently used for assessing light trap effect, this work has shown that under single choice bioassay conditions UV-A LEDs offer comparable risk protection against fly-borne disease in low light settings to fluorescent lamps with a fraction of the power consumption required to maintain an arc in a mercury phosphor lamps emitting light at the same peak frequency.

The use of a single light trap chassis allowed us to investigate the effect of changing the luminaire without affecting any other variable that would influence the catch rate of a trap in a room with a known number of house flies. Single choice bioassays should be the norm for insect light trap testing as LED luminaires become more common in the market. The alternative two choice testing will inevitably result in a bias towards high power units (regardless of luminaire type) being deployed in situations where a comparable level of disease risk reduction from house flies could be achieved using potentially lower UV-A light output more attuned to ambient light levels (Jones et al., 2017).

Further work will seek to define and optimise the relationship between an LED luminaire and the trap chassis to accentuate the directionality adaptive power modes unique to this technology. The results are also is in line with observations by Syms (1988) that sub-sets of flies within the released population are for unclear reasons do not display the same level of flight and attraction to UV-A than others. The behaviour of released sets house flies and identification and quantification of any sub-set therein that is more or less attracted to light traps shall be documented to improve the operational understanding and limitations of these units.

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PARASITOIDS FOR CLASSICAL BIOLOGICAL CONTROL OF *TINEOLA BISSELLIELLA* (LEPIDOPTERA: TINEIDAE)

[1]RUDY PLARRE, [2]HENRIK HERING, AND [1]MARTINA MATZKE
[1]BAM-Federal Institute for Materials Research and Testing, Unter den Eichen 87, 12205 Berlin, Germany
[2]BfR-Federal Institute for Risk Assessment, Max-Dohrn-Straße 8–10, 10589 Berlin, Germa

Abstract Two species of parasitoid wasps, *Apanteles carpatus* (Hymenoptera: Braconidae) and *Baryscapus tineivorus* (Hymenoptera: Eulophidae), are known to successfully parasitize larvae of tineid moths. We have evaluated each species potential impact on suppressing the pest *Tineola bisselliella* (Lepidoptera: Tineidae). Both parasitoids have shown high potential for biological control of webbing clothes moth larvae when tested on different host instars in the laboratory and field. Preferences for parasitizing older and larger host larval instars for egg largely overlap in both species. *Apanteles carpatus* is a solitary koinobiont and parthenogen, while *B. tineivorus* is a gregarious koinobiont, with a sex ratio of approximately 1:5 (male:female). Solitary reproduction in *A. carpatus* markedly suppressed the pest population early on but lagged large numbers of progeny in the F_1-generation. Gregarious reproduction resulted in a faster build-up of the F_1-generation but lagged the immediate control effect. Combining the release of both parasitoid species for pest control seemed to be the logical conclusion. We tested the reproductive success of both species when experimentally forced into intensive intraguild competition. For this, parasitoids had either to compete simultaneously or slightly time shifted on the same preferred host resource. Reproductive success of *A. carpatus* was not significantly influenced by the presence of *B. tineivorus*. *B. tineivorus* reproductive output was significantly reduced in the presence of *A. carpatus*. Overall suppression of host development was not significantly enhanced with both parasitoid species co-occurring when compared to their exclusive access to hosts. *B. tineivorus* does not significantly improve the control of webbing clothes moth hosts when occurring together with and having to compete against *A. carpatus*, at least in restricted laboratory conditions. Results show that *B. tineivorus* should be released with a temporal advantage of at least several days. For inundative release, reproduction strategies and even competition between parasitoids is negligible, and a deferred release strategy would not be required.

Key words *Apanteles carpatus*, *Baryscapus tineivorus*, biological control, inoculative release, intraguild predation.

INTRODUCTION

Biological control of pest insects is one cornerstone in modern concepts of IPM, not only in agricultural systems but also in museums, private households and the textile industry (Pinniger, 2001; Pinniger et al., 2016). *Tineola bisselliella* (Hummel) (Lepidoptera: Tineidae), webbing clothes moth, and *Tinea pellionella* (L.) (Lepidoptera Tineidae), case-making clothes moth, are important insect pests in these synanthropic environments (Cox and Pinniger, 2007), and parasitic wasps have been shown to be suitable for their biological control. One is *Apanteles carpatus* (Say) (Hymenoprera: Braconidae), a solitary koinobiont and parthenogen braconid wasp of approx. 4 mm in size (Fallis, 1942). This wasp is capable of parasitizing and completing development in all larval stages of *T. bisselliella* and *T. pellionella* (Plarre et al., 1999; Plarre and Balnuweit, 2003; Tibaut, 2005). The other is *Baryscapus tineivourus* (Ferriére) (Hymenoptera: Eulophidae), a gregarious koinobiont eulophid wasp of max. 2 mm and with a sex ratio of approximately 1 male to 5 females (Jotzies, 2011). For successful development, *B. tineivorus* requires older host larvae. Younger hosts can be parasitized in principle but these lack the resources to allow completion of larval development or pupation (Matzke, 2016). Table 1 summarizes for both species the present state of scientific knowledge relevant for controlling clothes moths.

For any applied pest control strategy, a mixed approach is considered advantageous, particularly if it results in a synergistic suppression of the pest. This also applies to biological means of control, where different species of pathogens, parasitoids or predators are released to reduce or eradicate pest populations (O'Neil and Obrycki, 2008). However, inter-specific competition between putative parasitoids or predators may be problematic (Brodeur and Rosenheim, 2000), especially when important biological niche parameters of the introduced beneficial species, like reproduction, are similar (De Moraes and Mescher, 2005). Although the reproductive strategies of *A. carpatus* and *B. tineivorus* differ, with each being solitary and gregarious respectively (Pennacchio and Strand, 2006), their host range and host larval instar preferences for egg laying largely overlap (Plarre et al., 1999; Matzke, 2016). Additionally, both species are koinobionts and endoparasitoids (Pennacchio and Strand, 2006). Indeed, *A. carpatus* and *B. tineivorus* can be regarded as members of the same guild (Hawkins and MacMahon, 1989), meaning that intraguild competition between these two beneficials is highly likely when the two species occur regionally and timely in sympatry (Ehler, 1992; Godfray, 1994; Pennacchio and Strand, 2006).

Intraguild competition could lower the reproductive success of a lesser competitor, hinder its sustainable build up of a residual population and reduce its efficacy in controlling the pest (Rosenheim et al., 1995; Brodeur and Rosenheim, 2000; Müller and Brodeur, 2002; Briggs and Borer, 2005). Competition in parasitoids during resource acquisition can be manifold (Godfray, 1994). For example, it may occur prior to oviposition during host searching and finding or by antagonistic behavior for individual hosts (Batchelor et al., 2005). Physical post-ovipostion competition may result in intraguild predation by larvae of the superior species inside the host (Polis and Holt, 1992; Rosenheim et al., 1995; Hunter et al., 2002; Müller and Brodeur, 2002; Arim and Marquet, 2004). Host or territory-marking allomones, produced by primary parasitoids or predators which repel secondary ones are non-physical forms of competition, resulting in the marker becoming the superior parasitoid or predator (Polis et al., 1989; Gnanvossou et al., 2003). The superior species is likely to out-compete the inferior one, ending in competitive displacement (Amarasekare, 2002; Reitz and Trumble, 2002). In the extreme, this could lead to pest control failure, when the competitors are of equal strength and restrain each other's reproduction.

Here, we experimentally compete *A. carpatus* and *B. tineivorus* in a restricted environment. This is achieved by simultaneously release of both species onto a limited number of accessible hosts, by narrowing the developmental stage of the host to be optimal for both parasitoid species, and by constraining the available space. Competition avoidance strategies, as described by Polis et al. (1989) and Hatcher et al. (2008), is thus ruled out by the overall experimental design. This scenario may well be realistic, when parasitoids are released initially after the first detection of the pest, e. g. through monitoring pheromone traps (Trematerra and Fontana, 1996). At such an early stage, the pest population presumably still has an age-uniform structure. In this study, we evaluated the reproductive success of either parasitoid species in the F_1-generation and their control effect on their common host *T. bisselliella*.

METHODS AND MATERIALS

Tineola bisselliella. Larvae of webbing clothes moth were derived from stock cultures of the BAM (Federal Institute for Materials Research and Testing) Berlin, Germany. Stock insects have been reared for more than 10 years on goose feathers, soaked in 10% brewer's yeast/water solution, oven-tried at 60°C and cooled down to room temperature. Rearing conditions are 27°C±2°C and 70%±5%r h.

Under these conditions developmental time from egg to adult moth lasts approx. 7 to 8 weeks (Griswold, 1944; Plarre et al., 1999). Larvae for experiments were 5 weeks old, prepared out of their feeding tubes and transferred onto patches of 100% worsted wool which had been placed into glass-jars of 780 cm^3 volume (ø10.5 cm x 9 cm height) and covered by a ventilated screw cap. During the next 24 hours, the larvae had spun new feeding tubes.

Apanteles carpatus. Wasps were reared continuously for more than 5 years on cultures of *T. bisselliella* as described above. Newly over night emerged adult wasps were used in experiments. *Apanteles carpatus* is thelytokous, and therefore all wasps were female.

***Baryscapus tineivorus*.** Wasps were reared continuously for more than 3 years on cultures of *T. bisselliella* as described above. Newly over night emerged adult wasps (males and females) were used in subsequent experiments.

Isochronic release of parasitoids. In each of six replicate experiments, 50 larvae of *T. bisselliella* were caged in glass-jars as described above, simulating realistic constricted host resource patches. After 24 hours, ten newly emerged *A. carpatus* females and 15 newly emerged *B. tineivorus* of mixed sex were simultaneously added to each replicate jar. Sexing live *B. tineivorus* is impossible without harming the insects. However, with a sex ratio of 1:5 (male/female) in *B. tineivorus*, it was assumed to have approximately even numbers of females from each parasitoid species. Both species had to compete with con-specifics and inter-specifics for host resources during the next 4 days, after which all parasitoids were removed. The patches with hosts were then incubated at rearing conditions to allow hatching of non-parasitized *T. bisselliella* larvae to adult moths, or in the case of parasitized individuals; development of each parasitoid species' F_1-generation.

For comparison and to evaluate the effect of interspecies competition, an equal number of set ups was prepared in the same way but with only one parasitoid species being released, respectively. For overall comparison an equal number of set ups was prepared in the same way without the release of any parasitoid species.

Metachronic release of parasitoids. In each of three replicate jars, 25 larvae of *T. bisselliella* were caged as described above. After 24 hours ten newly emerged *A. carpatus* females were added to each jar. Three days later, all *A. carpatus* were removed and 15 newly emerged *B. tineivorus* of mixed sex were added for the next 3 days. The same procedure was independently repeated but with introduction of parasitoids in reverse order. Direct pre-oviposition competition between the two parasitoid species was thus avoided. After removal of all parasitoids, the patches with hosts were incubated at rearing conditions to allow hatching of non-parasitized *T. bisselliella* larvae to adult moths or in the case of parasitized individuals; development of each parasitoid species' F_1-generation.

For comparison, equal numbers of replicates were prepared in the same way with either simultaneous release or no release of the parasitoid species.

Data Analysis

Data were analyzed statistically using two-sample t-Test for pairwise comparison of mean values. Differences at $p \leq 0.05$ were regarded as significant.

RESULTS

Isochronic Release Of Parasitoids

Development of clothes moth larvae to adults was reduced in all cases where parasitoids were introduced (Figure 1). This suppression, however, was statistically significant only when *A. carpatus* was present, either alone or in combination with *B. tineivorus* (Figure 1/II and 1/III). A significant reduction of the pest was not achieved when *B. tineivorus* acted alone under the above mentioned experimental condition (Figure 1/IV). Its contribution to moth mortality when both parasitoids species acted together was thus insignificant as well.

Reproductive success of *A. carpatus* in the F_1-generation was not influenced by the simultaneous presence of *B. tineivorus* (Figure 2/I and 2/II). Approximately 20 F_1 *A. carpatus* individuals completed development in each experimental set up. With 10 females of *A. carpatus* depositing eggs over 4 days, this corresponds to a reproduction rate of 0.5 per parental female per day.

On the contrary, reproductive success of *B. tineivorus* in the F_1-generation was significantly influenced by the simultaneous presence of *A. carpatus* (Fig. 2/III). Approximately 35 F_1 *B. tineivorus* completed development in the absence of *A. carpatus* (Figure 2/IV) but almost none when *A. carpatus* was present (Figure 2/III). Assuming a rate of 10 *B. tineivorus* females depositing eggs over 4 days, this corresponds to a reproduction rate of 0.9 and almost 0.0 per parental female per day, respectively.

Metachronic release of parasitoids

The development of clothes moth larvae to adults was significantly reduced in all cases where both parasitoids were present as compared to when parasitoids were absent (Figure 3). The order in which the parasitoids were released had no impact on pest population suppression (Figure 3/II, 3/III and 3/IV).

Reproductive success of *A. carpatus* in the F_1-generation was not influenced by the order of its release in relation to *B. tineivorus* (Figure 4/I, 4/II and 4/III). No difference in development was observed regardless of whether both parasitoids acted simultaneously or in succession. Approximately 12 new *A. carpatus* completed development in each experimental set up. With 10 females of *A. carpatus* depositing eggs over 3 days, this corresponds to a mean reproduction rate of 0.4 per parental female per day.

The reproductive success of *B. tineivorus* was strongly suppressed by the presence of *A. carpatus* (Figure 4/IV, 4V and 4/VI). However, with a 3-day time advantage *B. tineivorus* was able to slightly but significantly increase its reproduction rate (Figure 4/VI) when compared to 3-day time disadvantage or simultaneous release with *A. carpatus* (Figure 4/V and 4/IV).

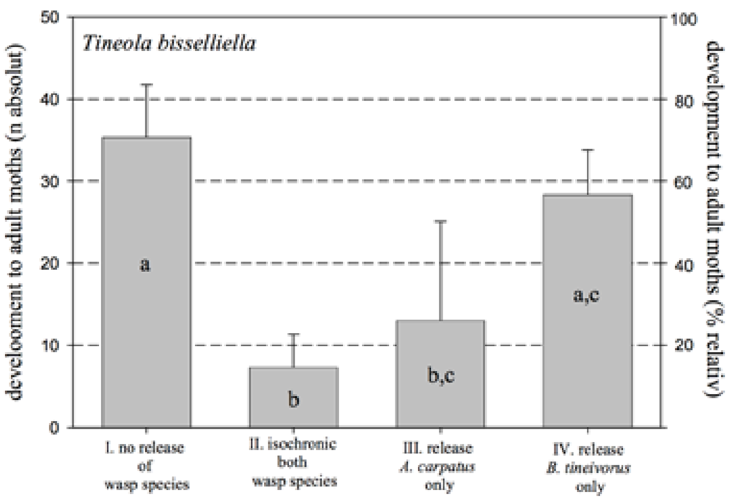

Figure 1. Mean absolute and mean percentile development of clothes moth larvae (*T. bisselliella*) to adult moths in respect to the presences of : I. no parasitoids, II. both parasitoids simultaneously, III. the koinobiont solitary larval parasitoid only, and IV. the koinobiont gregarious larval parasitoid only. Differences at the p≤ 0.05 level are indicated by different letters.

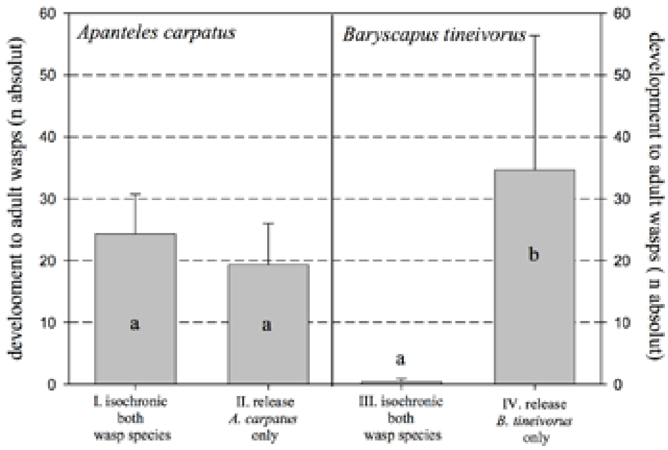

Figure 2. Mean absolute development of F_1-generation of *A. carpatus* from its host *T. bisselliella* in respect to: I. simultaneous presence of parental *A. carpatus* with *B. tineivorus* and II. single presence of parental *A. carpatus* as well as mean absolute development of F_1-generation of *B. tineivorus* from its host *T. bisselliella* in respect to: III. simultaneous presence of parental *B. tineivorus* with *A. carpatus* and IV: single presence of parental *B. tineivorus*. Differences at the $p \leq 0.05$ level are indicated by different letters.

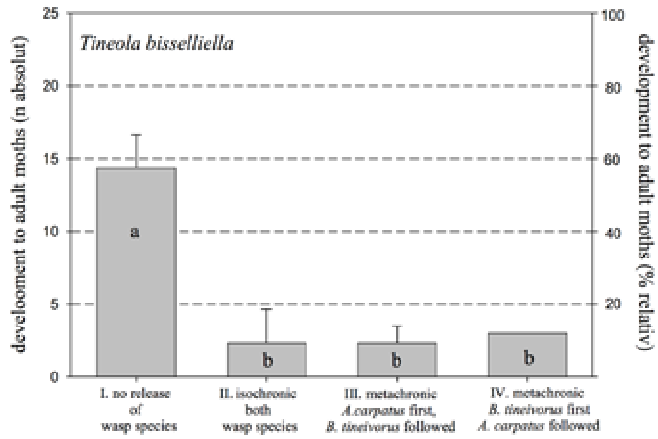

Figure 3. Mean absolute and mean percentile development of clothes moth larvae to adult moths in respect to: I. no parasitoids, II. simultaneous presence of parental *A. carpatus* and *B. tineivorus,* III. the metachronic presences of the larval parasitoid *A. carpatus* after 3 days by the larval parasitoid *B. tineivorus*, and IV. *B. tineivorus* followed after 3 days by *A. carpatus*. Differences at the $p \leq 0.05$ level are indicated by different letters.

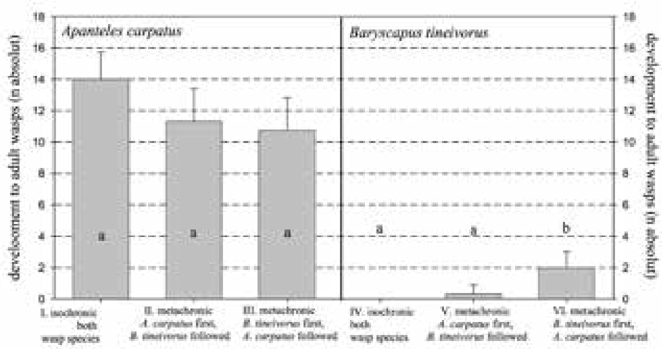

Figure 4. Mean absolute development of F_1-generation of *A. carpatus* from its host *T. bisselliella* in respect to: I. simultaneous presence of both parasitoids, II. metachronic presences of the larval parasitoid *A. carpatus* followed after 3 days by the parasitoid *B. tineivorus*, and III. metachronic presences of *B. tineivorus* followed after 3 days by *A. carpatus*, and mean absolute development of F_1-generation of *B. tineivorus* from its host *T. bisselliella* in respect to: IV. simultaneous presence of both parasitoids, V. metachronic presences of the parasitoid *A. carpatus* after 3 days by the larval parasitoid *Baryscapus tineivorus,* and VI. metachronic presences of *B. tineivorus* after 3 days by *A. carpatus.* Differences at the $p \leq 0.05$ level are indicated by different letters.

DISCUSSION

When parasitoids are released for biological control, two main objectives are pursued: a fast reduction of the pest below an economic threshold, and a rapid population build-up of the beneficial specie(s). *A. carpatus* and *B. tineivorus* differed considerably at our two main experimental restrictions on host use, which were limited time-window for parasitism and limited number of spatially confined hosts. In the absence of interspecific competitors, reproduction in the gregarious species, *B. tineivorus*, resulted in a higher number of F_1-progeny per parental female per day (0.9) than in the solitary *A. carpatus* (0.5). This would have led to a faster population build up of *B. tineivorus* over subsequent generations. Initial suppression of pest development by F_1-progeny was greater in *A. carpatus*. As a solitary parasitoid, a single egg is delivered to a different host individual, which resulted in 70% mortality of clothes moth larvae (Figure 1/III). Host mortality caused by *B. tineivorus* alone, by contrast, was only 40 % (Figure 1/IV), and did not significantly differ from the natural mortality of host larvae in our experiments (30 - 40%; Figure 1/I and 3/I) which is within the expected range for *T. bisselliella* (Griswold, 1944).

We hypothesized that the combined release of both parasitoids with their different reproduction strategies could have resulted in an additive effect on host mortality and a fast build-up of a residual population of at least one beneficial species. The highest host mortality in our experiments was recorded when both parasitoids acted together (Figure 1/II). However, host mortality caused by both parasitoid species did not significantly differ from that caused by *A. carpatus* alone (Figure 1/III). The simultaneous presence of *A. carpatu*s significantly reduced the reproductive success of *B. tineivorus* in the F_1-generation to almost zero (Figure 2/III) and therefore prevented its population establishment. This demonstrates that *B. tineivorus* suffers significantly from intraguild competition with *A. carpatus* under restricted laboratory conditions, whereas *A. carpatus′* developmental success is not influenced by the presence of *B. tineivorus* (Figures 2 and 4).

Although pre-oviposition competition between the two parasitoids cannot completely be ruled out, the deferred and successive release strategies adopted by each parasitoid respectively, indicates that interspecific competition is strongest during post-oviposition, among larvae inside the host. The most likely mode of action is predation (Rosenheim et al., 1995; Brodeur and Boivin, 2004), with *A. carpatus* larvae not only feeding on host tissue but also on *B. tineivorus* larvae.

Larvae of solitary endoparasitoids do not tolerate other internal feeders. If they encounter competitors, their feeding habits are likely to require significant aggression towards that intra or inter specific competitor in order to survive (Brodeur and Boivin, 2004). Solitaries such as *A. carpatus* also do not have to constrain their tissue consumption habits to smaller portions inside the host, unlike in gregarious endoparasitoids, where a large degree of immobility inside the host would have been selected for to avoid the encountering of conspecific competitors (Boivin and van Baaren, 2000; Pexton and Mayhew, 2001). This may explain why *A. carpatus* larvae are superior competitors. Under our metachronic experimental conditions, their reproductive success did not differ, regardless of whether they had to compete simultaneously with *B. tineivorus*, or following delayed introduction (Figure 4).

Solitary parasitoids may only slow down aggressive feeding activities or enter developmental dormancy when the host provides insufficient resources, such as in small hosts for example (Lawrence, 1990). For gregarious larvae this kind of behavior would be fatal. The life histories of *A. carpatus* and *B. tineivorus* fulfill these assumptions: Susceptible host instars for *A. carpatus* range from old (large) to very young (small), with prolonged developmental times resulting from infestation of the latter (Plarre et al., 1999; Harvey et al., 2000). In *B. tineivorus,* only older hosts facilitate successful development. Matzke (2016) has previously shown that younger hosts do not provide sufficient resources for the brood to complete the life cycle. Collective dormancy in gregarious endoparsitoids is unlikely to have evolved because of the difficulty associated with synchronizing behavior or physiology amongst competing members of the same brood. Complete extraction of host tissue has been reported for *A. carpatus*

(Harvey et al., 2000), whereas in *B. tineivorus*, depending on the actual number of progeny, parasitoid larvae may not completely hollow out a given host (Jotzies, 2011).

As the inferior competitor over a restricted range of host conditions, *B. tineivorus* was only able to successfully compete with *A. carpatus* when given a temporal advantage. We have demonstrated here that a 3-day advantage leads to significantly increased reproduction rate of *B. tineivorus* (Figure 4/VI)). It remains unclear whether *B. tineivorus* larvae successfully avoided secondary parasitism through direct physical defense against *A. carpatus* eggs or egg-larvae, or via the release of a repellent allomone. Evidence for the latter comes from studies in hymenopteran parasitoids where unparasitized and parasitized hosts could be discriminated prior to oviposition (Tillman and Powell, 1992; Godfray, 1994), but whether *B. tineivorus* mark their hosts during or after egg-laying remains to be shown.

CONCLUSION

Under restricted experimental conditions, *A. carpatus* clearly outcompetes *B. tineivoru*s. Simultaneous release of both species for biological pest control is therefore not recommended in situations where both parasitoid species are under severe competition for a restricted range of host conditions. Although such conditions are quite realistic in specific environments (e. g. new pest infestations), they do not represent the full range of host conditions found in nature. Nonetheless, in such conditions, we have shown that it may be effective to combine the benefits of a solitary reproductive strategy (*A. carpatus*) which has the advantage of higher initial host suppression, with a gregarious reproductive strategy (*B. tineivorus*) which has the advantage of a faster eventual build up of a residual population of beneficials. Deferred release of the two species, by which the inferior competitor is given a time advantage, represents a potential means of implementing such a mixed strategy (De Moraes and Mescher, 2005; Everard et al., 2009; Cusumano et al., 2011). Because *B. tineivorus* is gregarious, the consequent initial numeric superiority of this species' reproductive success in the F_1 generation is a significant factor that also should be considered when trying to give long term predictions (Table 1). *B. tineivorus* requires only 18 days to complete development in 5-week old, well fed *T. bisselliella* larvae (Matzke, 2012). This is in contrast to *A. carpatus,* which requires 34 days to complete development (Plarre et al., 1999).

Table 1. Reproduction life history of *Apanteles carpatus* and *Baryscapus tineivorus.*

<table>
<tr><th colspan="2">Life History Data</th><th>Apanteles carpatus</th><th>Baryscapus tineivorus</th></tr>
<tr><td colspan="2">Reproduction strategy</td><td colspan="2">endoparasitoid, koinobiont</td></tr>
<tr><td colspan="2">Solitary
parthenogenic (thelytoky)</td><td>Gregarious
males and females (1:5)</td><td></td></tr>
<tr><td colspan="2">Range of host species</td><td colspan="2">Tineola bisselliella, Tinea pellionella</td></tr>
<tr><td colspan="2">Range of host
developmental stage</td><td>All larval stages</td><td>Larval stages
5 - 7 weeks old</td></tr>
<tr><td colspan="2">Mean development time (days)</td><td>35, rang of 60 - 25 de-pending on host stage</td><td>18</td></tr>
<tr><td colspan="2">Parasitism rate (hosts/♀wasp)</td><td>Maximum 60</td><td>Mean 5</td></tr>
<tr><td rowspan="2">Mean Re-production capacity</td><td>F_1/host</td><td>1</td><td>4, maximum 20</td></tr>
<tr><td>F_1/♀wasp</td><td>30, maximum 65</td><td>38, maximum 75</td></tr>
</table>

Mean ♀ Longevity (days)	With food*	27, maximum 40	9, maximum 27
	With host**	Not determined	4, maximum 13
	With food* With host**	Not determined	11, maximum 16
	Without food* Without host**	Not determined	3, maximum 11

* Honey water; ** Larvae of *T. bisselliella*

Therefore, given a few days time advantage, in combination with a faster life cycle, *B. tineivorus* could in principle stably coexist alongside *A. carpatus*, and establish respectable population size (Amarasekare, 2002; Price and Morin, 2004; Briggs and Borer, 2005; Hatcher et al., 2008).

We emphasize, however, that our conclusion applies mainly to inoculative release strategies where the principal goal is to establish a sustainable population of the beneficial species in a uniform host environment. In many cases, a more heterogeneous and widespread pest (host) population in more diverse environments would create opportunities for coexistence through competition avoidance strategies (Godfray, 1994; Křivan, 2000; Borer, 2002; Revilla, 2002; Nakazawa and Yamamura, 2006; Amarasekare, 2007; Bampfylde and Lewis, 2007; Holdt and Huxel, 2007; Janssen et al., 2007; Cusumano et al., 2011).

For inundative release, in which a pest-infested environment is flooded with beneficials for immediate pest control, reproductive strategies and even competition itself between parasitoids, will have a negligible impact on the host (Rosenheim et al., 1995; Bográn et al., 2002), unless both species negatively influence each other in their host-finding abilities. But for *A. carpatus* and *B. tineivorus* this is not the case. Here, a deferred release strategy would not be required.

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PHYSICAL METHODS TO COMBAT MASS OCCURRENCES OF *CYLINDROIULUS CAERULEOCINCTUS* (DIPLOPODA: JULIDAE)

KLAUS ZIMMERMANN
inatura GmbH, Jahngasse 9, 6850 Dornbirn, Austria

Abstract In the small village of Röns (Austria) three families were confronted with a long lasting major mass occurrence of the snake millipede *Cylindroiulus caeruleocinctus* (Wood). Chemical, biological and physical methods were tested to force back those invaders and to protect the affected people. A three-stage defence-system containing different physical barriers was established to provide long-term protection. The inner circle was built up to prevent the millipedes from entering indoor areas. The middle circle was installed to keep them from climbing up the outer house walls. The outer circle was attached to protect the whole plot against new invaders.

Key words Snake millipedes, swarming behaviour, mechanical methods in pest management, silicate powder.

INTRODUCTION

Millipedes are a group of primeval arthropods characterized by two pairs of legs on most body segments. The biological knowledge about millipedes is rather sparse. On the other hand, their mass occurrences are rather common, and nobody can explain their biological reasons (Passig and Scholz, 2007).

In 2004, the biological advisory service (BAS) of the "inatura – Erlebnis Naturschau Dornbirn" was informed about the mass occurrence of *C. caeruleocinctus* in the village of Röns (Zimmermann, 2014b). Thousands of specimens were climbing up the outer house walls of three detached houses each night during a period of several weeks. Frequently singular millipedes managed to get into the houses, disgusting the tenants massively in their private sphere. This had already been remarked three years before, but in the beginning nobody had an idea of its dimension: the snake millipedes became part of the lives of the affected residents for more than ten years. As there was no chance of getting rid of them rapidly, it was the author´s goal to support the affected families in minimizing the disruptions caused by the invaders and to build up physical barriers to keep them away from more sensitive areas (Zimmermann, 2013).

The author has been asked for advice more than 100 times by people confronted with mass occurrences of *C. caeruleocinctus* and other millipedes in Austria and other European countries during the past ten years. None of these infestations reached the dimension of the one in Röns.

MATERIALS AND METHODS

Annoying Intruders

C. caeruleocinctus is a Julidae-species that is common in many parts of Europe (Bogyó and Korsós, 2010). It was also imported to the U.S.A. and Canada. Being a synanthropic ground dweller in open landscapes it can be found near the boundary of acres, in cemeteries and gardens – mainly under stones or wooden structures. The diet of this snake millipede contains leaves of several deciduous trees as well as litter of grass plants and moss. Being strictly hygroscopic the specimens show their main activity periods in spring and autumn (Voigtländer, 2005). During the hot summer as well as in winter they hide in deeper zones in the soil. They show a typical diurnal vertical movement pattern, their main activity period being during the night (Haacker, 1967). A few authors describe *C. caeruleocinctus* as a pest feeding on roots of carrots, sweet potatoes and other culture plants (Brunke et al., 2012). Much more relevant is its role as an annoying species, whenever thousands of individuals enter gardens and houses (Scott, 1958).

Evaluation Of The Pest

This mass occurrence of *C. caeruleocinctus* occupied an area of about two hectares of ground. It was completely impossible to count or assess the whole number of individuals. To get a proper reference to the dimension of the infestation, the numbers of individuals climbing up the house walls were counted frequently. This led to the experimental definition, that an amount of 500 or more individuals entering the walls of a house during one hour (at night) three times or more frequently during an activity period marks a major mass occurrence. This definition might also be useful to distinguish between an annoying occurrence and a real pest. Due to the author´s experience, an amount of 500 millipedes and more on the house walls leads to a significantly higher number of individuals trying to get into the houses. On the affected sites in Röns many singular counting (over a period of 7 years) with 2.000 individuals and more, climbing up the walls of each of the affected houses, were documented.

Methodical Attempts To Combat The Pest

At first a local pest controller tried to combat the millipedes using insecticides containing Pyrethrum or Chlorpyrifos, but none of these pesticides could stop these invaders. As a second step, different beneficial organisms like predatory mites (*Hypoaspis miles*) and nematodes (*Steinernema carpocapsae)* were tested to reduce the amount of millipedes (Schulte, 1989). Applied in situ, the mites showed a weak effect for a few days, whereas in laboratory experiments they showed no influence on the millipedes. Nematodes were tested in cooperation with the enema company (Germany), but also showed no effect on *C. caeruleocinctus*. As these chemical and biological methods did not help to reduce the swarming millipedes properly, various physical barriers and traps were tested to keep away these intruders. This led to the plan of an experimental three-stage defence system to force back the invading millipedes (Zimmermann, 2014a).

THREE STAGE PHYSICAL DEFENCE SYSTEM

The inner circle of the barrier-system was built up to prevent the diplopods from entering the indoor areas of the houses. To fulfill this purpose, insect screens were installed on windows and doors, several door and window seals were renewed, and other entrance facilities for the millipedes (ventilation slits etc.) were closed with tapes.

The middle circle was established directly on or near the plinths of the house walls to keep the millipedes from climbing up the outer house walls. It also enabled the protection of balconies, verandas, terraces, pergolas and other defined places near the houses. In a small distance from the ground a slippery plastic tape (7 cm high) was fixed with a double-sides adhesive tape to the house wall. Entering millipedes had no chance to pass this slippery barrier. The areas around doors and pathways were additionally protected with trails of silicate powder. To complete the protection masking tapes were applied as adhesive traps on the ground.

The outer circle was installed near the plot boundaries to protect the whole property against migrating millipedes. It was put into practice with a slug fence made of galvanized iron-plate. The vertical parts of this fence were coated with a liquid formulation of silicate powder to build a self-eroding surface. Whenever millipedes tried to climb up this chalky surface, small eroding silicate-crumbs stuck to their tarsi and made them lose their grip and slip down again (Mucha-Pelzer, 2010). This special surface coating had been developed by the Humboldt-University of Berlin and was tested in situ for the first time in Röns (Mucha-Pelzer et al., 2009). On both sides of this fence sets of barber-traps were posed to control the effect of this barrier.

EXPERIENCES AND DISCUSSION

It proved to be rather helpful to define three different protection areas. Each defence-circle had its specific functions and demands. But all these measures had to fulfill one goal, they had to ameliorate the welfare of the affected tenants.

Normally the interior areas indicate the most delicate space that has to be protected solidly. To achieve this, each single window and door seal had to be proved and – if necessary – renovated. Ventilation slits had to be taped carefully. All windows (and also balcony doors etc.) that might remain open during the nights had to be secured with insect screens. Wherever these inner defence-measures were realized properly, not even single millipedes were able to enter the rooms.

Keeping the house wall more or less free from millipedes is a further important step to keep the tenants calm. The applied plastic tape fulfilled this purpose sufficiently and free from service costs. Its only disadvantage was that it could damage the surface (painting) of the wall, when being peeled off again. Also barriers made of silicate powder showed a rather good performance against the millipedes. Part of the specimens was aware of the silicate trail and turned back. Others, that tried to crawl through the powder, were contaminated massively (Zimmermann and Duelli, 2007). Being animals with many joints and intersegments, the millipedes are predestinated to be controlled with silicate powder: The grains of the powder stuck everywhere on their bodies, destroying the waxy layer of their cuticule and making them unable to move or curl up. Most of the individuals died at least a few hours after being contaminated. But this lethal barrier needed to be maintained permanently: after strong rains or winds the trails had to be cleaned (especially if there were lots of millipede bodies covering the silicate) and new powder had to be applied. The installation of the adhesive traps made from masking tapes was rather tricky, and they had to be renewed very often. As soon as a few millipedes were already sticking to the tapes, the following individuals could cross them in climbing over their bodies.

The outer circle was established to keep migrating millipedes away from the whole plot. The slug fence and its special coating fulfilled this purpose perfectly. But the attempts to quantify this effect with barber-traps had to be cancelled because too many bycatches had been killed. The big disadvantage of this barrier was its permanent need for maintenance. After each grass-mowing, the fence had to be cleaned coated. During winter the fence had to be removed completely and then to be rebuilt in spring.

CONCLUSIONS

The tested set of physical barriers proved to be a proper long-term method to combat swarming and migrating masses of millipedes. The success of these measures is strongly depending on the attitude of the affected tenants, therefore a close contact between them and the pest advisors has to be established. According to the needs of the individual situation, this method can be adapted or only a part of the barriers can be installed. As this system does not help to reduce the stocks of the swarming millipedes (only the silicate powder partly does!), it could be combined with biological methods (nematodes and other beneficial animals) to get a quicker reduction of the problem for the tenants.

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WHAT IS EATING YOUR COLLECTION? DEVELOPMENT OF A WEB-BASED INSECT PEST RECORDING DATABASE

[1]JANE THOMPSON WEBB AND [2]DAVID PINNIGER
[1]Conservation Team Leader. Conservation Department, BM&AG, Chamberlain Square, Birmingham B3 3DH
[2]DBP Entomology, Cookham, UK,

Abstract There is very little information on the distribution of indoor insect pests, and particularly those that infest museum collections. In 2009, the authors developed a website using Renaissance in the Regions funding. The site www.whatseatingyourcollection.com was devised to be a source of information about IPM and insect pests with a photographic reference guide. The website grew out of requests for a reference tool from participants who attended our IPM workshops for people working in the Heritage sector. The participants also asked questions about the spread of insect pests in the UK and whether this was changing. Whilst a pest identification tool could be generated, it was not possible to answer the question regarding the distribution of insect pests other than via anecdotal evidence and personal experience. A second part of the website was therefore developed as an insect pest recording tool. IPM co-ordinators in UK heritage institutions were invited to record the insect finds from their trapping programme on a quarterly basis. There are also records from some domestic and commercial premises where the accuracy of identification can be assured. We have examined the results of all the recordings so far obtained and analysed the current distribution of many species of insect pests across the UK, based on geography and building type. We have also tracked changes in distribution and frequency of established pests and recorded the introduction and spread of new pests, such as Australian carpet beetle *Anthrenocerus australis*. With coordination, this recording system could be adapted for use in any country or geographical area and it is hoped that it will become a valuable tool to be used by all IPM practitioners.

Key words Museum pests, insect pest distribution, insect pest identification, database

INTRODUCTION

In 2003, Birmingham Museums Trust (BMT) was one of the recipients of Renaissance in the Regions funding from central Government. At the time, there was a strong emphasis on improving collections and BMT decided to use some of the funding to provide training in collection care for museums in the six counties of the West Midlands. A survey undertaken in 2002 by Porter identified that a gap had developed in the delivery of training in the heritage sector and that it was very difficult for smaller museums to access affordable, practical training. Where training was provided, the courses were often based in London, adding high travel costs to an already expensive course. These factors excluded many museums, especially those run by volunteers.

BMT planned a series of free collection care training for the West Midlands and courses began in 2004. After 12 years, the courses are still being run, although they are no longer offered free.

IPM courses were a major part of the programme offered and they proved to be some of the most popular courses. They engendered many questions, some about specific issues and some about IPM more generally. One of the early questions was - what pest insects are found in collections in my county?

This was closely followed by – how many are there? There was no way to answer these questions as the data did not exist. Information about the distribution of insect pests is very thorough for insects that affect crops – the outdoor insect pests, but there is less information regarding urban insect pests and even less about those that attack collections.

MATERIALS AND METHODS

There is some information available for indoor insect pests, but it is either highly specific or quite general. An internet search on varied carpet beetle, *Anthrenus verbasci* receives over 49,000 results. It is possible to find a map to show the distribution of this species, but that it favours the temperate regions. Superficially this is helpful, but it doesn't show if *Anthrenus verbasci* is more common in Wales than Sicily or if it is more common in museums than historic houses. For those people monitoring insect species and infestations in the heritage sector, this is the information we really need.

With this kind of information we could be on the alert for new species and perhaps change IPM practices to avoid a potential infestation. We decided that the heritage sector in the West Midlands needed a comprehensive resource to enable people to find out about IPM and to help them to identify what they were finding. We also wanted to create a database where we could start to gather information about what insects were being found and where they were. In addition we could record how many insects were being found and if the numbers or distribution were changing.

Website

Renaissance in the Regions funding was used to create a CD ROM that gave information on IPM and insect pest identification. This was found to be very useful, but the format was inflexible and could not be updated. We developed the concept into a website which contained expanded insect pest images and information to help identification and also enabled us to include a pest recording database. CD and website were called What's Eating Your Collection? (www.whatseatingyourcollection.com)

Confidentiality was an important part of the development of the website. To avoid embarrassment and to encourage heritage organisations to submit their data, each data enterer can only see their own data and on the public access side, the map is controlled so that it is not possible to zoom in to a large scale. This means that individual properties and institutions cannot be identified. The data enterer is required to enter information about their institution, date of check, trap type, insect found etc. This enables us to build up a picture about the kind of institution favoured by insect species, time of year when most are found and geographical spread. We only gather quarterly totals – the database is not meant to replace an institution's own pest recording, but be a supplement to it.

Managing The Website

Insect species are recorded using a drop down list. This was a later addition to the website. The field was originally free text and this gave us some spurious insect name combinations, such as *Stegobium smirnovi,* or useless information like "beetle". The drop down avoids this and thus provides consistent information. The scientific and common name drop downs are linked so that entering in one box auto fills the other – useful for those who can only remember one version of a name. The list contains all the insects illustrated in the identification section of the website and this gives a list of 49 insect species, although so far only 30 from the list have been recorded.

There are 32 data enterers registered representing a wide range of institutions. Birmingham Museums Trust, National Trust for Scotland, the National Trust and English Heritage enter data from across their portfolio of all types of property. There is also information from a commercial company, giving us data for commercial and domestic properties. It is essential that the data recorders are competent to identify the insects accurately as a wrong ID. would make any analysis worthless. All have been trained by the authors and receive refresher ID. information. In cases where the recorders are not sure of a species, they send specimens or images to one of the authors for confirmation. This has worked well and resulted in a number of unusual infestations being discovered. Examples include the first record of the museum nuisance beetle *Reesa vespulae* in a Birmingham Museum and the first record on the Australian carpet beetle *Anthrenocerus australis* in a National Trust property.

Analysing Data From The Website

The front end, accessible to anyone, allows one to search on a variety of terms. Drop down boxes allow entry of search terms. These are by county, building type, quarter/year, genus, species and stage of life cycle. A selection of filters allow the data to be ranked in different ways – it can be grouped by county, building type, quarter/year, genera, species, stages and shown on a map which allows for multiple permutations of data presentation.

RESULTS AND DISCUSSION

Examples of Pest Occurrence and Distribution.

Death watch beetle, *Xestobium rufovillosum.* Death watch beetles have been causing problems in buildings for hundreds of years. As they are particularly associated with oak timbers, it is not surprising that there are few historic buildings in England which have not had some damage. In recent years, improvements in design and good maintenance to prevent timbers getting damp, has reduced the fungal attack which the beetle larvae need to thrive. *Xestobium rufovillosum* (Figure 1) is widespread across most of England. However, there are very few records from Scotland and none from North of the Border area. The explanation is that there is very little oak in Scotland and therefore most wooden building structures are not prone to attack by death watch beetles.

Furniture beetle (woodworm), *Anobium punctatum.* Furniture beetle has also been associated with damage to wooden buildings and wooden objects, probably since Roman times. *Anobium punctatum* needs wood above 14% moisture content to thrive, it is not restricted to oak or wood with previous fungal attack. Furniture beetle (Figure 2) is widespread in the UK, even the far North of Scotland. *A. punctatum* it is associated with buildings with high humidity and is absent from most museums and other buildings with good heating systems which make the wood too dry (Ridout, 2012).

Figure 1. Distribution of Death watch beetle, *Xestobium rufovillosum*

Figure 2. Distribution of Furniture beetle, *Anobium punctatum*

Webbing clothes moth, *Tineola bisselliella.* *Tineola bisselliella* is the major pest of wool, fur and feather textiles in buildings in the UK. The data from the National Trust for Scotland shows that it has spread to all corners of the UK (Figure 3). It is increasing in numbers.

Two spot carpet beetle, *Attagenus pellio.* Also known as the fur beetle, *Attagenus pellio* is associated with damage to fur, wool and feathers in houses where infestations are often linked to blocked chimney flues. The data from historic houses (Figure 4) supports this and Figure 5 shows that it is very rarely found in museums which do not provide the right environment for *A. pellio* to thrive.

Figure 3. Distribution of Webbing clothes moth, *Tineola bisselliella*

Figure 4. Distribution of Two spot carpet beetle, *Attagenus pellio* in historic houses.

Vodka beetle or Brown carpet beetle, *Attagenus smirnovi.* This pest was probably introduced into the UK in the 1970's (Pinniger, 2011) and has become established in buildings the London area, firstly in museums and more recently in houses. Only a few specimens have been found elsewhere in the UK (Figure 6), but given its spread across mainland Europe, (Pinniger, 2013) the occurrence of *A. smirnovi* may be under-recorded in the UK.

Varied carpet beetle, *Anthrenus verbasci* and Guernsey carpet beetle, *Anthrenus sarnicus.* Varied carpet beetle *Anthrenus verbasci* is a pest of wool, fur and feathers in museum collections and domestic housing. It is widely distributed across the UK, apart from the North of Scotland (Figure 7). In the 1970's a new species, *Anthrenus sarnicus,* was found in London, having only previously been recorded from Guernsey. The spread of this species in London and elsewhere has been documented and it has continued to spread (Figure 8). We suspect that this species is also under recorded as it is difficult to distinguish *A. sarnicus* from *A. verbasci*. *A. sarnicus* thrives in warm environments so there may be more than one generation a year.

Figure 5. Distribution of Two spot carpet beetle, *Attagenus pellio* in museums.

Figure 6. Distribution of Brown carpet beetle, *Attagenus smirnovi*

Figure 7. Distribution of Varied carpet beetle, *Anthrenus verbasci*

Figure 8. Distribution of Guernsey carpet beetle, *Anthrenus sarnicus.*

Figure 9. Distribution of Australian carpet beetle, *Anthrenocerus australis*

Australian carpet beetle, *Anthrenocerus australis.* This was first recorded in the UK in 1938, few specimens were found until an infestation was discovered in a carpet in the Victoria and Albert Museum in 2012. Since then these carpet beetles have been collected in traps (Pinniger, 2014). The distribution map (Figure 9) will need to be updated regularly for new records of this species.

Although the distribution data covers a wide area of the UK, there are some obvious gaps where we do not have people recording or entering data. We hope to rectify this by recruiting more recorders to the scheme from the heritage sector and also from the pest control industry as the domestic and commercial data provided by Killgerm has been invaluable in providing a bigger picture of pests in all buildings across the UK. To do this we need accurate identification and greater awareness of new species. This has recently been highlighted by the first occurrence in the UK of grey silverfish *Ctenolepisma longicaudata*. Abby Moore at the Museum of London was the first to notice some unusually large silverfish on traps in her museum. The identification was confirmed as *C. longicaudata* and in 2016 a further infestation was discovered in a London art gallery (Moore et al 2016). We will now have to add *Ctenolepisma longicaudata* to our species list.

A number of people in other countries have expressed interest in our scheme and Austria and Scandinavia are currently discussing a similar recording system. It is hoped that maybe we will eventually have a European pest recording database, as insects certainly do not recognise national boundaries.

INSECTS IN DATABASE

Insect species listed on database

1. Australian carpet beetle *Anthrenocerus australis*
2. Australian spider beetle *Ptinus tectus*
3. Berlin beetle *Trogoderma angustum*
4. Biscuit or drug store beetle *Stegobium paniceum*
5. Black carpet beetle *Attagenus unicolour (megatoma)*
6. Booklouse or psocid *Liposcelis bostrychophila*
7. Brown carpet beetle/Vodka beetle *Attagenus smirnovi*
8. Brown house moth *Hofmannophila pseudospretella*
9. Brown-dotted clothes moth *Niditinea fuscella*
10. Carpet beetle larva *Anthrenus* sp
11. Carpet beetle larva *Attagenus* sp
12. Case-bearing clothes moth *Tinea pellionella*
13. Cigarette beetle *Lasioderma serricorne*
14. Common furniture beetle *Anobium punctatum*
15. Dark carpet beetle *Anthrenus fuscus*
16. Death watch beetle *Xestobium rufovillosum*
17. Firebrat *Thermobia domestica*
18. Fungus beetle *Cryptophagus* sp
19. Fungus beetle *Mycetophagus* sp
20. Furniture carpet beetle *Anthrenus flavipes*
21. Globular spider beetle *Trigonogenius globulus*
22. Golden spider beetle *Niptus hololeucus*
23. Guernsey carpet beetle *Anthrenus sarnicus*
24. Hide beetle *Dermestes maculatus*
25. Hide beetle larva *Dermestes* sp
26. House longhorn beetle *Hylotrupes bajulus*
27. Indian meal moth *Plodia interpunctella*
28. Larder beetle *Dermestes lardarius*
29. Mealworm beetle *Tenebrio molitor*
30. Museum nuisance *Reesa vespulae*
31. Obvious moth *Monopis obviella*
32. Odd beetle *Thylodrias contractus*
33. Peruvian hide beetle *Dermestes peruvianus*
34. Plaster beetle *Adistemia watsoni*
35. Plaster beetle *Dienerella* sp
36. Plaster beetle *Lathridiidae* sp
37. Powder post beetle *Lyctus brunneus*
38. Silverfish *Lepisma saccharina*
39. Six spot spider beetle *Ptinus sexpunctatus*
40. Spider beetle *Ptinus clavipes*
41. Tapestry moth *Trichophaga tapetzella*
42. Two-spot carpet beetle *Attagenus pellio*
43. Varied carpet beetle *Anthrenus verbasci*
44. Warehouse moth *Ephestia elutella*
45. Webbing clothes moth *Tineola bisselliella*
46. Wharf borer *Nacerdes melanura*
47. White-marked spider beetle *Ptinus fur*
48. White-shouldered house moth *Endrosis sarcitrella*
49. Wood weevil *Euophryum/Pentarthrum* sp

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BIOLOGICAL CONTROL WITH LARVAL PARASITOID *LARIOPHAGUS DISTINGUENDUS* IN MUSEUMS AND HISTORIC BUILDINGS

PASCAL QUERNER
University of Natural Resources and Applied Life Sciences, Department of Integrated Biology and Biodiversity Research, Institute of Zoology, Gregor-Mendel-Straße 33, A-1180 Vienna - Austria
Universitity of Applied Arts Vienna, Institute of Archäometrie, Expositur Salzgries, A-1010 Vienna, Austria

Abstract In this paper the biological control of biscuit beetle (*Stegobium paniceum*) and spider beetle (*Gibbium psylloides)* found in different museums, historic buildings and a monastery library in Austria is described. Six case studies are presented where the insect pest monitoring with sticky blunder traps showed the activity of biscuit beetles and spider beetles. It was not clear if objects were actively infested or the pests were coming from the building. Success of the release of wasps varies and no clear trend was found. In two cases the monitoring in the years after the release clearly showed the success of this control method. In the other cases the number of the beetles remained about the same. In those cases the beetles were probably not living in infested objects but were coming from the building. In the infested library other measures needed to be taken as the trapped number of beetles increased significantly. Biological pest control with mass release of parasitoid wasps might have a future in preventive conservation of museum objects, but success depends very much on the local circumstances and the type of the infested objects.

Key words Museum pests; Integrated Pest Management; IPM; treatment; costs.

INTRODUCTION

Integrated Pest Management (IPM) is an important part of preventive conservation in museums and aims to stop both damage to valuable museum objects and the spread of pests between collections. In IPM, different measures like sealing the building, regulating the climate, quarantine for all incoming objects, good cleaning and housekeeping, training museum staff and monitoring all help to prevent an infestation (see for example Brokerhof, 2007; Flieder and Capderou; 1999, Florian, 1997; Pinniger, 2015; Querner, 2015; www.museumpests.net). Most museums try to reduce the application of pesticides in their collections and use freezing, controlled heating, nitrogen or CO_2 fumigation or anoxic treatment to kill all stages of pests in wood, textile, natural history, ethnographic, modern art objects, historic libraries, archives or historic buildings (see Querner and Kjerulff, 2013 for an overview of treatment methods used in museums).

For some years, parasitoid wasps have been applied in biological pest control to reduce infestations in food storage and processing facilities (Schöller et al., 1997; 2006). Various parasitoid species are now commercially available and can be applied against different kinds of moths and beetles (Schöller and Flinn, 2000). Good results have been obtained from different experiments using various selected foods, breeding and releasing parasitoids against different stored product pests (Schöller et al., 1997; 2006).

The same pests as in the food industry like the drugstore beetle (*Stegobium paniceum*), the tobacco beetle (*Lasioderma serricorne*) and the webbing clothes moth (*Tineola bisselliella*) are also important pests in museum, therefore parasitiods could also be used to control them (see Schöller, 2010; Schöller and Prozell, 2011; 2016). Over the last years biological control of insects was tested in several museum collections (Querner and Biebl, 2011; Anheuser and Garcia Gomez, 2013; Anheuser, 2016; Querner et al., 2013; Biebl, 2013; Auer and Kassel, 2014; Querner et al., 2015) but most of these applications were carried out alongside other treatments such as freezing or nitrogen fumigation (Biebl and Reichmuth, 2013; Querner et al., 2015). Until now it is not clear whether a biological control using parasitoid wasps can be 100% successful in a museum environment. Compared with stored product protection, food storage and processing facilities, where biological control is used mainly to reduce infestations under a commercial threshold, in a museum context the infestation needs to be eradicated completely, as the objects are stored for long periods and cannot be replaced.

In this paper we report on the release of the larval parasitoid *Lariophagus distinguendus* against an active biscuit beetle infestation in a museum. See Table 1 for an overview of the most important pests and their natural predators (or predators accepting them as host), that are of relevance for biological control of museum pests.

Table 1. Different insect pest species found in museums or historic buildings (see Pinniger, 2015; Querner, 2015) and their biological control agents). Species marked with " * " are commercially available (Table modified from Biebl personal communication, Zimmermann, 2005; Al Kirshi, 1998).

Moth Species	**Egg and Larval Parasitoid**	**Reference**
Tineola bisselliella	*Trichogramma* spp. * *Apanteles carpatus* *	Zimmermann, 2005 Plarre et al., 1999
Tinea pellionella	*Baryscapus tineivorus* (Eulophidae)	Zimmermann, 2005
Beetles Species	**Predator or Larval Parasitoid**	
Stegobium paniceum *Lasioderma serricorne* *Niptus hololeucus* *Gibbium psylloides*	*Lariophagus distinguendus* * *Anisopteromalus calandrae* * *Cephalonomia gallicula*	Kaschef, 1961 Kaschef, 1955 Biebl and Reichmuth, 2013 Schöller, 2010
Trogoderma angustum *Anthrenus verbasci*	*Laelius pedatus* *	Al Kirishi, 1998
Attagenus unicolor *Attagenus smirnovi* *Anthrenocerus australis*	*Xylocoris flavipes * (bug)*	Al Kirishi, 1998
Anobium punctatum	*Spathius exarator* * *Cephalonomia gallicula*	Auer and Kassel, 2014

Larval parasitoid *Lariophagus distinguendus* (Hymenoptera, Pteromalidae).
A naturally occurring parasitoid of the biscuit beetle *S. paniceum* is the pteromalid parasitoid *Lariophagus distinguendus*. The larval parsitoid wasp has a cosmopolitan distribution and is reported to attack about 15 different beetle species from several families (Noyes, 2014), but not all equally successfully (Goodrich, 1921; König et al., 2015). Several of these pests are regularly active in museum collections or historic buildings and naturally become parasitized by *L. distinguendus*. Examples are: The larvae of the drugstore beetle *S. paniceum*, of the tobacco beetle *L. serricorne,* both occurring in museums and historic libraries and the hump beetle *Gibbium psylloides* and the golden spider beetle *Niptus hololeucus,* both frequently occurring in historic buildings.

The wasps find their hosts mainly by olfactory stimuli (Benelli et al., 2013; Steidle, 2000; Steidle and Ruther, 2000). *L. distinguendus* occurs naturally in central Europe and can also be found in high infested museum locations. The wasps lay up to 60 eggs on the larvae and pupae of the beetles, which are found within the infested materials. Parasitoid wasps should be released either monthly, or 2-4 times per year to treat an infestation.

MATERIAL AND METHODS

Monitoring insect activity in the storage or museums
In all locations monitoring with sticky blunder traps (type Catchmaster) was performed to collect regularly active insect pests and non-pest arthropods. Traps were replaced twice per year and checked six times per year between March and October. Traps were left in place also for the winter months but not checked regularly. The results and details from the monitoring data are not presented here. Table 2 presents the abundance of the pests *S. paniceum* and *G. psylloides* in the last years.

Parasitoid release after beetles were found
If an activity of pests like *S. paniceum* and *G. psylloides* was found, the release of the parasitoid wasps *L. distinguendus* was discussed with the conservator and the number of wasps and release dates decided on a case by case situation (see Table 2). There were released in the rooms where the beetles were found, usually close to the objects on the floor.

Table 2. Location (exhibition space in a museum = M, museum storage = S, historic library = L), pest species found and treated, years where the monitoring was performed, parasitorid release dates, number of parsitoid wasps *L. distinguendus* released each date.

Location and Pest Species	Year	Numb. of Pests	Parasitoid Release Dates	Number of Parasitoids Released	Success or Failure
Hofburg Wien (M) *G. p.*	2012 2013 2014 2015 2016	46 47 44 44 55	 2., 3., 4., 5. 2014 2., 3., 4., 5. 2015	 4 x 150 ind. 4 x 150 ind.	No effect on the biscuit beetles! -> can not reach the beetles underneath the floor boards
Monastery library (L) *S. p.*	2014 2015 2016	207 273 681	11., 12. 2014 4., 5., 7., 10., 10. 2015 3., 4., 5., 6. 2016	2 x 900 ind. 5 x 900 ind. 4 x 900 ind.	No effect on the biscuit beetles -> high infestation of books -> other treatment needed
Academy picture gallery (M) *S. p.*	2013 2014 2015 2016	5 8 4 8	 4., 5. 2014 2., 3., 4., 5. 2015	 2 x 120 ind. 4 x 180 ind.	No effect on the biscuit beetles ! -> they come from the building
KHM picture gallery (M) *S. p.*	2012 2013 2014 2015 2016	18 2 8 8 9	4., 5. 2012 4., 5., 6. 2013 3., 4., 5. 2014 2., 3., 4., 5. 2015	2 x 100 ind. 3 x 300 ind. 3 x 300 ind. 4 x 300 ind.	No effect on the biscuit beetles ! -> they come from the building
KHM old storage (S) *S. p.*	2010 2011	51 5	4., 5., 6. 2010	3 x 360 ind.	reduction of beetles, but all objects (paintings lined with starch paste linings) were treated with Anoxia before transport to new storage
KHM new storage (S) *S. p.*	2013 2014 2015 2016	51 ind. 0 0 0	monthly 7. 2013 until 9. 2014	6 x 1800 ind. 9 x 1800 ind.	no more biscuit beetles were found-> 100% success!

RESULTS AND DISCUSSION

In the two storages where old master paintings were infested by *S. paniceum* (see also Fohrer et al., 2006*;* Liu, 2013*),* the release of the wasp had a clear effect on the beetles resulting in much lower number of beetles found on the traps, or none at all. This result shows that biological control of museum pest is possible and can even result in 100% success.

No effect on the contrary was found in the infested library where the historic books already had quite a high infestation. The infestation was discovered in 2013 only by chance and the monitoring and release of parasitoids started soon after. We monitored the effect of the wasp released for two years, but after the beetle population increased significantly in 2016, the release of wasps was stopped and a alternative treatment method had to be found. Similar results were found by Anheuser and Garcia Gomez (2013) for biological control of the webbing clothes moths in an infested store. Also here the release of the wasps could not reduce the moth population. Anheuser (2016) describe pesticide residues in the objects as a potential reason for the failure of this method. This might also be the case in the historic library in Austria, where dead *L. distinguendus* were found close to the release tubes. Parasitoid wasps are known to be very sensitive towards historic pesticides (Mathias Schöller, oral communication) which might be a limitation of this method as many museum collections, especially natural history and ethnography, are still heavily contaminated. A second reason for the failure in the case study of the historic library is the already advanced and high infestation. Not a few beetles were found every year but hundreds (Table 2 presents only the number of beetles found on traps and by the windows, this probably reflects only a portion of the beetles hatching each year). As shown in other examples, parasitiod wasps are only effective when the infestation is still low (Schöller, 2010; Schöller and Prozell, 2011). Therefore a good monitoring and IPM program needs to be in place to monitor pest activity and be able to react when the population is still low.

No effect was also found in three locations where only a low number of *S. paniceum* and *G. psylloides* were found each year. Few beetles of both species were collected on the traps in each location, mostly in the same room year after year. Objects were checked for an infestation and none was found in all three locations. We therefore assume that the beetles were not coming from infested objects (this would have resulted in a clear increase of trapped beetles over the years), but from within the building. *S. paniceum* is known to be a generalist feeding on all kind of food. In museum and historic buildings it prefers food like starchy and dry plant material (see pest fact sheet on www.museumpests.net, www.whatseatingyourcollection.uk and www1.montpellier.inra.fr), but they can feed also on dust, debris, dead animals, bird nests or dead insects. In the historic location *G. psylloides* is probably coming from below the floorboards where it (and other spider beetles) is known to feed on plant material like straw that was historically used for insulation. The location is a palace in the centre of Vienna with a historic floor. When the beetles are coming from within the building, the parasitoid wasps don't seem to find their host larvae, which might be difficult to access underneath the wood parquet floor or in other areas like shafts, a hanging sealing or other dead spaces. If the source of the infestation is assumed to be here, other measurement like cleaning, sealing of gaps or acceptance of a low but stable pest level have to be considered.

We show that the release of parasitoid wasps as part of an Integrated Pest Management concept could have a future in museum environments. The method is cheaper than other methods like nitrogen fumigation, depending on the number of objects or room size. It is also easy to use by non-trained staff and some of the main museum pests can be controlled. The fact that objects do not require transportation to a nitrogen chamber or other facility is a big advantage, since the handling of museum objects bears risks and is also costly.

Advantages of the biological control in museums**: 1.** No chemicals are applied which could harm objects, staff or the environment; 2. Easy to use; 3. Cheaper than other treatment methods; 4. Objects do not need to be moved to another location for treatment..

***Disadvantages*: 1.** Natural enemies are only commercially available for specific pests; 2. They have to be regularly applied over a long period; 3. They are only effective in the early stages of an infestation; 4. Wasps need to be able to access all pest larvae in the objects; 5. The dead wasps need to be cleaned / removed after the treatment.

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PRACTICAL USE OF BRACONID WASPS FOR CONTROL OF THE COMMON FURNITURE BEETLE (COLEOPTERA: ANOBIIDAE)

[1]STEPHAN BIEBL AND [2]JUDITH AUER
[1]Ingenieurbüro für Holzschutz, Mariabrunnweg 15, 83671 Benediktbeuern, Germany
[2]Ostendstrasse 132, 90482 Nürnberg, Germany

Abstract This paper will show the practical use of a braconid wasp species (*Spathius exarator*) as a biological control method for *Anobium. punctatum*. The parasitic wasp parasitizes its host by piercing its ovipositor directly through the wood surface followed by oviposition onto the furniture beetle larva. Adult wasps emerge through a 0.5 mm wide hole, which can be distinguished from the 2 mm wide hole of *A. punctatum*. With the consultancy of an official expert for wood protection, the infestation of *A. punctatum* and the use of the braconid wasp *S. exarator* were controlled from 2014 till 2016 in a castle church (Schloßkirche Ellingen). Success was based on the count of new exit holes of wasps and furniture beetles on defined areas. The results of the three-year treatment period with the braconid wasp species showed improvement with a decrease of up to 80% emerging furniture beetles on monitored areas. The results of another representative chapel N., treated with *S. exarator* for two years, demonstrated a decrease of up to 100% emerging beetles with a slight rerise of *A. punctatum* activity after five years. These results show the promising state of affairs of biological control with the host-specific braconid wasp for *A. punctatum* infested objects. However, more practical experience will be required to optimise the treatment and rate of success. Furthermore, supportively integrated wood protection treatments, like the use of Anoxia, may be used to reduce partial tight infestations on removable objects.

Key words *Anobium punctatum*, *Spathius exarator*, castle, museum pests, integrated wood protection

INTRODUCTION

Many chemical products for wood preservation are currently in a review process and will be restricted from the European Biocidal Product Regulation (BPR). This will lead to a lower supply of chemical products for pest control and necessitates the use of alternative methods, like physical treatments or biological control. While physical methods for controlling wood boring insects, like heat treatment or anoxia, have a long tradition in knowledge and practical experience, the biological control using natural enemies to control wood pests had not been applied so far. Despite the knowledge of a wide spectrum of antagonists against different common wood boring species (Haustein, 2010; Schmidt 1952) only few statements of laboratory research as well as practical experience had been reported from scientists (Lygnes, 1955; Haustein, 2010).

Described predators of the common furniture beetle *Anobium punctatum* are amongst others the checkered beetles (Cleridae) *Opilo domesticus* and *Corynetes coeruleus*, the soft-winged flower beetle (Malachiidae) *Anthocomus bipunctatus* and the parasitoids *Spathius exarator*, *Sclerodermus domesticus* (Schmidt, 1952), *Cephalonomia gallicola* (Paul et.al., 2008) and *Cerocephala cornigera* (Becker, 1942). Laboratory breeding experiments with predators from the family of Cleridae showed, that they do not seem to be optimal candidates for biological control, since mass development required for the release in pest-infested buildings was not achieved (Haustein, 2010). Because of the lack of a bulk of natural enemies of *A. punctatum*, the commonly used parasitoid wasp *Lariophagus distinguendus* (Pteromalidae) to control storage pests was released at an infested historic altar in Erfurt (Paul et al., 2008). However, scientific investigations showed, that *L. distinguendus* has no potential for controlling the furniture beetle.

Moreover, it was recommended to focus on the natural enemies of *A. punctatum* for biological control (Steidle et al., 2007). Further practical monitoring (Ott, 2005; Paul et al., 2008; Schöller et al., 2008) revealed, that the clerid beetle *Corynetes coeruleus*, the bethylid wasp *Cephalonomia gallicola* and the braconid wasp *Spathius exarator* are the most common natural enemies of the furniture beetle in historic buildings in Germany.

With the scientific knowledge of Becker (1942) and Lyngnes (1956) the innovative pest control company APC AG from Nuremberg bred the braconid wasp *Spathius exarator* as a commercial biological control method against *A. punctatum* (Auer and Kassel, 2014). After successful mass rearing, first results of laboratory as well as several praxis tests were published (Kassel and Auer, 2015).

This paper will show the practical use of the parasitoid *S. exarator* in two representative churches, selected from currently more than 50 *S. exarator* treated objects. Additionally, as the infested wood in churches often is heavily painted, the difference in the parasitation rate of painted versus unpainted wood is presented in laboratory experiments.

MATERIALS AND METHODS

Life cycle of *S. exarator*

The parasitic wasp *S. exarator* parasitizes its host by piercing its ovipositor directly through the wood surface followed by oviposition onto the furniture beetle larva. After hatching, the larva of *S. exarator* feeds on the anobiid larva and kills the pest insect. *S. exarator* larvae pupate and, after eclosion, adult wasps emerge through a 0.5 mm wide exit hole, which can be clearly distinguished from the 2 mm wide hole of *A. punctatum* (Figure 1).

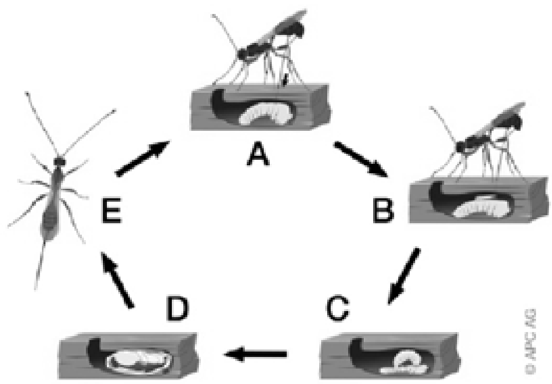

Figure 1. Life cycle of *S. exarator*. The female wasp drills its ovipositor through the wood surface (A), paralyses the *A. punctatum* larva and deposits one single egg on it (B). After development (C), *S. exarator* ecloses through a self gnawed exit hole with an average diameter of 0.5 mm (D).

Release protocol and monitoring

Between five and eight treatments at intervals of four weeks from Mai to October within a period of up to three years were performed by the company APC AG. About 100 breeded wasps of the species *Spathius exarator* were applied for each defined infested area inside the building. In general, a total of 500 wasps was released per object and treatment. Success was monitored by the intensity of the infestation by *A. punctatum* compared to the effectiveness of the parasitic wasps. On exactly defined areas, the new exit holes of wasps and furniture beetles were counted and documented after each treatment. From these data, the reduction of newly hatched *A. punctatum* beetles per year was calculated. Additionally, the ratio of parasitism was determined as quotient of number of new *S. exarator* and *A. punctatum* exit holes per year.

As the lifespan of the wasps without nutrition ended after three weeks (unpublished data), all living wasps, detected before releasing new wasps, had emerged in the church and were termed as second generation. These second-generation wasps were counted and documented.

Sample 1: Schloßkirche Ellingen

With an official expert for wood protection, the infestation of *A. punctatum* and the use of the braconid wasp *S. exarator* were controlled from 2014 till 2016. Treatments were performed by the pest control company APC AG with eight applications in 2014, seven applications in 2015 and five applications in 2016. Monitoring the count of exit holes was done on one infested part of the pipe organ (1 x 0.4 m). At each treatment 400-600 braconid wasps were released at five infested positions in the castle church.

Sample 2: Chapel N.

The pest control company APC AG performed six applications in 2012 and eight applications in 2013 in the Chapel N. From 2014-2015 a yearly monitoring, without treatments was performed. In 2016 one single treatment with monitoring was done. Monitoring with the count of exit holes was made on two benches of the pewage (total 4 x 0.4 m). At each treatment, about 250 - 400 braconid wasps were released at three infested places in the chapel.

Laboratory tests

To studying the effect of paintworks on the ability of *S. exarator* to pierce wooden surfaces and parasitize *A. punctatum*, a laboratory experiment was conducted.

Small test lumbers (5 x 1.5 x 1.5 cm), each equipped with two larvae of *A. punctatum,* were prepared by the Bundesanstalt für Materialforschung und -prüfung (Berlin, Germany). Ten of the test lumbers were painted twice with a commercial wood lacquer (Obi 2in1 Buntlack). Five painted or unpainted lumbers each were stored in a plastic box (23 x 15.3 x 16.5 cm) with air permeable but tightly closing plastic covers. Ten female and five male parasitoids were added to each plastic container. A second charge of wasps were added after a period of about 4 month. Ten unpainted lumbers without any addition of wasps served for controlling the optimal conditions for surviving and developing of *A. punctatum*.

The plastic containers were kept in the laboratory with 18 hours daylight, 6 hours darkness, 21° C room temperature and 70% relative humidity.

The number of new exit holes of *S. exarator* per lumber, representing the number of parasitized *A. punctatum* larvae, was counted monthly. After a period of six month, the test lumbers were splitted using a pry bar to determine the exact number of surviving anobiid larvae.

Data Analysis

For each date of the laboratory experiment, cumulative parasitation rates were evaluated per test lumber. Differences in the parasitation rates between painted and unpainted lumbers were analysed by Mann-Whitney U tests using the software PAST (version 2.17c; Hammer et al., 2001).

RESULTS

In both investigated churches, the number of new exit holes of *A. punctatum* clearly decreased after the release of the parasitoid *S. exarator*.

Sample 1: Schloßkirche Ellingen

Monitoring the defined area of the pipe organ revealed three hatching phases of *A. punctatum* during the three-year treatment period (Figure 2). The first hatching phase was long-lasting between June and October 2014, the second and the third hatching phase happened in July 2015 and 2016 and were well-defined. The number of emerged *A. punctatum* beetles decreased from eleven beetles in 2014 to four beetles in 2015 and to two beetles in 2016, implying an overall reduction of 64% after the first and of

82% after the second year of treatment. The reduction was caused by the parasitism of *S. exarator*, which killed 16 *A. punctatum* larvae in the monitored area during the entire treatment period, as identified by the exit holes of the wasps (Figure 3). The yearly ratio of parasitism was 1:2.75 after the first year of treatment, implying 2.75 emerged *A. punctatum* per emerged *S. exarator* on the monitored areas. This ratio was strongly reduced after the second year of treatment to 1:0.8 and even to 1:0.28 after the third year of treatment.

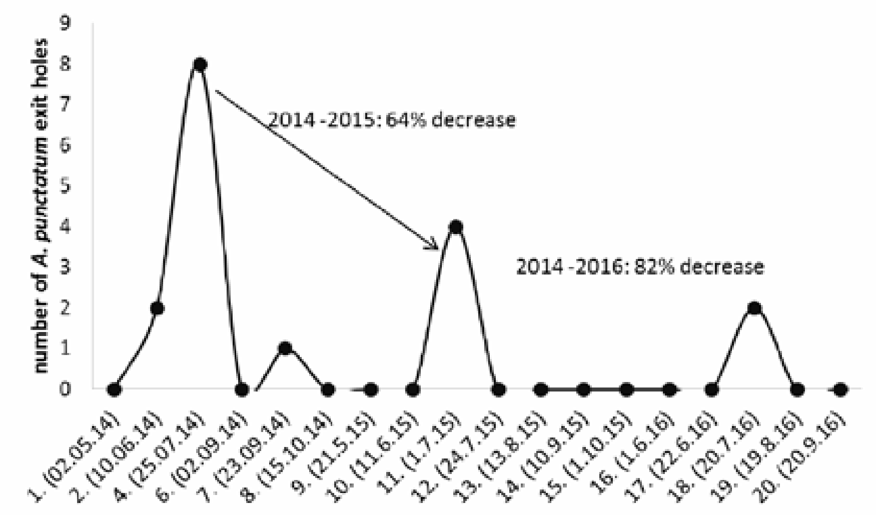

Figure 2. Number of new *A. punctatum* exit holes found at a defined Anobium infested area of the pipe organ between 2014 and 2016 in Schloßkirche Ellingen at the respective dates. Numbers before brackets indicate the number of treatment.

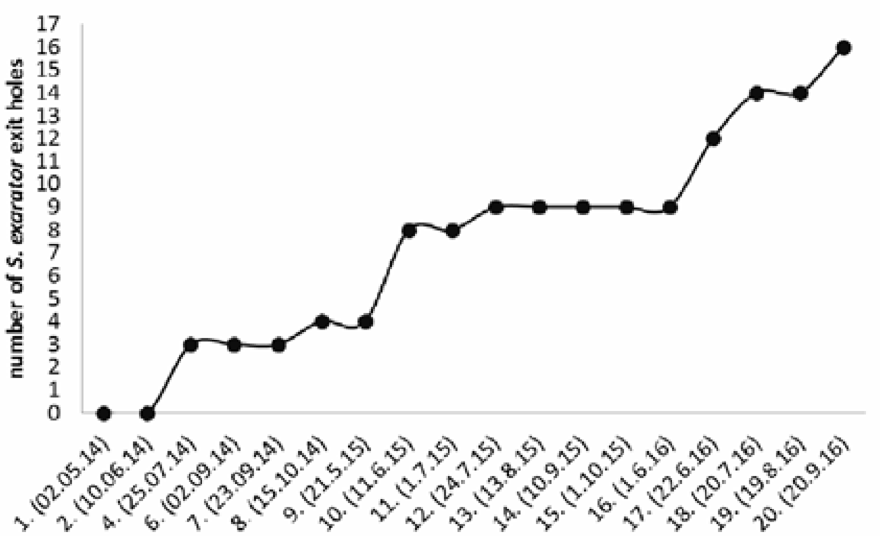

Figure 3. Cumulative number of *S. exarator* exit holes found at a defined Anobium infested area of the pipe organ between 2014 and 2016 in Schloßkirche Ellingen at the respective dates. Numbers before brackets indicate the number of treatment.

On selected infested places of the church, the second generation of *S. exarator* was searched and counted. The results show a continuous reproduction with a total of 148 autonomous developed parasitic wasps within the three years (data not shown).

Sample 2: Chapel N.
Between 2012 and 2013 the chapel N. was treated with *S. exarator*. After the two-year treatment period, yearly monitoring obtained long-term data of the infestation. Figure 4 illustrates the number of new exit holes of infested benches of the pewage. The results show a decrease from 25 exit holes of *A. punctatum* after the first year of treatment to 10 exit holes after the second year of treatment. In the third and fourth year without any additional treatment no new exit holes appeared, meaning a reduction of 100%. However, after two years without any application ten new exit holes of *A. punctatum* had to be recorded. Thus, a single treatment with the release of *S. exarator* was performed directly. As illustrated in figure 5 there was a strong parasitism of *A. punctatum* in the first two years, with a total of 128 parasitized *A. puncatum* larvae. In 2014, an overall number of 22 new exit holes (16 in spring, 6 in autumn) of *Spathius exarator* were found in the defined monitored area, even though no new wasps were released in this year. In 2015 no parasitism was detected in the monitored area, because of the weak infestation of *A. punctatum*. After the release of new parasitoids in 2016, the parasitation increased up to 167 new *S. exarator* exit holes within the monitored area.

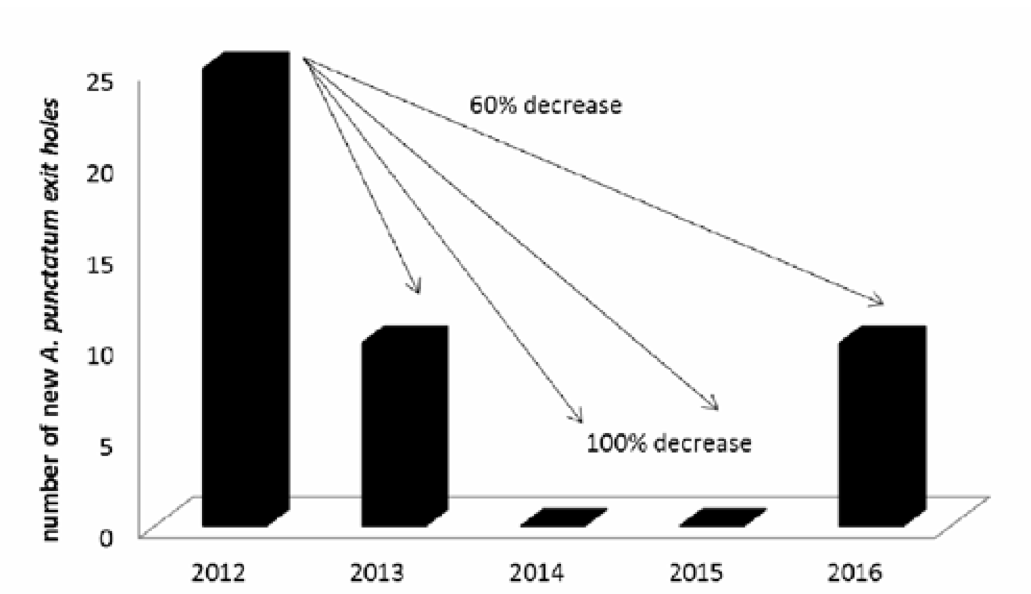

Figure 4. Number of new exit holes of *A.punctatum* over a period of 5 years for a defined area in chapel N. Treatments took place in 2012 and 2013. In the years 2014 and 2015 no treatments were performed. One further application occurred in 2016.

The yearly rate of parasitism in the church N. was 1:0.35 after the first year of treatment, implying 0.35 emerged *A. punctatum* per emerged *S. exarator* on the monitored areas. This rate was strongly reduced to 1:0.18 after the second year of treatment. In the third and fourth year, no beetles emerged. In the fifth year, an increased rate of 1:0.63 was documented.
The numbers of the second generation of *S. exarator* showed a continuous reproduction with a total of 39 *S. exarator* in the first and second year of treatment (data not shown). In the following years, no *S. exarator* were detected, because monitoring was done in late autumn.

Laboratory tests

After a period of six month, all *A. punctatum* larvae were parasitized and killed by *S. exarator* (Figure 6). In none of the pried tests lumbers living anobiid larvae were found. However, there are significant differences in the parasitation rates of painted and unpainted lumbers at three dates: while the parasitation rates in unpainted lumbers reached 100% after two months, the parasitation rates of painted samples is significantly lower in month 2, 3 and 4 of the experimental period (65%; Mann-Whitney U test, p < 0,01).

In the pried control samples without the addition of wasps 100% viable *A. punctatum* larvae were detected (data not shown).

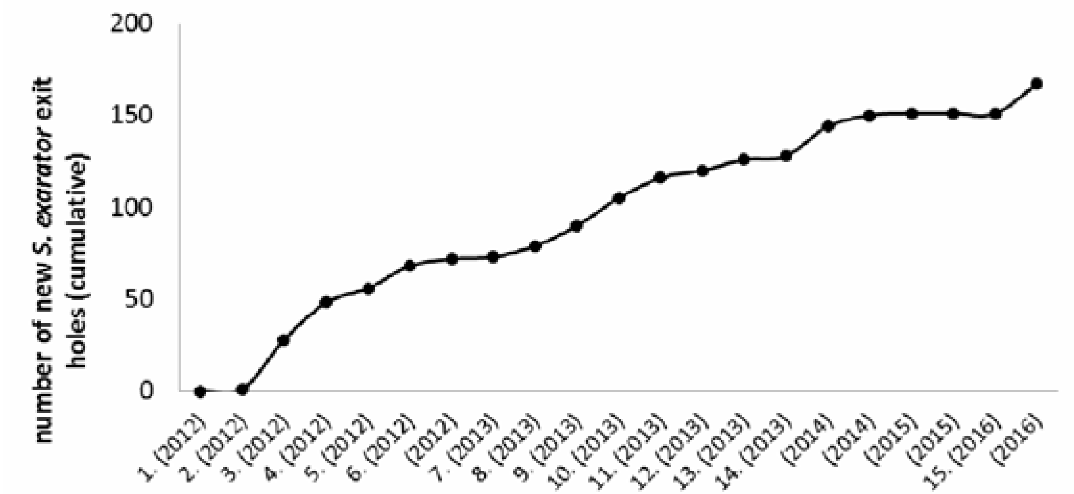

Figure 5. Cumulative number of *S. exarator* exit holes at a defined *A. punctatum* infested area in Chapel N. from 2012 till 2016 in chapel N. at the respective dates. Numbers before brackets indicate the number of treatment. In the years 2014 and 2015, no treatments were applied.

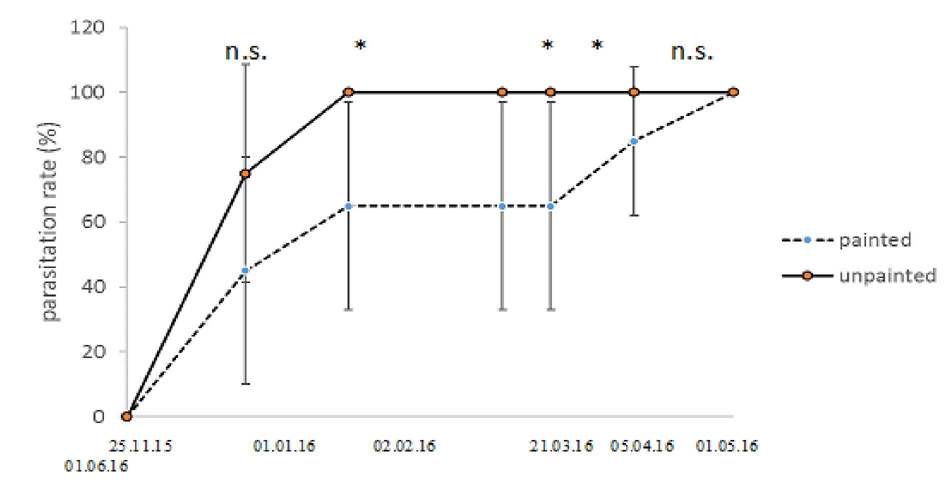

Figure 6. Cumulative parasitation rates of *A. punctatum* larvae in painted and unpainted test lumbers. (*: p < 0,01; n.s.:no significant difference; Mann-Whitney U test).

DISCUSSION

In both *Spathius exarator* treated churches, the number of new *A. punctatum* exit holes decreased over the treatment period. On the defined monitored area of the pipe organ in the Schloßkirche in Ellingen, a decrease of 82% of emerging *A. punctatum* was found over a period of three years. In Chapel N., a decrease of 100% was observed over a treatment period of three years. However, after two years without any treatment, ten new exit holes of *A. punctatum* appeared.

In both churches under investigation, the reduction of *A. punctatum* can be attributed to the parasitation of the released parasitoid wasp *S. exarator*, as indicated by the increased number of new *S. exarator* exit holes and the resulting higher parasitation rates. Monitoring of success in Ellingen revealed a short and well-defined hatching phase of the furniture beetle after the first treatment year. The phenomenon of a shortened anobiid hatching phases also occurred in other *Spathius* treated objects (Auer, personal communication). The number of second-generation wasps prior to the release of new wasps proves the ability of the parasitoids to survive and to develop autonomously within the treated objects. Moreover, the fact that new exit holes were found in chapel N. after an annual phase without any treatments demonstrates the settling of the wasps, thus proving the efficiency of the biological control method.

However, the recurrence of new *A. punctatum* exit holes after a two-year period without any releases also shows the necessity of a well-defined application program.

Monitoring was initially performed by the count of wood dust heaps. However, the number of heaps does not correlate with the intensity of infestation, as wood dust heaps are caused by furniture beetles, as well as by antagonists (e.g. Cleridae), secondary guest insects, vibrations or even swelling/shrinking after change of wood moisture (Biebl 2015).

It is well known that, due to the natural predator-prey relationships, biological pest control does not result in a 100% containment of pest organisms after a few treatment applications (Graf, 1992; Querner, 2017). Instead, a continuous monitoring program with periodic single treatments is strongly recommended. This is even more important in the light of the relatively long development period of *A. punctatum* which can take up to 5 years (Pinniger, 1996).

The results of the laboratory experiment show that *S. exarator* parasitizes furniture beetle larvae in unpainted as well as in painted wood. However, parasitation of painted lumbers seems to be delayed in comparison to unpainted wood. The hundred percent survival rate of *A. punctatum* larvae in the control lumbers without addition of wasps proves optimal laboratory conditions for the surviving and development of the furniture beetle. Further experiments with different materials (e.g. varnish, shellac, leaf gilding) will reveal more insights in the parasitation abilities of *S. exarator* and thus allow more sophisticated programs of application.

Nowadays some individual objects (buildings, artworks, etc.) with historic pesticide contaminated wood are known to have an active infestation with furniture beetles. However, parasitoid wasps are known to react very sensitive to historic pesticides (Querner, 2017). Therefore, in case of suspicion about pesticide contamination, infested wood should be checked using chemical analyses prior to biological control with parasitoids. In case of contamination, the application of braconid wasps could fail and other treatment methods should be applied after careful evaluation.

Fundamentally, the biological pest control results in a reduced infestation and keeps it below a certain minimum level, like longtime experiences from plant protection had shown (Haustein, 2010). After a period of intensive treatment, one or more further annual single-treatments will be recommended, to control the low infestation and hold it under a defined damage threshold.

Although research and application of biological control against the furniture beetle lasted only several years it showed already considerable success. Nevertheless, more practical experience will be required to develop a sophisticated, continuous application program to control the furniture beetle using its natural enemy.

CONCLUSIONS

The results show the promising state of affairs of biological control with the host-specific braconid wasp for *Anobium punctatum* infested objects. In comparison with chemical treatment methods and their negative effects, the success rate of this biological method up to 100% indicates the ability for effective wood preservation in historic buildings, for artworks, etc.

Integrated wood preservation always includes the combination of different methods (Graf, 1992). Elements of the integrated wood preservation are structural-, physical-, chemical- and biological methods. Supporting methods should also be applied in addition to the biological pest control to treat heavily infested removable objects as term of integrated wood protection.

However, more practical experience will be required to optimise treatment and rate of success with *Spathius exarator*, to fulfill the requirements of conservation without further damage of the historic objects by *Anobium punctatum* larvae.

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LYCTUS (COLEOPTERA: BOSTRYCHIDAE): A NEVER ENDING STORY

REINER POSPISCHIL
PMP-Biosolutions, Im Tiergarten 9, 50129 Bergheim-Erft, Germany

Abstract Alien true powder post beetle (Lyctinae) species started to be a concern with the growing worldwide trade after the Second World War. *Lyctus brunneus* arrived in Central Europe 70 years ago and replaced the native species *Lyctus linearis* as lyctine beetle of economic importance. But this situation changed at the end of last century. The author received numerous *Lyctus cavicollis* samples from different places of Germany between 2010 and 2017. Until 20 years ago this species was rare in Central Europe. However, in the meantime it established indoors and outdoors and reached a similar economic importance as *Lyctus brunneus*. Infestation by lyctine beetles was a typical indoor problem in the past and the source of introduction were infested woods. Nowadays outdoor populations of *Lyctus cavicollis* (and in the south west of Central Europe also *Lyctus brunneus*!) may invade homes (for example with firewood).

Key words *Lyctus brunneus*, *Lyctus cavicollis*, *Minthea rugicollis* true powder post beetles

INTRODUCTION

True powder post beetles of the subfamily Lyctinae (Family Bostrychidae) develop in the sapwood of seasoned timbers with high content of starch and are serious pests in furniture industry, in wooden doors, door frames and hardwood floors (Gay, 1953; Gerberg, 1957; Cymorek, 1969b). The economic importance of *Lyctus brunneus* (Stephens, 1830) in Central Europe started with the beginning of the 20th century. Until 1891 *L. brunneus* was very rare in Europe in contrast to the common *Lyctus linearis* (Fabricius, 1792). Up to 1923 this ratio reversed in UK (Hickin, 1960). On the European continent regular imports and fast propagation started after the Second World War with the increase of wood imports and world trade and *L. brunneus* replaced the native species *Lyctus linearis* as lyctine beetle of economic importance (Cymorek, 1969b, 1979). Until the end of the 20th century true powder post beetles which were sent for identification mainly belonged to *L. brunneus*. We had only one case with *L. linearis* from an outdoor wood storage place, an infestation with *Lyctus africanus* (Lesne, 1907) in souvenirs from the Ivory Coast, and one specimen of the genus *Trogoxylon*. But this situation changed dramatically during the past years. Lyctine beetles are now sent frequently for identification and the most prominent species are *L. brunneus* and *Lyctus cavicollis* (J.L. LeConte, 1805). A survey of these two species on their occurrence in Central Europe and habits until the year 2002 is given by Geiss (2002).

MATERIAL AND METHODS

Most samples came from pest management professionals, museums, scientific institutes and zoological gardens. Infestations were examined in detail and expertises were written in four cases. The specimens were included in the reference collection to save them for further studies. Wood samples with larvae were cultured at 25° C / 75% relative humidity. Identification was done according to Cymorek (1969a) and Gerberg (1957).

RESULTS AND DISCUSSION

Twenty three samples of true powder post beetles belonging to the genera *Lyctus*, and *Minthea* were submitted for identification since 2010 from different places in Germany, 13 cases with *L. brunneus*, 8 cases with *L. cavicollis*, 1 case with *L. africanus* and 1 case with *Minthea rugicollis* (Walker, 1858), respectively. Most samples of *L. brunneus* were collected from parquet flooring and some from museums and galleries.

A strong infestation of *L. brunneus* was found 2012 in at least 12 years old doorframes. Reason for this infestation were small North Indian Rosewood tables (*Dalbergia sissoo* Roxb.) from India, which after production were shipped directly to Germany and delivered to the customer, who found wood dust 5 months and exit holes 8 month later in several door frames and doors close to these new tables. Development of the larvae took place in a tropical hardwood inside the door frames. Doors and door frames were removed in autumn 2011 and stored in an unheated garage. The inspection of the infested furniture (door frames, doors and Indian tables) was carried out in February 2012 after a cold period of at least four weeks with temperatures between -10° C and -20° C during the nights. No exit holes were present on the surface of the tables, but beetles, exit holes and living larvae were found in the sapwood in hollow spaces inside the tables. Some areas of the doorframes were heavily infested and gnawing marks of the adult females were found on the surface. More than 100 adults hatched from the doorframes three months later in Mai and another generation followed 5 to 6 months later in October/November.

The common literature specifies that the development stages of *L. brunneus* can tolerate at least -7° C over night except for the eggs and the first larval stage (Cymorek, 1966). However, their high reproduction rate in 2012 after storage under temperatures below -10°C implicates a cold resistance to lower temperatures and longer periods than known from literature. Since the nutrient content in the wood decreases with the age, the risk of infestation by the lyctine beetles declines and more than 10 years old wood is less infested (Cymorek, 1979). The strong infestation of the door frames, the relatively short development time of the larvae and the body length of the beetles (4.5 - 6 mm) indicate that the wood is still susceptible to *L. brunneus* after a period of at least 12 years.

An unusual infestation with *L. brunneus* in railway sleepers during construction of a new underground railway line was inspected in October 2015. The responsibles had already removed 75 heavily infested railway sleepers. Many exit holes were found at the edges of the woods and below the thin surface layer the whole sapwood part was found to be totally destroyed by *Lyctus* over the entire length of the railway sleepers. The leftover few larvae and beetles of *L. brunneus* were used for identification.

According to the Federal Railways standards only beech and oak can be used as wooden railway sleepers in Germany (DBS 918 144, 2007). The standard allows the presence of a low sapwood portion. However, some inspected railway sleepers consisted of up to 20% sapwood.

Outside of the tunnel and on the first 100 meters inside the tunnel, the sleepers were treated with carbolineum against infestation by fungi and wood-destroying insects. Within the tunnel, no treatment with carbolineum was carried out in order to avoid an odor emission in the subway stations. The whole railway line was therefore thoroughly inspected and active infestation was noticed in some sleepers. Another 400 infested railway sleepers were replaced to avoid any risk for the rail traffic and the remaining woods were treated with a water based and odorless preservative.

The infestation with *L. brunneus* began during the manufacture in summer 2013 and the storage of the railway sleepers in the sawmill and later on at an outdoor storage place of the train. The likelihood of a future active infestation of *L. brunneus* in the tunnel was discussed. The temperature on the surface of the railway sleepers was about 14° C in December and January and 18° C during summer time. A development of *L. brunneus* is possible in less than 12 months under these temperature conditions depending on the nutrient content of the wood (Kuehne, 1981). According to Gay (1953) even a continuous temperature of 15° C is still sufficient for the development of *L. brunneus* with an extension of the development time to one year or even more. Vibrations and strong air flows by the rail traffic may also have a negative impact on the larval development and distribution of the adults in the underground tunnel.

An eight years old infestation of railway sleepers by *L. brunneus* was observed several months later in another town in North Rhine Westfalia. Both examples indicate that oak railway sleepers should have less than 10% sapwood and must be thoroughly examined for Lyctus infestation before their use in the railway line. These results indicate that the possibility of an infestation by *L. brunneus* in oak infrastructure of railway sleepers should be considered when planning new underground railway lines.

Eight infestations with *L. cavicollis* were discovered since 2013 in several Federal States of Germany. Beetles were twice isolated outdoors from dry Grape vine stems together with *Bostrychus capucinus* (Linnaeus, 1758) (Bostrychinae) and *Tarsostenus univittatus* (Rossi, 1792) (Cleridae). Another outdoor infestation was found in an outdoor children's playground in a zoological garden in North Rhine Westfalia (Gloyna, pers. com.). Indoor infestations of *L. cavicollis* derived from parquet flooring, door frames, a mahogany door and furniture. Only one sample of *L. africanus* arrived from a museum in Bavaria. *M. rugicollis* was discovered together with *Heterobostrychus brunneus* (Murray, 1867) (Bostrychinae) between picture frames which were sent from the Ivory Coast to a museum of modern art in Hessen. The exit holes of this species are only 0.8 mm in diameter and can easily be missed.

CONCLUSION

The examples demonstrate well that true powder post beetles are a concern for the pest management professionals and for insurance companies as well. Since the year 2000 *L. brunneus* is gradually being replaced by *L. cavicollis.* Other species of the subfamily Lyctinae are rarely found. In the past *L. brunneus* was a typical indoor pest in Central Europe which was brought into new buildings with infested wooden materials or furniture. Now first outdoor populations were detected in the southwest of Central Europe (Geiss, 2015). *L. cavicollis* is found indoors and outdoors and may enter buildings with infested fire wood from the forest. Outdoor populations were found in the south west of Germany, but also in Rhineland-Palatinate and North Rhine Westphalia. The intensified use of untreated wood which is susceptible to *Lyctus* also favours infestations by these species even in places where they were not recognized before (e.g. in underground railway lines or in outdoor children's playground). We already know a lot about *L. brunneus* (Cymorek, 1966, 1969b, 1979; Hickin, 1960; Kühne, 1980, 1981; Pospischil 2012), but information on the requirements of *L. cavicollis* is quite rare despite Geiss (2002, 2015). We need more efforts to understand the requirements of *L. cavicollis* as a drywood pest.

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CRYPTOTERMES BREVIS (ISOPTERA: KALOTERMITIDAE) CONTROL METHODOLOGY ON A HISTORIC BAROQUE CHURCH

EDER FLÁVIO REDE
Rua Júlio Prestes de Albuquerque, 405, Mairinque/SP, 18120 000,
Brazil/Tamanduá Bandeira Controle de Vetores e Pragas Urbanas Ltda.

Abstract The aim of this study is to present a methodology for *Cryptotermes brevis* control in such a fragile, old building environment. The described treatment was undertaken at Igreja Matriz Nossa Senhora da Candelária, a church built in 1780 at Itu city, São Paulo. Its presbytery is made of cedar and was being attacked by *Cryptotermes brevis*. It was decided to treat the structure by using a wettable granules formulation (Optigard LT® - thiamethoxam 25%) sprayed by an aerosol generator, with a needle injector. The application was made by using the holes made by the termites themselves. The product used dosage was 7g/1L of water and the insecticide solution reached about 1 meter distance into termites´ galleries. Twenty litres of insecticide solution were used to treat the infested area, showing that this is also an economic methodology. The used technic provided 100% *C. brevis* control after 2 years. This methodology, using thiamethoxam WG in an aerosol machine, showed effectiveness against drywood termites.

Key words Historic buildings, thiamethoxam.

INTRODUCTION

The West Indian drywood termite, *Cryptotermes brevis* (Isoptera: Kalotermitidae), infestation in historic buildings, mainly the ones from the baroque period, has been long known by the researches, as it is pointed by some authors: "Among the several threats to the historic built heritage, termite is one of the most harmful ones, as it was pointed by Paulo Duarte (1938) and Germain Bazil (1956/58). These insects reach the wood structures, wattle and daub and rammed earth walls, retables, altars, stairs and other wood pieces and many times is only noticed when the damages caused are greatly extended, often there are damages of large extension, frequently compromising the structural stability of the building itself." (Fontes and Filho, 1998).

Most of the infestation occurs due to negligence or lack of knowledge of owners and managers about the Isoptera. (Fontes and Filho, 1998). In Brazil, there is not enough reliable statistics regarding the degradation of cultural property caused by termite. However, a board presented on ICUP 2011, in Ouro Preto, showing a survey performed in baroque churches within the Serro area, in Minas Gerais, pointed that 83% of these buildings were attacked by termites (Silva et al., 2011).

Igreja Matriz de Nossa Senhora da Candelária, completed in 1780 in the city of Itu – the place where this work was developed – represents the social-economic and cultural development of prosperous cities in the colonial period in Brazil.

Since treatments that use toxic gases such as methyl bromide and aluminum phosphide are prohibited under the law, West Indian drywood termite control is done exclusively by means of application of insecticides solutions in Brazil. There are also the aerosol insecticides, marketed in the retail market, which can be also applied for termite control. However, all these techniques are limited as they present low productivity.

In baroque churches, the most difficult part in treating wood for controlling termite attacks has always been choosing the methodology and chemicals properly. Besides the difficulties for reaching 100% of structures, due to barriers and inaccessible confined spaces that limits the application and fast volatilization of insecticide solutions - and extremely high fire hazards due to the use of flammable products in pieces extremely dry and scarified by termites, which significantly increase the combustibility of wood pieces - there is also the enormous risks of damaging paintings, art elements made with natural pigment-based paints, and water and gliding made with the application of thin gold layers. Those factors impaired the use of treatment solutions formulated with oil solvents because they can stain or even remove the finishing. Even in little quantities, as in insecticide formulations of emulsifiable concentrates, which contain oil derivatives in their compositions, solvents can seriously damage paintings and ornaments, which also restricts the use of this type of compound.

This way, the challenge for performing these works that are highly specialized – where error margins are almost null – consist of the exact choice of insecticides, solvents, techniques of preparation for the woodwork, dosing and equipment that are able to ensure the efficacy of treatments without causing (minor) damages to wood components. For that, the insecticide shall be solvent-free, extremely efficient on termite elimination, high residual power and preferentially odorless. The solvent shall be completely inert to not damage ornaments, do not cause discomfort due to strong and long lasting odors in treated environments and to avoid the maximization of fire risks. Using water as a vehicle is the most recommended practice, although it has deleterious effects to wood in high doses. This way, treatments shall be suitable to be fully effective against West Indian drywood termite colonies elimination without damaging art or constituent elements.

The aim of this study is to present a methodology for *Cryptotermes brevis* control in such a fragile environment. The described treatment was undertaken at Igreja Matriz Nossa Senhora da Candelária, an ancient baroque church built in 1780 at Itu city, São Paulo. Its presbytery is made of cedar and was being attacked by *Cryptotermes brevis*. There are several old paintings on its walls, so the usage of formulations containing oil solvents is not appropriate.

MATERIALS AND METHODS

Chancel's woodwork comprises cedar wood, contemporary in this Church's construction. The treatments described as follows were performed considering all the aforementioned restrictions, and it was performed on the wood walls of the chancel, main altar, side altars, art and decorative elements and furniture of Igreja de Matriz de Nossa Senhora da Candelária, Itu, São Paulo, Brazil.

These described treatments occurred from June to September, 2014. By the end of the treatment, 10 visits with insecticides applications were done, totalizing 180 hours of work.

Being a building listed by the National Heritage Department, mechanical interventions, like perforation to allow insecticide application were considered aggressive due to the fact the attack was very disseminated through the architectural complex. Taking all considerations presented for this issue, the woodwork treatment by "extra-low volume" application of an insecticide solution of aqueous base and high concentration of active ingredient was selected. The application was made by using the holes made by the termites themselves, to avoid more damage to the wood. To perform this treatment, the pieces were checked, and the holes were previously identified and marked.

Before applying the insecticide, the internal part of the galleries was cleaned by compressed air jet. This procedure aimed to remove fecal granules inside the pieces, cleaning inside the attacked pieces, removing fecal granules, unclogging galleries and allowing the free flow of insecticide solution.

The structure was treated by using a wettable granules formulation (Optigard LT® - Thiamethoxam 25%), at 7g c.p./L dosage (higher than indicated by the manufacturer for subterranean termite control) sprayed by an aerosol generator (Micronizer®), with a needle injector, that allowed extremely low volumes application. The average flow measured for the injection nozzle was 80 ml/minute. This equipment also works with a low airflow that helps insecticide penetration into the wood.

RESULTS AND DISCUSSION

After treating such woodwork, there was a monitoring period of 24 months to assess the results. During this period, there was no occurrence of attacks by West Indian drywood termites regarding treated pieces. The technique used allowed the full Chancel treatment at Igreja Nossa Senhora da Candelária, and two years after applications, no West Indian drywood termite activity was found in the pieces. Monitoring visits were established every 60 days in the first year and every 90 days in the second year.

The low volume of insecticide solution used for the treatment, about 30 liters, was considered enough for this work, confirming one of the purposes of this test, which was to seek a higher efficacy in treatments and reducing the quantity of chemical agents used. This success is due to the set of methodologies: 1) Using a concentrated aqueous and low volume solution in order to not damage the pieces; 2) Using holes opened by termites as insecticide introduction points to reach the entire internal part of the pieces; 3) Previous clearance of galleries by using compressed air jet; 4) Using the galleries as conduction paths for the insecticide to go inside wood pieces; 5) Simultaneously inoculate both insecticide and compressed air, which enabled the expansion and propagation, and full distribution of insecticides through galleries; 6) Using insecticide with good residual and non-repellent effects.

In December, 2016, a West Indian drywood termite occurrence was diagnosed in one of the chambers of the Chancel. This attack is still under surveillance due to the fact traces are still arising from internal structural timbers of the walls not treated yet.

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A MULTI-STATE STUDY TO ASSESS THE EFFICACY OF CHLORANTRANILIPROLE IN CONTROLLING *RETICULITERMES FLAVIPES* (ISOPTERA: RHINOTERMITIDAE) IN INFESTED STRUCTURES

[1,2]T. CHRIS KEEFER, [3]SUSAN C. JONES, [2,4]EDWARD L. VARGO, [4]PAUL LABADIE, [2]ROGER E. GOLD, [1]CLAY W. SCHERER, AND [1,3]NICOLA T. GALLAGHER

[1]Syngenta Crop Protection, LLC.
[2]Department of Entomology, Texas A&M University, College Station, TX, United States
[3]Department of Entomology, The Ohio State University, Columbus, OH, United States
[4]Department of Entomology, North Carolina State University, Raleigh, NC, United States

Abstract The efficacy of Altriset® 20SC (AI, chlorantraniliprole) in controlling structural infestations of the eastern subterranean termite, *Reticulitermes flavipes*, was assessed in Ohio, North Carolina, and Texas. Prior to chlorantraniliprole treatment, termites were collected from the structure as well as from a grid of in-ground monitoring stations encircling each structure. Microsatellite markers were used to genetically fingerprint each of the termite colonies. The structures and stations continued to be monitored for 2 years after the termiticide treatment. Chlorantraniliprole provided effective structural protection as termite activity in and on the majority of structures ceased within ~1 month or less and they continued to be free of termites for the 2-year study duration; a follow-up spot treatment was done only at one house in Texas at 5-months post-treatment and at one house in North Carolina at 8 months post-treatment. A single infesting colony appeared to be associated with each structure prior to treatment.

Key words Termiticide, subterranean termite, colony, microsatellite.

INTRODUCTION

Chlorantraniliprole is an anthranilic diamide insecticide that has low mammalian toxicity and is considered a least risk pesticide (US EPA, 2008). Chlorantriliprole has a unique mode of action wherein it acts on the ryanodine receptor, where it stimulates the release and depletion of intracellular calcium, which causes paralysis of the target insect (Qi et al., 2011).

Altriset® with the active ingredient chlorantraniliprole is a liquid termiticide used as a sub-soil barrier to control subterranean termites. Chlorantraniliprole is a slow-acting non-repellent termiticide and studies have concluded that this type of termiticide allows for a high rate of transfer of active ingredient among nestmates (Bagneres et al., 2009). The objective of this trial was to evaluate the efficacy of chlorantraniliprole applied at 0.05% in controlling termites (*Reticulitermes flavipes* Kollar) in infested structures in multiple regions and to determine the fate of termite colonies in and around each structure post-treatment.

MATERIALS AND METHODS

All structures were infested with Eastern subterranean termites. At pre-treatment live termites were collected from each infestation point at each structure. At each structure, in-ground subterranean termite stations were installed in two concentric rings around the structure. All stations were monitored on a monthly basis for 3-6 months pre-treatment. All subterranean termites collected pre-treatment were preserved and held for subsequent analyses.

All termiticide applications followed the manufacturer's label instructions. Post-treatment inspections included the exterior of structure and in-ground termite stations. All structures were inspected on or about 1, 2, 3 months, and then quarterly for 24-months post-treatment.

From each station and shelter tube sampled, 10 workers (or all if fewer were present) were genotyped to determine colony affiliation. The DNA of individual workers was extracted and the genotypes for two microsatellite loci, *Rf 24-2* and *Rf 21-1*, were determined following established methods (Parman and Vargo, 2008; Vargo and Parman, 2012). Colony affiliation of groups of collected workers was determined by means of an exact test for genotypic differentiation as implemented in the program GenePop on the Web (Raymond and Rousset, 1995). Groups of workers from the same property that were not significantly differentiated were considered to belong to the same colony. This method has been used to determine colony affiliation in *R. flavipes* after insecticide treatments (Vargo, 2003; Vargo and Parman, 2012).

RESULTS AND DISCUSSION

Ohio Structures

At three of the structures, termite activity ceased within 1 month post-treatment. At the fourth structure, at 1 month post-treatment, several live termites were found among dead termites in a basement above ground station. After that inspection, there was no evidence of live termites found at any inspection.

Ohio Landscape

At each of the four structures, some the same colonies were present pre- and post-treatment. At one structure, the infesting termite colony was eliminated from the landscape during the second year. At three of the structures, termite activity, including the infesting colony, in the landscape persisted for 2 years

North Carolina Structures

Termite activity ceased within 1 month post-treatment at two structures. At the third structure, a spot treatment of chlorantraniliprole was required at 8-months post-treatment due to continued termite activity in damp floor joists. After this treatment, there was no evidence of live termites for the duration of the study. One structure was removed from the study due to a persistent water leak.

North Carolina Landscape

At each of the three remaining structures, some of the same colonies were present pre- and post-treatment in the landscape. At one structure, the infesting colony was eliminated from the landscape with the initial treatment of chlorantraniliprole. At the remaining two structures, the colony initially infesting the structures were last found at 6 months and 18-months post-treatment.

Texas Structures

Termite activity ceased within 1 month post-treatment at three structures. At the fourth structure a spot treatment was necessary in a window at 5-months post-treatment because several live worker and swarmer termites were present. After the spot treatment at 5-months post-treatment, no evidence of live termites was found at this structure.

Texas Landscape

At three of the structures, some of the same colonies were present pre- and post-treatment (Table 1). At three structures the infesting colony was eliminated from the landscape. Colony members were last found in in-ground stations at pre-treatment at two structures and 1-month post-treatment at the third structure. The fourth structure was removed from the study at 19-months post-treatment because the contracted PMP inadvertently performed a spot-treatment with a different termiticide.

Table 1. Results from structures treated with 0.05% chlorantraniliprole to control subterranean termites.

Site	Status at initial inspection		Status at 2 year inspection	
	Termites in structure	Termites in landscape	Termites in structure	Termites in landscape
Ohio	yes	yes	No	yes
North Carolina	yes	yes	No	yes
Texas	yes	yes	No	yes

Table 2. Summary of structures treated with 0.05% chlorantraniliprole to control subterranean termites.

Post-treatment Status	Ohio (4 structures)	North Carolina (3 structures)	Texas (4 structures)
Time to eliminate infesting colony from structure	<1-2 months at 4 structures	<1 month at 2 structures, 9 months at 1 structure	<1 month at 3 structures, 5 months at 1 structure
Spot treatment	0	1	1
Infesting colony eliminated from landscape	1 structure/4	1 structure/3	3 structures/4

Chlorantraniliprole effectively controlled *R. flavipes* infesting houses in OH, NC, and TX and resulted in apparent elimination of the infesting colony from the landscapes at 1 of 4 houses in OH, 1 house in NC, and 3 of 4 houses in TX (Table 2).

The reduction of termite activity in the landscape of the treated structures indicates that chlorantraniliprole is transferred among nestmates. This transfer of chlorantraniliprole has also been confirmed in laboratory trials (Puckett et al., 2012). Chlorantraniliprole has delayed mortality on subterranean termites. This allows donor termites to return to the nests and potentially transfer the active ingredient to recipient termites. Although mortality is delayed, paralysis of the mouthparts is immediate. Therefore, worker termites are unable to continue to feed and cause damage to a structure. Termites that come in contact with chlorantraniliprole have been noted to aggregate (Buczkowski et al., 2012). This increases social interaction, which increases exposure from donor to recipient termites.

CONCLUSIONS

Based on the elimination of termites from the structures, the objective of controlling termites in infested structures was achieved. The reduction of the frequency of termites in the landscape in the current study was noted. Therefore, it can be suggested that to some degree these three behaviors (feeding cessation, aggregation, donor to recipient transfer) are taking place in the field. Based on these facts and the data from the current study, it can be concluded that chlorantraniliprole when applied around a structure for post-construction control of subterranean termites does have some colony level effects in suppression of termites in the surrounding landscape.

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INTER- AND INTRASPECIFIC COLONY INTERACTIONS IN *RETICULITERMES* (ISOPTERA: RHINOTERMITIDAE)

MARK A. JANOWIECKI AND EDWARD L. VARGO
Department of Entomology, Texas A&M University, College Station, Texas, USA

Abstract Subterranean termites are the most economically damaging termites in the U.S. costing $11 billion annually for prevention, treatment, and repair of damage. However, termite colony spatial dynamics are an understudied topic. In previous laboratory studies, termite colonies of different species are aggressive while termite colonies of the same species are passive to each other. Conversely, in field studies, colonies of the same species have not been observed overlapping whereas congeneric colonies foraging ranges were observed overlapping. These seemingly conflicting findings require further research to clarify the inter- and intraspecific interactions of *Reticulitermes*. This project investigates these colony interaction dynamics in east Texas with three native species of *Reticulitermes: R. flavipes, R. virginicus,* and *R. hageni*. Two 14x14 grids of pine stakes spaced 2m apart were established in the Sam Houston State University Center for Biological Field Studies for a total of 392 stakes. These stakes were monitored monthly for active termite foraging and had a hit rate more than 10% in the first month alone. Collected termites will be genetically fingerprinted using microsatellites and colonies will be tracked for the duration of the study. Laboratory colonies will be established from termites at the field site and will be used for behavioral assays using both the petri plate and planar assay methods. Unique species and colonies will be paired in these assays to compare aggressive behavior. Finally, further sampling and investigation will occur at the areas of overlap to compare the behavior of different colonies of termites in the field to the results of the laboratory assays.

Key words Subterranean termites, genetic fingerprinting, behavior, planar assay.

INTRODUCTION

Subterranean termites, including *Reticulitermes*, primarily live in the soil and are the most structurally destructive family of termites in the United States (Suiter et al., 2002). While subterranean termites are urban pests, economically and environmentally, they are important because of their ability to consume cellulose materiel. Annually, Americans spend approximately $11 billion on prevention, treatment, and repair of subterranean termite damage (Su, 2002). Despite their pest status, termites recycle nutrients by decomposing wood and other nutrients back to the soil, thus providing a vital ecosystem service (Thorne and Forschler, 2001).

Although different termite species and colonies of the same species have often been observed in close proximity (Houseman et al., 2001; Deheer and Vargo, 2004), inter- and intraspecific termite colony dynamics and interactions have not been thoroughly studied in *Reticulitermes*. Houseman et al. (2001) investigated distributions of *R. flavipes* and *R. hageni*, but were unable to distinguish unique colonies. They attributed spatial distribution of these two species to temperature and soil moisture content with *R. hageni* preferring to forage in hot, dry periods and *R. flavipes* in cool, damp periods (Houseman et al., 2001). This confirmed an earlier laboratory study by Forschler and Henderson (1995) which found that *R. flavipes* is able to withstand water submersion the longest (LT_{50} of 19.6 h) of the several subterranean termites sampled. Laboratory aggression assays found a behavioral reaction between species of *Reticulitermes* (Thorne and Haverty, 1991), but no aggression between unique colonies of

R. flavipes (Bulmer and Traniello, 2002). In a field study, Deheer and Vargo (2004) found the opposite scenario, with overlapping colonies of different species, but no overlap in foraging ranges of colonies of the same species. This study tracked *Reticulitermes* colonies in Raleigh, North Carolina, U.S., for two years and determined colony identity using microsatellite DNA markers. Generally, they observed that low numbers of smaller colonies of *R. flavipes* never overlapped and fewer, larger colonies of *R. virginicus* overlap with colonies of *R. flavipes* ranges. In two cases, *R. virginicus* foragers were found in the same piece of wood that previously was inhabited by *R. flavipes* (Deheer and Vargo, 2004). *Reticulitermes* species and colony interactions are understudied and contain many discrepancies between laboratory and field trials investigating the same biological aspect. This study will examine these inter- and intraspecific colony interactions to determine what variables influence these boundaries and interactions. To investigate these colony interactions, I intend: 1) to characterize the species and colony boundaries of *Reticulitermes* in a given location, 2) to determine aggression between colonies of the same and different species in laboratory trials, and 3) to observe areas of actual or potential overlap of termite colonies in the field.

MATERIALS AND METHODS

Objective 1. A field site at the Sam Houston State University Center for Biological Field Studies was established in August 2016. Two 2-meter grids of pine stakes were created to provide similar clarity to previous studies that used a 2 meter and 2.5 meter grid, respectively (Houseman et al., 2001, Deheer and Vargo, 2004). Samples were collected monthly from any stake with active termite feeding, as done in DeHeer and Vargo (2004). Species will be determined genetically (Szalanski et al., 2003) for each sample. Colonies will then be characterized with previously established microsatellite DNA markers (Vargo, 2000; Dronnet et al., 2004) in a multiplex to differentiate and track colonies over time. The data obtained will be compared to previous studies (Houseman et al., 2001; Deheer and Vargo, 2004) and live termites collected will be kept in the laboratory for the next objective.

Objective 2. Although *R. flavipes* was previously shown to not be aggressive to other *R. flavipes* colonies (Bulmer and Traniello, 2002), this study assumed colonies were unique based on distance since it was before genetic markers were commonly used in termites. Now, with updated methods and in a different part of the species range (Texas rather than Massachusetts), I will examine interactions between termite colonies of the same and different species using the agonism assay outlined in Bulmer and Traniello (2002) as well as the planar areas from Chouvenc et al. (2011). Briefly, the Bulmer and Traniello (2002) method introduces 20 worker termites of each colony in an arena lined with moist filter paper and assesses mortality and injury at 24 hours after introduction. Termites are differentiated by feeding them dyed filter paper in advance of the experiment. This temporarily dyes the termite gut and is visible through its exoskeleton. Controls will consist of both 40 workers collected from the same wooden stake and combinations of workers collected from different stakes that were genetically determined to be the same colony. The second method in Chouvenc et al. (2011) has a modified arena consisting of soil sandwiched between two sheets of Plexiglas. This method has shown to have generally higher survival rates and provides a more realistic setup and allows the termites to tunnel (Chouvenc et al., 2011). These methods will be replicated with as many samples as possible obtained from the first objective.

Objective 3. After the termite colonies have been mapped out for each field site, the areas of overlap or near overlap will be investigated in greater detail. Additional wooden stakes will be added in these areas to make a 0.5 m grid that will provide more clarity of the overlap and ranges of each colony. Any additional wood debris present in these areas will be examined for potential foragers of either colony. Observations will be made at these overlapping areas as to how termites avoid or conflict with other colonies of the same or different species.

RESULTS

In the first five months at the field site (September 2016 to January 2017) there was an average of 48.4 active termite monitors per month, greater than 10% of the total stations. Currently, the genetic analysis is ongoing, but it appears that the abundance in the field station is directly correlated with the moisture in each site. The total number of active stations is approximately stable over the first five months, but the abundance in the two plots vary over time (Figure 1).

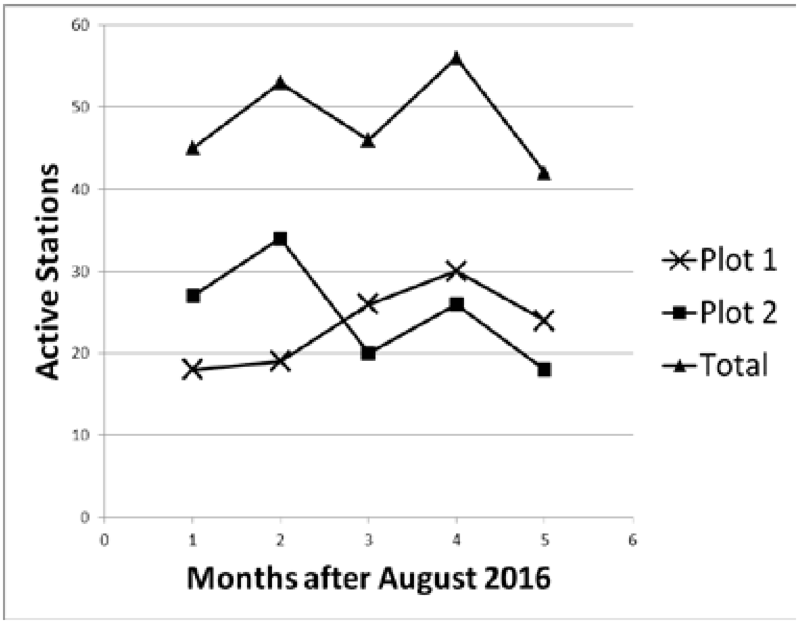

Figure 1. Number of active stations over the first five months for Plot 1 and Plot 2.

DISCUSSION

While much is known regarding termite biology, there are still many knowledge gaps that require further investigation. Although studies have scratched the surface of *Reticulitermes* colony interactions in the lab and field (Thorne and Haverty, 1991; Houseman et al., 2001; Thorne and Forschler, 2001; Bulmer and Traniello, 2002; Deheer and Vargo, 2004; Chouvenc et al., 2011), a consensus and understanding of the interactions is lacking. This project seeks to investigate these basic interactions between colonies of *Reticulitermes* of the same and different species to produce the foundation required to further study the mechanisms that mediate these interactions.

Termites are very significant economic pests, costing billions of dollars annually in the U.S. (Su, 2002). Colony interactions, as investigated in this project, have the potential to be applied to future pest management strategies. It has been suggested by Thorne and Haverty (1991) that the ability to understand and breakdown cues that termites use to distinguish colony members from outsiders could be manipulated to cause a “civil war” in the colony, and thus destroy the colony. Interfering with the cues that determine whether a colony fights or avoids another colony could also be used as a tool to control pest species.

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BUILDING CONSUMER TRUST WITH INTERNATIONAL PEST MANAGEMENT CREDENTIALING

CHRISTOPHER J. STELZIG
Entomological Society of America, Annapolis MD, 21401 USA

Abstract In 2004 the highly-successful Associate Certified Entomologist (ACE) program was developed by the Entomological Society of America (ESA) Certification Corporation (ESACC) as a means of providing a professional credentialing pathway for pest management professionals (PMP) who may not have the formal academic training of an entomologist. The ACE program and its partner program, the Board Certified Entomologist (BCE) are the two most dominant personal credentialing options for PMPs in the United States and around the world. However, they are not the only way that individuals and businesses in the pest control industry can differentiate themselves from the competition. From business credentials like QualityPro–a program offered by the National Pest Management Association (NPMA)–or the Confederation of European Pest Management Association's (CEPA) CEPA-certified directory, to third-party credentials such as Angie's List, today's pest management customer is faced with a dizzying array of credentials to sort through. This paper serves as an exploration of the various pest management-based credentials that exist, the reasons for personal and business credentials, and offers a deeper exploration of the ACE and BCE programs.

Key words Professionalism, certification, ACE, license.

INTRODUCTION

Professional credentialing is one proven way for PMPs to differentiate themselves from their competition. Research has shown that PMPs who hold a personal pest management credential feel high levels of personal satisfaction, feel more respected in the industry, enjoy a feeling of enhanced technical performance, have increased employment opportunities, and can assist their employers in securing more business contracts (McKinley, 2012). Studies have also shown that in general, certification can enhance salary potential, with one study indicating that overall individuals with at least one credential earned 18% more in salary (ICE, 2015).

Professional credentialing has long been an important component of many diverse industries, including automotive mechanics, computer technicians, lawyers, and medical doctors. Historically, entomology has been largely devoid of professional credentialing programs, though not for want of discussion and debate. The desire to create professional standards has often been a heated topic of conversation among entomologists. The controversy arises at least in part due to the inherent push-pull relationship between scientifically-rooted entomology and technologically-based entomology. (Smith, 1989). As evidenced by steadily growing ranks of certified individuals, operationally, today's ACE and BCE programs are far more recognized as a legitimate professional credential by ESA members and others. Both programs are directed by the ESACC Certification Board (CB), managed by the ESA staff, and overseen by the ESACC Governing Board.

Voluntary credentialing began to emerge as a business practice in the pest management industry in 1936 when the Purdue Pest Management conference–recognized as one of the first such programs in the United States–held its first conference. At that time, ESA leadership was considering what responsibility it had to develop a credentialing program. After decades of debate and discussion, ESA appointed a Standing Committee on Professional Training, Standards, and Status for Entomologists (Reed, 1964). The program's development took the next 13 years, and in 1968 the first certification program in the industry was launched with the adoption of the American Registry of Professional Entomologists (ARPE) program. At its height, approximately 25% of all ESA members were ARPE-certified (Smith, 1989) (Hines, 1975) (Sabrosky, 1969). In 1992, ARPE morphed into the BCE certification, which remains an active program today. Then, as now, the primary purposes of the program were to document professional training, promote ethical standards, and support the visibility of the profession (Cilek, 2010).

From the genesis of the first American entomological societies, there has been a call to better define the profession and professionalism of entomology. The American Association of Economic Entomology (AAEE)–one of the three Societies that merged to become today's ESA–stated in its founding documents "The membership shall be confined to workers in economic entomology. All economic entomologists employed by the general or State Governments or by the State Experiment Stations or by any agricultural or horticultural associations, and all teachers of economic entomology in education institutions may become active members of the association by transmitting proper credentials to the secretary." In other words, membership in AAEE was based on employment differentials rather than interest in the subject or academic credentials. Members were permitted to join who did not meet the standards, but they were only admitted as associate members (Osmun, 1989).

MATERIALS AND METHODS

The word "entomologist" has different definitions for different people. Popular culture would accurately describe an entomologist as a person who studies insects. Historically the ESA has felt that a minimum of a bachelor's degree in the science would be required to attain the title (Palm, 1958). In 1971, in an attempt to quantify the training required to attain entomologist status, ESA and ARPE created these two definitions.

Professional entomologist is a person who by academic training and/or practical experience has acquired a working knowledge in the science of entomology, who is recognized as an entomologist by entomologists, and who is engaged in entomological work as a substantial portion of his career.

Certified entomologist is an entomologist whose record has been reviewed by the committee on Professional Training, Standards and Status of the ESA and who is considered by that Committee to meet its requirements for professional entomologist.

Those definitions stood for the next 42 years. In 2013, the CB modified the ACE Code of Ethics in such a way as to clearly distinguish that, in the views of the Society, an entomologist is a person who has a minimum of a Bachelor's degree in entomology, or a closely-related discipline. All other practitioners certified by the Society are "associate certified," a continuation of the phrasing from the program's founding.

Regardless of business sector or industry, there are four primary types of credentialing and each serves a unique market need. They are:

1. Certificate program is one in which a person, after meeting minimum qualifications, voluntarily submits themselves to an organization for a determination of skills, competency, and/ or knowledge. A certificate of accomplishment is generally awarded.

2. Certification programs are also voluntary programs that are similarly structured for individuals, but they differ from certificate programs in that they also require ongoing proof of

training and competency to continue to hold the credential. Often this is evaluated through the submission of continuing education units (CEUs).

3. Accreditation programs are best defined as certification programs for businesses or organizations.

4. Licensure can define either a certificate, certification, or accreditation program, but the key difference is that licensure is mandatory, as defined by some type of a government agency. (Rops, 2011). Most jurisdictions regulate applicators that operate within their borders with a few organizations, such as the Association of Structural Pest Control Regulatory Officials (ASPCRO) serving as quasi-consultative bodies.

From a business accreditation perspective, NPMA's QualityPro was the first dominant business accreditation program to emerge. A 2004 NPMA pest management industry strategic sought to find a way to "raise the level of professionalism in pest management." Out of this plan grew QualityPro as a way to define professional business practices for the industry. The program serves only the North American marketplace and as of this writing there are 518 QualityPro Certified companies with 98% of them (508) based in the U.S.A. NPMA also offers service credentials for QualityPro certified companies. GreenPro (started in 2009) is held by 190 companies, QualityPro Schools (started in 2007) is held by 99 companies, and QualityPro Food Safety (started in 2007) is held by 35 companies. NPMA reports erratic growth for the first decade of the program, but a relatively steady growth of approximately 8.5% since 2012. Other U.S.-based accreditation programs, such as the IPM Institute's GreenShield are focused on specific aspects of pest control.

In 2015, CEPA launched EN16636 'Recommendations for the Provision of Pest Management Services' as well as implemented CEPA-Certified – a scheme to create awareness of and a desire for EN16636. They are both based on the development of a European standard for basic competencies for the operation of pest management companies in the European Union. The standard is an accreditation program and is only available in Europe.

Numerous licensing programs exist for both businesses and individuals. Additionally, a wide array of third-party review sites offer customers additional assurances of contractor competency. However, many of the review sites are not moderated and are thus subject to the capricious whims of the marketplace. Social media websites such as Yelp, Facebook, Porch, Houzz, Angie's List, TalkLocal, Judy's Book, Thumbtack, and HomeAdvisor are all places where customers can review, "like," attach comments or opinions to, or otherwise influence other people's perceptions and knowledge of a business or service individual's profile. These services serve a marketplace need, but are often are limited in their effectiveness to merely providing subject and anecdotal evidence, rather than certified and documented training and experience. However, at least partially due to low entry barriers to join as a member, social media platforms offer huge audiences and can serve as rapid change agents for businesses who receive either favorable or unfavorable reviews. Nearly 1.8 billion people use Facebook at least monthly and at least 1.18 billion people self-identify as daily users. 2013 data recorded 4.5 billion daily "likes" on the platform which is a 67% increase over the previous year (Zephoria, 2017). All of this indicates a vast, growing, and active marketplace with the potential to disrupt business models if not managed carefully.

DATA ANALYSIS

While many pest management certificate programs exist throughout the world, ACE and BCE are the only global and widely-accepted personal certification programs in the industry. BCE has been international from the program's earliest days. ACE began as a U.S.-only program; in 2014 ESA introduced the ACE International (ACE-I) program for non-US based PMPs. The U.S. version of the ACE-I program has grown an average >30% per year since introduction in 2004. (Figure 1).

Today the BCE program requires an advanced academic degree in entomology or a closely-related discipline to be considered as an applicant. Those without a degree in the science need to apply for ACE if they wish to become certified by ESA. In each case, however, the application process is similar and includes an application, letters of professional reference, documentation of work history, proof of professional competency, and approval by a program administrator. Once the application is approved, the applicant is permitted to take their examination(s) within one year. A key distinguisher of the ACE and BCE programs is that they are not in and of themselves a preparatory course. Instead, participants engage in self-study and take their examinations when they feel that they are sufficiently prepared. All examinations are administered by a proctor (generally an ACE or a BCE). Once an examinee attains a passing score, they do not need to retake that test again to maintain their certification. Continuing competency is documented via submitted CEUs at three-year intervals.

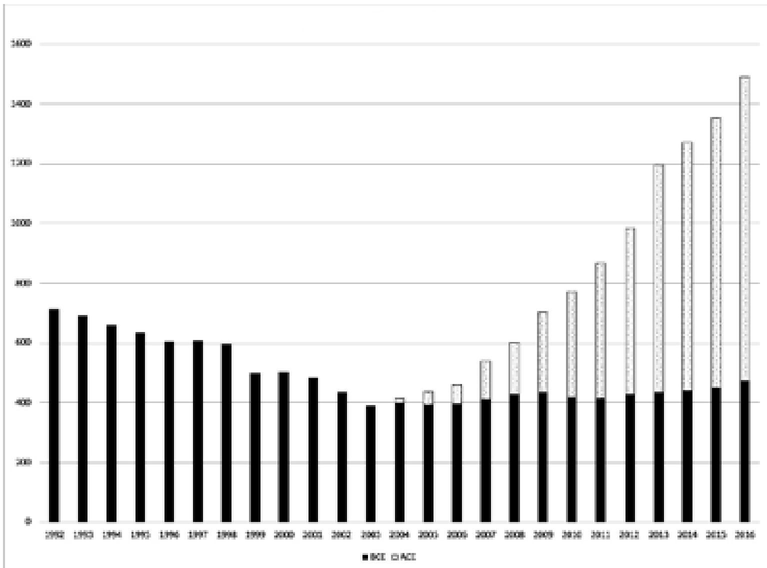

Figure 1. Growth of ACE and BCE since 1992.

CONCLUSIONS

Based on market adoption as a metric for market demand, the growth rate for ACE and ACE-I indicate that this is a credential with long-term potential for widespread adoption. The pest management marketplace is large and growing. In the U.S. alone, the industry is estimated to be approximately $7.8 billion annually with an estimated 20,000 firms in place and an estimated workforce of over 74,000 (GIE, 2017). The largest 100 firms in North America (by sales volume) posted an additional $304 million in sales in 2015 over 2014 and currently employ more than 57,000 individuals (BLS, 2017). While not all companies or individuals are qualified candidates for voluntary credentialing programs such as those described herein, the market for credentialing is clearly growing.

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CONTROL OF *PERIPLANETA AMERICANA* AND *BLATTELLA GERMANICA* OOTHECAE USING CHEMICAL AND BIOLOGICAL INSECTICIDES

[1]MARIAH V. BAGGIO-DEIBLER, [2]ROBERTO M. PEREIRA, AND [3]MARCELO DA COSTA FERREIRA

[1]Biologist, PhD in Agronomy (Agricultural Entomology), Department of Plant Protection, UNESP, Jaboticabal, SP, Brazil

[2]Agronomist, Associate Research Scientist, Entomology & Nematology Dept.,University of Florida, USA

[3]Agronomist, Professor, Department of Plant Protection, UNESP, Jaboticabal, SP, Brazil

Abstract *Periplaneta americana* and *Blattella germanica* are pests of great importance in urban areas, mainly due to their role as vectors of pathogens and capability of causing allergies. The interspecific differences between these cockroaches can lead to different results in their control. No chemical (liquid/powder application) or biological products have been successful in controlling pre-embryonic stages of cockroaches and little is known about their residual effect on nymph eclosion. The objective of this study was to analyze the potential of different synthetic chemical insecticides and entomopathogenic fungi in controlling oothecae of *P. americana* and *B. germanica.* Two experiments were conducted with the objective of determining nymphal mortality. First, the oothecae of *B. germanica* and *P. americana* were submitted to the following treatments: T1 – control, T2 – indoxacarb (0.03g/10mL) T3 –fipronil (62µL/10mL), T4 – lambda cyhalotrin (63µL/10mL) T5 – imidacloprid (31.70µL/10mL) and T6 – imidacloprid + β-cyfluthrin (21µL/10mL); and then the percentage of dead oothecae, the total number of nymphs eclosed and dead nymphs were compared. In the second bioassay, the same variables were analyzed after submitting oothecae to the following treatments: T1 – control, T2 – deltamethrin 0.05% (0.037g/0.015m^2), T3 – thyme oil 4.1% (0.046g/0.015m^2), T4 –imidacloprid + β-cyfluthrin 0.075% (1.22mL/0,015m^2), T5 – isolate 428 of *Beauveria bassiana* (2.6 x 10^8 conidia/0.015m^2) and T6 – isolate 2575 of *Metarhizium anisopliae* (1.7 x 10^8 conidia/0.015m^2). Each treatment consisted of 50 oothecae with 5 repetitions. Data were compiled and subjected to analysis of variance by the F test in a factorial (6 treatments X 2 cockroaches) and the means compared by the Tukey test ($p \leq 0.05$). *M. anisopliae* was the most effective strategy to control oothecae of both cockroach species. The chemical active ingredients fipronil, lambda cyhalothrin, deltamethrin and imidacloprid + β-cyfluthrin were most effective in controlling nymphs of these cockroach species.

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CONTROL OF TWO POPULATIONS OF *PERIPLANETA AMERICANA* BY SPRAYING CHEMICAL AND BIOLOGICAL INSECTICIDES IN URBAN SEWAGE

[1]MARIAH VALENTE BAGGIO-DEIBLER AND
[2]MARCELO DA COSTA FERREIRA

[1]Biologist, PhD in Agronomy (Agricultural Entomology), Department of Plant Protection, UNESP, Jaboticabal, SP, Brazil
[2]Agronomist, Professor, Department of Plant Protection, UNESP, Jaboticabal, SP, Brazil

Abstract The applicability of entomopathogenic fungi for *Periplaneta americana*'s control in urban sewage is still not well studied, even though its importance as an urban pest capable of disease dissemination being a constant. Females have an important role on dissemination of the species due to their oviposition capability and yet majority of researches focus on male control. The objective of this work was to verify the efficiency and viability of *Metarhizium anisopliae* and *Beauveria bassiana* fungi isolates applied by cold spraying on the control of two populations of *P. americana* females in urban sewage in comparison to chemical control. Twenty sewage galleries in Jaboticabal, Brazil and in Piracicaba, Brazil were used for the following treatments: T1- control (no application), T2 - Tween 80® 0.1% solution (suspension vehicle), T3- 3 x 10^8 conidia/mL suspension of JAB68 of *M. anisopliae*, T4- 3 x 10^8 conidia/mL suspension of IBCB35 of *B. bassiana*, T5- Insecticide with lambda cyhalothrin (2.5%) with recommended concentration (10mL/L). The applications were conducted by spraying a volume of 373.3 mL/m^3 with cold-air nozzle sprayer AT1000 and it was evaluated: total mortality, mortality by fungus, extrusion, time of death by the fungus and fungi viability before and after application. It was found that the isolate JAB 68 of *M. anisopliae* is more effective in controlling *P. americana* females in urban sewage when compared to IBCB35 of *B. bassiana*, and it doesn't differ from chemical control after second application. Conidia of this fungus are still viable after fogging, with 97% viability.

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GENETIC ANALYSIS OF TWO POPULATIONS OF *PERIPLANETA AMERICANA*

[1]MARIAH VALENTE BAGGIO-DEIBLER, [2]MARCELO DA COSTA FERREIRA, [3]ANDRESSA DE SOUZA-POLLO, AND [4]MANOEL VICTOR FRANCO LEMOS

[1]Biologist, PhD in Agronomy (Agricultural Entomology), Department of Plant Protection, UNESP, Jaboticabal, SP, Brazil.
[2]Agronomist, Professor, Department of Plant Protection, UNESP, Jaboticabal, SP, Brazil
[3]Agronomist, Assistant of Academic Suport IV, Department of Preventive Veterinary Medicine and Animal Reproduction, UNESP, Jaboticabal, SP, Brazil
[4]Biologist, Professor, Department of Applied Biology, UNESP, Jaboticabal, SP, Brazil

Abstract The control in the field of insect large geographical distribution as *Periplaneta americana* species, that is a mechanical vector of several pathogens to man, may be affected by genetic variability among their populations. They can be segregating, resulting in differences in susceptibility to the control agent. One way to get answers on genetic diversity of populations of some species studied, such as American cockroach, with lower cost and faster results is through the use of Random Amplification of DNA Polymorfic (RAPD) technique. The objective of this study was to determine the genetic distance between two populations of *P. americana* species. Twenty females of each area were selected, corresponding to F1 generation of Jaboticabal (Brazil) and Fn generation of Piracicaba (Brazil). Each adult had their third right leg removed for DNA extraction, followed by RAPD-PCR amplification. Out of the 192 tested primers, 14 were considered the most informative and used on the RAPD analysis. The RAPD bands in each sample were coded in binary matrix by PAUP software. Molecular Analysis of Variance (AMOVA) was performed and it was calculated the fixation index (Fst). There were 128 bands generated, of which 113 (88%) were polymorphic. Populations were separated in two groups corresponding to their locations. It was observed more subdivisions in Jaboticabal than in Piracicaba. The existing variation corresponded to 17.69% between cities and 82.31% within populations. The Fst of Jaboticabal was equivalent to 0.108 and 0.074 of Piracicaba. The gene flow (Nm) was higher in Piracicaba (3.15), followed by Jaboticabal (2.05) and between cities (1.16). Data show low differentiation within Piracicaba's population, moderate in organisms from Jaboticabal and high levels of interpopulation segregation, suggesting that the populations are different depending on their geographic location.

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COCKROACH MANAGEMENT, A MUNICIPAL APPROACH. CITY OF MADRID (SPAIN)

[1]CÁMARA JOSÉ-MARÍA, [1]TORRES PILAR, [1]REY-CARAMÉS CLARA, [2]BLÁZQUEZ ÁNGEL, [2]ÁLVAREZ-RUIZ SUJEILI, AND [2]CALDERÓN LUIS

[1]Carretera de La Fortuna, 33; 28054-Madrid (Spain) / Environmental Health Dpt., Health Madrid (City Council of Madrid).

[2]Calle Mar Mediterráneo 1, 28830, San Fernando de Henares, Madrid (Spain) / Rentokil Initial Spain.

Abstract Urban pest management in Spain, which involves prevention and control, corresponds to the municipalities. The data, key elements and conclusions of a continuous analysis of 10 years of the Madrid Municipality pest control program against sewer system cockroaches (*Blatta orientalis* and *Periplaneta americana*) are presented. Although most resources are focused on inspection and treatment of the municipal sewage system, integrated cockroach management requires other additional areas of work: (1) Information, education and citizen's responsibility; (2) Careful, continuous monitoring and treatment plan of the municipal sewer system; (3) Powerful data collection and integrated management of citizen complaints; (4) A comprehensive and well-designed action plan including environmental city monitoring to manage cockroach habitats; (5) Specific measures against *Periplaneta americana*, 'foreign' insect in Madrid but with increasing distribution in Spain and other Mediterranean countries in recent years; (6) Data management using the Geographic Information System tool of the city of Madrid; (7) Strengthen the research and collaborations with University and other research institutions; (8) Strong cooperation with pest control companies in the city. Overall, the continuous data analysis along the last 10 years has shown a decrease for the whole city (real citizen complaints). City districts revealed similar behaviour, only four of the twenty-one City Districts had experienced a very slight increase of the number of citizens' complaints (year 2016) with respect to the previous year 2015, but still showed a very strong and continuous decrease trend for the 10-year-period (-25,44 % reduction average; -17,82 to -36,16). Focusing on *Periplaneta americana*, data showed an increase in the last four years, the number of interventions in 2016 were 136 % higher than in 2015. Data analysis conclusions: (A) The sewer cockroach prevention and control require a strong involvement of municipal departments and resources. (B) Citizen's commitment and involvement in their private areas are critical. (C) It is necessary to implement new and effective application techniques and products in the sewer system compatible with chemical and environmental safety (i.e. effective and affordable baits). As a result, the application of these global approach procedures in Madrid Municipality, according to program indicators, has reduced notably cockroach infestation. Banning of conventional insecticide spraying practices in the Municipal sewer system has avoided the dispersion of cockroaches to alternative sewer system habitats (i.e. municipal or private subterranean infrastructures, etc.). (D) *Periplaneta americana* is the cockroach species with higher growth potential, therefore a strategic proactive plan to control and avoid the colonization of new areas must be designed and implemented as soon as they are detected or suspected. In this respect, the cooperation between municipal administrations and private property and pest control companies are entirely crucial.

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MULTIRESISTANT GERMAN COCKROACHES IN RUSSIA

OLGA EREMINA, VERONIKA OLIFER, AND ILKHAMIA IBRAGIMKHALILOVA

Scientific Research Disinfectology Institute, Nauchny proyezd, 18, Moscow, Russia.

Abstract From 2014 to 2016, in Moscow, Kaluga region, and Ural (Ekaterinburg) we have been monitored the susceptibility of male German cockroaches to topically applying 1µl insecticides of different structure. It was found that all cockroach populations are most resistant to pyrethroid insecticides, especially cypermethrin. Tolerance (3-13×) to pyrethroids in households and in the Moscow Zoo were registered, high resistance (140-300×) – in hospital, student dormitories or restaurant, and super-resistance we found in food market in Obninsk (>4000×). A number of the cockroach populations exhibited resistance to fipronil (10-54×). Tolerance or resistance to organophosphates (3-15×) are found in six Moscow, one Obninsk, and four Ekaterinburg cockroach populations. At the same time, these populations exhibit weak tolerance to carbamates (1-4×) and susceptibility or weak tolerance to neonicotinoids (0.7-3.5×) and avermectins (1-3×). A statistically significant delay in the expression of poisoning symptoms is found in all the studied populations upon contact male cockroach with the glass surface treated with 20 µg a.i./cm^2 of cypermethrin, chlorpyrifos, propoxur, and fipronil. A delayed insecticidal effect on resistant cockroaches or complete insecticide ineffectiveness has been established. In this regard, it is necessary to determine the level of insect resistance and develop the individual insecticide rotation scheme for each location. Analysis of the data obtained makes it possible to assume that we are dealing with the multiresistance accompanied with different genetic mechanisms - the occurrence of *Kdr*- and *Rdl*-mutations, changes in the permeability of cuticle and in the activity of enzyme systems, etc. Moreover, delay in cockroach poisoning after feeding baits based on isoxazolines, substances of new chemical class, having never been used in Russia, apparently demonstrates an increase in the level of detoxifying enzymes, in particular monooxygenases. In our situation, we can recommend to introduce into IPM system a number of baits based on aminohydrazones (hydramethylnon), avermectins (aversectin C and abamectin), and IGR (hydroprene which controls cockroach reproduction). It is necessary to use boric acid-based baits and agents with mechanical type of action - sticky traps, diatomaceous earth, which causes desiccation of cockroaches and their sufficiently rapid death.

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PERFORMANCE OF GEL BAIT INSECTICIDE IN THE SEWAGE SYSTEM OF BARCELONA FOR COCKROACH CONTROL

[1]FRANCO, S., [1]SIMIC, T., [1]VALSECCHI, A., [2]BUENO, R., [2]GARCÍA-HOWLETT, M., [2]LACOMBA, A., AND [1]PERACHO, V.

[1]Public Health Agency in Barcelona
[2]Lokímica, S.A.

Abstract The Public Health Agency of Barcelona (ASPB Catalan acronym) is in charge of the surveillance and control of pests, such as cockroaches, in public areas and sewage system of the City. As in other Mediterranean cities, *Periplaneta americana* has colonized the sewage system of Barcelona in last years, causing citizen complaints, economical costs and potential sanitary implications. Therefore, the control of this species in public environments is mandatory for multiple reasons. As 55% of Barcelona's sewage system can be visited and inspected the City counts with singular conditions to fulfil an Integrated Pest Management (IPM) based program. In order to test the efficiency of a gel bait insecticide in different situations of the sewage system, a comprehensive study was carried out from June to November 2016. According to current biocides registration in Spain and the gathered experience of routine control plans in the city, the product Ecorex Gel One® (Cypermethrin 40/60 at 1%) was selected for the assays. Four different treatment types with five replicas each (20 points) and two control points were chosen. Of the total 22 points 16 corresponded to accessible underground sewage system and 6 points to non-accessible underground sewage system. Accessible underground points included a 50m section with 3 manholes, inspection was made at underground level when possible, the difference in treatment consisted on whether the gel bait was applied at manhole level, underground level or at both levels. Each point was monitored every two weeks and information of cockroach activity (visual counting and trapping), as well as environmental humidity and temperature, was recorded every visit. Biocide was applied whenever activity was found, and an influence perimeter area was established where no other control actions could be taken. The efficacy, variation in efficacy associated with the point of gel bait application, as well as some interesting information about the ethology of *P. americana* in this urban habitat will surely be of great interest for future IPM sewage system cockroach control plans.

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INCIDENCE OF PATHOGENIC BACTERIA ON COCKROACHES COLLECTED IN DIFFERENT MUNICIPAL BUILDINGS OF BARCELONA

[1]MONTALVO T., [1]BARAHONA L., [1]PEPIÓ M., [3]BUENO R., [3]JARQUE J., [2]RAMÍREZ B., [2]SABATER S., [2]PLANELL R., AND [1]PERACHO V.

[1]Servei de Vigilància i Control de Plagues Urbanes, Agència de Salut Pública de Barcelona, Barcelona, Spain
[2]Servei de Microbiologia, Agència de Salut Pública de Barcelona, Barcelona, Spain
[3]Departamento de Investigación y Desarrollo, Laboratorios Lokimica, Barcelona, Spain.

Abstract Cockroaches (Blattodea) are important mechanical vectors for the transmission of many pathogens including bacteria, protozoa, fungi, and viruses, and can contaminate food, water, and affect animals and humans. These pathogens can be carried in different parts of the cockroach body (legs, mouthparts) as well as in their secretions (regurgitations and feces) so they suppose a major problem for public health. Transmission and storage of certain bacteria by cockroaches is an important risk factor for the spread of pathogens in hospitals, homes, restaurants and food establishments. Consequently, a suitable control of cockroach populations is needed to minimize the spread of several infectious diseases. To evaluate the pathogenic potential of cockroaches, a comprehensive study was carried out in municipal facilities of Barcelona analyzing the infestation level and prevalence of different pathogens. Exemplars of cockroaches were obtained from the pest surveillance and control programme conducted in 668 municipal buildings. Sampling was focused on two types of equipments with greater risk for public health in the context of likely alimentary contamination: municipal centers including a kitchen or a bar (alimentary manipulation) and municipal markets containing large quantities of food products (alimentary storage). Samples were taken every three months during one complete year (12 months). In each monitoring visit, cockroaches collected in sticky traps were carefully identified at species level and processed for pathogens screening in laboratory conditions. More than 80 samples were analyzed for those pathogens with major importance for public health: *Salmonella* spp, *Campylobacter* spp, *Listeria monocytogenes*, *Staphylococcus coagulase* positive and *Escherichia coli* beta-lactam resistant. The results show the presence of three synanthropic species of cockroaches in the equipments: *Periplaneta americana, Blattella germanica* and *Blatta orientalis* (in less amount). The analysis of the pathogens prevalence shows the ability of these insect species to transport and store bacteria of public health interest, certainly an important reason to increase efforts in pest monitoring and control programmes in these establishments and reduce the risks to public health.

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RESIDUAL EFFECTIVENESS EVALUATION OF INSECTICIDES AGAINST COCKROACHES ON POROUS SURFACE

[1]MARCOS R. POTENZA, [1]MARCIA DA F. VALBUZA, [2]WASHINGTON L.B. FERREIRA, AND [2]GUILHERME STAGNI

[1]Av. Cons. Rodrigues Alves, 1252, São Paulo/SP, 04014 002, Brazil/ Instituto Biológico/APTA

[2]Av. Nações Unidas, 18001 4ºandar São Paulo/SP 04795 900, Brazil/ Syngenta, Professional Pest Management LATAM

Abstract The residual effect of an insecticide is directly related to its formulation, active ingredient and application rates. The aim of this study was to evaluate the effectiveness of 2 distinct commercial insecticides products against *Blattella germanica* and *Periplaneta americana* during the following six months after their application on porous surface. The tested products were P1: 2.5% Lambda-cyhalothrin on a microencapsulated formulation (Demand 2.5 CS®) and P2: 3.5% Lambda-cyhalothrin combined with 11.6% Thiamethoxam on an EZ formulation – microencapsulated + suspension concentrate, respectively (Tandem®). The tested dosage followed manufacturers' indications: P1: 10mL/1L of water and P2: 8mL/1L of water. Each insecticide solution was sprayed on a 20cm x 20cm cement surface, following 1L/20m² proportion. Two hours after the application, 10 *Blattella germanica* individuals (5 males and 5 females) were exposed on the treated surface for 15 minutes, and, after that, 10 individuals of *Periplaneta americana* (5 males and 5 females) were also exposed to the same surface, for the same time. Five repetitions were made for each treatment. Residual effectiveness was evaluated at 30, 60, 90, 120, 150 and 180 days after application. These evaluations followed the same proceedings: individuals exposed for 15 minutes, 5 repetitions for each treatment. All the surfaces were kept in a laboratory, under room temperature and humidity. After exposure, all the individuals were moved to an insecticide free arena, with food and water available. Mortality was evaluated until 96 hours after exposition. Results showed that P1 and P2 provided 100% of control even 6 months after applications. For both products, LT50 (time to reach 50% of mortality) for *Blattella germanica* and *Periplaneta americana* was inferior to 1 hour at 0, 30, 60, 90, 120, 150 and 180 days after application. LT90 (time to reach 90% of mortality) for both species was also inferior to 1 hour for both products, even after 180 days after application. Microencapsulated formulations provided long lasting residual effectiveness even on porous surface. The obtained information is valuable when designing an effective cockroach control program where residual effectiveness is required.

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COMPARATIVE EVALUATION OF GEL BAITS PALATABILITY AGAINST *BLATTELLA GERMANICA*

[1]MARCOS R. POTENZA, [1]FABRÍCIO C. REIS, [1]THAIS L. LIMA, [2]WASHINGTON L. B. FERREIRA, AND [2]GUILHERME STAGNI

[1]Av. Cons. Rodrigues Alves, 1252, São Paulo/SP, 04014 002, Brazil/ Instituto Biológico/APTA/SAA
[2]Av. Nações Unidas, 18001 4ºandar São Paulo/SP 04795 900, Brazil/ Syngenta, Professional Pest Management LATAM

Abstract The control of *Blattella germanica* represents a major challenge for Brazilian PCOs (Pest Control Operators), due to their great capacity for reproduction and dispersal on tropical weather environments. Several gel bait insecticides have been developed and sold in Brazil, during the past few years, from a wide amount of multinational and local manufacturers. Although the active ingredient is a key component of baits, the food matrix is also a fundamental part of a product and it may vary from manufacturer to manufacturer due to the alimentary components used. The quality of the bait food matrix is directly related to its performance on the most challenging situations, such as kitchens and food processing areas, especially when competing with other food sources. The aim of this study was to compare the palatability of 9 distinct gel baits against a new product on Brazilian market – Advion Cockroach Gel Bait® (Indoxacarb 0.6%) – when offered to a population of *Blattella germanica*. The products tested against the Indoxacarb 0.6% bait, named here as P1, were: P2: a Hydramethylnon (2%) gel bait, P3: a Hydramethylnon (2%) gel bait, P4: a Fipronil (0.05%) gel bait, P5: a Fipronil (0.05%) gel bait, P6: a Imidacloprid (2.15%) gel bait, P7: a Imidacloprid (2.15%) gel bait, P8: a Imidacloprid (2.15%) gel bait, P9: a Imidacloprid (2.15%) gel bait and P10: a Indoxacarb (0.6%) gel bait, and all of them were present at Brazilian market by the time the trials were made. Ten repetitions were made for each P1 x Other Product trial. In an arena (33cm length, 22cm width, 10cm height) containing 30 individuals of *Blattella germanica* – 10 male, 10 female and 10 nymphs, fasted for 24 hours before the trials – 0.5g of P1 and 0.5g of other gel bait were offered simultaneously for 2 hours. After this period, both remaining baits were weighted on a high precision balance. P1 consumption was: 92.30% higher than P2, 233.33% higher than P3, 1000% higher than P4, 43.75% higher than P5, 62.5% higher than P6, 100% higher than P7, 300% higher than P8, 240% higher than P9 and 375% higher than P10. Results showed that P1 has superior palatability when compared to all its competitors. The obtained information is valuable when designing an effective cockroach control program where a palatable gel bait formulation is required.

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COMPARATIVE RODENTICIDES PALATABILITY AGAINST *RATTUS RATTUS* AND *MUS MUSCULUS* ON POULTRY FARM FACILITIES

[1]MARCOS R. POTENZA, [2]MARIA F. DOS SANTOS MENEGUETTI, [3]WASHINGTON L. B. FERREIRA, AND [3]GUILHERME STAGNI

[1]Av. Cons. Rodrigues Alves, 1252, São Paulo/SP, 04014 002, Brazil/ Instituto Biológico/APTA

[2]Al. Diamantina, 35, Fundos, Jd Esmeralda, Bastos, SP, CEP 17690-000, Brazil/ Meneguetti, Pest Control Operation

[3]Av. Nações Unidas, 18001 4ºandar São Paulo/SP 04795 900, Brazil/ Syngenta, Professional Pest Management LATAM

Abstract Rodents are frequent pests on a farm environment in Brazil. Among several species that may occur on this kind of facility, there are *Rattus rattus* and *Mus musculus*, also known as the roof rat and mouse, respectively. The aim of this study was to evaluate the comparative palatability of 6 distinct blocks against a recent released extruded block (Talon Blocos XT®), called here as P1, on both species mentioned above. The tested blocks were: P1: 0.005% Brodifacoum extruded block (20g), P2: 0.0025% Difethialone extruded block (15g), P3: 0.005% Difenacoum compressed block (20g), P4: 0.005% Bromadiolone extruded block (20g), P5: 0.005% Flocoumafen compressed block (20g), P6: 0.005% Brodifacoum wax block (20g) and P7: 0.005% Brodifacoum wax block (20g). Palatability tests against *Rattus rattus* were undertaken at Iacri city, São Paulo, in a quails creation facility. During sixty days, 20 bait stations were positioned on strategic points. Ten bait stations contained P1, P2, P3 and P4 simultaneously and the other ten bait stations contained P1, P5, P6 and P7 simultaneously. Consumption was evaluated by weighting the blocks every 15 days, with bait replacement at 15 and 30 days after the beginning of the trials. After that, bait replacement was made whenever there was block consumption noticed during the evaluations. P1 consumption was 122.05% higher than P2, 1950.52% higher than P3, 1950.52% higher than P4, 223.02% higher than P5, 2679.36% higher than P6 and 2114.06% higher than P7. Palatability tests against *Mus musculus* were undertaken at Bastos city, São Paulo, at a laying eggs chicken facility. During sixty days, 20 bait stations were positioned on strategic points. Ten bait stations contained P1, P2, P3 and P4 simultaneously and the other ten bait stations contained P1, P5, P6 and P7 simultaneously. Consumption was evaluated by weighting the blocks every 15 days, with bait replacement at 15 and 30 days after the beginning of the trials. After that, bait replacement was made whenever there was block consumption noticed during the evaluations. P1 consumption was 117.15% higher than P2, 324.43% higher than P3, 373.85% higher than P4, 144.76% higher than P5, 862.26% higher than P6 and 682.14% higher than P7. Results showed that P1 has superior palatability when compared to all competitors, for both species. The obtained information is valuable when designing an effective rodent program where an attractive bait product is required.

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BURROW TREATMENT WITH BIOCIDE BAITING IN URBAN GREEN AREAS OF BARCELONA

[1]FRANCO, S., [1]TONERA, J., [1]RISPA, R., [2]BUENO, R., [2]GARCÍA-ANGLÉS, A., AND [1]PERACHO, V.

[1]Public Health Agency in Barcelona [2]Lokímica, S.A.

Abstract The Public Health Agency of Barcelona (ASPB Catalan acronym) is in charge of the surveillance and control of pests, such as some rodents, in public green areas and sewage system of the City. In the context of recent legal restrictions on the use of rodenticides, the control in green surfaces through the unique employment of rodent bait stations has been insufficient to solve several problems linked to murids (*Rattus norvegicus* and *Mus musculus*) proliferation in urban environments. A specific petition was made to the Spanish Ministry responsible of biocides registrations to enable the use of rodenticide directly in burrows in green spaces of the City (tree pits, borders, etc.). The selected product was Lok Difenacoum Bloque ®, a rodenticide bait in block form with Difenacoum (0,005%) as active matter. After the positive response, a specific protocol was designed and implemented to maximize the security of the application technique for citizens and pets that frequent the affected areas: rodenticide was introduced 25cm (minimum) into the burrows from the ground surface and covered with soil, each site was checked twice a week until activity finished. This method was held during three and a half months in 2015 and eight months during 2016 in all the public green spaces in Barcelona where burrows were found. More than three hundred plans were carried out to control rodent infestations in parks, green spaces and tree pits, sometimes including just one burrow, and others even exceeding fifty burrows. The method proved to be more efficient and reduced the necessary control period compared to the use of bait stations, being small infestations the quickest to resolve. The use of bait stations comprises issues such as rats' rejection, vandalism or use viability (for example, it is not possible to use in tree pits) that hinder its success. Treating the burrows directly with rodenticide involves less time of exposure to biocides and less time of uncontrolled rat populations, thus it appears to be a more effective and safer method.

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DEVELOPMENT OF THE FIRST TRANSDERMAL RODENTICIDE SYSTEM, AN ALTERNATIVE TO ANTICOAGULANT MULTI-FEEDING

STEVE L GOODE

PiedPiper Consortium, Leominster, Herefordshire, HR6 9RW, United Kingdom

Abstract PiedPiper was born out of the desire to move rodenticide control away from the current multi-feed technology that leads to toxin resistant rats and mice. Our approach was to develop a single application methodology that would prevent the protracted times to death and also avoid the increase in tolerance to the anticoagulants. The primary step was to find a "suitable" toxin – i.e. a toxin which has had limited exposure in the field so as not to compromise its efficacy and could be developed into a transdermal application. Our compound of choice was cholecalciferol coupled with a solvent that readily traverses the dermis which could be formulated for aerosol use and dispensed as a single application treatment on to the back of a rodent that has been attracted into our custom designed Pest Control Device. The project has been funded by a number of grants such as EU FP7 R4SME and EU FP7DA – the first results were obtained in 2012 and these have been followed up with further independent trials in Romanville (France), Aston University (UK) and in the field at University of Nairobi (Kenya). All trials were successful and show that this is a highly innovative and effective system for rodenticide control in both urban and rural areas.

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A REVIEW OF THE CHALLENGES AND LESSONS LEARNED IN DEVELOPING PROACTIVE MANAGEMENT STRATEGIES TO COMBAT INFESTATIONS OF *CIMEX LECTULARIUS* (HETEROPTERA: CIMICIDAE) IN COMMERCIAL ACCOMMODATION ENVIRONMENTS

DAVID CAIN

Bed Bugs Limited, 3 Cobden Road, London, SE25 5NY

Abstract Having started working exclusively with bed bug infestations in 2005 and taking a unique science and fact lead approach to extermination we have always used the data produced in our work to refine the methods we use and to develop better processes. Initially focused on educational and raising understanding within our client's organisations we quickly realised the flaws in this approach, which by 2009 this lead to the creation of Passive Monitors for bed bugs taking our services to the hospitality industry from a purely reactive offering to one that is proactive and capable to dealing with infestations before they are able to develop and spread. While this has given us a viable tool to base solutions upon there have been significant challenges in the implementation of programs within a diverse range of hospitality providers from high end hotels through to hostels and residential care facilities. Through constant review and feedback, we have identified a number of key obstacles that must be overcome in the implementation of any successful program from the overcoming of natural entomophobia, the culture of pests being someone else's problem to deal with and the involvement of senior management. Having tackled 100% infested hotels (50+ rooms) through to entire student campuses (500+ rooms) we have established optimal detection parameters for different profiles of accommodation providers which can be adapted as the level of exposure risk changes while maintaining a foundation level of quality assurance. This review is best understood when presented as a timeline that illustrate when the challenges were first encountered, anticipated and overcome, from the changes in insecticide availability, emergence of widespread resistance through to the development of ProActive disciplines and green treatment solutions. The current conclusions to these projects have been that bed bugs continue to develop to treatment strategies and that without feedback and continual modification reactive solutions quickly fail.

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CASE REPORT: DESCRIPTION OF BED BUGS (HEMIPTERA: CIMICIDAE) INFESTATION IN PRISON, ESTATE OF SAO PAULO, BRAZIL

MARCIO RODRIGUES LAGE AND JOSÉ MARIA SOARES BARATA(in memoriam)

715 Av. Dr. Arnaldo, São Paulo / University of São Paulo, Dept. of Epidemiology, Brazil

Abstract The Cimicidae or bed bugs are sucking blood insects and *Cimex lectularius* and *Cimex hemipterus* are the main species cited with distribution in Brazil. They are nocturnal insects, remain protected in their hideout in most of the day and biting people during sleep, virtually any fissure or protected location serve as hideout to bedbugs colonize. Their importance to public health is due to on contribution to deterioration of human life quality. In Brazil it has been notified the presence of these insects in hotels and residences of high economic levels. The case to be treated in this research is located in a prison in the city of Mirandópolis, where was collected 62 bed bugs. The local infrastructure was very precarious, just not because it is a prison environment, but the place has been built for other purposes initially. It is noted that local conditions are clearly suitable for the existence of large infestation of bed bugs. The treatment carried out by the specialized company, it is observed that this was following the instructions of the manufacturers of the chemicals, as well as information from specialists. It is concluded this case may reflect a public health problem is still poorly noticed in Brazil.

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BED BUG MUNICIPAL MANAGEMENT. CITY OF MADRID (SPAIN)

CÁMARA, JOSÉ-MARÍA., TORRES, PILAR., ORTIZ, CARMEN., ARRIBAS, ALFONSO., PÉREZ, ANA., AND GARRASTAZU, CONSUELO.

Carretera del Barrio de La Fortuna 33, 28054 - Madrid (Spain) Environmental Health Dpt., Health Madrid (City Council of Madrid)

Abstract Initially infestations of houses by *Cimex lectularius* were sporadically detected in the middle of the previous decade and increased over the past years. Data obtained by the Environmental Health Department have shown events increase by 1000% in the past five years for the whole city. Among the 21 City Districts, *01-Centro* gathered the highest number of municipal interventions against bed bug infestations. In response to this situation, the environmental health services of Madrid City Council have quickly designed an action plan and specific procedures to carry out the best possible practices for preventing and controlling the numerous circumstances of bed bug infestation and spread. Some substantial actions are the following: (1) The promotion of information, education and reliability of citizens. (2) The information and promotion of the co-responsibility of the hotel industry. (3) Protection of municipal facilities susceptible to bed bug infestation, especially facilities and housing related to social care and temporary shelter of the homeless. (4) Special procedures and municipal intervention in social housing scenarios and other very complex cases such as bedbugs in multi-infested buildings, Diogenes Syndrome, etc. (5) Implementation of a special municipal service for collection of infested mattresses, furniture, etc. (6) Integrated monitoring and management through the vector/pest management geographic information system (GIS) of Health Madrid. (7) The cooperation and information sharing with private sector pest control companies. The implementation of these measurements and the analysis of the data obtained through the last five years allowed us to draw several conclusions, such as: (A) The lack of reliable indicators of infestation, then the important risk of underestimating real City situation and tendencies. (B) The importance that competent administrations, especially the health local authority, become aware of the relevance of this problem and of the necessity of a strong proactive and early response. (C) The transcendence and difficulty of implementing powerful strategies of citizen information and education regarding bedbug infestations, key aspect in an efficient prevention and control of the infestation spread. Media impact and information management. (D) The remarkable technical difficulties of the infestations treatments, especially in cases such as social housing, multiple-infested premises, squatted buildings, etc. Current problems for the use of new bedbug biocides and technologies in these complex situations. (E) The human and economic impact of bedbug infestations, especially in vulnerable social layers. (F) The importance of collaboration and coordination between Municipal Government (mainly Health, Environmental and Human Services) and pest control companies.

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SYNANTHROPIZATION OF MOSQUITOES (DIPTERA: CULICIDAE) OF SIBERIA AND AMUR REGION

ELENA BOGDANOVA

I.M.Sechenov First Moscow State Medical University, Moscow, Russia

Abstract Investigations were carried out in several climatic zones of Western and Eastern Siberia, and at the Amur region. Blood-sucking mosquitoes in these areas have a rich species composition and very high numbers. In addition to a significant irritating action in an attack on the people, they are vectors of many vector-borne diseases, and also cause allergic reactions and dermatitis. The research program included the study of the species composition and abundance of adults and larvae of mosquitoes. The surveys were carried out using standard methods in the neighborhood and in the territory of residential areas, had a long-existing and newly built. The species composition of mosquitoes of studied regions, according to our data, ranged from 22 to 31 species of the genera *Aedes, Culiseta, Culex, Anopheles.* Dominants in various accounting items were *Ae.communis, Ae.pionips, Ae.cantans, Ae.punctor*, *Ae.excrucians, Ae.cinereus, Ae.vexans, Ae.riparius, An.hyrcanus*. The number of mosquitoes in the new settlements was not significantly different from the number in their neighborhoods. With the development of localities in their neighborhoods and the outskirts it changed slightly. For example, in settlements at Western Siberia, in the years 1966-1968 the number of mosquitoes was 385 exemplars of a 5-minute account of entomological net, and in 1995-1998 - 265 exemplars of the records. However, the number of mosquitoes decreased from the village outskirts to its center to several tens of accounting. Of all the species in the settlements was met *Ae.communis, Ae.punctor, Ae.excrucians, Ae.riparius, Ae.cantans, Ae.diantaeus*, *Ae.cinereus, Ae.vexans* ssp, *An.hyrcanus*. In the localities with their development happens a reduction of the species composition of mosquitoes, but in different climatic zones and regions of different types, for example, *Ae.communis, Ae.punctor, Ae, cinereus, Ae.vexans* tended to the occupation of anthropogenic biotopes. This increases their epidemiological significance, since contacts of synanthropic species with the humans are increasing significantly.

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KNOWLEDGE, ATTITUDES AND PRACTICES ABOUT *AEDES AEGYPTI* (DIPTERA: CULICIDAE), VECTOR OF DENGUE, CHIKUNGUNYA AND ZIKA IN VILLAVICENCIO (COLOMBIA)

[1]GLORIA ISABEL JARAMILLO AND [2]LUZ STELLA BUITRAGO

[1]Carrera 35 N. 36-99 Barzal-Villavicencio / Universidad Cooperativa de Colombia, Medicine Faculty, Villavicencio-Colombia.

[2]Calle 37 N. 41 - 80 Barzal Alto-Villavicencio / Entomology laboratory, Health Secretary of Meta

Abstract Mosquito *Aedes aegypti* is the most efficient vector in the transmission of dengue, chikungunya and zika in Colombia. Villavicencio, located in the central part of the country, is one of the cities that report more cases of these pathologies. Despite the strategies of communitarian education to control the vector, the number of cases and larval index don't decrease. Then, it is important to know if the community recognize and apply this knowledge. The main objective was to determine the knowledge, attitudes and practices about *Ae. aegypti* in two low income neighborhoods in Villavicencio-Colombia. A descriptive cross-sectional study was conducted in 2016. The houses were selected randomly and in the event that nobody attended, the next house was selected. Only individuals aged 18 years and above were interviewed using a structured, pre-tested questionnaire. Informed consent (verbal) was taken from all the respondents and confidentiality was ensured. Additionally, entomological data were recorded in order to calculate the larval indexes at homes: house, container and Breteau index were calculated. 76 houses (309 people inhabiting them) were evaluated. 79,45% (IC95% 68,38-88,02%) of the people surveyed known that a mosquito transmits these diseases but unknown the scientific name and 68% (IC95% 56,22-78,31%) unknown that only the female bites. 81,36% (IC95% 69,09-90,31%) didn´t know the life cycle of the mosquito but are aware that the elimination of breeding sites and destruction of containers with water prevent transmission; 88,46% (IC95% 73,19-90,82%) recognize that the community should be responsible for these control actions at home. But the recommendations are not implemented due to lack of interest (39,51% IC95% 28,81-50,99%) or laziness (27,16% IC95% 17,87-38,19%). The house index was of 39,22% and 41,38% for each neighborhood, and Breteau index was 47,06 and 48,28. The highest percentage of positive breeding sites was for low tanks (62,5 % and 57,14 %) in both neighborhoods. It is necessary to review information campaigns, communication and education promoted in the municipality since they do not reflect the empowerment of its people. These programs need to translate population knowledge about vector borne diseases into positive preventive practices that lead to a reduction in the transmission of dengue, chikv, and zika in these communities. This will require more infrastructure and resources for long-term sustainability.

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INTER- AND INTRA-SPECIFIC LARVAL COMPETITION OF TWO CONTAINER BREEDING MOSQUITOES IN SOUTH TEXAS

[1]SAKSHI PURI, [2]FELICIA VAZQUEZ, AND [2]CHRISTOPHER VITEK

[1]Hidalgo County Health Department
[2]University of Texas Rio Grande Valley

Abstract There has recently been an observed increase in vector-borne disease in the Americas, including those diseases which are primarily vectored by *Aedes aegypti*. South Texas is at risk of vector-borne diseases due to the mild temperatures, year-round presence of the disease vectors, and proximity to Mexico where disease like dengue, Zika, and Chikungunya have been circulating. Longitudinal and seasonal patterns in abundance in South Texas of the two vector species, *Aedes aegypti* and *Aedes albopictus*, were hypothesized to be due in part to larval competition. We examined inter-specific and intra-specific interactions with *Aedes aegypti* and *Aedes albopictus* at 30°C with a 13:11h light:dark cycle, and 15°C with a 11:13h light:dark cycle, representing average summer and winter temperatures (respectively) for South Texas. Using F2 generation colony mosquitoes, we reared mosquitoes at varying densities and food availability. In experiment one, we kept the overall density and food levels the same, and varied the level of interspecific competition. In experiment two, we varied the starting densities and the food levels, but only included intraspecific competition. Temperature significantly influenced survivorship of both species regardless of density ($p < 0.05$), with increased survivorship in hot temperatures. In experiment two, food levels also significantly influenced survivorship ($p < 0.05$), and significantly interacted with temperature ($p < 0.05$) for both species. Starting density was not significant by itself in either experiment or interacting with any other variables in the first experiment ($p > 0.05$), but in the second experiment starting density of *Aedes aegypti* interacted with temperature to significantly influence *Aedes aegypti* survival ($p < 0.05$). Our results indicate that *Aedes albopictus* has an overall higher survivorship rate in all conditions, although in typical summer conditions the difference is minimal. At the densities tested, starting density of hetero- or con-specific larvae was not a primary influence on survivorship. These results may partially explain the differences observed in *Aedes albopictus* and *Aedes aegypti* abundance that was observed longitudinally in residential areas, although competition alone may not sufficiently explain the pattern of more *Aedes albopictus* in cooler, coastal areas. Development time and size at emergence are planned for future analysis.

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AEDES (STEGOMYIA) AEGYPTI AND *AEDES (STEGOMYIA) ALBOPICTUS* IN RUSSIA

[1]SVETLANA A. ROSLAVTSEVA AND [2]MIKHAIL A. ALEKSEEV
[1]Scientific Research Disinfectology Institute of Federal Service for Surveillance on Consumer Rights Protection and Human Well-being, Nauchnyi proyezd 18, Moscow 117246, Russia
[2]Scientific Research Disinfectology Institute of Federal Service for Surveillance on Consumer Rights Protection and Human Well-being, Nauchnyi proyezd 18, Moscow 117246, Russia

Abstract *Aedes aegypti* was first discovered on the territory of the former Soviet Union in Batumi as an introduced species in 1911. High numbers of these mosquitoes have been recorded on the Black Sea coast from Sukhumi to Batumi. Since 1950s, these mosquitoes are not detected in this region (Cherkasskyi, 2000). In August-September of 2001-2005, in the Central district of Sochi a few *A. aegypti* females were found (Ryabova et al., 2005). In 2007, the presence of this mosquito in the Greater Sochi was confirmed. Moreover, this mosquito was registered in the cities of Abkhazia – Gudauta and Sukhumi (Yunicheva et al., 2007). In Russia, *Aedes albopictus* females were first caught in the Greater Sochi (Khosta) in July 2011. Species identification of caught females was confirmed by PCR (Ganushkina et al., 2012). In 2013, in the Central district of Sochi, 100-400 meters from the sea coast, females, larvae and pupae of this species were found. By 2015-2016, range of *A. albopictus* has expanded rapidly, reaching Novorossiysk in the north and border between the Russian Federation and Abkhazia in the south. Recently, much attention is paid to Zika virus transmitted by *Aedes* mosquitoes, in particular, *A. aegypti* and *A. albopictus*. On February 1, 2016 WHO announced Zika as a threat to international public health. Knowledge of insecticide susceptibility of *Aedes* mosquitoes is required for drawing-up a plan on rational use of insecticides in mosquito control programs and for carrying out measures on sanitary protection of the territories of Russia and CIS countries. At the end of XX and beginning of XXI century, *A. aegypti* and *A. albopictus* mosquitoes were introduced on the Black Sea coast, apparently from different countries, and their insecticide susceptibility is not known. In 2016, we carried out the preliminary work for the establishment of diagnostic concentrations of larvicides commonly used for mosquito control in Russia and other countries. After literature analysis of the distribution, insecticide susceptibility of these species and our own experiments on mosquito larvae *A. aegypti* of insecticide-susceptible S_{NIID} strain, we obtained the diagnostic concentrations for some larvicides. Concentrations (mg/L) were: DDT – 0.1; temephos (abate) – 0.03; fenthion – 0.06; malathion – 0.155; chlorpyrifos – 0.016; cypermethrin – 0.119; alpha-cypermethrin – 0.086; deltamethrin – 0.048; lambda-cyhalothrin – 0.078; etofenprox – 0.05; larvicides based on *Bacillus thuringiensis* var. *israelensis*: «Larviol-pasta» – 0.013 and «Baktitsid» – 0.09. Diagnostic concentrations obtained for *Ae. aegypti* were used in July 2016 to determine the susceptibility of six sub-populations of *Ae. albopictus* from Loo, Khosta, Adler and Central districts of Sochi (most larvae were collected in cemeteries) by WHO method (1981). Larvae of all the populations studied were susceptible to cypermethrin, chlorpyrifos and larvicides based on *Bacillus thuringiensis* var. *israelensis*.

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IDENTIFICATION OF MOSQUITOES USING MORPHOMETRIC WING CHARACTERS

[1]ANDRÉ BARRETTO BRUNO WILKE, [2]RAFAEL DE OLIVEIRA CHRISTE, [2]LAURA CRISTINA MULTINI, [3]PALOMA OLIVEIRA VIDAL, [1]RAMON WILK-DA-SILVA, [1]GABRIELA CRISTINA DE CARVALHO, AND [1]MAURO TOLEDO MARRELLI

[1]Departamento de Epidemiologia, Faculdade de Saúde Pública, Universidade de São Paulo, São Paulo, Brazil
[2]Instituto de Medicina Tropical de São Paulo, Universidade de São Paulo, São Paulo, Brazil
[3]Departamento de Parasitologia, Instituto Butantan, São Paulo, Brazil

Abstract Mosquitoes are responsible for the transmission of several infectious diseases endangering approximately 3 billion people around the world. The precise identification of mosquitoes' species is decisive to understand epidemiological patterns of diseases transmission and to develop control strategies. The most commonly method used to the identification of mosquito relies on morphological taxonomic keys. Mosquito wing geometric morphometric is able to identify vector mosquitoes, even sibling and cryptic species. The objective of the present study is to accurately identify twelve mosquito species from the three main epidemiologically important mosquito genera using wing morphometric technique. Twelve mosquito species from three epidemiological important genera (*Aedes*, *Anopheles* and *Culex*) were collected and identified by taxonomic keys. The right wing of adult female mosquitoes was removed, photographed and the coordinates of eighteen landmarks at the vein intersections were digitized. The allometric influence was accessed followed by a canonical variation analysis, a thin-plate splines, a reclassification and cross-validated test were computed for each individual and a Neighbor Joining tree was constructed to illustrate species identification and segregation. The analyses and graphics were made with the following software: TpsUtil 1.29, TpsRelw 1.39, MorphoJ 1.02, Past 2.17c. The canonical variation analysis between *Aedes*, *Anopheles* and *Culex* genera showed a clear segregation of genera, subgenera and species in the morphospace correctly sorting out mosquitoes employed in this study. The pairwise cross-validated reclassification test regarding genera resulted in at least 99% identification accuracy scores, it also correctly identified subgenus resulting in a mean of 96%. Regarding species identification, 88 from 132 possible comparisons resulted in 100%, identification accuracy. Our results showed that *Aedes*, *Culex* and *Anopheles* were correctly distinguished by wing shape. When subjected to the analysis of lower hierarchical levels of subgenera (when present) and species, the wing geometric morphometric was also efficient resulting in high reclassification scores.

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SPECIES IDENTIFICATION OF THE *ANOPHELES FLUVIATILIS* COMPLEX (DIPTERA: CULICIDAE) USING PHYLOGENETIC ANALYSIS PCR-SEQUENCING IN SOUTHWESTERN IRAN

[1]GHOLAMHOSSEIN SHAHRAKI AND [2]ZEYNAB BARGHAMADI

[1]Faculty of Health, Yasuj University of Medical Sciences, Yasuj, Iran
[2]Tehran University of Medical Sciences, Tehran, Iran

Abstract *Anopheles fluviatilis* is one of the most important malaria vectors in Iran. The aim of this study was to identify *Anopheles fluviatilis* complex species using proliferation and sequence analysis of ITS2 and also 28.S- D3parts of rDNA gene in southwestern Iran. In this research *Anopheles fluviatilis* was caught from different areas of Kohgiluyeh and Boyer-Ahmad province at southwestern Iran in 2013. DNA was taken from 4 *Anopheles fluviatilis* selected samples and PCR tests of 28S- D3 part were done on these DNA samples. Then after the sequence results were obtained, these were identified and compared with similar samples of *Anopheles fluviatilis* based on data from a gene word bank. Phylogenetic tree and individual sequences of samples were calculated. PCR Phylogenetic analysis of r DNA 28S-D3 part of *Anopheles fluviatilis* confirmed that U genotype in Kohgiluyeh and Boyer-Ahmad province was identified. The gene length of r DNA 28S-D3 part between populations of this species was the same (333bp). This study showed that *Anopheles fluviatilis* has a species separate branch in southwestern Iran which is different to the branch of southeastern Iran (Hormozgan, Kerman and Sistaan & Baluchestaan provinces).

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FAUNA AND FREQUENCY OF PHLEBOTOMINE SAND FLIES (DIPTERA: PSYCHODIDAE) IN SOUTHWESTERN IRAN

GHOLAMHOSSEIN SHAHRAKI
Faculty of Health, Yasuj University of Medical Sciences, Yasuj, Iran

Abstract Determination of sand flies species is very important especially as vectors for Leishmaniasis in most provinces in Iran. This study was conducted to determine fauna and frequency of sand flies in Kohgiluyeh and Boyer-Ahmad province located in southwestern Iran. Sand flies samples were collected from five counts during May to November 2015 using sticky traps. Traps were installed in indoor and outdoor places of the rural selected areas after sunset and collected before the next day sunrise. Collected sand flies monitored for monthly activity and identified after mounting. From a total of 8500 collected specimens, 12 species of sand fly were identified. They were from two genera (*Phlebotomus* and *Sergentomyia*). Phlebotomus species (with 50.8% frequency) including: *Phlebotomus sergenti (58.3%), P. caucasicus* (26%)*, P.papatasi* (12.5%) and *P. kandelaki* (1%). Sergentomyia species including: *Sergentomyia tiberiadis* (49.5%), *S. clydei* (22.6%), *S. dentana* (6.5%), *S. sintoni* (10.7%), *S. theodori* (6.5%), *S. baghdadis* (2.1%), *S. parratomyia* (1.1%) and *S. iranica* (1.1%). The most trapped phlebotomus species were from indoor places (62.5%). *P. sergenti* observed the dominant species in the most study areas with relatively high frequency. Due to this species is the main vector for Anthroponotic Cutaneous Leishmaniasis (ACL) and the study province surrounded by three provinces which focuses of cutaneous leishmaniosis, it can be deduced that the potential of ACL in this province is considerable.

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MOSQUITOES (DIPTERA: CULICIDAE) IN THE CAPITAL OF THE CZECH REPUBLIC

FRANTIŠEK RETTICH AND MARTIN KULMA

National Institute of Public Health, Šrobárova 48, Prague 10, 142 00, Czech Republic

Abstract The occurrence of mosquitoes in Prague sub urban forests, gardens, parks, recreation areas, golf courses etc. was compared between flood and non-flood years. The monitoring was based on mosquito larvae collection and mosquito female catch by CO_2 traps. No massive larval populations of the anthropophilic species of flood mosquitoes (*Aedes vexans* or *Ae. sticticus*) appeared even after catastrophic floods (in 2002, 2006, and 2013). On the contrary, in drying out flood lagoons with water polluted by decaying vegetation, larvae of the mostly ornitophilic species *Culex pipiens/torrentium* hatched. No larviciding (often required by local authorities) was performed. In non-flood years, scarce populations of snow melt mosquitoes appeared regularly in remnants of natural habitats, with *Ae. cantans* being the dominant species. But rapid urbanization (fast spreading of the urban environment) caused the disappearance of many natural breeding sites. Some rare species recorded in the last two decades of the last century have not been collected again (*Ae. pulchritarsis, Ae. flavescens*, *Coquillettidia richiardii*, *Culiseta alaskaensis,* etc.). Of the sibling species *Cx. pipiens* and *Cx. torrentium*, it has been the latter that prevailed in most of the natural breeding sites. The breeding sites of an autogenous, anthropophilic form of *Cx. pipiens (molestus)*, which was frequently detected in flooded basements in Prague in the second half of the last century, have gradually disappeared owing to improved maintenance of buildings. *Cx. modestus* (a new species to Prague) larvae were caught on the edge of flooded meadows in the southern outskirts of Prague in 2013. *Culex* spp. females dominated in most of the CO_2 trap catches. No invasive mosquito species have been recorded yet. Data from the first half of 2017 will be added to the poster.

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REPORT OF *PAEDERUS* SPECIES IN IRAN, MAZANDARAN AND FARS PROVINCES, 2012- 2013

[1]SAHAR BAZRAFKAN, [2]SEYED MOHAMMAD ABTAHI, [3]ALI ASGHAR BAZDAR, AND [4]ALI MEHDINIA

[1]International Campus, Tehran University of Medical Sciences (IC-TUMS), Tehran, Iran
[2]Isfahan University of Medical sciences
[3]Department of Industrial Engineering, Faculty of Engineering, Golpayegan University
[4]Department of marine Living Science

Abstract Rove beetles of the genus *Paederus* cause dermatitis when they come in contact with human skin. Their hemolymph contains a blistering, vesicant and toxic amide named pederin. Dermatitis caused by stimulation of beetle *Paederus*, is a common health problem in Iran especially in Mazandaran (North of Iran) and Fars (South of Iran) provinces. *Paederus* is a genus of small beetles of the family Staphylinidae (rove beetles) with 622 valid species (Frank and Kanamitsu, 1987). This survey was carried out in several different districts of the Mazandaran and Fars provinces over a period of two years, during spring and summer 2012 and 2013. Rove beetles from selected areas were collected during every visit with the help of aspirator, during day hours (10:00-15:30) with hand catch method using. The greatest number of specimens (334) was collected from north (Mazandaran province) and identified as *Paederus fuscipes*. Other 154 beetles were collected from south (Fars province) were classified in two groups; *Paederus fuscipes* (28.07%) and *Paederus littoralis* (71.93%).

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QUANTITATIVE CHARACTERIZATION OF PEDERIN IN THE ROVE BEETLE, STAPHILINIDAE; *PAEDERUS*, IN MAZANDARAN AND FARS PROVINCES, IRAN.

[1]SAHAR BAZRAFKAN, [2]MOHAMMAD ALI OSHAGHI, AND [2]MANSOUREH SHAYEGHI

[1]International Campus, Tehran University of Medical Sciences (IC-TUMS), Tehran, Iran.
[2]Tehran University of medical Sciences, Tehran, Iran.

Abstract Rove beetles, genus *Paederus* have a vesicant toxin amid, in their hemolymph, named pederin, and caused lesions on human skin. It was first described by a procedure on 25 million *Paederus fuscipes* collected from the field. A few studies were conducted about pederin content of each beetle. It is very various in each specimen. According to a Pavan study in 1963, at least 1µg pederin per one male adult beetle is expected and 10 times more in females. Kellner and Dettner (1995) assessed a new method for quantification of pederin in each beetle for the first time. Before that, severity of dermatitis caused by *Paederus* beetles was the only method for determination of pederin content. They also reported 0.1-1.5 µg of the toxins in males and 0.2-20.5 µg in females, 10 fold more than males in most cases. Results: In this study pederin is quantified in field specimens of two province of Iran, Fars (south of Iran) and Mazandaran (north of Iran). Males contain 0.76µg, 0.73µg and 0.36µg for *P. Fuscipes* Mazandaran, *P. Fuscipes* Fars and *P. littoralis* Fars and females 10.89 µg, 10.31 µg and 3.29 µg of the toxin respectively.

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FLYING INSECT FAUNA OF HOSPITALS

[1]MATTHEW P. DAVIES, [1]MORAY ANDERSON, AND [2]ANTHONY C. HILTON

[1]Killgerm Chemicals Ltd, Wakefield Road, Ossett, WF5 9AJ
[2]School of Life & Health Sciences, Aston University, Birmingham, B4 7ET, UK

Abstract The collection and identification of flying insects associated with a number of UK hospitals was undertaken, in order to classify and enumerate the insects found and establish their seasonality and location in such premises, therefore informing pest control measures. A total of 19,937 individual insects (and other arthropods) were collected from seven UK hospitals over a 20 month period via ultra-violet light flytraps. Of these individuals, approximately 114 arthropod species were identified. Chironomidae were the most common flies, *Calliphora vicina* were the most common synanthropic fly and 'drain flies' were surprisingly numerous and represent an emerging problem in hospitals. Psychodidae were the most common of the 'drain flies' and were therefore the most important known insect vector of the 'hospital superbug' *Clostridium difficile* present in hospitals. Other known insect vectors of *C. difficile* present were *Musca domestica*, *Fannia canicularis* and *Drosophila* sp. Of the known insect vectors of *C. difficile*, *M. domestica* were surprisingly low in numbers. Another perhaps surprising finding was that 'occasionally encountered insects' (also known as occasional invaders / casual intruders) were actually the group most frequently found in hospitals. It was noted that presence of certain species, specifically some of the 'occasionally encountered insects' is diagnostic of proofing inadequacies in UK hospitals. Regarding seasonality, many species were present all year round and not all peaks in numbers were in summer, insect diversity was highest in spring and sheer numbers of insects were highest in summer. Location data showed that insects were found most often in food preparation areas. This study updates the knowledge base regarding flies in hospitals and contrasts with the general wisdom that houseflies *M. domestica* are the most numerous in such premises and that flies are mainly a summer problem. Furthermore, this work provides pest control and infection control staff with knowledge of the key flying insect species that are likely to be present in hospitals at certain times of year and in which hospital locations. This knowledge better informs the design of integrated flying insect management programs, in order to minimise the risk of disease transmission by flying insects, with pest control central to infection control.

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CHARACTERIZATION OF ANTIBACTERIAL ACTIVITIES OF A SUBTERRANEAN TERMITE AGAINST HUMAN PATHOGENS

XING PING HU, SANG-JIN SUH, AND YUAN ZENG

Auburn University, College of Agriculture, 107 Comer Hall, Auburn, Alabama

Abstract The emergence and dissemination of multidrug resistant bacterial pathogens necessitate research to find new antimicrobials against these organisms. We investigated antimicrobial production by eastern subterranean termites against a panel of bacteria including three multidrug resistant (MDR) and four non-MDR human pathogens. We determined that the crude extract of naïve termites had a broad-spectrum activity against the non-MDR bacteria but it was ineffective against the three MDR pathogens including MRSA. Heat or trypsin treatment resulted in a complete loss of activity suggesting that antibacterial activity was proteinaceous in nature. The antimicrobial activity changed dramatically when the termites were fed with either *P. aeruginosa* or MRSA. *P. aeruginosa* induced activity against *P. aeruginosa* and MRSA while maintaining or slightly increasing activity against non-MDR bacteria. MRSA induced activity specifically against MRSA, altered the activity against two other Gram-positive bacteria, and inhibited activity against three Gram-negative bacteria. Further investigation demonstrated that hemolymph, not the hindgut, was the primary source of antibiotic activity. This suggests that the termite produces these antibacterial activities and not the hindgut microbiota. Two-dimensional gel electrophoretic analyses of hemolymph protein spots indicated that a total of 38 and 65 proteins were differentially expressed at least 2.5-fold upon being fed with *P. aeruginosa* and MRSA, respectively. Our results provide the first evidence of constitutive and inducible activities produced by this termite against human bacterial pathogens.

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FREQUENCY OF KNOCKDOWN RESISTANCE MUTATIONS IN BODY LICE (PHTHIRAPTERA: PEDICULIDAE) FROM RUSSIA

[1,2]YULIYA V. LOPATINA, [2]OLGA YU. EREMINA, AND [3]LIUDMILA S. KARAN

[1]Moscow State University, Russia
[2]Scientific Research Institute for Disinfectology, Moscow, Russia
[3]Central Research Institute of Epidemiology, Moscow, Russia

Abstract One of the global problems of today is the insecticide resistance of the human louse *Pediculus humanus* L. to pyrethroids. The continuous application of pyrethroids (mostly, permethrin) resulted in the formation of local lice populations resistant to this group of insecticides. In the 2013-2015 body lice *P.h.humanus* were collected in various regions of Russia (European Russia, Ural, Siberia), both in big cities (Moscow, St. Petersburg, Novosibirsk, Perm), and in small towns (Kursk, Tambov). 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. The resistant haplotype was found in all of the body lice samples. The frequency of the resistant allele varied from 0.432 to 0.89 which indicates the different level of permethrin resistance. The resistance monitoring was carried out in Moscow in the 2008-2015. The frequency of the resistant allele was 0.488 in 2008; whilst a considerable part of the species was represented by susceptible homozygous (37. 5%) and heterozygous by *kdr* allele (27. 5%) individuals. The frequency of *kdr* allele which increased sharply in 2009 (0.763) has been gradually growing hereafter, and reached its maximum (0.879) in 2014. 80% of the insects were the homozygous resistant individuals at the time; the rate of susceptible homozygous individuals didn't exceed 3%. From 2011 onwards no colonies of exclusively permethrin-susceptible (SS) lice have been found. Our research resulted in the substitution of permethrin by fenthion in 2014 for the treatment of homeless people's clothes infected by lice, and for the liquidation of their head lice in the decontamination centers. This probably caused the decrease of the lice infestation rate among homeless people in 2014-2015, as compared to 2009-2013 (15% *vs* 30%). The other reasons of the improvement could also be the active efforts of charitable organizations in Moscow, and the increase of homeless people's awareness which allowed them to change clothing, infested by lice in particular, for the new one more often.

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UNDERSTANDING HOUSEHOLDERS' INTENTIONS TO ENGAGE IN PEST CONTROL: THE USE OF THE HEALTH BELIEF MODEL

[1]STEFAN A. LIPMAN AND [2]SARA A. BURT

[1]Department of Social Health & Organizational Psychology, Faculty of Social Sciences, Utrecht University, Utrecht, The Netherlands.

[#]Current address: Institute of Health Policy & Management, Erasmus University Rotterdam, Rotterdam, The Netherlands.

[2]Institute for Risk Assessment Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands.

Abstract Pests in the home are a health risk because they can be reservoirs or mechanical vectors for several bacterial, parasitic, fungal and viral disease agents. Other associated health risks are allergies and damage to building structure by gnawing. The aims of this study were to use a social cognitive model that is widely used in psychology, the health belief model, to investigate which factors influence householders' intention to control pests and to record which categories of pests are reported in homes in the Netherlands. An online questionnaire was developed to record the following variables: demographic variables, health motivation, perceived susceptibility/severity of contracting diseases through pests, and the costs/benefits of pest control. The survey was placed online between 11 September and 31 November 2015 and was completed by 413 respondents. Regression analysis revealed that perceiving the benefits of pest control and expecting severe consequences of zoonotic infections predicted higher intention to control pests. Intentions towards pest control were not influenced by perceiving oneself as susceptible to catching a disease from pests, health motivation (striving towards a healthy lifestyle), or by the expected costs of pest control. Interestingly, pet owners and farmers were less likely to control pests than others. A large majority of respondents reported pests in or around their home within the previous year. The prevalences were: rodents 62%, flying insects 98%, crawling insects 85%, birds 58%, and moles 20%. In conclusion, the findings of this study suggest that interventions aimed at improving the effectiveness of domestic pest control should focus on increasing the benefits that individuals associate with effective pest control and on underlining the severity of the diseases that pests may carry. That animal owners are less likely than other people to engage in pest control should be taken into account.

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LASER TECHNOLOGY FOR BIRD DISPERSAL

STEINAR HENSKES, TIM SPRANG, AND HENK VAN DIJKE

Bird Control Group, Molengraaffsingel 12, 2629 JD Delft, The Netherlands

Abstract Industrial sites are in conflicting with nature if it comes to birds. The roofs and ledges of factories and other buildings are an ideal habitat for birds to breed and forage. These places are often without human presence, making them an optimal place for birds to perch. The presence of birds and guano at industrial sites is a globally recognized problem that can seriously jeopardize safe working conditions and prove costly. Inspired by wildlife control programs at airports, we developed a new integrated approach that enables performance-based bird control. Results are significantly better than conventional methods. Many bird deterring products only focus on dealing with the symptoms of bird presence. They leave an important issue unaddressed: how can we integrate industrial activities into the natural environment in a sustainable way? Bird Control Group developed an integrated approach consisting of 3 main elements: 1. Continuous monitoring and analyzing of bird data using optical camera technology. 2. Avoiding birds entering the area by influencing the habitat and deploy automated lasers for bird repelling. 3. Direct intervention by wildlife officers. We furthermore developed the tools needed to put this approach into practice. The laser equipment is based on a scientific new technology: an automatic laser beam that makes use of the fear reflex of birds for green light. Built-in safety methods make sure that the laser can never dazzle aircraft, vehicles or the general public. The birds will stop seeing the industrial area as a safe haven since the system keeps firing its animal friendly laser 'attacks'. Bird Control Group has introduced a laser system that repels birds automatically from so-called 'bird hotspots'. The principle of repelling birds with a laser beam is inspired by nature. Birds perceive the approaching laser beam as a physical object. It appeals to the survival instinct, causing the birds to fly away. The continuous presence of the moving laser beam keeps areas free of birds, 24 hours a day. Numerous users in more than 70 countries make us of the technology. Some of these clients are leading international companies. Leading pest control companies around the world implement the approach at their clients. In a business case of a well-known tire warehouse, 40,000 square meter of roof is protected from birds with one laser system. The investment of about 10,000 Euro saves the company tens of thousands of Euro each year.

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INTENSITY-DEPENDENT SUCCESS OF FERAL PIGEON POPULATION CONTROL BY CULLING IN BARCELONA CITY (CATALONIA, SPAIN)

[1]J. PASCUAL., [1]T. MONTALVO., [1]V. PERACHO., AND [2]J.C. SENAR

[1]Servei de Vigilància i Control de Plagues Urbanes, Agència de Salut Pública de Barcelona, Barcelona, Spain
[2]Natural History Museum of Barcelona

Abstract Pigeon control actions in big cities are usually necessary to maintain the populations in such densities that do not generate conflicts with the human inhabitants. Pigeon control has traditionally been undertaken by culling. However, several papers have shown that this method might not be effective since it creates a gap in the capture locations that is rapidly filled by neighboring individuals. Nonetheless, many of these studies were based on culling actions over small or medium-sized cities, on culling over only some parcels of big cities or on culling over a small proportion of the whole population. In a former paper (Senar et al. 2009, Arxius de Miscel·lània Zoològica 7: 62-71) we showed that culling actions eliminating 227,479 pigeons from 1991 to 2006 (i.e.15,165 pigeons/year) were very few effective to the control of the whole pigeon population in Barcelona city, since the population increased from 183,667±14,914 to 256,663±26,210 individuals (CI 95%). Here we present the results of a study on the efficacy of culling actions from 2009 to 2014 over the same population, with an intensity of 42,363 pigeons caught per year, showing that the pigeon population dropped to 85,777± 10,028 individuals in 2015. These results clearly show that culling might be highly effective to the control of pigeon populations when its intensity is properly dimensioned. They also stress the importance of analyzing the effect of control actions over whole populations and not only to selected parcels in evaluation tests in order to overcome the influence of metapopulation dynamics.

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PHYTOMASS OF URBAN ECOSYSTEMS AS THE BASIS FOR ATTRACTION OF DESIRABLE AND UNDESIRABLE ORGANISMS

VALENTIN B. SAPUNOV

Saint-Petersburg State Agrarian University, St-Petersburg, Russia

Abstract Under increase of pressure on nature, many organisms have adapted to a new environment and began an active attack on new ecosystems. Urbanized communities are part of the biosphere and live according to the fundamental laws of ecology. Urban green plantations having both alive and dead phytomass are attractive for urban pests. Under increase of natural hazard activity and anthropogenic pressure, biological indication as cheap monitoring methods becomes necessary. Urban plants are natural indicators of ecological pressure: float asymmetry is a measure of mutagenic pollution. Abnormal form of three is criteria of teratogen pollution. The more plants have abnormality, the more resources for pests. Importance of such an indication is increased by Chernobyl and Fukushima incidents etc. The ratio between number of species and their biomass has mathematical description available to the prediction of the number of weed plants and phytophagous, which will occupy urban plantations. Urban populations have a higher phenotypic variation than wild ancestors. According to data of ICUP (1993 – 2014), 3 important processes during the 22 years took place: 1. Development of fundamental knowledge about urban pests. 2. Development of practical methods of pest control. 3. Ecological and evolutionary processes in pests that allowed them to take extensive ecological niches. The latter occurred most rapidly and efficiently, and the pests have proved to be stronger than program of pest management. Some organisms in the urban environment were more populated than their ancestors in the wild. Dead plant phytomass is resource for the breeding of termites. These insects produce gases that significantly affect global climate. In some cases, increase in the number of pests occurs after natural disasters. So, tsunami 2011 in Japan washed up on the shore a huge biomass of marine animals. It has become a breeding ground for the development of many pests and sharply worsened sanitary conditions in coastal areas. The first ICUP conference was dominated by reports offering chemical methods of control and rotation of pesticides. These methods have been, and will continue to apply, despite their possible negative environmental consequences. However, an increasing number of works devoted to environmentally friendly methods of struggle, reproducing the processes occurring in nature without human influence. This is the use, along with pesticides, repellents, including those of natural origin. An important aspect of pest management under policy of "green economy" – the shift from synthetic pesticides to substances derived from natural materials and organisms, and synthetic analogs of such substances. Accordingly, the fight against plant pests and pathogens for humans can only be effective based on the latest achievements of scientific ecology and on control of their resources.

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CONTROLLING INVASIVE TREE PESTS IN THE URBAN FOREST USING TREE MICRO INJECTION

ALEXANDER CORNISH AND PETER WYSS

Syngenta Crop Protection AG, Schwarzwaldallee 215, CH-4058 Basel, Switzerland

Abstract It is well documented that several waves of invasive tree pests are advancing across the European continent and there are few practical solutions to halt their progress. Pests include Horse Chestnut Leaf Miner, Red Palm Weevil, Asian Longhorn Beetle, Pine Wood Nematodes and Pine and Oak Processionary Moth. Studies carried out over the last 10 years using specially formulated emamectin benzoate have shown that the invasive pests listed above can be controlled effectively via tree micro injection. Examples of be effective control will be shown, often for several years following a single application. The application method is particularly suited to treatment of urban trees.

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LURING BISCUIT BEETLES (COLEOPTERA: ANOBIIDAE) AWAY FROM DRIED ORNAMENTAL PLANTS

[1]CHRIS SWINDELLS AND [2]YVETTE HARVEY

[1]Acheta Consulting Ltd, Church View, Front Street, Churchill, North Somerset, BS25 5NB
[2]Royal Horticultural Society Garden Wisley, Woking, Surrey, GU23 6QB

Abstract Monitoring of insect pest activity with the use of sticky blunder traps (sometimes with an attractant) is routinely carried out in the food industry and other businesses. Biscuit beetles (*Stegobium paniceum*) can be a significant pest but, there have been few commercially available species specific traps. Monitoring for *Stegobium paniceum* has been limited to visual inspection, the use of generic sticky blunder traps, or captures in electric flying insect control units. In collaboration with Acheta Consulting Ltd, The Royal Horticultural Society's (RHS) herbarium at RHS Garden Wisley has recently taken part in a very small trial of a pheromone lure, currently commercially unavailable in the UK, to attract male *Stegobium paniceum*. The herbarium contains over 83,000 ornamental plant specimens that have been pressed, dried and mounted on thick card. Most specimens have not been treated with any pesticide, and are contained within sealed metal cabinets in a small room that has open access to other rooms. The herbarium suite is not environmentally controlled. Although the specimens are annually frozen (a rolling programme), a population of *Stegobium paniceum* have been able to survive in the collection area. This made the herbarium ideal to trial the pheromone lure. The trial was undertaken during August and September 2016. As part of the trial, the pheromone lures were not stuck on glue traps provided by the manufacturer of the lure, or presented in the orientation suggested by the manufacturer. Lures were stuck on to alternative commercially available crawling insect monitor glue pads, held within a hanging frame and hung around the collection area. Identical traps lacking the lures were placed near the lured traps. Results indicate that *Stegobium paniceum* were attracted to the lured traps in preference to the traps that were free of the pheromone lures. Beetles were still being caught after the manufacturers recommended renewal time, indicating the potential effectiveness of the pheromone lure. Captures within the traps have indicated where the problem is likely to be and has shown the pheromone to be a promising tool in the fight against *Stegobium paniceum* in a herbarium collection.

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AUSTERITY BITES: LOCAL AUTHORITY PEST CONTROL SERVICES

MATTHEW AUDLEY, SIMON FORRESTER, AND PARMINDER THINDAL

British Pest Control Association, 4A Mallard Way, Pride Park, Derby DE24 8GX

Abstract The landscape of public health pest control is changing. Budgets are being cut across the country, with most local authorities (LAs) still being the main source of dealing with public health pest problems as they did before, but with significant reductions in resourcing. Through analysing the UK pest control call-out data of LAs and comparing them to commercial (private) pest control companies, it is apparent that under-resourcing public councils places significant pressure on the public to self-finance public health pest control. Data indicated that UK LAs have been gradually contracting out their pest control services within the last five years. For the financial year 2011-2012, 17.9% of LAs contracted out their pest control services. This has risen significantly, emphasising the notion that LAs are no longer financing public health pest control, and suggesting that pest control services at LAs are under-resourced due to the government austerity measures within their local districts. Many services are under threat from future cutbacks, and in most cases these cuts have already had an impact on pest control staffing levels and services. This highlights a worrying trend; that UK LAs can no longer provide pest control services to the public due to the current measures and changes to LA financial models, thus leading members of the public and private pest control companies to solve the public health pest control needs of the nation. The majority of the general public perceives that the first call for pest-related problems is their local council. However many will discover their calls being rejected from councils that no longer offer these services, and therefore will rely on DIY treatments and commercial pest control providers. It's thought that analysis of BPCA referral data demonstrates that areas where cutbacks are prominent, there are resultant increases in demand for commercial pest control activity. This exposes the vulnerable to unscrupulous and potentially unqualified pest operators. The British Pest Control Association believes these data highlights the need for greater cooperation between LAs and the private sector to guarantee an affordable and safe pest control for the public.

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ABILITY OF TRAINED SCENT DETECTION DOGS TO DETECT GRAIN WEEVIL IN WHEAT SAMPLES

A JUSON AND C JUSON
Merlin Environmental Solutions Ltd. PO BOX 112, Carshalton, Surrey SM5 4XT. UK

Abstract Cereal grains are the major source of food for humans and most domesticated animals. In many developing countries, overall post harvest losses of cereals of between 10–15% are fairly common. The grain weevil, *Sitophilus granaries* is a pest of wheat, oats, rye, barley, rice and corn. Grain weevils cause significant damage to harvested stored grains and may drastically decrease yields. They are hard to detect and usually all of the grain in an infested storage facility must be destroyed. In common with other grain boring species, its larval stage is concealed within the grain, feeding on the germ, only becoming apparent after pupation. Currently on farms, manual samples, traps, and probes have been used to determine the presence of insects. These methods are not efficient and are time consuming. At the industrial level, acoustic detection, carbon dioxide measurement, uric acid measurement, near-infrared spectroscopy, can be used to detect insects in grain samples however these methods are cost prohibitive and not widely available in the developing world. The use of scent detection dogs for the monitoring of wood destroying insects and certain parasites of man is widespread and highly successful, however their role in stored product pest management has not been investigated. Dogs were trained as part of this study to detect grain weevil larvae and adults using a toy and verbal/physical interaction reward system. Their efficacy was tested with adult grain weevils and larvae placed in vented polycarbonate containers under controlled test conditions. Dogs were able to discriminate grain weevil samples from *Blattella germanica*, and *Callosobruchus maculatus*, with a 96% positive indication rate (correct indication behaviour from dog when target present) and 0% false positives (incorrect indication of when not present). Under controlled conditions using infested wheat samples from captive colonies, dogs were 92% accurate in locating live grain weevil larvae prior to pupation at infestation levels as low as 1 infested grain per 100ml of wheat. If trained properly, dogs can be used effectively to locate live grain weevil larvae in stored wheat crop prior to pupation. They could offer a fast and cost effective addition to integrated pest management programmes and subsequently reduce grain losses.

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INSECTICIDE CANDLE

BOEY WAI LOON
Plot 10.01, Jalan PKNK 1/3, Kawasan Perindustrian Sungai Petani, 08000 Sungai Petani, Kedah, Malaysia.

Abstract Insecticide candle is the optimum human lifesaving innovation that kills flying vectors, particularly mosquitoes - the most lethal of all insects in the world. Over one million people worldwide die from mosquito-borne diseases every year. The candle is a special vegetable wax-encased formulated candle which uses pyrethroids as the active repellent ingredient. An evaluation of the candles' repellent effect was done by the National Institute of Malariology, Parasitory and Emotology, Vietnam, after a field efficacy trail of the candles' formulation with transfluthrin was done using the human bait technique as the criteria in measuring the population of night biting mosquitoes, *Anopheles epiroticus* and *Culex vishnui*. The field trial was done in an indoor setting, conducted at the brackish residential area, An Thoi Dong, Can Gio District, Vietnam where the predominant population density species was the *Anopheles epiroticus* reported to be resistant to the pyrethroids class. The field trial results showed that in spite of mosquito resistance to pyrethroids the repellent candles provided a highly substantial protection effect from biting. The average protection effect of the candles in 6 hours biting of *Anopheles epiroticus* was 71.92% and *Culex vishnui* at 76.36%. In comparison, the results showed an increase of protection against a previous bioassay result done in July 2014 where the protection rate was only 60% with alphacypermethrin. This suggests that the candles had proved better to limit mosquito biting and has gathered a high degree of acceptance by the community (98%) for the candle. Further prevailing results were gathered from a laboratory evaluation research at the Vector Control Research Unit, Universiti Sains Malaysia using the Peet Grady chamber method indicating a response after 5 hours burning of a single candle. After-burned-time measured for 8 hours showed the efficacy for knock-down (KT50 and confidence limit) at 4.09 minutes and 100% mortality. This candle is a new revolutionary solution. It is a scientifically tested and proven tool in fighting mosquitoes and the diseases they transmit**.** Essentially applicable for both indoor and outdoor use, it is smokeless, odourless and adequately doubles up ideally as a light providing source especially useful in areas with no electricity. It stands decidedly as an effective, acceptable, affordable, accessible, and available source of household protection against many widespread vector diseases.

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DEVELOPMENT OF A BIOLOGICAL TICK TRAP BASED ON ATTRACT-AND-KILL STRATEGY

[1]KERSTIN BÜCHEL, [1]HANS DAUTEL, [1]BJÖRN PÖTSCHKE, [1]MARIELLA JONAS, [2]MARION WASSERMANN, [2]UTE MACKENSTEDT, [3]ELISA BEITZEN-HEINEKE, [3]WILHELM BEITZEN-HEINEKE, [3]MICHAEL PRZYKLENK, [4]SISSY-CHRISTIN LORENZ, [4]PASCAL HUMBERT, AND [4]ANANT PATEL

[1]IS Insect Services GmbH, Haderslebener Straße 9, 12163 Berlin, Germany
[2]University of Hohenheim, Dept. of Parasitology, Emil-Wolff-Straße 34, 70599 Stuttgart, Germany / cooperation partner
[3]BIOCARE company of biological plant protection mbH, Dorfstr. 4, 37574 Einbeck, Germany / cooperation partner
[4]Bielefeld University of Applied Sciences, Faculty of Engineering Sciences and Mathematics, Fermentation and Formulation of Biologicals and Chemicals, Wilhelm-Bertelsmann-Str. 10, 33602 Bielefeld, Germany / cooperation partner

Abstract The most frequently reported vector-borne diseases in the temperate zone of the Northern Hemisphere are related to ticks, e.g. borreliosis and tick-borne encephalitis. In Europe, *Ixodes ricinus* (Acari: Ixodidae) is the most abundant species infesting a wide range of hosts. Increased public awareness concerning infectious ticks has raised interest in an effective tick control. The aim of this project is the development of a tick trap based on an attract-and-kill strategy. Therefore, we screen long and short range attractants as well as aggregation pheromones. For the first time we demonstrate behavioural assays with *I. ricinus* nymphs using a novel y-olfactometer for screening compounds of, inter alia, the classes of aldehydes, lactones, and terpenoids. We demonstrate a significantly attractive effect of CO_2 on *I. ricinus*. Further we screen aggregation pheromones using a refined static assay. Up to date we detected aggregation pheromones of the classes of purines and their derivatives. These substances combined (attractant component) will be released through a capsule-based biopolymer system, which is coupled with a kill component, a naturally occurring entomopathogenic fungus. The fungus *Metarhizium* spp. is able to infect the ticks as they come into contact with the capsules. The intention of the trap is to provide protection against *I. ricinus* in areas frequently used by humans. Future research should investigate whether similar results are obtained when adult *Ixodes* ticks or other tick species are tested.

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PEST ODYSSEY UK – A GROUP TO PROMOTE IPM IN CULTURAL HERITAGE IN THE UK

[1]SUZANNE RYDER, [1]ARMANDO MENDEZ, [2]PHILIP BAXTER, AND [3]DAVID PINNIGER

[1]Natural History Museum, Cromwell Road, London SW7 5BD,
[2]The British Museum, Great Russel Street, London WC1B 3DG,
[3]DBP Entomology, Cookham SL6 9DE

Abstract The Pest Odyssey group consists of professionals from the cultural heritage sector with a wide experience of IPM, a personal interest and demonstrated dedication to the program and its applications. We effectively represent the cultural sector and members are from a wide group which includes; National Museums, Historic Houses and independent consultants. It has its origins in a programme set up by English Heritage in 2008 to encourage transmission and exchange of IPM skills. The group really came together in its present form to organise and run the very successful 2011 Pest Odyssey Conference at the British Museum. Our mission is to provide a trusted platform to communicate, advise and promote best practise in Integrated Pest Management for cultural heritage. This multi-disciplinary group provides expertise in reducing pest risk, thus protecting valuable collections. We advocate the use of IPM as an essential cost-effective and sustainable tool to serve the cultural heritage industry, creating collaborative networks and sharing relevant information. There is a small steering group of members who must show active participation within the group. Some of the achievements over the last 10 years include: development of guidelines and strategies for IPM programmes, advice to legislative bodies directing control and treatment products and developing the Pest Odyssey website. The group aims to organise one open meeting each year for interested museum and pest professionals to encourage presentation of new information and discussion on best practice IPM strategies in the heritage sector.

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THE FATE OF TICK POPULATIONS (ACARI: IXODIDAE) AFTER INTRUSION OF AN URBAN SETTLEMENT INTO WILD NATURE (A CASE STUDY)

IGOR USPENSKY
The Hebrew University of Jerusalem, Israel

Abstract The construction of a hydroelectric power station on Zeya River near the town of Zeya (Amur Region, Russian Far East) in the early 1970s spurred the development of a new urban settlement in the town for construction workers. Natural conditions of this area supported the persistence of populations of 3 species of ixodid ticks: the taiga tick *Ixodes persulcatus* (*I.p.*), the main vector of a number of tick-borne pathogens, as well as *Haemaphysalis concinna* and *Dermacentor silvarum*. The preferred host species for adult ticks in the area were Siberian roe deer, wild boar and numerous mountain hares. The abundance of *I.p.* adults in the peak of their activity reached 12.0 specimens per 1 km of dragging at the initial stage of construction of the settlement. Numerous cases of tick attacks and bites (mainly by *I.p.* adults) were registered in this and adjacent areas at that time. The prevalence of the tick-borne encephalitis virus in *I.p.* females was found to be up to 1.0%. Aerial treatment of the area by DDT dust was performed in 1970, which resulted in the disappearance of adult ticks of all 3 species. The effect on the larval and nymphal stages was weaker, 40-60% reduction according to the data of tick collection from small mammals. In the following 4 years, no adult ticks were detected in the area, while the preadult occurrence decreased every year; tick attacks were rarely documented, and then only near the border of the treated territory. These results are in contrast to the data obtained in a number of studies, in which a gradual recovery of tick populations was observed in the years following a single acaricidal treatment. The unexpected results of our study may be explained by the strong anthropogenic pressure over the territory of the settlement and its surroundings. Each year following the acaricidal treatment, the size of the settlement and its human population increased significantly, which resulted in intensive trampling of the grass, uncontrolled fires, destruction of vegetation as well as displacement of wild animals from the territories contiguous to the settlement. As a consequence, the territory was transformed into a very unfriendly environment for tick development and feeding.

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PRODUCTION, CHARACTERIZATION AND EFFICACY OF ETHYL CELLULOSE-BASED NANOCAPSULES CONTAINING IMIDACLOPRID FOR CONTROL OF *MICROCEROTERMES DIVERSUS* (SILVESTRI) (ISOPTERA: TERMITIDAE) UNDER LABORATORY CONDITIONS

[1]BEHZAD HABIBPOUR, [1]REZA ABOLFARSI, AND [2]ALI ASHRAFI

[1]Department of Plant Protection, College of Agriculture, Shahid Chamran University of Ahvaz, Iran.

[2]Department of Materials Engineering, College of Materials Engineering, Isfahan University of Technology, Iran.

Abstract *Microcerotermes diversus* (Silvestri) (Isoptera: Termitidae) is a major species of termites that attacks cellulosic materials and is an economically important pest, damaging buildings in Khuzestan Province (Iran). Neonicotinoids, most notably imidacloprid, have recently been introduced for pest control programs. The main purpose of this study was to develop new techniques for termite control by environmentally friendly formulations. Nanotechnology has become important in a number of fields during the past decade. The use of this new technology in the preparation of nanocapsules made of different materials in a slow and controlled release of drugs and toxins and increase stability within the nanocapsules has been recently reviewed. In the present study, we report for the first time the emulsification synthesis of ethylcellulose-based nanocapsules containing imidacloprid. In this method, we prepared the polymer and the polymer solution (including: ethylcellulose, imidacloprid, benzene and ethanol) was added drop by drop to an aqueous solution (including: deionized water, nitric acid, sodium dodecyl sulphate and polyethylen glycol). Finally, produced ethylcellulose-based nanocapsules containing imidacloprid and determined some of the quantitative and qualitative characteristics such as shape and size of the particles by Scanning Electron Microscope (SEM) using a secondary electron detector (SE) and Infrared spectroscopy (IR). The effects of ethylcellulose-based nanocapsules without imidacloprid and those containing 50, 100, 500 or 1000 ppm imidacloprid were evaluated against *M. diversus*. The ethyl cellulose in this formulation did not induce lethal effects against the termite and showed feeding attractancy. Treated filter papers with this formulation caused a gradual increase in mortality during the 14-day trial. Our results suggest that ethyl cellulose-based nanocapsules containing imidacloprid could be an effective termiticide formulation.