

Proceedings of the

2012

National Conference on Urban Entomology



May 20-23, 2012 Atlanta, Georgia

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PROCEEDINGS OF THE 2012 NATIONAL CONFERENCE ON URBAN ENTOMOLOGY

Edited by Daniel R. Suiter

ACKNOWLEDGEMENTS

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PROCEEDINGS OF THE 2012 NCUE, ATLANTA, GA

DISTINGUISHED ACHIEVEMENT AWARD IN URBAN ENTOMOLOGY

ARNOLD MALLIS MEMORIAL AWARD LECTURE:

Science of 'Omics in Urban Pest Management

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University of Nebraska, Lincoln, NE 68583-0816

Dear NCUE organizing committee members, Arnold Mallis memorial award selection committee members and colleagues:

I am indebted to you for giving me an opportunity to serve the science of urban entomology. It is an honor and privilege to join my colleagues who received this award from 1988-2010. I extend my salutation to Dr. Roger E, Gold for opening the door of urban entomology. Special thanks to my graduate students who entered through that opportunistic door, received their education and are enjoying the rewarding professional career. In terms of my background, I come from a very small town in India that did not even have an elementary school when I started my education. I have been fascinated with education and constantly observed my teachers and other professionals. I set the goal early in life to earn the highest degree. So, I have been very hungry for education. To satisfy my big appetite for education, I opted for a mantra/slogan/motto: "if he/she can, I can---". I have always tried to associate with progressive people with character and find solutions to challenges. I received my B.S. in agricultural sciences and M.S. in entomology from Nagpur University in India. I started my Ph.D. in entomology at the Indian Agricultural Research Institute in New Delhi, India but left for USA to acquire novel educational experience. Subsequently, I received my Ph.D. in Entomology in 1974 from the North Dakota State University, Fargo. Upon graduation, I opted to continue my professional life in urban entomology. My career in urban entomology has been very rewarding and I had the opportunity to interact with many kind and progressive urban entomologists. I had the pleasure of training 8 M.S. and 12 Ph.D. students. Three postdocs also worked in my laboratory.

In 2010, the Arnold Mallis memorial award recipient, Mr. Donald A. Reierson has provided outstanding historical perspectives in urban entomology. Therefore, I wanted to address the upcoming scientific developments with a topic on "Science of 'Omics in Urban Pest Management". To proceed further, I feel that it is important to review the historical urban pest management milestones involving insecticides. History of insecticide developments can be broadly categorized as follows:

- 900 AD: Arsenical insecticides were used in China to control insect pests garden plants/crops;
- Prior to 1940's: Use of arsenicals (Lead arsenate), botanical insecticides and fumigants (HCN);
- 1940-1950: Introduction of chlorinated hydrocarbon insecticides (e.g., DDT, Methoxychlor, dicofol/ Kelthane, benzene hexachloride/BHC etc.);
- •1950-1960: Era of Cyclodienes (aldrin, dieldrin, chlordane, heptachlor, toxaphene, mirex bait), etc.), Organophosphates (diazinon, malathion, dimethoate/Cygon, chlorpyrifos/Dursban, dichlorvos/DDVP, trichlorfon/Dipterex, acephate/Orthene), Carbamates (carbaryl/Sevin, propoxur/Baygon, bendiocarb/Ficam), and botanical insecticides (nicotin sulphate, pyrethrum powder, etc.);
- 1960-1970: Mostly, organophosphates, carbamates, pyrethroids (allethrin, resmethrin, tetrame thrin, cypermethrin, fenvalerate, esfenvalerate, deltamethrin, permethrin, bifenthrin, cyhalothrin, cyfluthrin, etc.);

- 1970s: Era of third generation insecticides (hypothesized by Dr. Carroll M. Williams) including insect
 - growth regulators (juvenile hormone mimics, e.g., methoprene, hydroprene, fenoxycarb, Chemosterilants, Pheromones (aggregation pheromones and sex pheromones);
 - 1980s: Introduction of Avermectin (Merck Co.); Microbial Pesticides: *Bacillus thringiensis* (Bt) (gamma endotoxin includes Cry 1-6 proteins, Cyt proteins and Vip 1-3 proteins);
 - •1980s: Amidinohydrazone: Hydramethylnon (cockroach bait), Sulfonamides: Sulfluramid (bait products);
 - •1990s: Compounds with new chemistry Benzoylphenylureas (diflubenzuron, hexaflumuron, noviflumuron, teflubenzuron, lufenuron, pyriproxyfen);
 - •1990s: Neonicotinoids: (imidacloprid, acetamiprid, dinitefuran, thiamethoxam) Phenylpyrazoles: fipronil, (Termidor), Pyrroles: chlorfenapyr (Phantom); and
 - 2000s: Oxadiazines: Indoxacarb (Advion, Arilon); and Anthranilic diamides: chlorantraniliprole (Altriset).

Now, there is a wave of new science: "Science of 'Omics" which includes proteomics, genomics, functional genomics, structural genomics and comparative genomics. To understand these concepts, the training in entomology will have to be modified to encompass formal courses in molecular biology, insect biochemistry, general molecular genetics, insect molecular genetics, molecular phylogenetics, bioinformatics, signal transduction, gene expressions/replication and knowledge of cell structure (Figure 1).

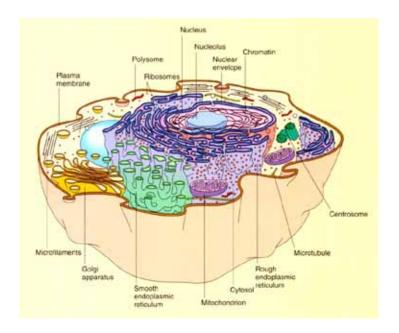


Fig. 1. Cell Structure (Ref. Robert F. Weaver. 2002. Molecular Biology. McGraw Hill Co., 859 pp.)

The cell contains nucleus which is the repository of genetic information encoded in deoxyribonucleic acid (DNA) and organized in chromosomes. DNA composition includes four nitrogenous bases [Adenine (A), Cytosine (C), Guanine (G), Thymine (T)], phosphoric acid, and sugar (deoxyribose). The composition of DNA double helix is presented in Figure 2.

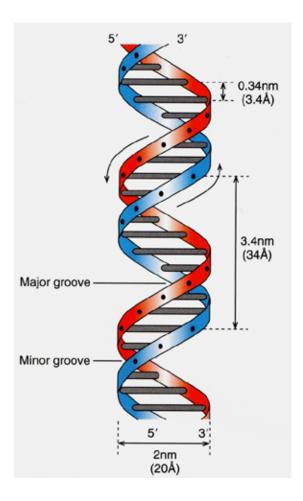


Fig. 2. The DNA double helix is presented as a twisted ladder with sides representing the sugar-phosphate backbones of the two strands and rungs representing the base pairs. (Ref. Robert F. Weaver. 2002. Molecular Biology. McGraw Hill Co., 859 pp.)

The ribonucleic acid (RNA) is also a major component of cell structure and it delivers genetic information from genes to the ribosomes. The RNA composition also includes four nitrogenous bases [Adenine (A), Cytosine (C), Guanine (G), Uracil (U)], phosphoric acid and sugar (ribose).

While conducting molecular research, in 1941, the Nebraska scientist, Dr, George Beadle (native of Wahoo, Nebraska) discovered the relationship between genes and enzymes. He hypothesized that there is one gene per one enzyme. In 1958, Dr Beadle was recognized for his novel discovery and awarded a noble prize (Figure 3). Subsequently, a Beadle Center for molecular research was constructed on the University of Nebraska-Lincoln Campus.



Fig. 3. Dr. George Beadle (native of Wahoo, Nebraska), noble prize winner for his research on discovery of one gene/one enzyme hypothesis.

Application of 'Omics Techniques

In agriculture, proteomics (Cry-1-6, Cry-1a, Cry-1b, Cry-1-c) have been adapted in transgenic BT corn to suppress of European corn bore and corn rootworm populations. Similarly, transgenic cotton has been developed to control cotton bollworms.

In Urban entomology, 'omics techniques cover following areas:

- Study of Insect gut microbes Metagenome sequencing and gene expression;
- Genes in arthropods vectors for immune response to disease organisms;
- Genes to detoxify insecticides e.g., P-450;
- Microarray analysis of transcriptome's response to pesticides, climatic stress, host-defense, infection by viruses and microbial pathogens;
- Genetic transformation of insects-Gene inactivation in malaria refractory mosquitoes:
- Genetic transformation of *Metarhizium* spp. fungus;
- Genetic transformation of insect parasites; and
- Microbial diversity and ecology of the termite gut and genetic engineering of gut bacteria for termite control (e.g., paratransgenesis).

Genome Sequenced

There has been substantial progress made in sequencing the genomes of key insects (Figure 4). This information will be utilized by scientists to discover new techniques to manipulate insect biology for beneficial purposes.

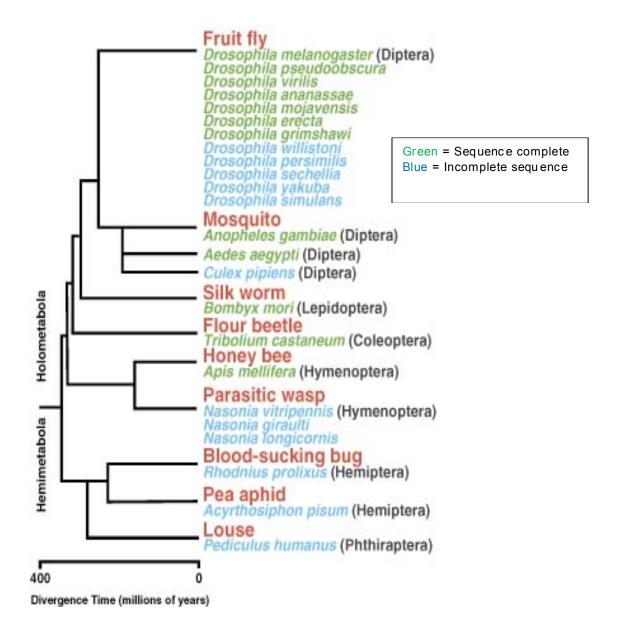


Fig. 4. Insect Genome sequenced (Ref: Grimmmelikuljen et al. 2007. Pest Management Science. 63: 413-416).

RNA Interference

The RNA interference is defined as the ability of exogenous single-stranded RNA (ssRNA), double-stranded RNA (dsRNA), small interfering RNAs (siRNAs) and/or small hairpin RNAs (shRNAs) to suppress the expression of the gene which corresponds to the dsRNA sequence. This RNA interference process is also known as:

- gene silencing;
- post-transcriptional gene silencing (PTGS);
- RNA silencing; and
- quelling in fungi.

In 1990, Dr. Richard Jorgensen and colleagues attempted to produce a petunia with a deeper color by inserting the gene for purple pigment into its genome under the control of a stronger promoter. Instead of turning dark purple, the new petunias were either entirely white or streaked purple and white (Figure 5). Jorgensen surmised that the additional copy of the gene suppressed both itself and its endogenous counterpart, an event he called co-suppression.



Fig. 5. Coloring patters in petunia resulting from RNA research. Ref: http://www.fas.org/biosecurity/education/dualuse/FAS_Hannon/index.html

For discovery of RNA interference, Andre Fire and Craig Mello received a Noble prize in 2006 (Figure 6) for their research on the gene silencing by double-stranded RNA.



Fig. 6. Picture of Dr. Fire and Dr. Mello
Ref. http://www.nobelprize.org/nobel_prizes/medicine/laureates/2006/
Dr. Fire Affiliation at the time of the award: Stanford University School of Medicine, Stanford, CA.
Dr. Mello Affiliation at the time of the award: University of Massachusetts Medical School, Worcester, MA.

The RNA interference in termites, *Reticulitermes flavipes* through ingestion of double-stranded RNA was reported by Xuguo Zhou, Marsha M. Wheeler, Faith M. Oi and Michael E. Scharf (Ref. Zhou et al. 2008. Insect Biochem. Mol. Biol. 38(5): 805-815). These authors reported the morphological deformities in subterranean termites when fed on hex-2 dsRNA (Figure 7).



Figure 7. Morphological impacts in *R*. flavipes termites after Hex-2 dsRNA feeding, both with and without co-application of ectopic juve nile hormone (JH). Photographs were taken of alcohol-preserved individuals using a digital Syncroscopy system (see text for details).

- (a) "Normal" or wildtype caste phenotypes as would naturally appear in termite colonies, or as induced by feeding on Hex-2 dsRNA alone or JH alone: worker (left), presoldier (middle), and soldier (right).
- (b) An extreme example of a worker that underwent a lethal status-quo molt immediately after ingesting a second dose of Hex-2 dsRNA+JH.
- (c) Workers and presoldiers after lethal molts as induced by two doses of Hex-2 dsRNA+JH. Despite the range of malformations, all individuals were lethally affected. View high quality image (238K)

Research on RNA interference-mediated knock-down of Bla g 1 in the German cockroach, *Blattella germanica* L. was published by A. Suazo, C. Gore and C Schal. (Ref.: Insect Molecular Biology (2009) Volume: 18 (6): 727-736). These authors used RNA interference (RNAi) to silence the expression of a gene encoding Bla g 1, a human allergen produced by *B. germanica* to study its function in cockroach physiology. Females injected with 1 microg of double-stranded RNA contained 64% less Bla g 1 protein and Bla g 1 mRNA abundance was reduced by 91.4% compared to shaminjected females.

RNA Interference of NADPH-Cytochrome P450 Reductase results in reduced insecticide resistance in the Bed Bug, *Cimex lectularius*. These research findings were reported by Fang Zhu, S. Sams, T. Moural, K. F. Haynes, M. F. Potter and Subba R. Palli in 2012 (Ref. PLoS ONE 7(2): e31037. doi:10.1371/journal.pone.0031037).

The research objective was to clone cDNA and knockdown the expression of the gene coding for CPR (NADPH-Cytochrome P450 Reductase) to determine whether or not P450s are involved in bed bugs insecticides resistance. These authors found that P450-mediated metabolic detoxification may serve as one of the resistance mechanisms in bed bugs.

Recently, C. Tittiger (Department of Biochemistry and Molecular Biology, University of Nevada, Reno, NV) published a paper on "Functional Genomics and Insect Chemical Ecology". Dr. Tittiger used different terminology (Ref. Journal of Chemical Ecology 30(12): 2335-2358, 2004) such as:

- Gen'ome'
- · Prote'ome'
- Transcript'ome'
- · Phen'ome'

He ended with suffix "ome" which is commonly used in meditation.

Future of Urban Entomology in Relation to 'Omics or 'Omes

It appears the science of "omics" or "omes" is very intriguing and promising. The urban entomologists will have to adapt to this science. These adaptions may include for:

- · Entomology Professors involved in teaching;
- Urban Research Entomologists Re-tooling;
- Entomology Graduate Students
- Extension Entomologists;
- · Consultants, including Forensic Entomologists
- · Contract Research entomologists
- · Technical Directors/Entomologists Major Firms; and
- · Federal and State Government Entomologists

My thanks to all the researchers mentioned in this presentation for their contributions. I am grateful to Ralph Narain for his assistance. Once again, I appreciate your attention to this presentation.

Thank you.

STUDENT SCHOLARSHIP AWARD PRESENTATIONS

BACHELOR OF SCIENCE AWARD:

Population Growth Characteristics of Incipient Colonies of the Eastern Subterranean Termite, *Reticulitermes flavipes* (Isoptera: Rhinotermitidae)

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In May 2011, ~800 colonies of *Reticulitermes flavipes* were initiated with paired alates originating from a single swarm that took place in Pickaway Co, Ohio. Each male and female pair was placed in a "nest" container (3.8 cm ht x 5.4 cm dia) provisioned with moist mulch. These colonies were maintained in an environmental chamber in the dark (26° C, 85-90% RH). Fifteen different colonies were censused monthly for a 1-yr period, but only established colonies headed by a single queen and king were examined. Data were recorded for all castes in each colony including the number of individuals and their collective mass; the mass of the king and queen was individually obtained.

Egg production was intermittent, with the greatest number of eggs (mean = 14.5) produced during the first month. There were three cohorts of eggs; the first was the largest and occurred during the first and second month, the second was very small and occurred in the sixth and seventh month, and the third was moderate, spanning months nine through twelve. Egg production was not constant, most likely due to the initial brood care tasks performed by the king and queen. These tasks are later taken over by workers, enabling the reproductives to focus on egg production.

Larvae were observed at 1 mo and workers (> second instar) were first observed at 2 mo. The survival rate of the initial brood was less than 100%, since the average number of eggs, larvae, and workers in the first two months decreased during the following months. At the 6-mo census, a soldier was observed in each of two colonies with total populations of 12 and 13 individuals. Soldiers were sporadically present in colonies thereafter and increased in abundance at the 12-mo observation. One-year-old colonies ranged in size from 20 to 40 individuals, including the king and queen.

The mass of both the king and queen greatly decreased during the first two months, coinciding with egg production and colony foundation, and their mass remained relatively constant through the 12-mo reading. These results suggest that the reproductives initially use their fat stores to produce young, with a corresponding decrease in reproductive biomass. Total mass of each colony gradually increased over time. Offspring biomass was equal to that of the reproductive pair at the 2-mo census, double at 3 mo, and quadruple at 11 mo.

Overall, this study's results are consistent with previous models and suggestions regarding colony growth (Grube and Forschler 2004, Suiter et al. 2002), but this study provides the numerical data.

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MASTER OF SCIENCE AWARD:

Extraction Efficiency of *Dermatophagoides pteronyssinus* from Several Substrates Using Two Techniques

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The term House Dust Mites (HDMs) is used to describe a functional group that includes several species of very small arthropods found worldwide within human habitations. These mites are considered a major cause of allergic disease, including conditions such as allergy, hay fever, rhinitis, asthma, and atopic dermatitis (Nadchatram 2005). Asthma alone leads to 500,000 hospitalizations a year, a large percentage of which involve children (Sharma et al. 2011). Numerous studies have sampled for HDMs inside homes with the intent of determining species distribution and numbers of mites for a variety of purposes involving, but not limited to, indoor air quality and/or allergy and mite management (Arlian et al. 1982, Arlian et al. 1992, Caplin et al. 2009). It is therefore important to know if the methods employed to sample HDM populations provide a realistic estimate of HDM numbers. The correlation between several weight categories of *Dermatophagoides pteronyssinus* and the actual number of mites in that weight category was tested as the basis for examination of the efficiencies of two common HDM extraction methods - heat escape and flotation. A strong correlation between HDM weight category and number of mites was demonstrated using linear regression ($R^2 = 0.9928$). The heat escape method was tested using 2.0 mg of mites. This method removed a mean of 4.34-6.97% of the mites. Flotation, the most frequently cited method, also provided low extraction efficiencies, especially if HDM's were combined with a substrate such as dust (mean = 6.53% ±5.30% when using this method with 1.0 mg of mites, and mean = 6.85% ±6.91% when using this method with 5.0 mg of mites) or kapok fibers (mean = 0.52% ±0.46% when using this method with 1.0 mg of mites, and mean = 0.27% ±0.24% when using this method with 5.0 mg of mites). These studies indicate there may be many more mites in homes than reported by the literature.

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DOCTOR OF PHILOSOPHY AWARD:

Effect of Heat, Surface, and Aging on Bed Bug-Labeled Residual Insecticides

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Heat is an effective method for treating bed bug infestations because it has the ability to penetrate hidden harborages and bed bugs are not heat resistant (Usinger 1966). However, if not performed properly, heat treatments can leave cold spots within the treated area, leading to treatment failure (Pereira et al. 2009). Heat treatments can also cause bed bugs to move (Hulsare et al. 2010), possibly outside of the area being treated, and heat leaves no residual (Pinto et al. 2007). Therefore, insecticides are often sprayed to supplement heat treatments (Miller 2010), but no protocol for this method has been established. In addition, it has been demonstrated that product efficacy can vary from surface to surface (Toews et al. 2003, de Arias et al. 2003). Bed bug mortality was tested with carbamates and organophosphates on metal, wood, cotton cloth, polyester cloth, and corrugated cardboard, demonstrating product efficacy differences (Fletcher and Axtell 1993). Because these active ingredients are no longer used indoors, an evaluation of current products is warranted.

We exposed bed bug-labeled insecticides to 135 °F (~57 °C) for 7 hours on three surfaces (carpet, metal, unfinished wood), and applied field and susceptible strain bed bugs to determine the impact of high temperature and surface on mortality. This method was repeated except products were aged 2 weeks before bed bugs were placed on panels. Mortality was recorded every 24 hours for 2 weeks. Based on the results, whether a product should be sprayed before or after a heat treatment varies with the surface being sprayed and the product being used. Different products on different surfaces vary in their toxicity under different conditions. These results should be taken into account when planning a heat treatment protocol. Additional research is being conducted to provide specific recommendations for commonly used bed bug control products on various surfaces, heated or unheated, and aged for up to six months.

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STUDENT PAPER COMPETITION

Subterranean Termites (Isoptera: Rhinotermitidae) of Alabama: A New Identification Tool Using the Worker, Soldier, and Imago Castes

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Subterranean termites (Isoptera: Rhinotermitidae) are eusocial, colonial insects that profoundly impact the economy of the southeastern USA. Unequivocal identification of these insects is critical to their control, yet identification remains a chronic problem. Morphology-based identification is greatly limited because the few existing keys not only do not include all species known to occur in the state, but also employ only the imago and soldier castes, which are either not available year-round or when available are typically present only in low numbers. The most numerous and year-round available worker caste may only be unequivocally identified using molecular sequencing, but this barcoding approach has been hampered by difficulties with matching sequence data and traditional taxonomy. To address this issue and expedite the processing of an ongoing state-wide survey, we developed morphological keys for the worker, soldier, and imago castes, using an approach integrating DNA barcoding and morphology. Specimens used in constructing the keys were sampled from multiple colonies located across the state's geographic area in both urban and natural environments. Specimens were first identified by sequencing using COII and by unequivocal morphological characters, and then exhaustively searched for novel morphological characters that consistently separated the five species. This was repeated for the worker, soldier, and imago castes. The resulting keys to Alabama's Rhinotermitidae comprise the first morphological tool in the United States to utilize the rhinotermitid worker caste, and the first keys for the state to separate the five species reported to occur in Alabama: Coptotermes formosanus, Reticulitermes flavipes, R. hageni, R. malletei, and R. virginicus.

Effects of Point-Dusting Foraging Tunnel on *Reticulitermes flavipes* (Isoptera: Rhinotermitidae)

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The efficacy of tunnel treatment on a group level, the dosage effect of powdered fipronil, a nonrepellent termiticide and the tunneling activity of *Reticulitermes flavipes* (*Kollar*) (Isoptera: Rhinotermitidae) was determined in laboratory. Termite workers were tested in bioassay units consisted of group site and feeding site connected with tygon tubes. *R. flavipes* were exposed to two doses (0.15 mg and 0.30 mg) of powdered fipronil. The treatment was applied to single tunnel 30 cm away from the feeding site and its impact was directly observed using termatrac T3i. We used five replicates for each of the treatment and control. After the treatment the chambers were monitored every other day until 100% mortality was reached. Dose 0.30 mg resulted in 100% mortality at day five where 0.15 mg at day seven. On average abnormal behaviors and lack of locomotion, were faster for groups of termites treated with 0.30 mg compared with termites treated with 0.15 mg of treatment. Post treatment tunneling activity showed untreated termites constructed more and longer tunnels in the feeding site compared to those treated with powdered fipronil. Our results provide strong evidence for effective group effects of powered fipronil on *R. flavipes* when a single tunnel is treated leading to group suppression and elimination under laboratory conditions.

Genetic Engineering of Gut Bacteria from the Formosan Subterranean Termite, Coptotermes formosanus Shiraki, to Serve as "Trojan Horses" for Termite Control

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The Formosan subterranean termite (FST), *Coptotermes formosanus* Shiraki, is one of the most devastating urban pest species in the United States and causes an estimated loss of \$500 million annually in Louisiana. Termite control relies heavily on chemical treatment of soil and structures. Though successful, most of the termiticides used are known to have unfavorable effects on the environment and human health. Biological control has been considered as an environment friendly option for termite control; however, social behavior and immune response are thought to prevent the spread of pathogens in termite colonies (Chouvenc et al. 2011). Paratransgenesis, which involves the use of genetically modified symbionts to kill the termite, has the potential to overcome the issues associated with both chemical and biological control strategies.

FST workers harbor a complex microbial community of protozoa and bacteria in their hindguts on which they depend for survival. The protozoa help the termite digest cellulose, and in their absence, termites die of starvation. It has been previously shown that a lytic peptide (Hecate) kills the termite gut protozoa, ultimately killing the termites (Husseneder & Collier 2009, Husseneder et al. 2010b). Attachment of a ligand that binds Hecate to protozoa increases the efficiency and specificity of the lytic peptide towards the gut protozoa, thus minimizing non-target effects. Our objective is to find a target specific and environmentally safe delivery system, i.e. a "Trojan Horse", to express ligand-Hecate and spread it throughout the termite colony.

Materials and Methods

Termite collection and sample preparation: Workers and soldiers were collected from three different FST colonies in New Orleans, LA in Fall 2009. Termites were fed in the lab on an artificial cellulose diet in Petri dishes kept in an incubator at 25±2°C and 85% R.H. Twenty workers from each colony were euthanized in 70% EtOH. Whole guts of the termites were extirpated using sterile forceps and were immediately homogenized in 1ml sterile BHI broth (pH 7.0).

Bacterial isolation: The gut homogenate was serially diluted in sterile BHI, and dilutions were plated on McConkey, Phenylethyl alcohol and MRS agar. Plates were grown anaerobically, using H2/CO2 BBL GasPaks™ at 30°C for 48 hrs. The isolated bacteria were screened based on the colony characteristics (size, shape, color, margin, elevation) and gram staining. In total, 192 bacterial colonies, representing 15 morphotypes, were processed for identification (Sethi & Husseneder unpubl.)

16S rRNA gene sequencing: All 192 isolates were grown overnight in 500 μ l of sterile BHI. Next, 250 μ l of the overnight culture was stored as glycerol stock, and the remaining 250 μ l of culture was used to extract DNA using DNeasy® 96 Blood & Tissue Kit (Qiagen). The 16S rRNA genes were amplified using universal bacterial primers 27f and 1492r. PCR products were sequenced at Beckman Coulter Genomics facility, MA.

Sequence analysis: Nearly full length sequences of ~1400bp were obtained. The 16S rRNA sequences were initially processed in ChromasPro (v. 1.5) to check sequence quality and assembly. The sequences were checked for orientation using OrientationChecker software. Sequences were trimmed and edited using the software DAMBE (v. 5.2.65). Sequences were compared with those present in the

GenBank/NCBI database using BLAST 2.0, and sequences with≥ 97% similarity were considered to belong to the same species.

Minimum inhibitory concentration (MIC): The minimum inhibitory concentration for Hecate and ligand-Hecate was determined for *Lactococcus lactis*, *Pilibacter termitis*, *Enterobacter cloacae* and *Trabulsiella odontotermitis* as proposed by R. E. W. Hancock for testing antimicrobial peptides (http://www.interchg.ubc.ca/bobh/MIC.htm). The final concentration of peptides ranged from 0.19μM-100μM. The cultures were grown overnight, and the concentration at which no visible was observed was considered as the MIC. The experiments were carried out in triplicate.

Transformation of *Trabulsiella odontotermitis*: *T. odontotermitis* was transformed according to standard protocols. Plasmid PTrcHis 2-ELGFP6.1–TOPO (provided by Dr. Kato, LSU) containing ELGFP gene, under the control of a Trc promoter and an ampicillin resistance gene, was used for transformation. Transformation of bacteria was confirmed by observing the colonies on selective media (LB agar with 100 μg/ml ampicillin and IPTG) under a Lumar fluorescent microscope. Bacterial cells from the fluorescent colonies were observed at 100x magnification using a Decon- Leica DM RXA2 fluorescent microscope. Cultures of transformed *T. odontotermitis* (*T. odontotermitis*-gfp) were maintained as glycerol stocks at -80°C.

Feeding experiment: Ten workers were placed in Petri dishes at $25\pm2^{\circ}$ C and 85% R.H. and were allowed to feed on cellulose discs containing ~ 106 cells of *T. odontotermitis*-gfp. For controls, termites were fed on cellulose discs containing ~ 106 cells of *T. odontotermitis* (non-transformed) and cellulose discs without any bacterial cells. The experiment was carried out in triplicate. Guts of five workers from each set were extirpated and homogenized in 500 µl sterile PBS. The homogenate was serially diluted to 10^{-3} and spread on LB agar with 100 µg/ml ampicillin and IPTG. Plates were incubated overnight at 30° C. Fluorescent colonies were counted under a Lumar fluorescent microscope.

Results

Bacterial identification

After initial screening of the 192 sequences, 137 sequences with full length and high quality were processed for identification using the NCBI BLAST tool. Bacteria showing ≥97 % sequence similarity, matching colony characters and gram nature were assigned to the same species (Table 1). Out of the 132 isolates, 15 different species were identified. Most of the species belonged to the family Enterobacteriaceae (66.6%); other isolates belonged to the families Enterococcaceae (33.3%) and family Streptococcaceae (33.3%). One bacterium was similar to an uncultured firmicute bacterium.

Table 1: List of bacteria identified based on 16s rRNA gene sequences

Phylum Proteobacteria			
Family Enterobacteriacea	Number of isolates	Accession Number of closest match	% Similarity
Klebsiella sp.	4	DQ831003.1	99
Enterobacter hormaechei	9	JF690889.1	99
Enterobacter aerogenes	25	AB244472.1	99
Enterobacter asburiae	6	HQ242719.1	99
Enterobacter cloacae	10	CP001918.1	99
Serratia marcescens	1	HQ242736.1	99
Escherichia coli	1	EF560789.1	97
Trabulsiella odontotermitis	1	DQ453130.1	99
Enterobacter sp. SD-A	2	JF968609.1	99
Uncultured bacterium	1	DQ068900.1	98
Phylum Firmicutes			
Family Enterococcaceae			
Enterococcus faecalis	1	GQ337884.1	99
Pilibacter termitis	22	NR_042949.1	98
Family Streptococcaceae			
Lactococcus garvieae	27	HQ407256.1	99
Lactococcus lactis	15	GQ337893.1	99
other			
Uncultured firmicute bacterium	12	GQ502553.1	99
Total	137		

Minimum inhibitory concentration: Of the bacteria identified, four bacteria (*L. lactis, P. termitis, E. cloacae* and *T. odontotermitis*) were selected for further screening. The MIC revealed that these four bacteria species are able to tolerate higher concentrations of ligand-Hecate than Hecate (Table 2).

Transformation of *Trabulsiella odontotermitis*: Transformed *T. odontotermitis* conferred ampicillin resistance and fluorescent phenotype when grown in presence of IPTG. Bacterial cells from fluorescent colonies of *T. odontotermitis*-gfp observed under Decon- Leica DM RXA2 showed brightly fluorescent cells and gave additional confirmation of fluorescent phenotype. These results confirmed the transformability of *T. odontotermitis* and its ability to express foreign proteins.

Feeding experiment: The gut homogenate of workers fed on cellulose discs with T. odontotermitisgfp for 48 hrs showed the presence of fluorescent colonies when spread on LB agar containing ampicillin and IPTG. Numbers of fluorescent bacteria isolated per gut ranged from 1.2 to 4.6 \times 103. The negative controls did not show presence of fluorescent colonies. These results show that T. odontotermitis-gfp is ingested by the termite survives in the gut at least for 48 hrs.

Table 2: Minimum inhibitory concentration (MIC) of Hecate and ligand-Hecate

Bacteria	Hecate MIC (µM)ª	ligand-Hecate MIC(µM) ^a	
Enterobacter cloacae	25	50	
Trabulsiella odontotermitis	25	50	
Pilibacter termitis	6.25	25	
Lactococcus lactis	12.5	<100	

a: Represents mean MIC values of three experiments

Discussion

In the current study, we isolated 15 species of bacteria from the guts of FST workers and began testing four of them for their usefulness as a "Trojan Horse" in a paratransgenesis system. A potential "Trojan Horse" should be 1) a symbiont of the target insect, i.e. inhabit the termite gut, 2) tolerant to the effects of the toxin it will be spreading, i.e. ligand-Hecate, 3) genetically modifiable to express foreign proteins and 4) able to survive in the gut of the termite upon reintroduction and able to spread in the termite colony.

We selected a combination of two gram-positive and two gram-negative bacteria, L. lactis, P. termitis, E. cloacae and T. odontotermitis, respectively, as candidate "Trojan Horses". All four species have been previously reported to be found in the termite gut and can thus be considered symbionts (e.g. Higashiguchi et al. 2006, Chou et al. 2007, Husseneder et al. 2010a). E. cloacae has been used to proof of the concept of paratransgenesis (Husseneder and Grace 2005, Zhao et al. 2008). In addition, these bacteria proved to be tolerant against ligand-Hecate. The MIC of ligand-Hecate was always higher than Hecate for all the bacteria tested. These results show that attachment of a ligand to a lytic peptide decreases its efficacy against the non-target bacteria. This is expected since the ligand was designed to attach to protozoa and not to non-target cells, such as bacteria (Husseneder et al. 2010b). The MIC of all the bacteria tested for ligand-Hecate tolerance was at least 25 times higher than 1 µM, which is the concentration sufficient to efficiently kill the gut protozoa (Husseneder et al. 2010b). Hence, all the bacteria tested were able to tolerate the concentration of ligand-Hecate required to kill the gut protozoa and fulfill second criteria for a "Trojan Horse". Of the four bacteria that fulfilled the first two criteria, we selected *T. odontotermitis* for transformation because it is a gram negative-bacteria belonging to the family Enterobacteriaceae, and standard protocols are available for genetic manipulation of the members of this family. Also, *T. odontotermitis* is found exclusively in the termite gut which made it a preferred choice for environmentally safe genetic manipulation. The transformation of *T. odontotermitis* with a plasmid containing the GFP gene showed that the bacteria can be engineered to express foreign proteins and thus fulfills the third criteria for the "Trojan Horse". The feeding experiment confirmed the ingestion of *T. odontotermitis* and its survival in the gut for at least 48 hrs which partially completed the fourth criteria of a "Trojan Horse". The expression of GFP by *T. odontotermitis* and its rapid ingestion by the FST are comparable with the previous experiment with E. cloacae (Husseneder and Grace 2005). Future work involves studying the long-term survival

and spread of *T. odontotermitis*-gfp in a termite colony. Ultimately, the application of *T. odontotermitis* as a paratransgenic Trojan horse will involve genetic engineering to express ligand-Hecate and applying transformed *T. odontotermitis* as bait for termite control.

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Evaluation of Three Termite Bait Materials: Pine Wood, Cardboard and Corn Cob

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The consumption and chemical transfer efficiency of two commercially used termite bait materials, wood and cardboard, and one potential bait material, cob of maize, were evaluated for use against the Formosan subterranean termite (*Coptotermes formosanus*) in the lab. No-choice and choice tests determined termite consumption of the three materials. In the no-choice test, the consumption of wood and cob was similar and significantly more than cardboard. Tunneling under the food sources was similar, which indicated that activities were equally centered around the food sources. In the two choice test, the consumption was: wood > cardboard, cob > wood, cob > cardboard, and the tunneling actually under these choices was: wood = cardboard, cob > wood, cob = cardboard. In the multiple choice test, no significant difference was detected in consumption, but the amount of tunnels made by termites directly under the cob was significantly more than wood and cardboard.

Nile blue A was used to study the potential chemical transfer between bait and termites. 0.1% dyed cardboard, cob and wood were provided to termites as food. Termites feeding on wood showed a significantly higher rate of turning blue in the 6h compared to cardboard and cob, but there was no significant difference after 12h. The blue termite feeding on different bait materials were then collected and combined with untreated termites to evaluate the efficiency of Nile blue transfer between nestmates being fed or starved. The results showed that there was no significant difference in percentage of white termites turning blue when food was provided, whereas in starvation treatments, the rate of white termite turning blue was dramatic; wood treatment was causing more termites turning blue than that of cob and cardboard. Our study indicated that, as a waste product of food and biofuel industry, cob could be used as a cheap resource of termite bait, at least as efficient as wood and cardboard.

Population Genetics and Breeding Structure of Subterranean Termites (Reticulitermes flavipes) From Infested Urban Structures in Nebraska

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Reticulitermes flavipes is the most common subterranean termite species infesting urban structures in Nebraska. Ten termite samples from twenty infested urban structure infested with R. flavipes were genotyped at seven microsatellite loci. Heads from individual workers were pulverized in 0.2 ml reaction tubes and deoxyribonucleic acid (DNA) was extracted using the Gentra Systems Puregene DNA purification kit protocol, except Proteinase K solution was not added and extracted DNA was rehydrated in 80 μ l of 1x TE solution. Samples were sequenced on a Beckman Coulter CEQ 8000 Genetic Analysis System using GenomLabTM Fragment Analysis Protocol. All seven microsatellites were polymorphic with 1-6 alleles per locus with the frequency of most common allele within 0.11-0.60 which indicated high level of genetic variability on a local scale. We compared the colony breeding system and population genetic structure among twenty infested urban structure across Nebraska. Our data shows 17 out of 20 infested urban structures were simple family colonies and highly inbred (FIC = -0.684). The other three urban structures were mixed family colonies. There was a high level of genetic differentiation (FCT= 0.459) between all the twenty urban structures.

Biology and Management of the Dark Rover Ant (Hymenoptera: Formicidae)

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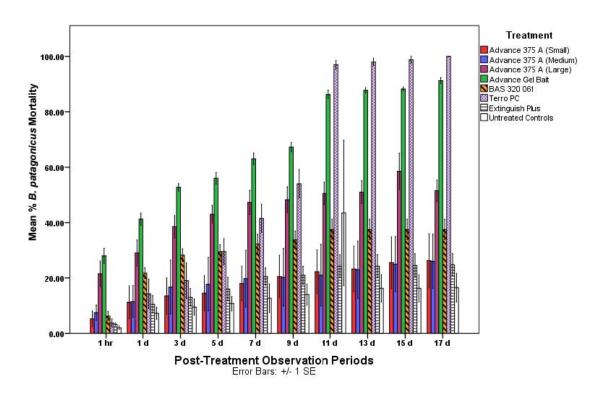
A laboratory study was initiated at the Center for Urban and Structural Entomology at Texas A&M University in College Station, TX. Arenas, 38 X 68 cm fluon-lined plastic pans, were provisioned with food (honey water solution and dead crickets), water and harborage. After a 24 hr starvation period, *Brachymyrmex patagonicus* (100 workers, 3 queens and 1 g brood) were introduced to arenas. Data regarding worker mortality was collected at 1 hr, and 1, 3, 5, 7, 9, 11, 13, 15, and 17 d after treatment. Four replications of each of the following treatments were conducted:

- 1. Advance 375 A (small grit) 0.70 mm-Abamectin B1 0.011%
- 2. Advance 375 A (medium grit) 1.0 mm-Abamectin B1 0.011%
- 3. Advance 375 A (large grit) 1.4 mm-Abamectin B1 0.011%
- 4. Advance Ant Gel-Sodium Tetraborate Decahydrate 5.4%
- 5. Experimental Granular Bait
- 6. Terro PCO Gel-Boric Acid 5.40%
- 7. Extinguish Plus Granular Bait-Hydramethlynon/S-methoprene 0.365%/0.250%
- 8. Untreated Controls

At the 1 hr observation period, the mean mortality of *B. patagonicus* was significantly greater in the Advance 375A (Large Grit) and Advance Ant Gel Bait treatment groups than that of any other treatment; or untreated control groups. By 1 d, mortality in all treatment groups was greater than that of the controls (Fig.1). At the 3 d, and until the 9 d observation period, mortality was significantly greater in the Advance Ant Gel Bait treatment group than all remaining treatments and controls. At the 11 d observation period, mortality was greatest in the Terro PC and Advance Ant Gel Bait groups, but was not significantly different between each other. Mortality was not significantly different between the Terro PC and Advance Ant Gel Bait groups, and the mean mortality of these treatments was significantly greater than all other treatment and control groups at, and beyond the 13 d observation period (Fig.1).

With the exception of the 1 hr observation period and until the 11 d observations, mortality associated with Advance Ant Gel Bait was significantly greater than that of other treatments and untreated controls during this trial. At and beyond day 11, Advance Ant Gel Bait shared its top mortality rank with Terro PC, another gel bait. It is of interest to note that the two gel baits outperformed the granular baits in this study. With regards to the granular baits, mortality associated with the large sized Advance 375A was consistently significantly greater than that of the other sizes of Advance 365A, remaining granular baits, and untreated controls. While the gel baits outperformed the granular baits in this study, it is important to note that the large sized Advance 375A performed very competitively against *B. patagonicus* ants.

Fig.1. Mean % mortality of *B. patagonicus* at 1 hr, and 1, 3, 5, 7, 9, 11, 13, 15, and 17 d after exposure to baits.



Instar Determination of the Dubia Cockroach (*Blaptica dubia*): A Maximum Likelihood Approach

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Instar determination is fundamental to both basic research and its application. The Dubia cockroach, *Blaptica dubia*, is a popular pet and an excellent feeder insect for many reptiles and amphibians. A general method using Gaussian mixture models to determine the number of instars in this species is developed. Application of the methods is illustrated by analysis of data collected on the *B. dubia*. The analysis indicated that there are seven instars in *B. dubia*. The growth ratio in the analysis follows the Brooks-Dyar rule. The growth ratio of pronotum length, pronotum width, and head width are 1.26, 1.24, and 1.19, respectively. Since *B. dubia* shares a similar growth model with other paurometabolous insects, this method maybe applicable to other insects as well.

Optimizing Pest Management Curricula for Use in K-12 Classrooms

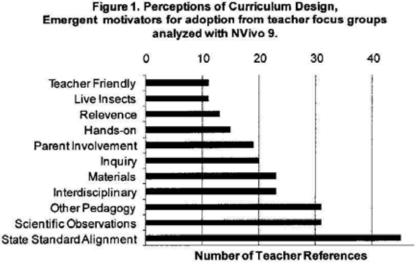
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"Educate to Eradicate" is a K-12 curriculum project using termite biology and control as the basis for science education (Grace et al. 2007, 2008) that has been implemented in over 350 Hawaii public school classrooms with more than 11,800 students; and is coupled with community outreach efforts. This study was initiated to (1) identify factors that influence the adoption and continuation of pest management curricula in public school classrooms, and (2) evaluate the efficacy of community education efforts. Teacher focus groups were organized to assess "Educate to Eradicate" curriculum design and professional development implementation. Perceptions of key project components and supports were recorded during teacher focus groups. Project supports useful for continued curriculum implementation were summarized and rated. Secondly, we evaluated changes in instructional practice and student learning. Efficacy of the program in promoting termite suppression was measured through student engagement in extension activities and changes in prevention knowledge. Findings will inform modifications to curriculum, professional development, and project supports. Resources will be optimized to maximize teacher continuation and student learning.

Partner teachers during the 2011-2012 school year were recruited to participate in focus groups (n=22, 41%). Five homogeneous groups were organized based on grade level, years of partnership (early adopters ≥ 3 years, late adopters ≤ 2 years), and school location (Morgan 1997). All groups were recorded, transcribed, and analyzed using content analysis (NVivo qualitative data analysis software; QSR International Pty Ltd. Version 9, 2012). The study design, procedures, and instruments were approved by University of Hawaii Institutional Review Board (CHS#18356).

Keys to Project Success

Focus group participants identified essential aspects of curriculum design, training, and support. Overall, the curricula's alignment to Hawaii state standards, incorporation of scientific observation, and use of pedagogy (reinforcement, kinesthetic songs, and crafts) were most frequently cited as motivators. Additionally, use of live organisms, hands-on activities, visuals, inquiry activities, and parent involvement were described as keys to project success (Figure 1).



In addition to curriculum design, teachers reflected on the project's professional development. Educate to Eradicate has employed a range of professional development techniques throughout its lifetime. Early-adopting elementary teachers felt their weekend training went beyond what they could utilize in class, however they enjoyed mastering content and conducting inquiry laboratories. Birman et al. (2000) argue that professional development that focuses on science content while providing opportunities for active learning positively affects teacher adoption (2000). Late-adopting elementary teachers valued in-class lesson modeling, which did not require additional hours beyond the workday. At some schools, entire grade levels were trained this way, increasing opportunities for collective participation. Teachers given the opportunity to discuss concepts and problems associated with new curriculum are more likely to continue with collegiate support (Birman et al. 2000, Ni 2007). Early-adopting middle school teachers utilized and valued in-class lesson modeling. Late-adopting middle and early-adopting high school teachers were more autonomous, requiring limited training (~ 2 hours), curriculum resources, project materials, and access to project staff for question/answer sessions.

While weekend training allowed teachers to explore "Educate to Eradicate" content deeply, at-school lesson modeling allows the project to reach more teachers. White (2005) argues that effective professional development deepens teachers' content knowledge, while minimizing additional time demands. Future creation of "Educate to Eradicate" videos may efficiently hybridize professional development to include science content and lesson modeling. Supports beyond professional development were also discussed.

Teachers cited help from their grade level colleagues most often as a key support to project adoption and continuation (15 references). Grade level members helped one another by creating/adapting project materials, preparing copies, setting-up laboratories, issuing grade level reminders, and serving as a project point-of-contact. Assistance from the UH Termite Project staff was also valued by teachers (13 references). Teachers indicated prompt communication, material drop-off/pick-up, curriculum modeling, field trips, and visits from entomologists as favorable staff services. Teachers noted that administration helped by scheduling grade-level planning time and granting teachers fiscal and curricular autonomy. Exciting entire grade levels or departments about curricula, providing technical support, and creating user-friendly lessons that minimize teacher time inputs (White 2005) have the potential to increase curricula continuation.

Continuation Needs

Each focus group brainstormed then ranked project continuation needs. Live termites and habitats were essential. Teachers would not continue the project without live termites. Preserved termites and damage samples were also vital project components. All groups were willing and able to store these samples indefinitely. High and middle school teachers valued laboratory kits. These teachers were willing to house, maintain, and restock kits from year-to-year. One high school teacher had already purchased all materials used during unit instruction and only required live termites each year to continue. All teachers valued project staff services, materials/kits, and were interested in additional resources. However, these were not considered essential for project continuation. Establishing an efficient distribution system, which allows for drop-offs to distant schools, will help insure curriculum continuation.

Change in classroom time allocated to science

At the elementary level, teachers were asked to quantify changes in classroom time devoted to sci-

ence. Early adopters emphasized the importance and extensive use of science instruction throughout their teaching careers. They indicated that "Educate to Eradicate" motivated students while honing observation and questioning skills. One teacher stated, "When we... create[d] our other units, we knew that the termite one was so hands-on that we tried to make the other [science units] hands-on because we wanted to have some of those same things that get the kids so excited." Late adopters reported increasing science instruction to 1.5 hours per week. This was an increase from 45 minutes per week, twice a quarter, or whenever it fit into instruction.

All middle/high groups indicated the UH Termite Project was currently their only project-based unit. Teachers would like to partner with similar standards-based projects that provide hands-on activities and project materials. All middle school teachers indicated partnership with the project resulted in increased technology use and note taking.

Student knowledge and behavior

Partner teachers administered pre- and post-project surveys as part of instruction. A total of 4,750 paired-surveys were received from students in grades 1-12 (48% return rate). Four prompts were consistent across grade levels (Figure 2).

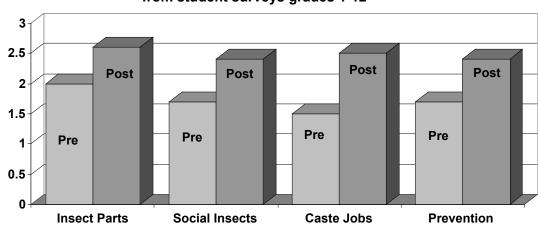


Figure 2. Changes in Student Knowledge, from student surveys grades 1-12

Survey responses from different schools were combined as split-plots. Years of teacher participation was included as a covariate (Table 1).

Table 1. ANOVA for Prevention Prompt					
Source	df	Mean Square	F	P	
School	30	2.4	1.1	0.4	
Teacher (School)	63	2.2	4.8	< 0.0001	
Curriculum	1	386.4	18.6	0.0002	
Curriculum X School	30	20.8	45.6	< 0.0001	
Years of Teacher Participation	1	1.7	3.8	0.05	

Participation in the "Educate to Eradicate" curriculum had a significant effect on student scores. Average pre-program responses to the prompt "I can list the six prevention steps scientists suggest

my parents take to keep worker termites from damaging our home" averaged between "Not Really" and "I Think So" at 1.7. After curriculum participation, the average response was between "I Think So" and "Yes" at 2.4. Effects of teachers within schools and the interaction between curriculum and school were also significant. Effects were consistent across prompts.

As a culminating activity, students survey their homes with their parents, searching for termite signs and/or termite-conducive conditions. Students use a home survey sheet for the inspection, which includes an area for parent/guardian feedback and comments.

Return and signature rates of this home survey were tallied during the 2010-2011 school year. The home inspection survey was returned by 86% of students. Of those returned, 74% were signed by a parent/guardian.

Conclusions

Teacher adoption of pest management curriculum can be maximized by tightly coupling lessons to the target groups' state standards. Incorporation of live organisms, inquiry, parental involvement, and grade-appropriate pedagogy can increase appeal to teachers. Posters and videos are desirable unit resources, which may reduce the need for project personnel and in class modeling. Curricular materials should to be readily available at low or no cost.

To date, the UH Termite Project has reached over 11,800 Hawaii public school students. Participating students have had significant gains in unit-specific content and prevention knowledge. Additionally, students have communicated their learning to parents/guardians while inspecting their homes for termite signs and/or termite-conducive conditions. The goal of this program is a self-sustaining curriculum that will require limited institutional inputs, increase science literacy in Hawaii schools, and help to protect current and future homeowners from termite damages.

Acknowledgments

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Pyrethroid-Resistant Bed Bugs, *Cimex lectularius* L.: Characterization of Cuticle Using Molecular, SEM, and GC-MS Methods

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There are two types of insecticide resistance that have been reported in bed bugs: target site insensitivity (kdr) caused by the two-point mutations of the subunit genes of the voltage-sensitive sodium channel (Yoon et al. 2008), and enhanced detoxification enzyme caused by up-regulation of the Cytochrome P450 genes (Adelman et al. 2011). However, a third type of insecticide resistance caused by reduced cuticular penetration has not been reported in bed bugs. The insect cuticle is the outer layer of insect exoskeleton that prevents water loss and protects from physical injuries. In the urban environment, the insect cuticle also serves as the physical barrier to insecticide exposure.

 ${\rm LD_{50}}$ values for the resistant strain and susceptible strain bed bugs were calculated after exposure to technical grade pyrethroid insecticides using injection and topical application methods. The ${\rm LD_{50}}$ values for both topical and injection application were compared within each strain. As expected, the topical ${\rm LD_{50}}$ was many times greater than that of the injection in both strains. However, we found resistant strain bed bugs had greater differences (>1000-fold) in ${\rm LD_{50}}$ values between the two application methods than that of the susceptible strain bed bugs. The greater differences in ${\rm LD_{50}}$ values between the two application methods in resistant strain bed bugs suggested that the cuticle plays a greater role as a physical barrier to the insecticides in the resistant strain than in susceptible strain bed bugs.

We analyzed cuticle of resistant strain bed bugs using molecular methods, scanning electron microscopy (SEM), and gas chromatography-mass spectrometry (GC-MS). The molecular analysis using real-time quantitative PCR revealed many genes that code for cuticular proteins were significantly upregulated in resistant strain bed bugs. Measurements of cuticular thickness (SEM) also determined that the cuticle in certain regions of the body was significantly thicker in resistant strain bed bugs than in the susceptible strain bed bugs. Also, preliminary analysis of the bed bug cuticular hydrocarbons using GC-MS revealed resistant strain bed bugs had a higher concentration of some cuticular hydrocarbons than the susceptible strain bed bugs. While all of the studies presented here are still preliminary, they present evidence to suggest that the resistant strain bed bugs also have the reduced cuticular penetration type of insecticide resistance.

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Effect of Various Blood Alcohol Concentrations (BAC) on Bed Bug Feeding and Reproduction

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Recent resurgence in the common bed bugs (Cimex lectularius L.) infestation worldwide has created a need for renewed research on biology, behavior, population genetics and management practices. At present, majority of the research is focused on insecticide resistance and control methods, while a negligible percent focus on other areas of interest. Humans consume alcohol on a regular basis at social events. Alcohol is transported and stored in the blood, blood alcohol concentration (BAC) impact individuals differently. The bed bug requires a blood meal to grow, molt and reproduce. One of their main hosts is humans. This research focuses on determining the effect of alcohol adulterated reconstituted human blood (RHB) on bed bug biology including feeding, reproduction and fecundity. Four BAC levels (0.010, 0.025, 0.050 and 0.100%) were tested and a control with no alcohol. The experimental design was a complete randomized design consisting of 20 adult bed bugs (10 males and 10 females) per treatment and 6 replications per treatment. The groups of 20 adult bed bugs were weighed and allowed to feed for 45 minutes then weighed again. They were placed in a growth chamber (set at 23 ± 2°C, 50 ± 10% RH and 12:12 L: D) undisturbed for 7 days. The average bed bug mass increased at 105.60% (± 8.12). Bed bugs fed on 0.100 BAC showed an average mass increase of 12.5% (± 0.80). A total of 266 (average 44.3 ± 10.86) eggs were produced by bed bugs in control and 72 (average 12.0 ± 1.88) eggs by bed bugs fed on various concentrations alcohol adulterated blood. There was a negative correlation between the BAC and average mass percent increase and between the numbers of eggs produced. Nymph emergence was greater than 80% for all treatments. with control having the highest nymph emergence at 95.11%. These results suggested that increasing BAC negatively impacted bed bugs capacity to feed to repletion and resulted in reduced fecundity.

Egg Surface Morphology and Morphometrics of Bed Bug (Cimex lectularius L.) Eggs

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Scanning electron microscopy was used to distinguish morphological features of bed bug, *Cimex lectularius*, eggs. Efforts were made to distinguish morphological and morphometric features of eggs from a susceptible, laboratory strain (Harlan) and two pyrethroid susceptible strains (Richmond and Royal Oaks) collected in the field. We did a one second application of Bedlam to filter paper and allowed females to lay eggs directly on the filter paper. We found that 57% more eggs from the Royal Oaks resistant strain hatched compared to the Harlan susceptible strain. Eggs from resistant strains are more difficult to kill using pyrethroid insecticides and we assume there must be morphological differences of eggs between strains that enhance their resistance.

External morphological features of the egg shell were observed using a scanning electron microscope. Eggs from all three strains were cigar shaped and tapered on both the anterior and posterior portions. The operculum is covered with polygonal structures and is located on the anterior portion of the egg shell. The outer chorion is reticulated, consisting of many spine-like projections. We found no significant differences in our preliminary analysis among strains in respect to length and width of the eggs. Studying the morphological features of eggs from both susceptible and non-susceptible strains may be helpful in understanding bed bug egg biology.

Sustainable Bed Bug Management for Vulnerable Housing Facilities

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For residents of multi-unit low-income housing facilities, the cost of professional bed bug control is unaffordable. A professional bed bug treatment for a single apartment unit typically costs between \$500 (for a single application of both non-chemical and chemical methods; three treatments are recommended) and \$2000 (whole unit heat treatment. In combination with other practical control methods, such as heat treatment, vacuuming, and the installation of bed bug monitoring devices and mattress encasements, appropriate diatomaceous earth applications are fundamental to a complete integrated approach to preventing and managing bed bug infestations. Because of its broad application and low cost, diatomaceous earth is a practical and effective tool for bed bug control that, with the proper training and certification, housing facilities staff can apply preventatively in order to help reduce the cost of controlling multiple infestations. Diatomaceous earth applications were made along the perimeter of each apartment unit in a 120-unit government-funded complex in Harrisonburg, Virginia. Mean treatment time per unit was 36 minutes. Mean treatment volume per unit was 65.19 grams. Preventative diatomaceous earth applications have the potential to reduce the cost of bed bug control in multiunit low-income housing facilities.

Ruining Your Picnic: Prevalence of Ants in Urban Parks in Tucson, AZ

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We surveyed the ant communities in three contrasting environments in Tucson, AZ. We considered the ant assemblages in recreational parks, natural Sonoran desert and remaining fragments of the desert within the city. Here, we describe the initial results of this survey related to the prevalence and abundance of pest ants. Three species of pest ants were found to be more successful in urban environments than natural areas. The Southern fire ant (*Solenopsis xyloni*) was found in all three environments but was significantly more abundant in parks and urban desert fragments than natural desert. The Black Rover ant (*Brachymyrmex patagonicus*) was found almost exclusively in parks. Argentine ants (*Linepithema humile*) were only found around permanent sources of water. Additional sampling around an urban pond revealed that they were only present at a short distance from it. In summary, these results suggest that pest ants in southern Arizona are limited by the availability of water in the environments they invade.

Introduction

We surveyed the ant communities in three contrasting environments in Tucson, AZ. Here, we show some preliminary results of an ongoing study of the ant diversity in urban environments. We specially considered urban parks. Very few studies have sampled the ant communities inside recreational urban parks, although the few that are available agree that parks include diverse ant communities (Pacheco and Vasconcelos, 2006; Yamaguchi, 2004). The most relevant pest ants we found were the southern fire ant, Black Rover ant and Argentine ant (*Solenopsis xyloni, Brachymyrmex patagonicus* and *Linepithema humile* respectively).

Southern fire ants are a native species that is important as a pest in the southern US. They possess a painful sting and have relatively large colonies of up to a few thousand individuals. In a recent survey, they were the pest ant species that generated more calls to pest management companies in Phoenix, AZ (Field et al., 2007). The Argentine ant and the Black Rover ant are both exotic species originating in South America and are considered invasive pests. Argentine ants are a dominant species that forms supercolonies and are known for their capacity to displace native ant populations in the locations they invade (Holway and Suarez, 2006). Black Rover ants are a nuisance pest that has recently expanded its exotic range to much of the southern United States (MacGown et al., 2007).

Materials and Methods

Tucson, AZ is inside the Sonoran desert biome and therefore is considered an arid environment. We sampled ants in 3 kinds of environments in and around the city: urban parks, urban desert fragments and natural desert. We defined urban parks as habitats within the city that are used for recreational purposes and are maintained by the city administration. They include open areas with turf, as well as a mixture of native and exotic plants. They have artificially enriched soils and enjoy irrigation during most of the year. Sites considered desert fragments were places surrounded by the urban matrix that maintained most elements of natural desert communities primarily due to being undeveloped. In most cases, they could be considered vacant lots, although we also included sites that had purposefully been maintained in a semi-natural state. Natural desert sites were locations in the nature parks surrounding the city which were meant to exemplify the natural communities in that region of the Sonoran desert. They were chosen to have elevations similar to those of the study sites within the city between 2400 and 2700 ft (732 and 823 meters).

Twelve sites were selected for each environment kind. All sites had an area between 1.5 and 3 acres. Each site was sampled with 12 to 16 pitfall traps, depending on its size. Pitfall traps consisted of 8 oz plastic jars. Propylene glycol in the form of environmentally safe antifreeze (Sierra Brand®) was used as a killing agent. Traps were placed in a rectangle of 3x4 traps separated from each other by a distance of 65.6 ft (20 meters). In many parks, attaining this arrangement was impossible since heavily used areas had to be avoided. However, we always maintained a minimum distance among traps of at least 65.6 ft (20 meters). Traps remained in each site for 72 hours, after which the resulting samples were taken to our laboratory. Sampling was carried out in October of 2010. Ants were collected from the samples and put into vials with 95% ethanol. They were later counted, identified, and a representative sample for each site was pinned. Analysis of the samples is not yet complete, and results included here were obtained from data of four sites from each kind of environment.

Additional sampling was carried out in Reid Park in April of 2012 after the discovery of Argentine ants (*Linepithema humile*) in that location. Reid Park is an urban park that includes a large pond. Pitfall traps were placed in groups of 6 at four distances 30.5, 492, 984 and 1,476 ft (10, 150, 300 and 450 meters) from the edge of the pond. Groups or traps consisted of a rectangle of 2x3 traps. Distances to the pond were measured from the edge of the pond to the trap closest to it in each group. As in previous sampling, traps remained in the ground 72 hours, after which their contents were analyzed in our laboratory.

Statistical analyses consisted of ANOVAs of different aspects of abundance and prevalence of pest ants found in our samples. All analyses were carried out with Jmp, version 8.0 (SAS Institute, Inc.).

Results

Samples considered for analysis included a total of 6,341 ants in 30 species. Clear patterns of prevalence and distribution were observed for two important pest ants: the Southern fire ant (*Solenopsis xyloni*) and the Black rover ant (*Brachymyrmex patagonicus*). Southern fire ants are a native species and were found in all three environments. They were the most abundant ant species collected, including 38.7% of all ants. They were present in 11 out of 12 sites under consideration, the only exception being one natural desert site. No significant differences were found in the numbers of southern fire ants per trap in the different environments (One way ANOVA, F= 1.99, df=2.64, p=0.144). However, significantly more traps contained these ants in parks and desert fragments than they did in natural desert sites (One way ANOVA, F=7.28, df=2.9, p=0.0132) (Figure 1).

Black rover ants were found almost exclusively in traps collected from parks. The only exception was one individual found in a trap from a desert fragment. They represented 6.4% of all ants collected. Although they were never the numerically dominant species, they were present in most of the traps from parks. Between 55 and 88% of the traps in each park contained these ants.

The additional sampling carried out in Reid Park produced a clear distribution pattern for the Argentine ant *Linepithema humile* (Figure 2). Argentine ants were only found in samples placed at 30.5 and 492 ft (10 and 150 meters) from the pond but were completely absent from samples taken at greater distances. On the other hand, southern fire ants were absent from samples taken at 30.5 and 492 ft (10 and 150 meters) from the pond but were abundant at 984 and 1,496 ft (300 and 450 meters) from it (Figure 2). Black rover ants were present in varying numbers at all distances from the pond. Besides Southern fire ants, other 10 species of native ants were found in small numbers. The abundance of native ants, including *S. xyloni*, was significantly lower at 30.5 and 492 ft (10 and 150 meters) from the pond, were Argentine ants were present (One way ANOVA, F=9.93, df=3,18,

p=0.0004). Total species richness was also lower at 30.5 and 492 ft (10 and 150 meters) from the pond than at 984 and 1,496 ft (300 and 450 meters). Five ant species were found at 30.5 ft (10 meters) from the pond and seven at 492 ft (150 meters), while both at 984 and 1,496 ft (300 and 150 meters) we found 8 species.

Discussion

We found that pest ants in Tucson, AZ are more abundant in parks than in natural and semi-natural environments. Southern fire ants were found in more traps in parks and urban desert fragments than natural environments. This suggests that their nest density is greater in these habitats. On the other hand, we did not detect differences in the intensity of foraging activity that would be suggested by differences in ants found per trap. Greater numbers of fire ant nests in an area might result from greater water availability in urban environments. It has been shown that fire ant abundance may increase up to two orders of magnitude when water is provided through irrigation (Menke et al., 2007).

The fact that Black rover ants were almost exclusively found in parks is likely also the result of greater water availability. Black rover ants are a tropical species, and as such require stable sources of water (MacGown et al., 2007). Infestations of these ants inside structures are usually related to excessive moisture. When moisture problems are corrected, infestations such disappear without the need for further control (Klotz et al., 2008).

Argentine ants seem to be even more dependent on moisture for their survival. In Tucson, we have only found them around two permanent sources of water: the pond in Reid Park and a residential pool. High soil moisture has been identified as the main abiotic factor mediating the invasion of Argentine ants and their displacement of native species (Menke et al., 2007). We found Argentine ants up to 500 ft (150 meters) away from the pond in Reid Park, but they were absent from samples at a greater distance. Interestingly, southern fire ants were not present wherever Argentine ants were found. Since southern fire ants were very abundant in parks otherwise, this strongly suggests that they were being displaced by competition with the Argentine ants. Native ants in general were also less abundant where Argentine ants were present and had lower species richness. This observation is consistent with previously reported effects of Argentine ant invasion (Holway and Suarez, 2006). Interestingly, Black rover ants were abundant even in areas with Argentine ants. It is likely that rover ants are successful invaders due to their small size that allows them to avoid confrontation with more aggressive species such as Argentine ants and southern fire ants.

Although in Tucson, AZ water availability seems to be the main limiting factor for pest ant populations, other environmental factors are probably more important in different cities. For example, sampling in urban environments in Raleigh, NC, suggested that higher temperatures inside the city had a strong influence on the composition of ant communities (Menke et al., 2010). Understanding how habitat modification as a result of urbanization affects pest ant populations could become a valuable tool for preventing their invasions in the future. In southern Arizona, future research could explore modifying irrigation regimes to reduce or suppress ant pest populations.

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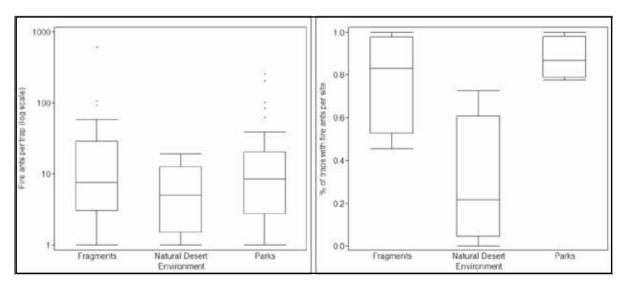
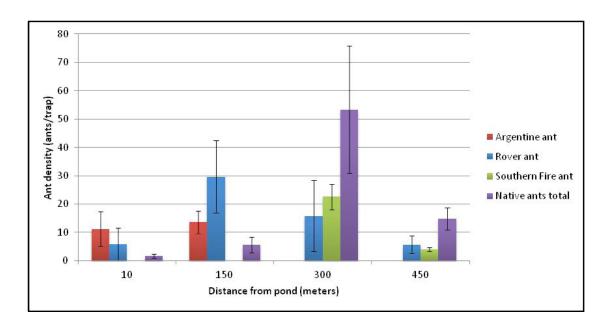


Fig. 1: Comparisons of abundance and prevalence of the southern fire ant (*Solenopsis xyloni*) in urban desert fragments, parks and natural desert. The numbers of fire ants in each pitfall trap were compared (left), as well as the percentage of traps per site that included fire ants (right).

Fig. 2: Comparison of ant density at different distances from an artificial pond inside an urban park. Results shown are the mean for each group of traps, and error bars were constructed with the standard error of the mean. The numbers for native ants include Southern fire ant counts.



Tracking Argentine Ant Foragers Using Sandwich ELISA Tests

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Linepithema humile (Mayr), the Argentine ant, is an invasive ant species and a significant pest in natural and managed habitats (Holway 2002). In South Carolina, Argentine ants have become a major problem in campsites and natural areas in some state parks (Ellis 2009). For this study, IgG rabbit serum (Sigma Chemical Co., St. Louis, MO, USA) was used as a protein marker, and a Sandwich ELISA test (Hagler 1997, Hagler and Jackson 1998) was used to detect the markers to determine foraging distances and patterns of *L. humile* in natural areas. The major objectives included; 1) determination of the optimal protein marker concentration for detection in individual ants; 2) determination of protein marker longevity in individual ants in both lab and field settings and 3) determination of foraging distance of *L. humile* in relation to detecting protein markers in individual ants. This information was used to evaluate distances in ant bait station placement at Greenwood State Park in Greenwood, SC.

To determine the optimal protein marker concentration in an individual ant, *L. humile* foragers were collected from the field. In the laboratory, the ants were fed with three IgG serum protein concentrations of 0.01, 0.001, and 0.0001 mg/ml of sugar water (30% sucrose). After 3 days, the Sandwich ELISA test was performed. Data were analyzed using the Dunnett test (SAS 2003) which compares the mean of each treatment with that of the control. Protein levels in ants fed with either 0.01 or 0.001 mg/ml were significantly higher than control (ant fed only sugar water).

To determine IgG serum longevity in individual *L. humile*, ant foragers were collected in the field. In the laboratory, collected ants were placed into Petri dishes and fed with 0.001mg/ml IgG rabbit serum in 30% sugar water. After 3, 5, 7 days post-feeding, the Sandwich ELISA test was performed to evaluate protein longevity in individuals. Data were analyzed with a Dunnett test. At three days, the average protein marker levels were significantly different than control ants fed sugar water only.

To determine the longevity that IgG rabbit serum could be detected in individual ants in the field, one *L. humile* colony was offered 300 mls of 30% sugar water containing 0.001mg/ml of IgG rabbit serum. For a control, another *L. humile* colony was offered only 30% sugar water. For both treatments, KM AntPro Bait Stations (KM Ant Pro LLC, PO Box 967, Nokomis, FL, USA) were used. Ants from both colonies were collected at 2 hr, 5 hr, 10 hr, 1 day, 2 days, and 3 days post-treatment and evaluated using the Sandwich ELISA test. The data from control colony and treated colony at varying time were analyzed with the Dunnett test. At all times, including the three days post-treatment, the protein levels in the ants exposed to the IgG rabbit serum were a significantly greater than control.

A fourth test was performed to determine the distance at which the protein marker could be detected in *L. humile* colonies in the field. Treatment and control stations were prepared as previously described. After three days, ants were collected at 5, 10, 15, 20, and 25 m from the bait stations and tested using the Sandwich ELISA test. Treatment data and data from control were analyzed with the Dunnett test. Ants collected up to 25 m were found to have significantly different protein levels than ants from the control colony.

The previous tests were used to determine an optimal distance of ant bait stations placement at Greenwood State Park in Greenwood, SC. In one area of the park, nine bait stations were placed in three equidistance rows at 10 m apart. In another area of the park, nine bait stations were placed in three equidistance rows at 20 m apart. In both areas, each station contained 300 ml of sugar water with 0.001 mg of IgG rabbit serum/ml. After 3 days, *L. humile* samples were collected half-way between stations in each plot. Levels of the protein marker found in individual ants from both areas were determined using Sandwich ELISA. Analysis of Variance was used to compare optical density for the two distances. There was no difference in protein levels in ants between the sites with bait stations placed at either 10 m or 20 m apart. This information is being used to evaluate liquid baits containing insecticides in stations placed at 20 m intervals. Ultimately, these results should enable the development of an improved baiting program for *L. humile* in South Carolina state parks.

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Fact or Fiction: Evaluating Claims of Repellency of Various Plants to Argentine Ants

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In a laboratory bioassay, we tested various claims for the use of raw plant material (soybean, cucumber, tansy, and rosemary) for repelling Argentine ants, *Linepithema humile*. Repellency of each treatment, including positive and negative controls, was evaluated at 2 and 4 hours after the addition of 20 ants in each container. Each treatment was replicated 12 times. In Petri dish assays, freshly cut disks of tansy leaf, soybean leaf, and cucumber peel were found to not demonstrate repellency against the ants after 2 and 4 hours. However, rosemary and spearmint leaves appeared to show some repellency after 2 and 4 hours, with around 50% of ants remaining outside of the spearmint treated Petri dishes and around 75% of ants remaining outside of the rosemary treated Petri dishes. Controls consisted of water as a negative control and a 1% peppermint oil solution as a positive oil control. Controls were effective after 2 and 4 hours with greater than 90% of the ants entering the water treated Petri dishes and less than 3% of the ants entering the peppermint oil treated Petri dishes.

Efficacy of Professional-Use Insecticides Against the Invasive "Kudzu Bug", Megacopta cribraria

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The recently introduced "kudzu bug," *Megacopta cribraria* (Hemiptera: Plataspidae), was first discovered in its invasive range because of overwintering behaviors that result in nuisance aggregations on homes and structures. This insect's alarming rate of range and population expansion after its recent arrival in the southeastern United States has led to a demand for research-based management recommendations. We tested the immediate and one-week residual efficacy of several professional-use insecticide products against adult kudzu bugs on common exterior structural materials. Generally, products containing pyrethroid active ingredients applied at the highest labeled rates showed good immediate efficacy against this insect, with lowered but still effective residual activity up to a week after application. Future work will examine the efficacy of these materials at longer time intervals.

SUBMITTED PAPERS GENERAL

Factor Affecting German Cockroach Control in Low-Income, Multi-Unit Housing

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Low-income multi-unit housing is continuously plaqued with large populations of German cockroaches. With so many effective bait formulations (and negligible cockroach resistance to baits), how do these populations continue to exist? One reason is that low-income facilities are often mandated to hire the lowest bidder for pest management contracts. The break-even cost for pest control companies in the US is ~\$1.00 /minute of technician time. In cases where the pest control contract for the apartment community averages \$6/unit, the technician must spend < 6 min in a single apartment to make any profit for his company. In highly unsanitary and cluttered environments, the technician struggles to find places where gel bait can be applied without contaminating non-labeled surfaces. Consequently, the technician does not have the time (or space) to put out enough bait to reduce the cockroach population. In highly infested units in Richmond, VA, cockroach populations in apartment units were monitored prior to their quarterly pest control service in June 2011. Three weeks after treatment, the same units were monitored again, only to find that the populations had doubled in size. We hypothesize that the populations were so large, and the amount of bait applied was so small, that the cockroaches' annual summer growth cycle was completely unaffected by the pest control service. The purpose of this study was to develop an application method where large amounts of gel bait (60-90 g; enough to impact the cockroach population within a unit), could be placed in a very short period of time (minutes), without contaminating surfaces.

Discovery and Development of a New Bait for Silverfish Control

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Silverfish (Thysanura (now Zygentoma): Lepismatidae) species such as the firebrat, *Thermobia domestica* Packard and *Lepisma saccharina* (L.) are cosmopolitan pests of stored goods. These pests are omnivorous, feeding on carbohydrates (starches; flour, cereal, pasta, dextrin glue, book bindings, cellulose), proteins (pet food, dead insects, other silverfish, silverfish eggs, exuviae), and textiles (cotton, linen, silk, and synthetic carpet fibers).

Our goals were to: 1) Review the efficacy of commercial silverfish baits including those containing boric acid, hydramethylnon, and indoxacarb, 2) Evaluate the potential of other chemistry as bait actives, and 3) Develop a new bait formulation attractive and effective against pest silverfish.

Silverfish bait research dates back to the 1920's. At that time, the actives (e.g. arsenic trioxide, so-dium fluoride) were quite toxic and used at high rates (> 4%). None of the old actives are registered today. Laboratory and field trials on different silverfish species showed variable results and field studies sometimes suggested repellency of the bait formulations. Recent work of Rust and Millard (2009) indicated that current baits labeled for silverfish are ineffective.

We used the following methods to study silverfish bait products and active ingredients. The test species was the firebrat, *Thermobia domestica*. All baits were tested two ways: A "choice" test using alternate food (ground oats) and treated food and a "no choice" test containing only treated bait. We studied commercial products labeled for silverfish control and screened new active ingredients (mixed into ground oats) for efficacy. Commercial products contained boric acid, hydramethylnon, or indoxacarb as active ingredients.

The three boric acid products tested were ineffective against the firebrat. Firebrats consumed very little of the boric acid baits; the presence or absence of competitive food made no difference. The LT50 values for all boric acid bait products were extremely long and not significantly different from the control LT50. Products containing hydramethylnon and indoxacarb were more acceptable to the firebrats but were rather slow acting (see table below)

Treatment	Competitive food	LT50 days (95% CI)	Bait consumed (mg)	Food consumed (mg)	Total consumed (mg)
Control	n/a			42	42
Advion	no	23 (20-29)	16		16
Advion	yes	26 (22-33)	23	31	54
Maxforce	no	14 (11-17)	20		20
Maxforce	yes	34 (27-59)	14	17	31

In the screen for new bait actives, abamectin, dinotefuran, and fipronil did not appear to be good candidates. Novaluron, a chitin synthesis inhibitor was also relatively ineffective. However, metaflumizone was reasonably good and chlorfenapyr was excellent. (see table below).

Treatment, ai and % in matrix	Competitive food	LT50 days (95% CI)	Bait consumed (mg)	Food consumed (mg)	Total consumed (mg)
Control	no			50	50
Abamectin, 0.01	no	71 (45-172)	15		15
	yes	58 (34-104)	Not record	ded Not reco	rded 20
Chlorfenapyr, 0.20	no	2.2 (1.8-2.6)	7		7
	yes	3.1 (2.3-4.4)	Not record	ded Not reco	rded 21
Dinotefuran, 0.05	no	47 (34-78)	13		13
	yes	32 (24-51)	13	10	23
Fipronil, 0.001	no	22 (17-51)	29		29
	yes	59 (40-119)	Not record	ded Not reco	rded 58
Metaflumizone, 0.063	no	8.5 (7.7-9.3)	18		18
	yes	8.0 (7.5-8.5)	Not record	ed Not reco	rded 36
Novaluron, 0.25	no	36 (28-55)	48		48
	yes	30 (25-39)	19	18	37

We tested a commercially available 20% boric acid based bait station (Dekko) and compared it to a prototype station containing 0.20% chlorfenapyr oat granules attached to corregated carboard using dextrin glue. The results, shown below, confirm the ineffectiveness of boric acid products and support the effectiveness of chlorfenapyr as an bait active.

Treatment	Competitive food	Total sample size	14 day mortality (%)
Control Dekko – 20% boric acid Prototype station w/ 0.20% chlorfenapyr	n/a	60	7%
	yes	60	4%
	yes	60	53%

There are several factors that could affect the efficacy of silverfish baits. They include bait matrix attraction (influences the amount of bait consumed) and effectiveness of the active ingredient (active ingredients may be antifeedant or repellent). In addition, species-specific differences in behavior and susceptibility are very likely.

In conclusion, we found that:

- >Commercial products labeled for silverfish control were ineffective against firebrats in laboratory trials.
- >Some active ingredients such as boric acid appeared to be antifeedants.
- >Several active ingredients were only moderately toxic even when ingested.
- >Chlorfenapyr (0.20%) was very effective against firebrats and could be used in a bait station.

Fifty Years of Innovation for Structural Fumigation with Sulfuryl Fluoride (Vikane® gas fumigant)

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Sulfuryl fluoride (SF), trademarked as Vikane® gas fumigant (Dow AgroSciences, Indianapolis, IN), was first commercially marketed in the United States in 1961. Since then, more than 2 million structures, including historical landmarks, churches, museums, medical and research facilities, courthouses, hotels, ships, transportation vehicles, and multi-unit dwellings (Binker 1993, Thoms et al. 1998, Thoms and Mensing 2006), have been fumigated using Vikane. Vikane has unique attributes as a structural fumigant, including broad spectrum efficacy (Thoms and Scheffrahn 1994), excellent penetration, low sorption, relatively inert and nonreactive with structural contents, and rapid aeration (Thoms and Phillips 2004). These unique properties are why Vikane continues to serve as an important tool for the pest control industry fifty years after its market introduction.

Sulfuryl fluoride can be toxic to people, pets, and other non-target organisms and has no intrinsic warning properties; it is colorless, odorless, and tasteless. For these reasons, the label for Vikane requires the use of chloropicrin as a warning agent during fumigation of buildings, appropriate respiratory protection for fumigation personnel, and gas-specific detectors for confirming clearance of the fumigant after aeration. Continuous improvements have been made in safety-related equipment and procedures used with Vikane since its market introduction, and these were reviewed by Thoms (2000). Since 2000, additional enhancements have been made in the use of chloropicrin, SF detection technology, and aeration procedures.

Prior to 2007, the dose rate for chloropicrin was fixed at 1 fl ounce per 10,000 – 15,000 ft³ of fumigated space. Fumigators observed that even the lowest label rate of chloropicrin often required additional or extended ventilation to aerate from large buildings. From 2000-2007, Dow AgroSciences sponsored and conducted extensive field research to determine if lower chloropicrin doses could provide sufficient warning concentrations throughout the fumigant exposure period. Rudolf Scheffrahn, University of Florida, and Dr. Robert Williams, Dow AgroSciences in California, used detection devices, including the MIRAN infrared analyzer, to accurately measure chloropicrin concentrations adjacent to and distant from introduction locations in sealed buildings throughout a range of exposure times. Eighty buildings, representing slab and crawl space underseals, residential and commercial buildings ranging from 12,000 to 2,700,000 cubic feet in volume, were tested in FL and CA. The results of the field testing were used by Dr. Scott Ray, Dow AgroSciences, to develop a new model for calculating chloropicrin dose rates based on exposure time, structure volume, and building underseal (slab or crawlspace). The model calculates chloropicrin doses that are generally less than the previous fixed dose rates. In 2007, the model was incorporated into the Fumiguide® (Dow AgroSciences), a handheld calculator which determines dosing for Vikane and chloropicrin.

From 2003-2005, Dow AgroSciences collaborated with Spectros Instruments in the development of the SF-ExplorIR, a rugged, hand-held, portable gas detector which can accurately measure low concentrations of SF using a nondispersive infrared sensor. Dow AgroSciences developed the performance specifications for the gas detector and conducted extensive laboratory trials on accuracy of five prototype units to measure a range of concentrations (0, 1, 3, 5, 7, and 10 ppm of SF), in varying temperatures (59, 75, 86oF) and relative humidity (35, 50, 90%), and during continuous operation for 8 hours. This testing verified the accuracy of SF-ExplorIR to measure low SF concentrations (1 ppm

± 1 ppm) in a variety of conditions. Dow AgroSciences then sent the units to five fumigators in CA, FL, and HI for a one month field evaluation, during which the fumigators logged hours and conditions of use. At the end of the field trial, fumigators completed a 15 question survey rating equipment performance and returned units to Dow AgroSciences for laboratory evaluation, measuring 1 to 10 ppm SF as described above. Fumigators commented that the digital, backlit display was easy to read, the reading response time was fast, and the unit was easy-to-handle. They liked the audible alarm, data storage, and extended battery life. Minor drawbacks were 15 minute warm-up time and the intermittent, automatic rezeroing using fresh air from an internal air bag. Changes were made to the SF-ExplorIR, such as locking out some of the menus to prevent tampering, based on fumigator recommendations from the field trial. The SF-ExplorIR requires annual calibration verification, has proven to be a reliable, robust detector for Vikane, and is now widely used by fumigators.

The clearance (permissible exposure limit, PEL) threshold standard for SF changed from 5 ppm to 1 ppm in 2006. Dow AgroSciences, in collaboration with California fumigators, conducted research to develop the California Aeration Plan (CAP) to meet criteria established by CA Department of Pesticide Regulation (DPR). CAP aerates the structure for a minimum of 12 hours while the tarpaulins, or seal, remain in place. A fan attached to ducting exhausts fumigant at least 10 feet above the ground, away from nearby people and properties. Seals on this ducting are removed remotely from ground level and fans attached to ducting activated from outside the fumigated space to prevent worker exposure above the 1 ppm PEL. Screened vents, on sides of the structure opposite the exhaust ducting, are opened for fresh air intake. Dow AgroSciences validated CAP during comprehensive trials at 14 single family residences, 4 multi-unit residential complexes and 1 school. Additional trials were conducted at a two story strip shopping mall, a commercial office building and a waste water treatment facility. After DPR accepted CAP in May 2010, the California fumigation industry transitioned to this aeration procedure through 2010.

Dow AgroSciences was awarded the 2009 Commissioner's Award for Pesticide Stewardship by the Florida Department of Agriculture and Consumer Services (Anonymous 2010a). The award was given in recognition for the company's ongoing Caretakerssm program for training and stewardship to support proper use of Vikane. The Caretakers program was established in 1995, and is one of the longest running product stewardship training programs in the pest control industry. The program is updated and revised annually based on input from experts in fumigation at Dow AgroSciences, the fumigation industry, and regulatory officials.

Vikane gas fumigant is being used to control emerging pests and prevent the establishment of exotic, introduced pests. Dow AgroSciences has been the innovator in developing procedures and technical literature to assist fumigators with fumigating structures and contents to eliminate bed bugs, a re-emerging pest (Anonymous 2007, 2008, 2010, Thoms 2010, Thoms and Scheffrahn 2008, Walker et al. 2008). Vikane is used to eliminate infestations of exotic termites, such as the arboreal termites *Nasutitermes corniger* (costalis) and *N. acajutlae*, on pleasure boats (Thoms 2004, Scheffrahn et al. 2004). Pleasure boats are postulated to be an important source for introduction of exotic termite species into the United States, based on historical records and a recent study correlating the proximity of marine dockages to confirmed infestation sites of *Coptotermes* species in Florida (Hochmair and Scheffrahn 2010). Vikane is also used to eliminate structural infestations of medically-important spiders, including the first documented infestation of the Chilean recluse spider, *Loxosceles laeta*, established in a FL residence in 2002 (Thoms 2004). This residence was fumigated with 10-fold the drywood termite dosage rate of Vikane to eliminate the spider infestation.

In conclusion, after 50 years of innovation with SF, Dow AgroSciences remains committed to re

search, development and stewardship of Vikane gas fumigant. Use of this fumigant continues to evolve to meet society's needs for control of re-emerging pests, newly introduced exotic pests, and pests in a diversity of infested structure types.

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Megacopta cribraria (Hemiptera: Plataspidae) in North America: Numts, Heteroplasmy and the Population Genetics of a Rapidly Dispersing Urban Nuisance

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Plataspid stinkbugs (Hemiptera: Plataspidae) from Asia were first discovered in North America in October 2009 in Jackson County, Georgia USA feeding on kudzu, *Pueraria montana* Lour. (Merr.) variety *lobata* (Willd.) and clinging to the sides of houses (Suiter et al. 2010, Jenkins et al. 2011, Zhang et al. 2012). In March of 2010 the bugs had been confirmed in nine northeast Georgia counties and identified as *Megacopta cribraria* (Eger et al. 2010) from morphological characters. Shortly thereafter a 2336 bp mitochondrial DNA (mtDNA) fragment, which included the complete sequences of the tRNA-Tyr gene, cytochrome c oxidase subunit I (COX1) gene, tRNA-Leu gene, and the partial sequence of the cytochrome c oxidase subunit II (COX2) gene, had been PCR amplified and sequenced with the chain-termination or Sanger methodology and put into the GenBank databank (www.ncbi. nlm.nih.gov/genbank/) as haplotype GA1 (GenBank # HQ444175) (Jenkins and Eaton 2011).

As these bugs spread across Georgia and into South Carolina in 2010 (Figure 1) the collaborative work among entomologists paid off. We knew that *Megacopta cribraria* had been introduced into North America and generally from where they had come, Asia. There was also now a one-to-one correlation between morphological taxonomy and an accessible mtDNA sequence marker in GenBank. As we processed the randomly sampled DNA from the ethyl alcohol (EtOH) preserved M. cribraria collected from 2009 through 2011 and into 2012, however, we consistently found a single female haplotype, GA1. Since this was unexpected we began to look more closely at the sequence chromatograms processed in Sequencher 5.0 software (Gene Codes Corporation) for clues of introgression and other phenomena which would indicate more than one female line existed or had existed at introduction. Sometimes we noticed a smaller peak within a larger peak at specific sites. Nuclear mitochondrial pseudogenes or sequences (numts) are often coamplified with mtDNA orthologues when universal primers are used (Moulton et al. 2010). Suspecting the presence of numts, we designed M. *cribraria* specific primers. These were used to reamplify the gene fragment from a more dilute sample. Whenever we observed the phenomenon of a smaller peak inside of a larger peak with the smaller peak being at least half the size of the larger one (Figure 1), we reamplified the fragment using the species specific primers. We then diluted the original sample, reamplified with species-specific primers and a slightly more dilute sample. The observed smaller peak disappeared and we generally got a clear 2336 bp GA1 haplotype.

Recently we observed the multiple peak phenomenon and initially were unable to clear it, even using the above technique or stringent PCR conditions. Our first thought was heteroplasmy. Heteroplasmy was defined by Magnacca and Brown (2010) as the "coexistence of multiple mitochondrial haplotypes in a single organism." It is a difficult phenomenon to demonstrate even though you expect to find individuals with closely related, transcribable, single copies of both alleles. Its incidence may also be lower than reported by other studies (Goto et al. 2011). Although the heteroplasmy did not persist, we conducted a phylogeny analysis with this "GA2." Because most sites were transitions, indicating it was unlikely that there would be an amino acid change, and the sequence was not too different from GA1, the sample fell into the GA1 clade. This showed that the two likely had the same origin.

We will soon add nuclear DNA fingerprinting technology to complement the mtDNA sequence from 269 individuals across time and space with the GA1 haplotype which has dispersed from GA into SC, NC, VA, AL, TN, FL and Honduras. We continue to look for genetic diversity and/or genes within *M. cribraria* and between *M. cribraria* and the endosymbionts that foster the adaptive potential of these bugs. Understanding the interactions between and among this stink bug's genes and among symbiotic complexes may provide insights for controlling the spread of *M. cribraria*.

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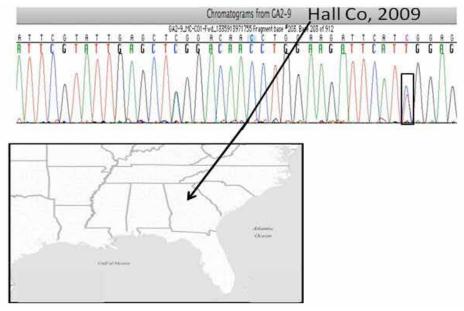
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Fig. 1. Collection from Hall County in 2009. Notice a smaller peak inside the larger peak (black rectangle to the right). Could be heteroplasmy or numts. It proved to be neither upon closer examination. Arrow points to Hall County.



Novel Approach for Public Education about Structural Fumigation

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Structural fumigation is unique among pest control options within the urban environment in its visibility to the general public. While the public may only be aware of pesticide applications occurring in homes and other commercial structures if they see pest control operator vehicles or the individual(s) making the application, it is difficult to miss the colorfully tented structures that are undergoing fumigation. This heightened awareness often results in questions and concerns from the public about the risks associated with fumigation.

Individuals have many options available to obtain answers to questions about pests and pest control options including fumigation. These options can include newspaper articles or columns, magazine articles, television and radio programs and increasingly, the internet. Today almost 80% of the population in North America uses the internet to obtain information (Internet World Stats 2011). Individuals asked to identify their anticipated main source of information in 5 years, overwhelmingly selected the internet (82%) as the primary source for information, followed by television (13%) and newspapers (0.5%) (Reuters 2009). Use of the internet to obtain information is fairly stable across age groups with the exception of the oldest age group (41%). However, as the population ages, this difference is likely to become less evident (Zickuhr, K & A. Smith 2012). Most individuals across all ages utilize search engines to locate information rather than entering a specific URL (Purcell 2011). Studies have also shown users utilize specific criteria to determine the credibility of websites including website design, information structure, and navigation. Information usefulness, while mentioned, was not a key criteria utilized by users (Fogg et al. 2002).

Credible information presented to internet users by credible researchers in the style of a journal publication often contains terminology not well understood by the general public. Extension publications, while removing most jargon, are still written for a well educated audience. However, studies have demonstrated that the US public reads at an 8th grade level (Kaestle et al. 2001). To ensure current scientific information reaches the broadest audience, we must utilize the internet to its fullest. This can be done by understanding how the internet and search engines function, user search behavior and how to format information so that it is easily read by the general public. This presentation reviews research related to internet use, user search behavior and document formatting to maximize availability and usefulness of information to internet users.

The internet provides the public with easy access to a large amount of information which can be viewed as both a benefit and a detriment. While most information provided through traditional communication methods is often vetted by other knowledgeable individuals and contains references to known sources, information presented on the internet may or may not be based in fact or subjected to peer review. Dr. R. Krieger, extension toxicologist at University of California (UC) Riverside, became concerned that the information available on the internet about structural fumigation was often inaccurate, biased and from questionable sources. In 2010, he along with research assistants from the Personal Chemical Exposure Program (PCEP) at UC Riverside partnered with Dow AgroSciences' researchers to evaluate ambient air concentrations of Vikane® gas fumigant (sulfuryl fluoride) during fumigation of two residences. The new California Aeration Procedure (CAP) was used. The data were used to assess potential exposure of workers, bystanders and residents to fumigant during the

fumigation process.

To maximize public access to the study results, the internet was chosen as the main delivery method. Study results were written using easy to understand language, included a number of images and formatted to take advantage of whitespace. Additionally, frequently asked questions were collected from pest control operators, Dow AgroSciences researchers and sales personnel. Answers were written to avoid use of technical terminology and jargon, and images were included where appropriate. All documents and information related to the study were made available to the public through a popular, existing website hosted by Dr. Vernard Lewis, UC Berkeley (http://nature.berkeley.edu/upmc/home.php). Fumigators, pest control operators (PCOs) and Dow AgroSciences personnel provided initial input on content and functionality to ensure maximum usefulness of the site. Research results were published in PDF format to ensure easy access and printing for users regardless of computer platform used. FAQs were structured on the website to allow for easy viewing incorporating show/hide functionality. Structuring the FAQs in this fashion also allowed for the webpage to be viewed in its entirety without extensive scrolling by the user. Additional feedback from PCOs, fumigators and other users of the site will be used to continue to refine website structure and enhance and expand the content.

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Recent Trends in Urban Spiders in Georgia, Including an Update on The Brown Recluse Spider, *Loxosceles reclusa* Gertsch and Mulaik

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Arthropod data was taken from samples sent via mail to the Homeowner Insect and Weed Diagnostics Lab and from homeowner samples sent to the Distance Diagnostics through Digital Imaging (DDDI) system. Samples brought directly to the lab by homeowners or sent via e-mail were not included. All arthropod samples were categorized by type of sample (such as stored product pest, wood destroying pest, occasional pest, etc.) and by the month the sample was collected. Spiders were counted as their own category, irrespective of whether they were domestic or occasional species. Data for categories was expressed as a percentage of the total sample number while seasonal data was expressed as total number of samples per month.

The percentage of arthropod samples from homeowners which were spiders remained between 7 and 9% for the years 2005-2007 with an increase to 16% in 2008. In 2009 the percentage remained up at 14%, the second largest category sent in by homeowners for that year, with garden and lawn pests being the largest at 29%. Despite the larger percentage from earlier years, no brown recluse spiders were identified in the samples for 2009. There was instead an increase in the number of brown widow spiders from previous years.

In 2010, the percentage of homeowner samples that were spiders fall back to the 2005-2007 levels at only 7 %, one of the lowest categories. Garden and Lawn pests again had the largest percentage at 21% with stored product pests coming in second at 16%. As in 2009, no brown recluse spiders were found in the samples sent to the lab for identification.

Even though total numbers of samples were down across the board, the percentage of arthropod samples sent in by homeowners which were spiders increased a little in 2011 with 10% of the total. This however was still one of the lower categories, with nuisance pests (ants, cockroaches, flies, etc.) taking the top spot at 19% and garden and lawn pests coming in a close second at 18 %. Along with the slight increase in percentage, brown recluse spiders were identified in the homeowner samples for the first time in over two years.

In addition to the lower total numbers of spiders in 2011, the peak month(s) for spider sample submissions also differed from previous years. Unlike 2009 and 2010 which showed the typical peak of spider samples submitted in the late summer and early fall (between August and October), the largest number for 2011 occurred in June, with numbers decreasing sharply after that and never recovering.

Despite sometimes lower numbers, some of the species sent in for 2009 - 2011 were new ones for the identification lab. These included a wafer-lid trap door spider in 2011, Amphinectidae for 2009 and 2011, and wall spiders for 2011. Also in 2011 was the first submission of the brown recluse spider for more than two years.

SUBMITTED PAPERS TERMITES

Toxicity and Horizontal Transfer of a Dry Formulation of Fipronil (Termidor® Dry Termiticide) Against Formosan Subterranean Termites

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The toxicity and horizontal transfer of a new formulation of fipronil, Termidor® Dry Termiticide, was tested against Coptotermes formosanus Shiraki in the laboratory. The formulation was applied in 3 different ways: 1) directly applied to termites (donors) and mixed with untreated termites (recipients) at 3 ratios, viz., 50 donors: 50 recipients, 20 donors: 80 recipients and 10 donors: 90 recipients. 2) applied onto the surface of 3 mm thick sand or soil substrate in a Petri dish and then topped with another 3 mm thick sand or soil layer whereupon 75 termites were released, and 3) applied to the inner surface of a tube (either 5 cm or 15 cm long) which connected 2 foraging dishes, one containing dry sand and the other moist sand plus a wood block and termites (100) were released into the dry sand dish. In the direct treatment experiment, mortalities were recorded at 2, 5, 20 and 42 hours after treatment (HAT). All donors and >93% of the recipients were dead by 42 HAT. Significant mortalities of both donors and recipients were observed at 5 HAT at all donor: recipient ratios. During this period, the mortality of the recipients (but not donors) at 10: 90 was significantly lower than those at the other two ratios. At 20 HAT, the mortalities of both donors and recipients were significantly lower than the other ratios tested. In the substrate treatment experiment, mortalities were recorded at 2, 5, 15, 25, 40 hours after exposure (HAE) and every 25 h interval thereafter until termites were dead. All termites were dead at 65 HAE on the sand treatment and at 190 HAE on soil treatment. More than 96% mortality was observed at 40 HAE on the sand treatment as compared to only 6% mortality on soil treatment during the same time period. In the tube treatment experiment, mortalities were recorded at times as in the substrate treatment experiment. More than 97% mortality was observed at 90 hours after release (HAR) for both tube lengths as compared to <3% mortality in controls. About half of the termites were dead by 15 HAR regardless of the tube length.

Field and Laboratory Evaluation of Altriset® in Southern Arizona

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Subterranean termites have the potential to cause extensive damage to cellulose, wood products and structures in temperate and tropical areas. Chemical manufacturers are constantly evaluating products in order to protect structures. The product Altriset® by DuPont is one of these products that have been approved by EPA to be used as a termiticide. Evaluations of field applications of Altriset® within a grid study, bioassay data and the use of Experimental Use Permit information in southern Arizona are reported here. For the grid study, we established six plots consisting of 51 collecting stations set out along the circumference of five annuli. The annuli were centered around an original active central feeding station (CFS) with radii of 1.5, 2.0, 4.0, 7.0 and 10.0 m. Collecting stations were placed at equidistant points along each respective annulus approximately 3.13 m apart to from the sampling grid. On January 30, 2009, we treated 3 plots designated "treatment plots" at full labeled rates with Altriset ® and three plots designated "control plots" with water. In addition to the treatment to the 6 plots, a 10-foot "residue" strip was treated approximately 30 m from plot 3. It was identical to a 10-ft treated side of a plot but was used to extract soil samples for bioassays in the lab. The samples were used only for bioassays and not residue. We took samples on 3 different dates 3, 15 and 27 months post application. Field evaluations were conducted under a EUP of Altriset® 200SC termiticide to determine efficacy against the key termite species, Heterotermes aureas, in Arizona. These evaluations were conducted periodically over a 24 month period. Eleven homes in the Tucson and Phoenix area were selected based on the protocol criteria that homes selected had an active infestation of live termites inside or on the exterior of the structure. A 0.05% dilution of Altriset® was applied to the structures during a timeframe from 5/28/08 thru 9/18/08. The results of the grid study analysis of the six plots nearly one year post application indicated there was a significant difference in the mean foraging population per station between stations internal to the treatment zone (X, A and B) then those outside the treatment zone (C, D, and E). The mean foraging population per station for stations outside the treatment zone was significantly greater than the mean for stations within the treatment zone. In the bioassay study, in general, for all time intervals of 3, 15 and 27 months post application of Altriset® appeared to be very effective against *H. aureas* workers regardless of their colony origin or percent moisture of the soil. There was no termite survival in any of the treatment replicates. In the EUP portion of the study, after 24 months the project was terminated with eleven structures being treated over a 3-month period in 2008. Each structure averaged about 3,032 sq ft, with 4.4 termite tubes and just over 281 linear ft of treatable area. An average of 112 gallons was used at each structure with treatment being applied to 7 post-tensions, 3 floating slabs a structure with multiple additions over a 40 year period. Eight of the 11 structures had no additional treatments with 3 structures receiving additional treatments but none in any of the previously treated areas.

Performance of Recruit® HD in Field Trials against Heterotermes aureus (Snyder) in Arizona

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Seven trials against *Heterotermes aureus* (Snyder) were completed in Arizona as part of a nationwide protocol. The protocol was designed to evaluate the performance of Recruit® HD termite bait for control of subterranean termites in the United States as a requirement for registration. Of the seven trial structures, four were remedial sites (termites were found in the structure prior to study initiation) and three were considered preventive sites (termites were found on the property but not in the structure). Monitoring stations were installed at the test structures according to the label directions for Recruit® IV termite bait and a similar number of additional stations were installed between the monitoring stations. Recruit HD baits containing 0.5% noviflumuron and weighing approximately 150 grams were installed into each of the additional stations. Stations were checked quarterly; however individual baits were only replaced at one year if greater than one third was consumed. This is consistent with the current Recruit HD label which permits an annual inspection interval. Termites were collected from the structures and stations during inspections and colonies were characterized using microsatellite markers. Structural inspections were conducted at study initiation, when colony elimination was determined and six months post colony elimination.

Fourteen colonies of *Heterotermes aureus* in total were identified; seven at the preventive sites and seven at the remedial sites. Twelve of the initial 14 colonies were eliminated within one year of feeding on Recruit HD; most were confirmed eliminated at the second or third quarterly checks. Of the two colonies that persisted beyond one year, one colony could not be confirmed eliminated until the sixth quarterly check due to the lack of timely colony characterization, and one colony remained in a wood monitor after consuming 5% of one bait when dampwood termites invaded the bait. Recruit® HD provided structural protection at all sites as all structures were termite free at inspections six months after elimination of initial colonies or at time of study conclusion.

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Performance of Altriset[™] (Chlorantraniliprole) Termiticide Against Formosan Subterranean Termites, *Coptotermes formosanus* Shiraki, in Laboratory Feeding Cessation and Collateral Transfer Trials

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Belonging to a new class of chemical insecticides, chlorantraniliprole (Altriset™) was recently developed and marketed by DuPont Crop Protection. Chlorantraniliprole is currently classified as a 'reduced-risk pesticide' by the Environmental Protection Agency (EPA, 2008). The compound is an anthranilic diamide, and exhibits a novel mode of action in which insect ryanodine receptors are activated, resulting in rapid paralytic muscle dysfunction (Hannig et al. 2009, Cordova et al. 2006, Cordova et al. 2007, Lahm et al. 2005, and Lahm et al. 2007). Regulation of the release of internal cell calcium is affected by activation of ryanodine receptors. The downstream physiological effect of this disruption of calcium homeostasis results in feeding cessation, and eventual death of the insect (Teixeira et al. 2008). The effectiveness of this compound has been demonstrated in mortality trials against a variety of insect species belonging to the orders Coleoptera, Diptera, and Hemiptera (Kuhar et al. 2008, Palumbo 2008, and Schuster 2007). We designed laboratory trials to study the efficacy of chlorantraniliprole on Formosan subterranean termites (FST) Coptotermes formosanus Shiraki, feeding rates and collateral transfer of chlorantraniliprole among FST nestmates. Laboratory and field trials were conducted to quantify mortality of FST Coptotermes formosanus Shiraki resulting from chlorantraniliprole treated soil, the degree to which the termites curtail feeding intensity post-exposure to chlorantraniliprole treated soil, collateral transfer of chlorantraniliprole among nest mates, and the effectiveness of chlorantraniliprole as a remedial treatment against structural infestations of FST. Termites which were exposed to treated soil consumed significantly less paper than unexposed FST. The mean percent mortality of those termites exposed to chlorantraniliprole treated soil was significantly greater than that of unexposed FST. Depending on donor:recipient ratios, the mean mortality of recipients ranged from 14.65 – 90.00 % in the collateral transfer trials. There was a positive correlation between increased donor density and recipient mortality.

Termite Feeding Cessation- FST were field-collected from Beaumont, TX approximately 2 weeks prior to the initiation of this study. Sandy-loam soil was prepared for these trials by treating it with chlorantraniliprole using the following procedures. A 1,000 ppm stock solution of chlorantraniliprole and water was made by adding 0.10 g of technical grade chlorantraniliprole to 100 ml of deionized water. Next, a serial dilution of the stock solution was accomplished by adding 15 ml of deionized water to 15 ml of stock solution. This final solution was added to 270 g of soil and distributed by mixing the soil with a stir rod within a 750 ml plastic beaker. The treated soil was allowed to rest for a period of 24 hrs. Glass test-tubes (10 cm in length and open at both ends) served as arenas for this experiment and followed the design of Gold et al. 1996. After preparation, the tubes were stored vertically in test-tube racks (untreated-agar end at top). Tubes were stored at 25°C overnight to allow moisture to become uniformly distributed in the matrix. There were 10 replications of each treatment and untreated control groups. After the 24 hr period had elapsed, 20 FST workers and 5 soldiers per replication were randomly selected from laboratory stock and introduced into the "untreated-agar end" of the test-tube arenas, and the introduction time was recorded. The time at which the tunneling termites reached the treated soil was recorded as the exposure 'start' time. Cohorts of termites were allowed to tunnel in the soil for four different time periods (1, 2, 4, & 8 hr). These four time periods represent four distinct treatments. After the termites had tunneled for the pre-determined time period, the rubber stoppers were removed and termites and soil were carefully tapped out through the untreated end

of the arena into a clean Petri-dish. Pre-feeding digital images of 5 X 5 cm pieces of brown paper towel (Cormatic-Georgia Pacific, Atlanta, GA) were taken for comparison to post-feeding images. Termites were then carefully moved to a smaller Petri dish containing the paper towel, half of which was covered by 50 g of play sand, moistened with 10 ml of water. This arrangement of paper towel and moistened sand provided ambient humidity within the arenas, as well as constantly moist paper towel, on which the termites fed. Petri dishes were sealed with ParaFilm®, transferred to an environmental chamber, and maintained at 25 ± 5° C and 85 ± 5% RH. Untreated control tubes were constructed in the same manner as the treatment tubes, but the soil remained untreated. Termite cohorts were allowed to tunnel for the same time as the treatment groups periods (1, 2, 4, and 8 h). Termites were transferred to identical feeding dishes, and feeding rates were then calculated in the same manner as in the treatment groups. These control groups are referred to as 'Tunneling Controls' (TC). An additional set of controls which were not allowed to tunnel, were established in feeding dishes (as above) to compare the amount of feeding for the same time period as the treatment and TC groups. Twenty termite workers were introduced directly into these Petri dishes without subjecting them to tunneling, and observation periods were identical to the treatment and untreated control groups. This control group is referred to as 'Feeding Controls' (FC). Termite mortality was recorded daily for 12 d postexposure. At the end of the trial, the remaining paper towel was allowed to dry in the laboratory, after which, digital images of the paper were taken with a Canon EOS 50D 15.1 megapixel digital camera fitted with a 28-135 mm lens (Canon U.S.A., Inc. Lake Success, NY). Pre-feeding and post-feeding images were then compared using SigmaScan PRO v.5.0 photo-editing software, and the surface area (cm²) differential was calculated and statistically analyzed (calibration of the photography technique was made for each image prior to area measurement).

Collateral Transfer- As in the feeding cessation study detailed above, termites used in this study were field-collected from Beaumont, TX approximately 2 weeks prior to the initiation of the trial. Ten replications of each treatment (donor:recipient ratios) and untreated controls were conducted for this trial. Arenas consisted of a 15 cm Petri dish, each with a a 7.6 X 7.6 cm piece of brown paper towel (Cormatic-Georgia Pacific, Atlanta, GA) placed on the floor of the Petri dish and moistened with 8-10 droplets of water from a 25 ml Samco Scientific Corporation pipette (San Fernando, CA). The paper served as food and harborage for termites. Donor:recipient ratios in this trial included 0:20 (untreated controls), 1:19, 5:15, 10:10, 15:5, 19:1, and 20:0. Donor FST were marked using orange Rust-Oleum Marking Paint (Vernon Hills, IL). The methodology used to mark the donors was similar to that described by Forschler 1994. A stock solution of 50 ppm was made using formulated chlorantraniliprole. Donor FST were treated on the thorax with 0.3 µl of 50 ppm chlorantraniliprole using a Hamilton 700 series micro syringe pipette (Reno, NV). Two untreated FST soldiers were added to each arena. Additionally, two sets of post-treatment observations were made to observe mortality at 4 h then daily through 7 d. Feeding intensity was measured in these trials by taking pre-feeding images of the 7.6 X 7.6 cm brown paper towel and post-feeding images at the end of these trials (7 d after treatment). As described above, pre- and post-feeding images were compared using SigmaScan PRO v.5.0 photoediting software, and the surface area (cm²) differential was the metric used for statistical analysis. Calibration of photography was made for each image prior to area measurement.

Termite Feeding Cessation- All treatment cohorts that were exposed to chlorantraniliprole treated soil (regardless of exposure time) consumed significantly less paper (F = 21.37; df = 8,89; P < 0.01) than the 'Tunneling Controls' (TC) and 'Feeding Controls' (FC) (Fig. 1a). Additionally, the mean amount of paper consumed by FST after exposure to chlorantraniliprole treated soil was negatively correlated with time of exposure (Fig. 2). A similar trend was not observed in the TC (Fig. 1a). With the exception of the 1 and 4 hr Tunneling Control groups, the mean % mortality of FST remained below 10% in the untreated controls through 11 d after exposure to untreated soil (Fig. 1b). At and beyond 3 d post exposure, the mean % mortality of all treatment cohorts that were exposed to chloran-NCUE 2012

traniliprole treated soil (regardless of exposure time) was significantly greater (F = 9.64; df = 8,89; P < 0.01) than the TC and FC (Fig. 1b). Additionally, with the exception of the 12 d observation period, the mean % mortality of treatment cohorts that were exposed to chlorantraniliprole treated soil was positively correlated with time of exposure.

Collateral Transfer- Percent donor mortality in all of the treatment ratios was significantly different (F = 17.73; df = 8,89; P < 0.01) than that of the 0:20 donor:recipient ratio (untreated controls) at 7 d post-treatment. However, there were no significant differences in donor mortality between the different treatment ratios at the 7 d observation period, and mean mortality ranged from 50.00 - 76.00 %. There were significant differences (F = 17.73; df = 8,89; P < 0.01) in recipient mortality levels among the different treatment ratios at 7 d post-treatment, and mean mortality ranged from 14.65 - 90.00 %. Regarding total mortality (donors and recipients) for each donor:recipient ratio, there were significant differences (F = 7.58; df = 8,89; P < 0.01) at the 7 d post-treatment observation period. The greatest percent total mortality (74.5 %) occurred in the 15:5 ratio at 7 d, followed by 67.5% mortality in the 19:1 donor:recipient ratio. Mortality in the untreated and unmarked group was 9.00 %, and was not significantly different from that of the untreated and marked group (21.00 %) starting at 24 h thru 120 h post-treatment (F = 0.067, df=19, P = 0.31). There were significant differences in consumption of paper among the different treatment groups and the 0:20 (untreated control) donor:recipient group (F = 15.33; df = 8,89; P < 0.01) (Fig. 1c). The greatest consumption occurred in the 0:20 ratio, followed by the 5:15, and then the 10:10 (Fig. 1c).

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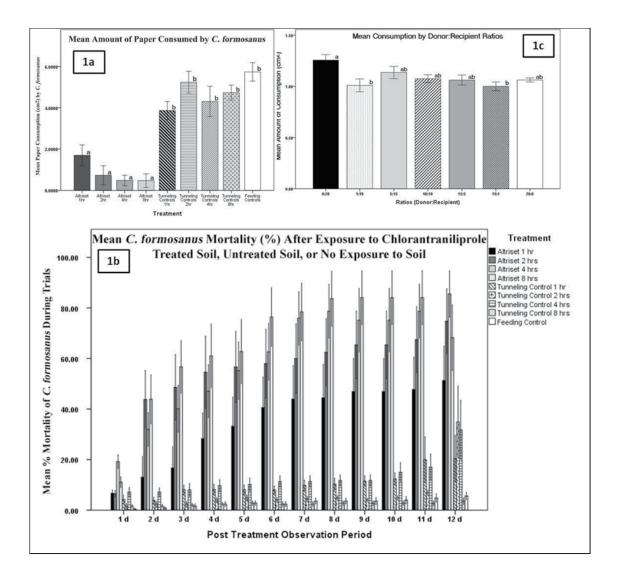


Fig. 1. (a) Mean paper consumed (cm2) by FST through 12 d after exposure to chlorantraniliprole treated soil, untreated soil, or no soil. Treatments were replicated 10 times. Bars with the same letter are not significantly different using Analysis of Variance (ANOVA) and Tukey's HSD mean separation test at P < 0.05, (b) mean accumulated FST % mortality through 12 d of observation, and (c) Mean amount of consumption of substrate (cm2) by FST (donors and recipients) in each donor:recipient ratio through 7 d post-treatment. Bars with the same letter are not significantly different using Analysis of Variance (ANOVA) and Tukey's HSD mean separation test at P < 0.05.

Altriset[™] (Chlorantraniliprole) Termiticide Performance Against Formosan Subterranean Termites, *Coptotermes formosanus* Shiraki, in Field Trials Through 3-Years

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Field trials were conducted to quantify the effectiveness of chlorantraniliprole as a remedial treatment against structural infestations of Formosan subterranean termites (FST). Through 24 mo post-treatment, 27.3% of the structures which were treated in field trials were observed to have infestations of termites that required re-treatment; however, no active FST were observed to be infesting any of the structures during the 30 and 36 month post-treatment inspections. Additionally, a novel scoring rubric was developed that will allow standardization of field study sites with respect to dissimilarity in site variables, and will allow for more consistent comparison of results across disparate field experiments. An explanation for the lack of successful remediation of many of the structures involved in the field trial is proposed and is based on our novel scoring system.

For the purposes of this field trial, Center for Urban and Structural Entomology (CUSE) and DuPont personnel jointly inspected and agreed upon 11 structures with monolithic slabs or pier and beam construction, each of which had at least one active FST shelter tube on the exterior of the structure. All of these structures were located in southeast Texas. Chlorantraniliprole treatments were made by a pest management professional at each property according to the manufacturer's label directions. These treatments were overseen by CUSE personnel. All structures were treated between May and July 2008. Post-treatment exterior (and interior when possible) inspections were made on or about 2 wk, and then at 1, 3, 6, 12, 18, 24, 30, and 36 months. This monitoring schedule is more robust than that which would generally be incorporated into professional pest management protocols. If active termites were discovered during post-treatment inspections, termite samples were collected and preserved in 100% ethanol as voucher specimens. DuPont authorized personnel were notified of any post-treatment activity before any supplemental treatments (ST) or re-treatments (RT) were performed. A supplemental treatment (ST) in this study is defined as:

- (a). Chlorantraniliprole spot treatments to active termite areas that were not originally treated at the initial application. This is not a failure of chlorantraniliprole, since the termiticide was not applied to that area, and termites penetrated through an untreated zone.
- (b). Treatment of infested elements of construction where termites survived due to conducive conditions, such as leaking pipes, unusual construction elements that did not allow application to reach the infested area, or an isolated above ground colony with no soil contact.
- (c). Treatment where a conducive condition existed which contributed to or allowed termites to remain active and penetrate the treated zone. Thus, it was not considered a chlorantraniliprole failure.

A re-treatment (RT) in this study was defined as: the application of chlorantraniliprole as a spot treatment to active termite areas that were originally treated at the initial application. This was considered a failure of chlorantraniliprole. That is, termites were able to penetrate through the treated zone; however, if that area of penetration had conducive conditions (or other issues as above) that allowed termites to penetrate, and the condition was not corrected, then this was considered a supplemental treatment (ST) as described above.

In this study, all structures were ranked based on several parameters related to the difficulty of the structure-specific treatment procedures. The parameters used to populate the ranking rubric included: termite species (1-desert termite, 2- drywood termite, 3-*Reticulitermes*, and 6-*Coptotermes*); number of mud tubes and location; the number of conducive conditions present at each structure; and, construction type (1 monolithic slabs, 2 pier and beam, and 3 floating slabs). The Total Difficulty Score (TDS) of each structure was calculated by summing all points assigned for each category. It is presumed that difficulty to control termites at structures is positively correlated with higher TDS.

The mean number of pre-trial exterior termite mud tubes per structure was 3.91, and ranged from 1 – 10. The mean volume of finished solution applied to structure exteriors was 302.15 L, and 10.33 L on the interiors. During the first 24 mo of the 36 mo trial duration, 27.27% of the structures were infested with FST and required re-treatment (RT), and 18.18% required supplemental treatment (ST). This includes Structure #3 in which FST were not completely controlled until a re-treatment (RT) was made after the 1 mo inspection, when FST were discovered on the exterior at the original site of infestation (Fig. 1 and Table 1). The re-treatment (RT) was performed with 15.14 L of chlorantraniliprole. Due to damage caused by hurricane lke, access to eight of the structures was limited at the 3 mo post-treatment inspection, and only three of the eleven structures were inspected. No termite activity was found at those three structures. At the 6 mo inspection, two structures were found to have termite activity (Fig. 1 and Table 1), including Structure #2, which had active termites on the exterior and received a re-treatment (RT) with 15.14 L of chlorantraniliprole. Additionally during the 6 mo inspection, active termites were found swarming from an interior wall within structure #9. After further investigation of the swarm, a previously unknown cold joint was discovered and this structure received a supplemental treatment (ST) with 37.85 L of chlorantraniliprole. At the 12 mo inspection, Structure #8 had active FST swarming from a previously treated bath trap. Upon further examination, it was determined that there was a water leak in the bath trap area, this leak was repaired, and the area received a supplemental treatment (ST) using 22.71 L of chlorantraniliprole. No subterranean termite activity was found during the 18 mo inspections. At 22 mo post-treatment, Structure #9 homeowners notified us of FST swarming again from the same cold joint, but in a different area. This area received a supplemental treatment (ST) with 189.27 L of chlorantraniliprole. This ST is included in the 24 mo post-treatment observation period on Fig. 5. Also during the 24 mo inspection, active FST were discovered at Structures # 2 and 5 (Fig. 1 and Table 1). Both structures had active termites on the exterior, and both were re-treated (RT) with 15.14 L of chlorantraniliprole. No subterranean termite activity was found on any of these structures at the 30 or 36 mo inspection. After ranking, using the rubric described in the Material and Methods section, the mean total difficulty score (TDS) of the structures in this study was 12.36, and ranged from 9 – 18 (Table 1). At least one ST was required in 18.18% of the structures, and all were made to structures that ranked below the mean TDS. Of the re-treated structures (RT), all but one occurred in structures which ranked above, or just below the mean TDS (Table 1).

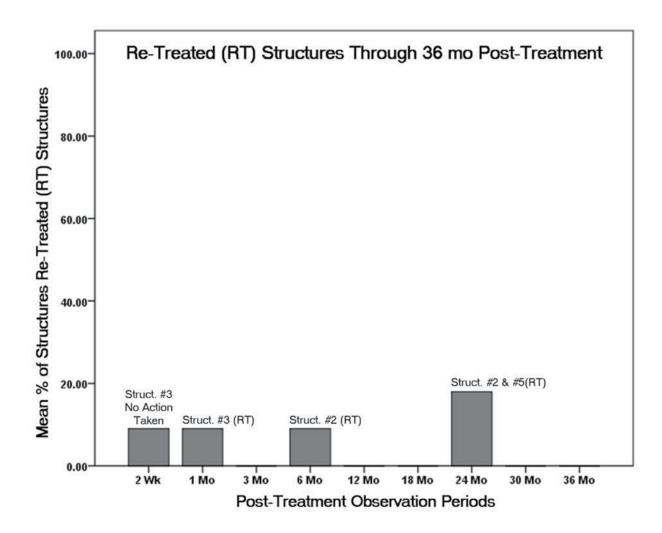


Fig. 1. Mean % of re-treated (RT) structures infested with FST after a post-construction treatment with chlorantraniliprole through 36 mo post-treatment. Nomenclature above bar: Individual structure number and RT (re-treatment). NOTE: Active termites were found at Structure #3 at 2 weeks post-treatment, but no corrective action was taken until 1 month post-treatment.

Table 1. Data-populated scoring rubric developed for, and used in these trials to standardize the total difficulty score (TDS) associated with treatment of each structure.

Structur #	re Termite Species	# of Mud Tubes	<i>y</i> .	# of Inditions Inducive	Total # of ST's # of RT's Difficulty Score (TDS)		
1	C. formosanus=6	10 Exterior	Monolithic=1	1	18.0	0	0
2	C.						
	formosanus=6	10 Exterior	Monolithic=1	1	18.0	0	2
3	C.						
	formosanus=6	1 Exterior 1 Interior	Monolithic=1	1	10.0	0	1
4	C.						
5	formosanus=6 C.	3 Exterior	Pier & Beam=2	2	13.0	0	0
	formosanus=6	2 Exterior 1 Interior	Pier & Beam=2	1	12.0	0	1
6	C.						
7	formosanus=6 C.	4 Exterior	Monolithic=1	1	12.0	0	0
8	formosanus=6 C.	5 Exterior	Monolithic=1	1	13.0	0	0
9	formosanus=6 C.	3 Exterior	Monolithic=1	1	11.0	1	0
9	formosanus=6	2 Exterior	Monolithic=1	1	10.0	2	0
10	C.			·		_	J
11	formosanus=6 C.	2 Exterior	Monolithic=1	1	10.0	0	0
	formosanus=6	1 Exterior	Monolithic=1	1	9.0	0	0
Mean &	6	3.91	1.18	1.09	12.36	0.27	0.36
(S.D.)	(0.00)	(3.24)	(0.40)	(0.30)	(3.07)	(0.64)	(0.67)

NOTE: All structures were ranked on the difficulty of the treatment based on several parameters which included, termite species, number of mud tubes and location, and the number of conducive conditions present at each structure. The table details the ranking of termite species based on colony size: 1 desert termite, 2 drywood termite, 3 *Reticulitermes*, and 6 *Coptotermes*. Each structure received one point for every mud tube located on the structure, and one point for each conducive condition found to be present at the structure. Construction type was also given a treatment difficulty ranking as follows: 1 monolithic slab, 2 pier and beam, and 3 floating slab. The Total Difficulty Score (TDS) of each structure was calculated by summing all points assigned for each category.

Powdered Cellulose Additive Influences Distribution of Liquid Termiticides in 'ABC' Gravel Foundation Fill

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A field study was conducted to determine the influence of powdered cellulose additive on vertical distribution of active ingredient (AI) of six different termiticide formulations at different depths in 'aggregate base course' (ABC; Baker and Weeks 2002), and for loamy soil from Mississippi and sandy Florida soil (Table 1). Three of these termiticides contained powdered-cellulose additive and three did not. Cellulose-augmented termiticides were retained in significantly greater concentrations in uppermost ABC partitions compared with their counterpart non-cellulose termiticides containing the same active ingredient. Active ingredient residues decreased with increasing depth. Cellulose-augmented termiticide applied to the surface of loamy soil from Mississippi and sandy soil from Florida also resulted in decreasing active ingredient concentrations with depth. ABC perimeter trenches and trenches around upright utility pipes penetrating through the fill received by-the-label termiticide applications, resulting in relatively greater variability of distribution in fill compared with vertical filtering associated with surface applications.

Arizona Chemical Group, Inc. (AGC), Mesa, AZ, owns unique liquid formulations of termiticides that are made, in part, from insecticide active ingredient plus a finely powdered cellulose additive. Percentages of cellulose and additives in these formulations are proprietary and not available for description. Cellulose additive may influence termiticide sorption to foundation fill materials, which could alter Al longevity and biological availability to foraging termites (McCall et al. 1980; Smith and Rust 1993; Watson et al. 1998).

Termiticide formulations contained bifenthrin, cypermethrin, or permethrin. Standard aqueous mixtures alone as well as ACG's proprietary formulations with the same Als plus powdered cellulose additive were compared in this side-by-side study. Times-Up®T/C was compared with Tengard®SFR (permethrin); Grenade®T/C was compared with Demon®TC (cypermethrin); and Silencer® was compared with Talstar® (bifenthrin). Times-UpT/C, GrenadeT/C, and Silencer all contain powdered cellulose additive.

Objectives: Determine 1) termiticide residues in ABC fill (surface applications); 2) termiticide residues in ABC fill (trench applications); 3) Times-Up T/C residues in construction fill soils from Mississippi and Florida (surface applications).

Objective 1. Each termiticide was applied uniformly over the surface of three 31.5-m² side-by-side blocks of evenly distributed, level, non-compacted 15-cm-deep ABC fill.

Objective 2. A 13-cm-deep by 15-cm-wide trench was excavated along the inside face of each block's form-board frame, and a by-the-label termiticide application was applied into perimeter trenches as well as into trenches around a 5.1-cm dia. PVC service pipe for each termiticide used.

Objective 3. Commonly used sub-concrete slab foundation fill soils from Mississippi and Florida were contained and lightly tamped down to a level surface within their individual 0.50- by 0.50-m-square

by 10.2-cm-deep wooden frames, and triplicate partitioned samples later extracted. Times-Up T/C (0.27% AI, w:w) aqueous permethrin mixtures were uniformly applied to the soil surface from a height of ≈15-cm using 4.0 L watering cans. Application rates were equivalent to 3.8 L per 0.93 m² of soil surface per label directions.

Following surface and trench applications, termiticides were allowed to disperse and soak into ABC and soils for 24 h. For surface applications, separate triplicate 0.0- to 1.3-, 1.3- to 2.5-, 2.5- to 5.1-, and 5.1- to 10.2-cm-deep vertical core partitions were then extracted in sequence from ABC and soils. For trenches, a 7.6-cm-deep vertical core of fill was extracted from the trench within each block and consolidated into one composite core. This procedure was repeated for each utility penetration and termiticide, producing a non-partitioned triple composite trench fill sample for each termiticide formulation.

Results

Objective 1 Results. Times-Up T/C and Tengard SFR. Permethrin residues from 0.27% and 0.50% Times-Up T/C applications (749±20 and 1,872±361 ppm) were significantly greater compared with residues from 0.27% and 0.54% Tengard SFR (456±23 and 1,127±158 ppm) in the top 0.0- to 1.3-cm partition, respectively (P ≤ 0.0001). These results demonstrate that the formulation augmented with cellulose powder exhibited reduced downward advection and greater AI residues in the top partitions of ABC compared with the non-cellulose formulation. For both termiticides, $\geq 95\%$ of permethrin was retained in the top 2.5 cm.

Grenade T/C and Demon TC. In the top 0.0- to 1.3-cm and 1.3- to 2.5-cm partitions, cypermethrin residues from 0.12% Grenade T/C applications (551±73 and 191±20 ppm) were greater compared with 0.25% Demon TC applications (507±41 and 159±46 ppm), although not significantly different. However, Grenade T/C was applied at half the Demon TC rate. These results demonstrate that cellulose augmentation facilitated reduced advection and decreased hydrodynamic dispersion of termiticide, leading to increased AI concentrations in the upper partitions of the soil matrix (Watson et al. 1998). For both termiticides, ≥94% of cypermethrin was retained in the top 2.5 cm.

Silencer and Talstar. In the top 1.3-cm-deep partition, bifenthrin residues from 0.06% Silencer applications (123±20 ppm) were significantly greater compared with 0.08% Talstar applications (85±6 ppm; $P \le 0.0001$). Recovery of AI, even when applied at a lower rate, was significantly greater in the top partitions when cellulose was present in the formulation compared with the non-cellulose formulation. For both termiticides, ≥92% of extractable cypermethrin was retained in the top 1.3 cm.

Objective 2 Results. Trenches and Pipe Penetrations. ABC from trenches contained >200 to ≈2,300 ppm AI, distributed variably throughout the entire trench depth, as sampling cores were not partitioned. Due to mixing during applications, there was no apparent trend of greater ppm being recovered from cellulose-augmented termiticides compared with non-cellulose formulations. Applications of 0.50-0.54 percent AI resulted in 1,233 to 2,030 ppm residues, whereas 0.27 percent AI applications produced 387-1,288 ppm in ABC. Applications of 0.06-0.08 percent AI produced 201-576 ppm. However, inherent to physical digging and then applying termiticide into the trench as well as during backfilling, variations in AI concentrations occurred throughout the ABC vertical profile. Termiticide residues in perimeter trenches were more variable than residues from utility pipe trenches. Utility pipe trenches were 0.7-m-long compared with 6.9- and 13.7-m-long perimeter trenches. Thus, there was less ABC volume treated in utility pipe trenches, resulting in less residue variability between corresponding counterpart treatments.

Objective 3 Results. Mississippi and Florida Fill Soils. Permethrin residues from Times-Up T/C were significantly greater in the 0.0- to 1.3- and 1.3- to-2.5-cm-deep partitions for both Mississippi (672±129 and 335±69 ppm) and Florida (1,224±221 and 739±73 ppm) soils compared with deeper 2.5-5.1- and 5.1-10.2-cm-deep partitions (27±12 to 0.0 ppm Mississippi; 136±27 to 0.0 ppm Florida). Top-to-bottom decrease of ppm was apparent ($P \le 0.0001$). For both soil types, ≥92% of residue was retained in the top 2.5 cm of soil.

Overall, cellulose powder augmentation facilitated enhanced retention of termiticide in the near-surface partitions of ABC fill. Additionally, cellulose-augmented pyrethroid termiticides applied at reduced rates resulted in residual AI concentrations known to be efficacious against subterranean termites in ABC as well as loam and sand fill soils (Beal 1986; Forschler 1994; Forschler and Townsend 1996; Gold et al. 1996; Baskaran et al. 1999; Saran and Kamble 2008; Mao et al. 2011). Detailed residue results and analyses of all applications and partition depths have been submitted to a peer-review journal for publication. Results demonstrate that applications of the cellulose-augmented termiticide formulations used in this study, when applied at the same or reduced rates to ABC fill, provide ppm residues greater than standard pyrethroid termiticides without cellulose in the upper areas of this aggregate foundation fill.

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Mention of a company or trade name, or termiticide name does not imply endorsement by Oklahoma State University or any of its associates or agents, or by the authors or their affiliates or companies.

Table 1. Classification of aggregate and soil, building construction foundations.

Textural fractions - % w:w										
Fill	pН	Sand	Silt	Clay	OM ^a	Texture Class				
Arizona ABC	8.8	85	5	10	0.29	Coarse Gravel + loamy-sand fractions ^b				
Mississippi	6.9	40	37.5	22.5	0.37	Loam				
Florida	7.7	90	5	5	1.19	Sand				

^a Organic matter content, % w:w.

^b≈40-50% coarse gravel, ≥2.0- to 5.2-cm maximum size; remainder loamy-sand fractions as described.

Evaluation of Two Ready-To-Use Termidor Formulations and Premise Foam for the Control of the Drywood Termite *Incisitermes snyderi* (Kalotermitidae) Using Localized Treatments to Naturally Infested Lumber

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Twenty-one 2.6-cm ×15.8-cm x 3.3-m (D:W:L) custom cut cypress boards infested with drywood termites were examined for activity using a Termatrac® (65 Christensen RoadStapylton, 4207 Queensland, Australia) microwave motion detector. Boards were numbered and a tape measure was placed lengthwise down the middle of each board. On either side of the tape measure, readings were taken every 15 cm creating 22 separate sections for recording data. The Termatrac measurement sites for each board therefore remained consistent throughout the study.

Immediately prior to treatment a resistograph drill (IML-Instrumenta Mechanic Labor GmbH, 1275 Shiloh Road, Ste. 2780–30144 Kennesaw, GA, USA) was used to locate at least one gallery at two locations (61-cm mark and 122-cm mark) regardless of the site of activity in that board. Resistograph drill holes were placed within 5-mm of the 61-mm or 122-cm mark on the edge of a board. The resistograph drill hole that indicated at least one gallery at that respective location was used as the point of application of the appropriate treatment. Treatments included three formulations and two active ingredients; Premise® Foam (0.5% imidacloprid)¹, a Termidor® Dry (0.5% fipronil)² and a BASF experimental formulation in a pressurized can (fipronil).

This was a highly rigorous test given the length of the boards, the degree of infestation and the fact that only two treatment injection points were made per board, 61-mm from each end. Average Termatrac termite motion detector readings for each treatment at 65 days after treatment indicated a 5.9% reduction in activity for the untreated control (N=5), 41.7% reduction in activity for Premise foam (N=5), 86.3% reduction in activity for Termidor Dry (N=6) and 90.6% reduction in activity for the BASF fipronil experimental formulation (N=5). Destructive sampling at 65 DAT revealed three of six boards in the fipronil dry formulation treatment and two of five boards in the fipronil experimental formulation had complete control of termites. No boards for the Premise Foam or the control treatments had complete control of termites. The results also indicated that the Termatrac was excellent at locating termite activity but provided 9.5% false negatives.

- 1 Premise® is a registered trademark of Bayer Environmental Science
- 2 Termidor® is a registered trademark of BASF

Molecular Evolution of Reticulitermes from the Southeastern USA

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The evolutionary pattern of *Reticulitermes* speciation has always been mysterious. Molecular markers from mitochondrial and non-coding DNA regions were employed to determine the evolutionary pattern for this group of insects. Sequences obtained were analyzed using phylogenetic (MEGA 5, PHYML, MrBayes) and molecular sequences program (BEAST and DNASP) to determine the evolutionary pattern and estimated divergence sequence. Preliminary results indicate that *R. flavipes* is the ancestral species. There appears to be an extinct ancestral species from which *R. virginicus*, *R. hageni*, *R. malletei* and *R. nelsonae* diverged. Of those species, *Reticulitermes hageni* is the most recently diverged species. Questions remain about the exact sequence of the evolutionary divergence of the remaining three species although the data support an earlier divergence for *R. malletei* and *R. nelsonae* than *R. virginicus*.

Dispersal Dynamics of the Exotic Arboreal Termite Nasutitermes corniger

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Nasutitermes corniger is an exotic arboreal termite endemic to the Neotropics and an economically important structural pest. This termite was first discovered in the United States in Dania Beach, Florida, in May 2001, and was estimated to have been established for 5-8 years. Due to the proximity of the infested area to seaports it is assumed to have arrived shipboard from an unknown location in the Caribbean Basin. In order to understand the dynamics of exotic termite introductions, a study using polymorphic microsatellite markers was conducted. This was a step in understanding the number of propagule pairs and the number of generations on land.

Termite specimens were collected from various infestation sites in Dania Beach and preserved. Comparison of individuals from these subpopulations for their microsatellite loci estimated the population structure and potentially also the parentage of the colony. Predictions were made whether the subpopulations were merely simple families headed by a single pair of unrelated or related reproductives, or extended families resulting from inbreeding of existing individuals and mixed families resulting from the fusion of two or more genetically unrelated colonies. Furthermore, percent polymorphism, number of alleles per locus, and comparison between observed and expected heretozygosites were made.

Using the Advance® Termite Bait System to Monitor Termite Populations and Provide Community-Wide Structural Protection

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The Advance Termite Bait System (ATBS) was installed around sixteen structures in Gainesville, Florida, to test efficacy in compliance to the Florida Adminstrative Code (FAC) 5E-2.0311 (Performance Standards for Preventative Termite Treatments for New Construction), which requires a 90% reduction in 90% of the structures within 12 months or 90% reduction in 90% of the monitors within 12 months. Prior to ATBS installation, visual and canine inspections confirmed termite activity in seven structures and stake monitors confirmed termite activity in close proximity to seven others while two structures served as unbaited controls. ATBS stations were monitored by University personnel on a monthly or quarterly basis, depending on the stipulations of the protocol. Structures were inspected for four years via annual visual and canine inspections and no termite activity was found in any structure, including those that were infested at the time of installation.

With an average of 12.2 stations per structure, the average number of stations and monitors that experienced termite activity was 7.3 and 4.6, respectively. The time to feeding cessation for stations around infested structures and structures with associated activity only differed by 4 days (121 vs 124 days). Throughout the duration of the study, termites often reappeared quickly around structures, indicating that ongoing monitoring and station maintenance in areas of high termite activity is important to ensure structural protection.

Intercepting Treatment of Shelter-Tube with Termidor Dry: Effects on Populations in Colony Nest and Satellite Feeding Site

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Subterranean termites build a network of tunnels to connect various food resources and locations inand above-ground. Current products are to be applied around/beneath structure or to active infestations. This study is to evaluate the colony efficacy and efficiency of shelter-tube spot-treatment with a powder formulation of fipronil on subterranean termites. We tested the recipient colonies of the Eastern subterranean termites. The bioassay set-up was comprised of three parts: a container hosting termite colony (named nest-site) and a container hosting food-source (named feeding-site) connected by a 2-m soil-filled Tygon tube. Treatments involved two doses: 0.15 and 0.3 mg/colony. After the tunnel network was established in the bioassay set-ups, the designated doses were puff-injected, at an acute angle, into a pre-identified tunnel 0.5-m from the feeding-site. A specially designed applicator was used to execute the treatment. Termidor dry was puff-injected to an average of 12.33 cm into the tunnel. Visual observation recorded termite tunneling activity and estimated mortality. Termatrac T3i was used to verify termite motion and detect the likelihood of hiding activities. Significantly decreasing termite activities was observed at feeding site first followed by inside tunnels, and at colony-nest the last, regardless of the dose. Treatments 0.3 and 0.15 mg/colony eliminated satellite-feeding-site populations at 4 and 8 d post-treatment, and achieved complete mortality at 18 and 24 d, respectively. These results indicate that treating shelter-tube offers a new approach to gaining a guick and targeted control of termite infestation with significant insecticide reduction.

Evaluation of Novaluron For Use as a Termite Bait Active

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Laboratory and field studies were conducted to evaluate the bait transference and efficacy of Trelona™ Compressed Termite Bait (0.5% novaluron) against both the Eastern subterranean termite, *Reticulitermes flavipes* (Kollar) and the Formosan subterranean termite *Coptotermes formosanus* Shiraki. Results of laboratory transfer assays indicate significantly higher recipient mortality for novaluron at low donor:recipient ratios (1:19, 5:15) when compared to diflubenzuron. The ability of Trelona™ Compressed Termite Bait to effectively eliminate populations of *C. formosanus* infesting standing, live trees in New Orleans, LA was evaluated using in the Advance® Termite Bait System. Elimination of all detectable termite activity was achieved at all study sites. The mean time to elimination of termites was 198 days and 96 days for fall and spring baited sites respectively. Results of trials in and around Galveston and Houston, TX demonstrated the ability of Trelona™ Compressed Termite Bait when used in the Advance® Termite Bait System to control populations of *C. formosanus* infesting structures at all sites evaluated with an average time to elimination of 5.3 months.

SYMPOSIUM: NEW TECHNOLOGIES FOR TERMITE MANAGEMENT

Development and Commercialization of Recruit® HD, the First Durable Bait for Subterranean Termites (Isoptera: Rhinotermitidae)

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Recruit® HD (noviflumuron) is a new durable subterranean termite bait that has been in development since 2004. Our durable bait concept employs bait that is durable for multiple years while retaining palatability and toxicity to all important subterranean termites. It provides structural protection from subterranean termites when serviced only once annually. A durable bait formulation should possess specific attributes to be effective. These attributes and results of research conducted to address them are discussed below.

Economically important termite species should readily consume the bait in the laboratory, and consumption must cause mortality. Laboratory studies were conducted to determine the consumption and toxicity of Recruit HD to Reticulitermes flavipes (Kollar), R. virginicus (Banks), R. hesperus Banks, Coptotermes formosanus Shiraki, and Heterotermes aureus (Snyder). Consumption of this bait was generally equal to or greater than that of a less durable refined cellulose bait (Recruit® IV). In addition, the bait was toxic across termite species.

The bait should be durable in the field under a variety of conditions and retain palatability and toxicity. The durability of the Recruit HD bait matrix was tested initially in a single trial conducted in Florida. Blank baits were left in the field in excess of 5 yr with samples periodically removed and tested for consumption in the laboratory. Termites (*R. flavipes*) consumed significantly more aged bait than southern yellow pine wood or fresh bait. A larger scale durability study was initiated in 2007 with multiple baits installed at research sites in IN, MS, and SC. Data collected quarterly over a 4 yr period indicates that *R. flavipes* generally consumes more of the aged bait than fresh bait and this difference is frequently significant. In addition, analytical results indicate that there was no loss of active ingredient over the course of this study.

The bait must eliminate termite colonies.

In late 2006, preliminary colony elimination studies in the field demonstrated that termites fed on this bait and 12 colonies (*Reticulitermes* spp. and *C. formosanus*) were eliminated in an average of 168 days. Additional colony elimination was documented in the regulatory field validation study discussed below.

Termites in the field should readily detect and consume the bait. Field trials to assess the discovery and consumption of the Recruit HD bait matrix compared to wood monitoring devices were conducted with six termite species (Reticulitermes spp., C. formosanus and H. aureus) in ten states. The trials were monitored quarterly for one yr. When data were summarized across all species and locations, termites discovered and consumed significantly (p < 0.01) more bait than wood monitors. Differences

were not always significant when results from individual species or locations were analyzed, but the trends were consistent.

Finally, the candidate bait formulation had to demonstrate structural protection and perform according to standards developed by regulatory agencies.

Beginning in 2007, 111 baited structures were evaluated for termite control and structural protection under a protocol approved by the Termiticide Scientific Review Panel and the United States Environmental Protection Agency (EPA). The results of this study exceeded requirements of the protocol and supported registration of Recruit HD by the EPA and state regulatory agencies. A total of 245 termite colonies were eliminated in this study and all structures were protected from termite infestation. The mean number of Recruit HD baits installed at structures in this study was 21.6 and mean consumption was equivalent to 1.0 bait device. Hence, an average of 95% of installed bait was still present after elimination of termite colonies at these structures.

The development activities described above were conducted to support registration in the United States where Recruit HD was introduced commercially in late 2010. It was launched in Japan in 2010 as well, following field studies that confirmed efficacy on *C. formosanus* and *R. speratus* (Kolbe), the most important termite structural pests in Japan. Field studies in France, Malaysia, Indonesia, Thailand and Australia were initiated in 2011 with a hexaflumuron-based durable bait similar to Recruit HD. Trial sites were selected to represent economically important species of termites, with opportunities for in-ground and above-ground bait placements. Early results from these trials have been encouraging; data will continue to be developed to support commercialization in selected global markets.

Performance of Recruit HD® versus *Coptotermes formosanus* Shiraki in Trials Conducted by the City of New Orleans Mosquito and Termite Control Board

Claudia Riegel and Barry Yokum
City of New Orleans Mosquito and Termite Control Board

Eight properties with structures infested by the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, were baited and evaluated by the City of New Orleans Mosquito and Termite Control Board (NOMTCB) as part of a Termiticide Scientific Review Panel (TSRP) and United States Environmental Protection Agency (EPA) approved research protocol using the Sentricon2® Termite Colony Elimination System with Recruit3® HD bait. Sites were monitored approximately quarterly. Elimination of all Formosan colonies was achieved at all structures and was confirmed via DNA analyses. Most colonies were eliminated within the first two quarters after initial bait consumption. At one site, two new Formosan colonies, confirmed by DNA analyses, invaded nine months after the initial colony was eliminated. Each was eliminated at the time of the first quarterly check after feeding on Recruit HD remaining at the site. One of the new Formosan colonies was eliminated after feeding on one of the same Recruit HD bait devices previously fed on by the original eliminated colony. Two other sites had *Reticulitermes flavipes* colonies invade and feed on Recruit HD after the initial Formosan colonies were eliminated and both *R. flavipes* colonies were also eliminated within the first or second quarter following their infestation of the bait.

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Termidor® HE High-Efficiency Termiticide Copack with Termidor HE Technology: An Overview of BASF Corporation's New Termiticide

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For more than a decade, Termidor® termiticide/insecticide has set the standard for termite control efficacy. Now, Termidor®® H•E High-Efficiency Termiticide Copack with Termidor®® HE Technology redefines termiticide application precision, volume, labor, and time.

Termidor H•E Copack is the result of more than five years of collaboration among BASF Corporation ("BASF") researchers, from a variety of disciplines, who were charged with improving the dispersal of Termidor within the soil treatment zone without compromising the efficacy of Termidor. The new molecular technology of Termidor H•E Copack creates an "Enhanced Protection Zone" by dispersing product more evenly through the soil, while using less water, smaller trenches, wider drill hole spacing, and shallower treatment depths¹.

Termidor H•E Copack temporarily forms a weak association between the active ingredient ("a.i.") and the water carrier, boosting the a.i.'s movement through the soil. This permits more a.i. to pass through the upper few inches of soil before becoming bound, (when compared to standard termiticide treatments). This temporarily enhanced movement allows for shallower trenches (four inches wide by two inches deep vs. six inches by six inches²) and wider drill and rod spacing (eighteen inches vs. twelve².) It also allows treatments to be performed using less water – approximately one-half to one-quarter the volume of water required to perform standard termite treatments. This is due to: (1) a labeled application rate of two gallons per ten linear feet¹ vs. the standard termiticide treatment rate of four gallons per ten linear feet and (2) a labeled minimum required treatment depth of two feet vs. four. For a typical slab treatment, the amount of a.i. applied with the Termidor H•E Copack is the same as a standard termiticide treatment because Termidor H•E Copack is applied at twice the concentration, but with half the volume (0.125% at 2 gallons per 10 linear feet instead of 0.06% at 4 gallons per 10 linear feet¹.)

To field test the efficacy of Termidor H•E Copack, 150 naturally infested structures located across the United States were treated according to the Post-construction Exterior Perimeter/Localized Interior (EP/LI) Structural Treatments use directions on the Termidor HE Copack label. Applications were performed by licensed pest control operators and were observed and inspected (initial and follow-up at 1, 3, 6, 12, 18, and 24 months) by university- or industry- associated professionals. Treatments were made against multiple genus of subterranean termites and on a variety of construction types, and at a range of soil and climatic conditions. As of March 2012, the 75 structures installed in 2009, the 49 structures installed in 2010, and the 26 structures installed in 2011³ all exhibited 100% control. All of the soil samples taken one year after treatment from a subset of the 2009 installations exhibited 100% efficacy in laboratory bioassays.

A field-based time and motion study was performed during 2011 resulting in more than two miles of trenching across more than 60 structures with a variety of foundation types. Major time savings were found to occur during tank filling (50% less time), trench excavation (58% less time), and material application (55.6% less time for trench application, 50% for sub-slab injection.) Based on rates calculated from this field work, a Termidor H•E Copack application at a slab home with 150 linear feet of trenching and 50 linear feet of sub-slab injection would require 49% less time (80 minutes vs. 159)

minutes) to trench, drill, treat, and patch when compared to a standard termite treatment.

¹For most labeled applications.

²Some states may have other, superseding requirements.

³Final report through 12 months not yet submitted to U.S. Environmental Protection Agency.

Performance of Termidor HE® Termiticide Against Subterranean Termites in Laboratory Collateral Transfer Trials and Field Applications

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In the laboratory study there were four replications of each donor:recipient ratio and untreated controls were constructed for this trial. The following ratios of donor:recipient termites were utilized in these trials 20:0, 15:5, 10:10, 5:15, 1:19, 0:20 (untreated marked control), and 0:20 (untreated unmarked control). Recently collected Reticulitermes flavipes and Coptotermes formosanus were utilized in this study and allowed to adjust to laboratory conditions for at least 48 hrs before beginning study. Worker termites were placed in labeled Petri dishes that corresponded to a final untreated arena to allow determination of collateral effects. These worker termites of both species were marked using fluorescent paint. Marked termites were allowed to adjust to laboratory environment for 48 h. Treatments to the soil (sandy loam) were made with the following products: Termidor® SC (BAS 350) 95 I) Lot No. 1219502FI and Termidor HE® polymer (BAS 270 00 S) Lot No. 502012. This product was used as a Co-pack and was mixed just prior to study initiation. Treatments to soil were made according to the manufacturer's label, and untreated controls were 'treated' with water only. Both the treated and the untreated control soils were allowed to dry for 24 h. Initial arenas consisted of Petri dishes measuring 9.0 cm x 2.5 cm. Approximately 34 g of treated soil were placed in each Petri dish. Worker termites of either R. flavipes or C. formosanus were added to each arena after assembly, and were allowed to contact treated soil for 30 minutes. After the contact period elapsed, these termites (donors), were placed in an untreated, clean Petri dish (9.0 x 2.5 cm) with moistened #4 Whatman filter paper, and unexposed termites (recipients). Two soldier termites of corresponding species were placed in all arenas. Observations of mortality of worker termites (donors and recipients) were taken at 1, 4, 24 h and then daily until 100% mortality was reached in each treatment. Analysis was performed using SPSS v 18, and means were separated with Tukey's HSD.

At the 1 h observation period for *C. formosanus*, the 10:10 treatment was the only one that showed mortality, but it was not significantly different from the other treatments or untreated controls. There were significant differences (P=0.05) in total mortality beginning at the 4 h observation period where the 10:10 treatment showed a significant difference (P=0.05) from the rest of the treatments and the untreated controls. Starting at the 72 h observation, all of the treatments were significantly different (P=0.05) from the untreated controls, but there were no significant differences between any of the treatments. At the 1 and 4 h observation periods the only donor populations with any mortality were in the 10:10 treatment. At the 72 h observation, all donor populations in the treatments showed 100% mortality and the untreated controls showed minimal mortality. At the 1 h observation, there was no mortality in the recipient populations in the treatments. At the 96 h observation, all recipient populations in the treatments showed 100% mortality and were significantly different (P=0.05) from the untreated controls, which had less than 10% mortality throughout the study.

At the 1h observation period, *R. flavipes* had no significant differences in mortality between the treatments and the untreated controls. At the 4 h observation period, the 20:0 treatment caused the greatest mortality followed by the 15:5 and the 10:10 treatments, respectively. These three treatments were significantly different (P=0.05) from all of the other treatments, and untreated controls. By the 48 h observation, all of the treatments were significantly different (P=0.05) from the untreated controls. At the 1 h observation period, there was no mortality in the donor populations in any of the

treatments. At the 48 h observation all of the donor populations in all the treatments showed 100% mortality, and were significantly different (P=0.05) from the untreated controls. At the 1 h observation period there was no mortality in the recipient population in any of the treatments. At the 48 h observation period the recipient populations in the15:5 treatments showed 100% mortality, and at the 72 h observation period all recipient populations in the treatments showed 100% mortality. At the 72 h observation all of the recipient populations were significantly different (P=0.05) from the untreated controls, which had less than 10% mortality throughout the study.

In this study all of the treatments showed 100% mortality in all the different ratios of donor:recipient termites in both species by 96 h post-exposure. *C. formosanus* had a slower response to the treatments than did *R. flavipes*. The higher donor:recipient ratios (20:0 and 15:5) in both species of termite were the fastest to reach 100% mortality. There was evidence of transfer of active ingredient from donor to recipient termites for both species of termite tested. This was evident based on the fact that the treatments showed 100% mortality, while the untreated controls showed less than 10% mortality for the duration of the study.

In the field study eleven structures infested with *R. flavipes* and one infested with *C. formosanus* were utilized. Center for Urban and Structural Entomology and BASF personnel jointly inspected and agreed upon 12 structures to receive treatment. All structures were located in the Houston/Galveston, TX area. Soldier termites were collected from all 12 structures, identified with termite identification keys, and stored in 100% ethanol as voucher specimen. It was verified through interview of the structure owners that none of the 12 structures had received a subterranean termite treatment within the past 12 months. Four of the structures were pier and beam construction which included the one structure infested with *C. formosanus*, the other eight structures were monolithic slab construction. All 12 structures had at least one active subterranean termite mud tube leading from the soil into the structure that was associated with an exterior wall. A diagram was made of each structure prior to treatment to include all known points of subterranean termite infestation. All subterranean termite mud tubes were documented and marked relative to a permanent benchmark (corner of the foundation). There was a mean of 2.3 subterranean termite mud tubes per structure with a range of 1-8.

Under the supervision of personnel from the Center for Urban and Structural Entomology and BASF, all chemical applications were made by a licensed pest management professional. All termiticide applications were made following the Termidor® SC Exterior Perimeter/Localized Interior directions for use according to the label. With the following exceptions: all exterior drilling was done on 46 cm centers and all trenches were 5 X 10 cm. Volume and concentration of finished solution applied varied according to the treatment specifications. At each of the structures, one half of the desired volume of water was added to the tank and then the appropriate volume of Termidor® HE Co-pack was introduced to the tank, and the remaining volume of water was added to ensure thorough mixing of the solution. In setting up for the study, the linear footage for each structure was measured prior to the treatment to ensure the proper volume of finished solution was applied. The mean linear feet perimeter of the 12 structures was 57.86±18.43 m. Six structures each received one of the following treatments.

- 1. Dilution of 0.63% w/w Termidor® HE and 0.63% w/w of Termidor® HE Technology in water. Applications were made at 7.5 L of finished dilution per 3 linear m /0.30 m of depth.
- 2. Dilution of 1.3% w/w Termidor® HE and 1.3% w/w of Termidor® HE Technology in water. Applications were made at 7.5 L of finished dilution per 3 linear m / 0.30 m of depth.

There were no interior applications made at any of the 12 structures. The mean volume of finished solution applied at each structure was 162.76±52.35 L. A Great Plains Industry Inc. 01N Series (Wichita, KS) digital flow meter was utilized during this trial to ensure proper volumes of termiticide were applied at each structure. All structures were treated between August 10 and August 13, 2009. Post-treatment inspections were made on or about 1, 3, 6, 12, and 24 months. Post-treatment inspections included visual and research techniques such as the use of a Termatrac®, borescope, and/or infrared camera. Thus the objective of this field work was to determine effects of Termidor® HE Termiticide Co-pack against two species of subterranean termites when applied as a post-construction treatment to infested structures.

The mean volume of finished solution applied at each structure was 162.76±52.35 L. There has been no detection of subterranean termite activity on any of the 12 structures at any time post-treatment through 24 months.

Altriset™: A New Termiticide with Unique Post-Exposure Effects, Delayed Toxicity & Favorable Environmental Profile

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Altriset[™] (18.4 SC) contains chlorantraniliprole as an active ingredient, is recently registered as a termiticide. Its unique mode of action, post-exposure behavioral effects on termites, non-repellency, and favorable environmental profile makes it an ideal termiticide. Worker termites exposed for different time periods (1, 5, 15, and 30 min) to Altriset[™] treated sand (50 ppm, w : w), exhibited delayed toxicity. Tunneling in treated soil/sand caused "Feeding Cessation" and "Aggregation Effects". After exposure to treated sand (1, 5 & 10 ppm, w : w), termite workers readily picked up lethal doses of chlorantraniliprole but were able to walk and move normally for at least 3 hr after the exposure.

Delayed Impacts of Altriset Termiticide on the Eastern Subterranean Termite

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Chlorantraniliprole, a recently discovered insecticide from DuPont, belongs to a new chemical class, anthralinic diamides. In 2010, DuPont registered chlorantraniliprole for termite control under the brand name Altriset Termiticide. This is the first liquid termiticide to be classified as "reduced risk" by the Environmental Protection Agency. In continuous-exposure and limited-exposure laboratory bioassays, Altriset 200 SC caused delayed mortality of Reticulitermes flavipes workers. Continuous exposure to Altriset-treated soil affected the behavior of termites within 24 h at concentrations ≥25 ppm as evidenced by the significantly large numbers of sluggish, ataxic, moribund, and/or dead termites compared to those in the control. Furthermore, in these bioassays, Altriset significantly affected R. flavipes feeding behavior as no cellulose consumption was evident at 25, 50, and 100 ppm. The time to achieve >95% termite mortality in continuous exposure bioassays using labeled rates of three liquid termiticides indicated that Termidor (60 ppm) was the fastest-acting termiticide taking 3 d to achieve mortality, Premise (50 ppm) was slowest acting taking 31 d, and Altriset (50 ppm) was intermediate acting taking 14 d. Another study was conducted using two whole colonies wherein a small percentage of colony members were exposed for 4 h to Altriset-treated soil (treatment) or untreated soil (control) then returned to their respective colony. For the Altriset treatment, subsequent termite foraging activity in cellulose feeding monitors was negligible. At the 10-week colony breakdown and census, the Altriset-treated colony exhibited a population decline of ~75% after only ~7% of the colony members had been briefly exposed to Altriset. This suggests that whole colony decline may occur through the horizontal transfer of Altriset Termiticide from briefly exposed workers to unexposed nestmates.

SUBMITTED PAPERS ANTS

Impact of Systemic Thiamethoxam on Argentine Ant Foraging Patterns in Aphid-Infested Pepper Plants

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Materials and Methods

This experiment was conducted in a greenhouse on the University of Georgia's Griffin campus. During the studies, greenhouse temperatures were 60-75°F and 60-80% relative humidity. The four trials were initiated in September – October, and each trial ran for 21 consecutive days; each trial consisted of five replicates, so data (means) in this report are based on 20 replicates (n = 20).

Potted pepper plants (6-12 inches high) were placed inside a glass dish (15 cm diameter by 7.5 cm high), the inside and outside walls of which were coated with Fluon to prevent the escape of ants (the inside Fluon coating) and the introduction of ants (the outside Fluon coating) from the arena floor. As needed, plants were watered by simply adding water to the glass dish. A plastic saucer (30 cm diameter) was then placed, like a collar, around the stem of each plant while resting on the rim of the planter. The collar was added to keep foraging ants from moving into and nesting in the moist soil. The vertical, inside lip of the saucer was coated with Fluon to prevent the escape of ants. The floor (area directly underneath foliage) of each plastic saucer was lined with a paper towel to prevent the deposit of excessive honeydew and subsequent growth of sooty mold on the floor of the saucer.

Several thousand (estimated visually) Argentine ants, *Linepithema humile*, freshly-collected from the field, were placed in a plastic box (14 x 19 x 9 cm) half-filled with leaf litter and soil. The box of ants was then placed in the center of an arena (75 x 75 x 6 cm high). The inside walls of the plastic box and the arena were coated with Fluon to prevent ant escape. Two inverted plastic "pizza savers" were hot glued to the bottom of each plastic box to serve as an attachment point for each of three 65 cm long wires (1/8 inch diameter soft wire) that served as bridges from the box containing the ants to each plant (Fig. 1).

Treatments. Three plants (treatments), infested or not with green peach aphids (*Myzus persicae* (Slulzer)), were used in this study. In the last of four trials, about 10% of the aphids were the melon/cotton aphid, *Aphis gossypii* Glover. Plant A was purposefully left without aphids (a clean, aphid-free plant) for the duration of the 21-day test, while plants B and C were purposefully "seeded" with aphids about three weeks prior to the start of the test, so that at the start of the test (day 0) they both had healthy aphid infestations.

To begin the test (i.e., day 0), the three plants were placed in three corners of the test arena. A wire bridge, from the plastic box containing the ants, was attached to the stem of each plant, thereby connecting each plant to the box containing the ants. The wire bridges were installed to facilitate the movement of ants between their nest box and the plant, where they fed on honeydew produced by

aphids. The foraging system was, in a sense, closed in that ants could forage only between their nest box and any one of the three plants, but nowhere else.

On day 9 of the test, Plant B was provided 75 mls of 0.10% thiamethoxam (Optigard Flex) by injection into the soil, at the base of the plant, with a plastic syringe; Plants A (aphid-free) and C (aphid-infested) remained unaltered.

Data Recorded. The number of ants found anywhere on each plant was counted daily for the duration of the test (21 days). In addition, daily aphid infestation on each plant was estimated using a 10-point scale, as follows (Fig. 2):

- 1 0 aphids 6 60% infestation
- 2-20% infestation 7-70% infestation
- 3 30% infestation 8 80% infestation
- 4 40% infestation 9 90% infestation
- 5 50% infestation 10 100% infestation/dead plant

Data Analysis. For each replicate, data were expressed as the percentage of ants feeding among the three plants during two periods: 1) days 1-9 (before thiamethoxam was added to the soil of plant B), and 2) days 10-21 (after thiamethoxam was added to the soil of plant B). Percentages were acsin, square root transformed (to normalize the data) and the transformed variable then analyzed by one-way analysis of variance (ANOVA) among treatments before (days 1-9) and after (days 10-12) the introduction of thiamethoxam into the system (Figs. 3 and 4). Furthermore, the transformed variable was analyzed by t-test to compare ant foraging activity before versus after the introduction of thiamethoxam for each of the three treatments (Figs. 5-7).



Fig. 1. Test arena with the box of ants containing several thousand Argentine ants in the center and wire bridges connecting the box of ants to the plants which were or were not infested with aphids.

Systemic thiamethoxam (0.10% Optigard) eliminated aphids from infested pepper plants in two days (Fig. 2).

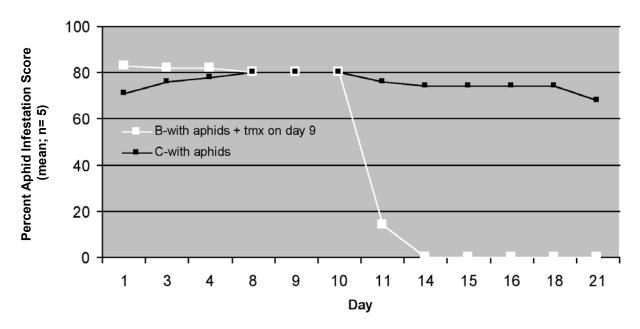


Fig. 2. Aphid infestation score on plants B and C.

For the 9 day period *before* the introduction of thiamethoxam, Argentine ants foraged equally and significantly (P < 0.0001) more in aphid-infested plants (plants B & C) than in plants not infested with aphids (plant A) (Fig. 3, black bars). For the 12 day period *after* the introduction of thiamethoxam, Argentine ants foraged significantly (P < 0.0001) more in the one remaining plant infested with aphids (plant C) and less in the thiamethoxam-treated plant (plant B) (Fig. 4, white bars).

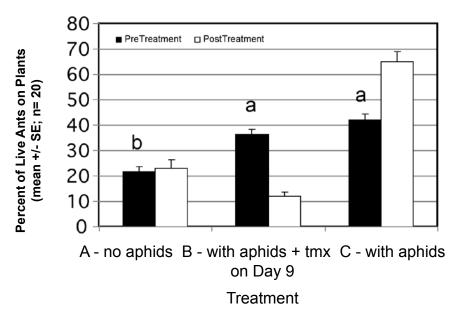


Fig 3. Ant foraging in plants for the 9 day period *before* thiamethoxam treatment (one-way analysis of variance; F = 22.0; P < 0.0001; df = 2, 57).

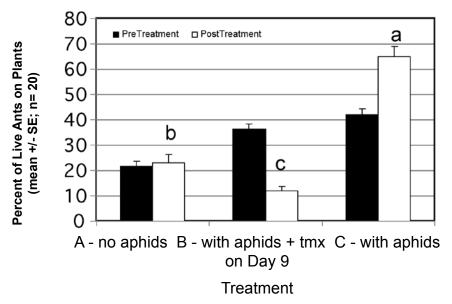


Fig. 4. Ant foraging in plants for the 12 day period *after* thiamethoxam treatment (one-way analysis of variance; F = 72.5; P < 0.0001; df = 2, 57).

Ant foraging activity did not change (P = 0.7195) in plants that did not contain aphids (Plant A) (Fig. 5). Ant foraging activity was reduced (P < 0.0001) in aphid-infested plants treated with thiamethoxam on day 9 (Plant B) (Fig.6). Ant foraging activity increased (P < 0.0001) in plants infested with aphids but never treated with thiamethoxam (Plant C) (Fig. 7).

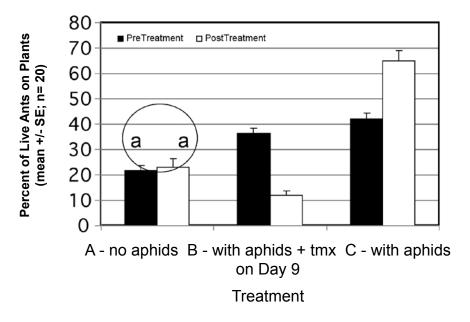


Fig. 5. Ant foraging activity in plant A: t = 0.36; P = 0.7195; df = 38.

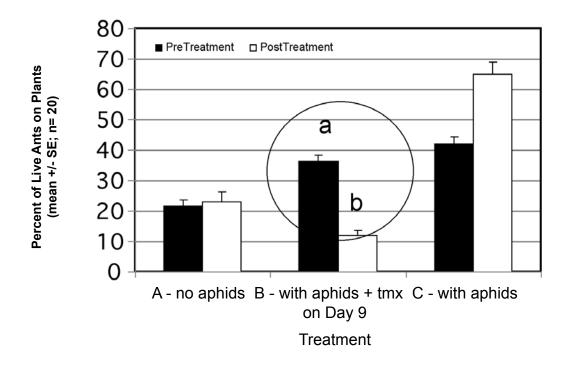


Fig. 6. Ant foraging activity in plant B: t = -8.24; P < 0.0001; df = 38

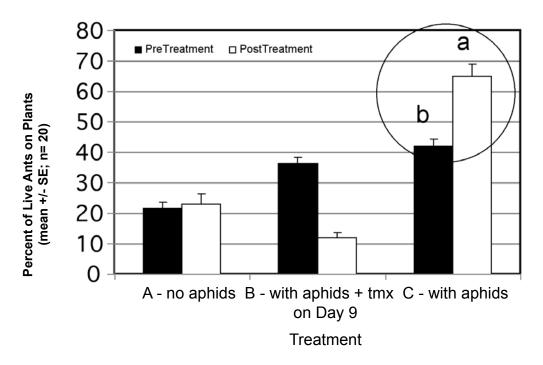


Fig. 7. Ant foraging activity in plant C: t = 4.94; P < 0.0001; df = 38

Dupont[™] Arilon®: A New Residual Insecticide Formulation With Higher Durability and Flexibility

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Arilon® (20 WDG, Indoxacarb), a new residual spray formulation, demonstrated excellent efficacy against cockroaches and ants. Adult male German cockroaches (GCR) and Argentine ant (AA) workers exposed for as little as 1-10 minutes to surfaces (ceramic tiles and wood panels) treated at labeled rate of 0.05 and 0.1%, resulted in 100% mortalities within 3 d. In simulated rain studies, GCR, American cockroaches, and AA, exhibited 100% mortality within 5 d, when confined to (constant exposure) Arilon® (0.05 & 0.1%) treated tiles and wood panels. Arilon® treated tile and wood panels remain active against ants and cockroaches for 3 months even though every 15 d these surfaces were subjected to a 0.25 inch simulated rain event.

Exposed German cockroaches and Argentine ants were able to transfer lethal doses to unexposed individuals. In our studies we were able to show that ants (10 min & 60 min) and cockroaches (60 min) to Arilon® treated substrate picked up more than lethal amounts of Arilon® and contaminated harborage, food and water. When unexposed ants and cockroaches were added to these contaminated containers, 95-100% mortality was observed by day 3. Thus, Arilon® is highly efficacious against different urban pests as it is being rapidly picked up by the insects and is transferred to other unexposed individuals as well as nesting sites.

Evaluation of Arilon® for the Control of Ants and Other Perimeter Pest Species

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Arilon® (20 WDG) is a broad spectrum, non-repellent residual spray containing the active ingredient indoxacarb (20% wt:wt). The Arilon® formulation is designed to be used both indoors and outdoors; hence a low odor and non-staining formulation in addition to a flexible outdoor perimeter band application of up to 10 feet. Furthermore, Arilon® has the potential to transfer among insects. Indoxacarb has a unique mode of action as insect enzymes are required to convert indoxacarb from the parent compound into the MetaActive™ form. The MetaActive™ form binds to the insect's target site, keeping the sodium ion channel closed which results in paralysis and death. Arilon® was evaluated for the ability to control perimeter pests including ants and earwigs. In field trials conducted in Griffin, GA, Arilon® (0.05%) applied at 4 gallons per 1000 square feet provided a 99% reduction in extremely heavy Argentine ant, *Linepithema humile*, trails within one week of application and continued to prevent ants from returning to high numbers for 8 weeks. In field trials conducted in Blacksburg, VA, Arilon® was applied at 1 gallon per 1000 square feet at 0.05% or 0.1% to control the odorous house ant. Taponima sessile. Arilon® (0.05%) provided the greatest reduction of odorous house ant numbers at 7 days and 60 days after treatment at 99.6% and 99.1% respectively. Arilon® applied at 0.1% provided the greatest reduction in odorous house ant numbers at 7 days (90.7%) and 14 days (98.2%) after treatment. In field trials conducted in West Lafayette, IN, trees infested with the carpenter ant, Camponotus pennsylvanicus, were used as models to test the efficacy of Arilon®. A 5 foot band of Arilon® (0.05% or 0.1%) was applied to the base of infested trees at 1 gallon per 1000 square foot. Both the low rate and high rate of Arilon® provided excellent control and carpenter ant activity was reduced by 100% for 28 days at which point the study ended.

In a laboratory study, Arilon® was an effective contact and residual insecticide against the European earwig, *Forficula aricularia*. Within 16 hours of being directly sprayed with indoxacarb, ≥90% of earwigs from two populations displayed symptoms of severe intoxication, and 100% were either ataxic, moribund, or dead at 1 day. Brief exposure (5 minutes or 1 hour) to dried residues on either a porous or non-porous substrate also was sufficient to cause severe intoxication of earwigs within 1 day. In all bioassays, indoxacarb-treated earwigs showed no signs of recovery during the 21-d observation period. In outdoor habitats, intoxicated earwigs would be more vulnerable to desiccation, predation, or pathogens leading to higher mortality than in a laboratory setting.

Acknowledgments

We thank Drs. Dini Miller, Daniel Suiter, Grzegorz Buczkowski, Gary Bennett, Susan C. Jones and Mr. Josh Bryant.

Argentine Ant Management on Santa Cruz Island

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Argentine ants (Linepithema humile) are a highly invasive species that pose a serious threat to ecologically intact systems worldwide. Their aggressive interspecies interactions may be linked to observed declines in the diversity and abundance a wide variety of invertebrate species inhabiting Argentine ant infested areas. Pollination success and seed dispersal may be reduced as native invertebrate activity declines, with ensuing negative repercussions for native plant reproduction and ecological community composition. Argentine ants were first discovered on Santa Cruz Island, California in 1996 at two locations formally used by U.S. Navy contractors. Thereafter, Argentine ants established at two additional locations in the island's central watershed. The island managers, The Nature Conservancy and National Park Service, are beginning a treatment protocol in spring of 2012 targeting two of these infestations. We will treat one entire isolated infestation which encompasses five acres of scrub and oak vegetation. This complete site treatment will eliminate the common confounding variable of Argentine ant re-infestation from untreated edges, and will allow us to more precisely measure the efficacy of our protocol. The second infestation site is 33 acres, but due to current state and federal permitting, we will treat only 10 acres in 2012. Our treatment protocol consists of six applications of sucrose- attractant bait: two applications with S-methoprene and four applications spaced one month apart with thiamethoxam. Prior to each treatment we will conduct rigorous monitoring to detect ant activity at 65 stations in both the treatment and control sites. Data from the trial in 2012 will be submitted to state and federal regulatory agencies. In 2013 we plan to treat both infestations in their entirety. Island managers are also working with other landowners, such as the US Navy, to implement this protocol in other Channel Islands containing isolated Argentine ant infestations.

Ants Up North: Survey of Urban Ants with Pest Management Personnel

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Ants were collected by pest management professionals across Canada during 2011 in a project funded by Orkin Canada. Pest Management Professionals (PMPs) were requested to collect and submit samples for identification. In addition a collection trip was made through British Columbia with Orkin Canada personnel. Collections on this trip were made at disturbed sites and residential areas. A total of 346 samples were collected from 9 provinces.

The most common pest ants in the samples included: carpenter ants (7 species of *Camponotus*) in 56 samples: 5 out of 9 provinces; moisture (or cornfield) ants (6 species of *Lasius*) in 70 samples: 6 out of 9 provinces; thatching ants (17 species of *Formica*) in 70 samples: 7 out of 9 provinces; pavement ants (*Tetramorium caespitum*) in 44 samples: 3 out of 9 provinces

The European red fire ant (*Myrmica rubra*) was submitted from five sites in Ontario. After the collections were tallied, personal communication with Robert Higgins, Thompson Rivers University, indicated another four sites where this ant has been collected in 2011 in British Columbia. This ant has also been reported in New Foundland and Prince Edward Island in a survey completed by the University of Maine. Additional samples are needed to update the information on this important pest species, introduced from Europe and seemingly spreading into urban areas. It is an invasive species that swarms when disturbed and stings.

Another myrmicine ant (tentatively identified as *Manica hunteri*) was collected at 13 sites in British Columbia. This stinging ant was found next to foundations and at doorsteps of residential areas and garages. It has not been reported as a pest or nuisance ant however it is described as 'not aggressive, but will sting promptly'.

The velvety tree ant (pine tree ant), *Liometopum luctuosum*, was collected at six sites in British Columbia. This ant is only known in western provinces of Canada and western states in the United States. It is an important ant to the pest management industry because it is a wood-destroying ant. These ants excavate wood similar to carpenter ants, however, the wood that is excavated is extremely fine. The colonies are large and very mobile. This ant also nests in and excavates foam insulation.

Ants found in samples that were not expected included the ghost ant (*Tapinoma melanocephalum*), ponerine ants (three provinces), and acrobat ants.

Other nuisance ants included pharaoh ants, thief ants, odorous house ants, and small honey ants. In these samples across Canada, 47 different species were identified. Some ant species are common around structures some are not. Identification keys to the ant genus, *Formica*, are cumbersome and in need of revision. Many people working with this genus, group the ants into 7 major *Formica* groups. These ants are not usually problems around structures and are important foragers on other insects; however, some make piles of unsightly thatch in yards, bite, and irritate homeowners.

SYMPOSIUM: CARIBBEAN CRAZY ANTS

Morphological and Molecular Diagnostic Identification of *Nylanderia* sp. nr. pubens

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Paratrechina and Nylanderia genera have seemingly increased their ability to invade non-indigenous areas due to their synanthropic behaviors. A lack of cohesive taxonomic cosmopolitan description of the genera has led to confusion as to the status of some invasive populations. This lack of complete generic descriptions have direct effects on research funding and federal actions.

Populations of *Nylanderia pubens* previously described have included possibly extant populations of Florida and the Caribbean. Some of these data are based upon *N. pubens* as an occasional pest with few numbers. Since the original descriptions in the period of 1953-1973, populations of a similar species have arisen in both Texas and Florida with tremendous population levels never before described and are now known as a major pest.

Morphological analyses were conducted on *N. sp. nr. pubens* populations of Texas and compared to *N. pubens* populations described by Trager (1984). Statistically significant differences were found in numerous morphologies (Meyers and Gold 2008). Canonical discriminant functions analysis was performed and also found significant differences among populations.

The analyses of mtDNA COI of *Paratrechina* spp. revealed several robust clades from the various trees. Within these clades are two or three distinct clades of *N. pubens* and *N. sp. nr. pubens*, including a clade of *N. pubens* from Florida. The clade of *N. sp. nr. pubens* and *N. pubens* from St. Croix may support identification of an undescribed species of *N. pubens*.

More research regarding behavior, mating compatibility, phylogeny, or other analyses of these populations should be conducted before raising the *N. sp. nr. pubens* Texas populations to the status of an undescribed species. However these morphological and molecular diagnostic data, and additional behavioral and population size differences, and personal communication (J. Trager) indicate the *N. sp. nr. pubens* populations may be a likely invasive, undescribed species.

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Biological Aspects of Nylanderia pubens (Forel) in Florida

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Nylanderia pubens (Caribbean crazy ant) is rapidly spreading throughout Florida, Texas, Louisiana and Mississippi's landscapes and natural areas. The abundant populations of *N. pubens* makes it an indoor and outdoor nuisance pest. Three aspects of *N. pubens* biology were examined in this study. First, ant nests collected in Florida and censused monthly for over a year revealed higher prevelance of alates in fall and winter. Secondly, three potential viruses were discovered in *N. pubens* sample from Florida. Insect parasitic viruses were replicating on the RNA of *N. pubens*. Finally, *N. pubens* interactions with honeydew-producing hemipteran insects in landscapes was observed. Plant samples were collected monthly to identify the commonly-tended hemipteran species (three aphid species, two mealybug species, gall, lacebug and three scale insect species) and host plants. Seasonal variation in ant and hemipteran densities per plant species (holly, sugarberry, magnolia and live oak) were positively correlated over time. These studies enhance our knowledge about the development, biology, and management of this invasive pest ant species.

Fecundity of Nylanderia sp. nr. pubens Under Laboratory Conditions

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Since 2002, robust densities of *Nylanderia sp. nr. pubens* have been found in localized infestations in southeast Texas. This invasive species is a tremendous nuisance, causing electrical equipment failure, and is ecologically dominant. The extreme polygynous nature of *N. sp. nr. pubens*, has undoubtedly been a major factor in the invasive success of this species in southeast Texas. Determining the reproductive potential of *N. sp. nr. pubens* is a vital step in determining biological idiosyncrasies and invasive potential of the species.

In a laboratory study modeled after Arcila et. al. (2002), 30 colonies were collected ≥ 10m apart in East Columbia Texas from May 2nd-May 8th of 2012. Experimental colonies consisted of either one queen (monogynous), three queens (trigynous), or six queens (hexagynous) with 100 workers per queen. Colonies were provisioned with harborage, water, 10% honey water, 0.5g of hot dog, and two crickets. Colonies were kept at 27°C and 80% R.H. with a 14:10 light to dark photoperiod. The colonies were allowed to function normally for three days, at which time, the colonies were placed into a -20°C freezer for 12 hrs. to stop egg production. Eggs were counted to determine fecundity of queens and queen weight was determined. A general linear model was used to determine statistical significance, mean separation, and the relationship between queen weight and fecundity.

Results showed that as queen number increased, individual fecundity increased (Fig. 1). However, statistical significance was not achieved (p = 0.06) due to 80% of monogyne colonies not producing any eggs, which resulted in a large standard error for that treatment. In the field, *N. sp. nr. pubens* colonies always consist of multiple queens. Therefore, the hexagyne colony fecundity of $0.25 \pm .0.12$ eggs/hr. should be considered most accurate. However, it is common to find between 15-20 queens per colony under each landscape article, and it is yet to be determined if laboratory colonies with this extreme number of queens will yield a higher production of eggs. The positive correlation between queen number and individual fecundity suggests that there is a synergistic affect. Furthermore, the fact that monogyne colonies rarely (20% of the time) produced eggs, this species may be considered functionally polygyne. The average queen weight was 3.65 ± 0.39 mg but no significant relationship was found between queen weight and individual fecundity.

Worker egg load, the number of eggs carried by a single worker, was an average of 31.97 ± 21.06 eggs. However, the largest egg cluster found was 113 eggs. It is unknown whether this worker was actually carrying that egg cluster or if she attached to the cluster as she was dying. Therefore, removing this outlier, the corrected mean worker egg load was 29.52 ± 15.68 eggs.

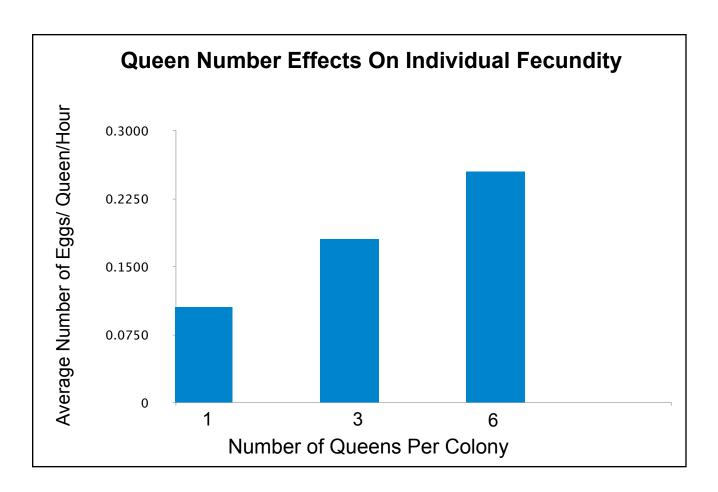


Fig. 1. Depiction of individual fecundity through all treatments. As queen number increased, individual fecundity increased. The general linear model determined that p = 0.06 (Alpha = 0.05).

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Field Biology and Nutritional Studies for Caribbean Crazy Ants

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The tropical and subtropical super colony ant, *Nylanderia pubens* (Forel), the Caribbean Crazy Ant (CCA), has spread throughout Florida and the states along the Gulf of Mexico. These ants invade homes and urban lots but also disrupt natural ecosystems. In order to better control these pest ants, we need to understand their spatial and temporal dynamics in their habitat, and develop new baits specifically design and to attract and kill CCA. Most current ant control tools are not very effective in reducing populations of *N. pubens*, and bait formulations specific for CCA have not been investigated.

The spatial distribution and density of CCA was examined in urban lots in North Central Florida. The ant population was sampled on 6 lots in 2 locations over the course of 2 years. In early spring, CCA were restricted to discrete areas around the lots, where heavy leaf litter or other material offered abundant nesting sites and protection from colder temperatures. As the season progressed into summer and the temperatures increased, the CCA infestation spread into broader areas, and the ants dominated the lots with an increase in number of ants per area, as well as the area occupied by CCA. In late summer and into early fall, the infestation started to shrink back to the original infestations sites. The number of ants at each location also decreased as temperature decreased. Similar pattern was observed during the 2 years the CCA population was observed. However, in the second year, CCA increased its dominance with almost total elimination of other ant species from the urban lots.

N. pubens preferences to a variety of granular bait components were tested in laboratory and field experiments. The studies examined preference for: granular size, matrix type, and additives on to the bait matrix, including sugar, oil and cricket tissue. The results from the preference experiments led to the formulation of a granular bait consisting of a dog food matrix with preferred granule size around 1 mm to which macerated crickets were added in the form of a slurry.

Active ingredients were added to the granular bait matrix and tested against laboratory CCA colonies. The formulation containing fipronil at 0.000225% caused the quickest and highest mortality of CCA compared to formulations containing indoxacarb and imidacloprid. The fipronil bait cause delayed toxicity in the ants, which helped with distribution of the active ingredient within the ant colony and, as with the other active ingredients tested, did not discourage foraging from the ants. Future field work with the formulated granular ant bait with fipronil will test the efficacy of this bait against field populations of CCA.

Integrated Pest Management of the Caribbean Crazy Ant in Florida

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The Caribbean crazy ant, *Nylanderia pubens* is an invasive ant species established in Florida and Texas and has recently been reported from Louisiana and Mississippi. In Florida, this pest is found in over 20 counties and is spreading rapidly throughout the state. This ant has clearly been designated as an urban pest, infesting lawns and homes and reducing the recreational and aesthetic value of residences. The Caribbean crazy ant has not yet been declared an industrial or agricultural pest but there is a clear opportunity for the Caribbean crazy ant to infest manufacturing facilities and agricultural machinery. Anecdotally, the ant is reported to be an emerging ecological pest by competitive displacement of native ant species and attacking ground nesting birds, mammals and reptiles.

Researchers at the University of Florida are interested in developing an Integrated Pest Management (IPM) strategy for this pest. As a first start, they are studying the biology, ecology and behavior of this ant, testing commercially available products and standard methodologies and exploring novel techniques for control.

To study the demography of Caribbean crazy ants' nests over an annual cycle, ant nests were collected and returned to the laboratory. The ants were separated from the nest material and slowly transitioned to artificial nest cells. The numbers of queens, workers, male and female alates, pupae and larvae were counted and recorded. Figure 1 details the mean percentage of each in the nests each month. From this we conclude that mating occurs in late winter as evidenced by the appearance of male alates in the nests. Brood production increases significantly in the spring and remains fairly strong throughout the summer. At the onset of autumn, brood production drops off and workers make up the greatest percentage of nest members.

The macronutrient preferences of Caribbean crazy ants were explored by the use of food assays in the field. Paper disks were provisioned randomly with six macronutrient choices (tuna in water, pureed beef, 10% sucrose solution, honey, peanut oil and lard) and placed on the ground near ant foraging activity. After 15 minutes, the numbers of ants on each food was counted and recorded. The overall macronutrient preference of Caribbean crazy ants was protein (Figure 2). The ants' dietary preference shifted to carbohydrates in the fall, corresponding to the decrease in brood production and higher percentage of workers in the nests.

Laboratory bioassays were used to test commercially available insecticidal granular baits for attractiveness and efficacy. Twenty ants were placed into a test arena constructed of a plastic deli cup with a Castone[™] covered bottom and lined on the interior walls with Fluon[™]. Ants were provisioned with water and harborage. After an acclimation period of 24 hours, granular baits or 10% sucrose solution (control) were introduced into the test arenas. The number of ants on the bait was recorded every ten minutes for one hour. Mortality was recorded daily for 7 days or until 100% mortality. The baits tested were: Maxforce® Granular Bait (Bayer Environmental Science, Research Triangle Park, NC), Maxforce® Fine Granular Bait (Bayer Environmental Science, Research Triangle Park, NC), InTice[™] Granular Ant Bait (Rockwell Labs, Ltd., N. Kansas City, MO), Advance® 375A Select Granular Ant Bait (BASF, Research Triangle Park, NC), Advance® Carpenter Ant Bait (BASF, Research Triangle Park, NC), Amdro Pro® (BASF, Research Triangle Park, NC), Niban® Fine Granular Bait (Nisus Corporation,

Rockford, TN), Extinguish® Professional Fire Ant Bait (Wellmark International, Schaumberg, IL), Esteem® Ant Bait (Valent USA Corporation, Walnut Creek, CA). Each bait bioassay was replicated ten times and the entire study repeated in the spring, summer and fall. The optimal ant bait should consist of an attractive and palatable matrix and contain an active ingredient that acts slowly enough that the toxicant is distributed through the colony via trophallaxis but also with sufficient rapidity that the Pest Management Professional sees an impact within three days to a week. If the data for bait attractiveness, number of days until 100% mortality and percent mortality in 3 days after treatment are displayed together in a bubble chart, the result is Figure 3. According to the defined criteria of this study, the best performing baits are displayed in the lower, right quadrant. Amdro Pro and Maxforce Complete Insect Bait, both containing the active ingredient hydramethylnon, were the best performing baits in this study. Although not necessarily the most attractive of the baits tested, they provided quick and complete control of test ants. Ant baits with protein containing matrices were highly attractive to Caribbean crazy ants while those formulated for fire ants and thus containing large amounts of oil, were not. These data align with the results of the previously reported macronutrient preference studies.

In summary, an effective IPM strategy would take advantage of monitoring and early bait placement before populations reach critical levels. Ant bait selection should focus on protein based matrices during the spring and summer months while carbohydrate containing baits may be considered for the fall.

Fig. 1. Mean percentage of queens, workers, male and female alates, larvae and pupae in field collected Caribbean crazy ant nests over a one year period.

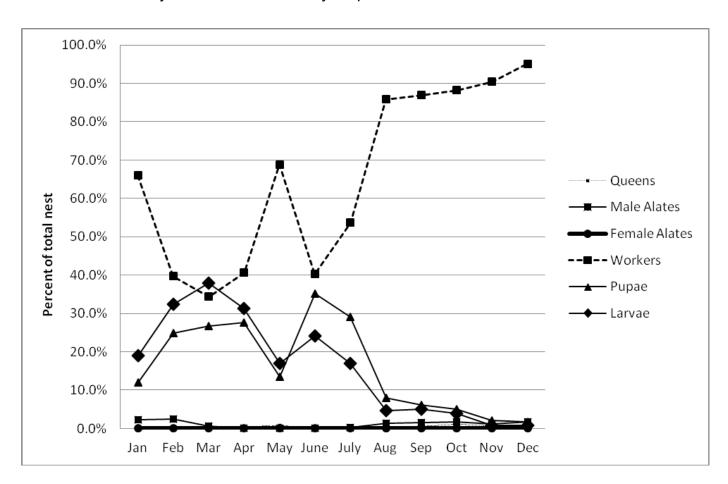


Fig. 2. Mean percentage of foraging Caribbean crazy ant workers on each of three macronutrient choices in 15 minute field assays.

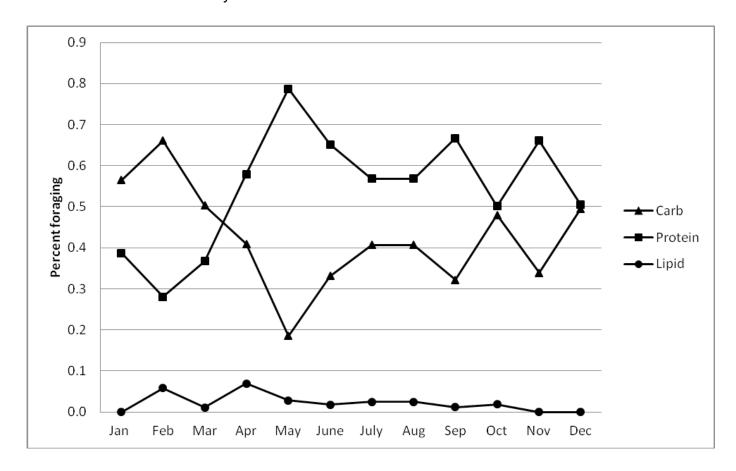
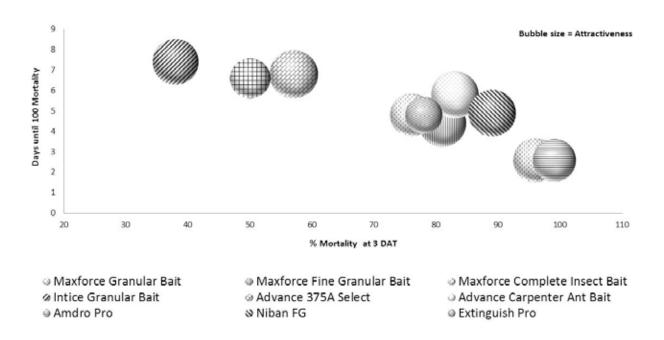


Fig. 3. Bubble chart depicting attractiveness (size of bubble), number of days until 100% mortality (y-axis) and mortality at 3 DAT (x-axis) for commercially available granular baits against Caribbean crazy ant in laboratory bioassays.



SUBMITTED PAPERS BED BUGS

A Novel Method for Artificially Feeding Bed Bugs, *Cimex lectularius* L. (Hempitera: Cimicidae)

Eva Chin-Heady, Ronda L. Hamm, Joe J. DeMark, Steve Nolting, Gary Bennett and Kurt Saltzmann Dow AgroSiences and Purdue University

Bed bugs, *Cimex lectularius* L. (Hemiptera: Cimicidae), require a blood meal in order to develop and reproduce. Bed bugs can be difficult to rear in the laboratory and options to feed bed bugs artificially are limited. The most widely used artificial feeding method utilizes an artificial membrane system and a circulating water bath to warm the blood. Several drawbacks exist, including the possibility of flooding bed bug rearing jars with water or blood and the need for expensive custom made glassware. In this study, a bed bug feeding system using a hot plate and Petri dish was developed and compared to the water bath method. The Petri dish method was simple to use compared to the water bath method. The two methods resulted in similar numbers of bed bugs that fed at each feeding and no difference in the time required for adults to develop from first instar nymphs.

Submitted for publication

Exploring a Novel Detection Technique for the Common Bed Bug

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It is often difficult to detect bed bug infestation when the infestation level is low, and the harborages of bed bugs are hard to reach for investigation (e.g., narrow wall crevices, nail holes). In fact, many detection devices available today target starved bed bugs that are likely to be attracted to various cues associated with their food source, warm-blooded animal. When these devices or visual investigation are impossible or not practical to use, we can use chemical signature of the bed bugs for their detection. Because it is known that some well-trained dogs can detect hidden bed bugs by sniffing, it might be reasonable to expect that bed bugs produce certain signature odors. Here, we investigated if we can determine bedbug infestation by detecting their chemical signatures. In studies of chemical ecology, SPME (solid phase microextraction) and activated charcoal adsorbent have been used as extremely simple but powerful methods to collect various volatiles. We examined whether the bedbugs produce specific volatiles and those can be collected with SPME or activated charcoal filter connected with aeration apparatus for analysis with CG-MS (gas chromatography-mass spectrometry). Because the release rate of the chemical compounds is context-dependent (i.e., emit more when they are disturbed), we also used CO₂ to maximize their production of the characteristic volatiles, thereby enhancing our ability to detect small numbers of bed bugs.

Lessons Learned in Bed Bug Rearing

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Laboratory rearing of bed bugs (Cimex lectularius) using an artificial feeding system requires a reliable blood source for population maintenance. Our research laboratory acquired chicken blood from a biological supplier for almost a year without incident, but in November 2011, large numbers of recently fed bed bugs were found dead. In-depth follow-up with the biological supplier revealed that their practice was to treat chicken pens and bedding bi-weekly with Sevin® (carbaryl), and the blood had been drawn 3-d post-treatment. Follow-up bioassays were conducted to determine if carbaryl was the causative agent of mortality in our bed bug populations. In these bioassays, bed bugs were fed with blood drawn from a single pen of chickens at 1, 4, 6, and 13 d after pens and bedding had been treated with carbaryl. Mixed-stage bed bugs originating from three bed bug populations (Harlan, Arundel, and Marcia) fed upon all blood samples regardless of draw date, with Harlan and Arundel bugs having the highest feeding rates (89.2% and 82.5%, respectively). Marcia bugs fed at a much lower average rate (25.8%) and thus mortality of this population is not reported. Bed bugs from Harlan and Arundel experienced significantly higher mortality when fed on blood drawn on 1 and 4 d after treatment (DAT) compared to 6 and 13 DAT. Furthermore, differences in mortality were evident among bed bug populations, with Arundel experiencing significantly higher mortality than Harlan. Subsamples of the blood subsequently were analyzed, and 0.003 ppm carbaryl was evident at 1 DAT, whereas carbaryl was undetectable at 6 DAT. Based on these findings, increased scrutiny is warranted when interviewing and selecting a blood supplier given that pest management practices in animal husbandry can greatly impact bed bug rearing success.

Introduction

Rearing bed bugs (*Cimex lectularius*) in the laboratory presents unique challenges because either a suitable live animal host or an artificial blood-feeding system is required for population maintenance and growth. An overriding consideration when using a live host is the possibility of general discomfort, allergic reaction at the bite site, and anemia. Using a live host is an expensive approach that requires considerable oversight with regard to animal use protocols and appropriate care of host animals; there are strict regulations for the use or maintenance of vertebrates in the laboratory. In order to feed bed bugs on human volunteers, a protocol approved by an institutional review board (IRB) is required. If many bed bug populations are to be maintained, consideration must be given to obtaining a long-term, consistent group of volunteers.

Many laboratories use custom built or commercially available artificial membrane feeders for rearing bed bugs. A blood-feeding apparatus with mouse skin as a membrane through which bugs could feed was described by Ogston and Yanovski (1982). Montes et al. (2002) subsequently described a system using a blood-filled reservoir covered with a stretched piece of Parafilm M that served as a membrane. Both of these custom built systems used a recirculating hot water bath to control blood temperature. Precise regulation of temperature is important since optimum feeding is achieved when blood is maintained at 37-38°C, and bed bugs do not feed on blood that is cooler than 34°C or warmer than 42°C (Bell et al.1966). Our laboratory uses a commercially available apparatus, the Hemotek 5W1 system (Discovery Workshops, Accrington, England), which utilizes an electric heating unit wherein the temperature may be adjusted by a set screw. The Hemotek system uses several metal reservoirs, each of which is covered by a membrane then screwed onto individual heating units for subsequent

filling with blood.

An artificial feeding system requires selecting an appropriate host to supply the blood. Although humans are the primary host of bed bugs, laboratory studies have shown that these insects can successfully feed on the blood of a number of warm-blooded animals including chickens, pigeons, bats, mice, rats, guinea pigs, rabbits, and horses (De Meillon and Golberg 1947; Usinger 1966; Araujo et al. 2009). However, all of these researchers found that bed bug development and reproduction were quite variable on these different hosts. Furthermore, blood preparation methodology appears to greatly influence rearing success. De Meillon and Golberg (1947) found that bed bugs did not successfully develop on fractioned or hemolyzed, defibrinated blood of humans, rabbits, or horses. Bell et al. (1966) had unsatisfactory results when feeding rabbit serum or erythrocytes to adult female bed bugs—no viable eggs were produced following the first feeding and the bugs did not survive a second feeding. Montes et al. (2002) investigated the effects on anticoagulation methods on blood meals provided from cattle, sheep, and chickens, and they found that heparinized whole blood was more suitable than defibrinated blood because bed bug nymphs and adults achieved better engorgement weights and more eggs were produced.

A reliable provider for blood is another consideration when using an artificial feeding system. Blood can be readily procured from biological supply companies, but one downside to using off-site facilities is that their animal husbandry practices can impact rearing success. During the process of selecting a blood provider for our bed bug rearing program, companies were interviewed as to their animal care practices, particularly with regard to their use of medications and insecticides. The selected biological supply company indicated that they did not use insecticides on their animals nor did they not draw blood from animals that were being medicated.

In mid-November 2011, ~1 yr after initially procuring blood from our biological supplier, our lab received a new shipment of chicken blood, which was promptly fed to numerous bed bug populations. Large numbers of dead, engorged bugs were found when rearing containers when examined several days later. Follow-up correspondence with the vendor revealed that the chicken pens and bedding had been dusted on a bi-weekly basis with Sevin® (carbaryl), and the lot of blood was drawn 3-d post-treatment. A follow-up bioassay was conducted to determine if carbaryl was the causative agent of mortality in our populations.

Materials and Methods

The biological supplier provided blood samples, which were drawn from chickens at 1, 4, 6, and 13 d after their pen and bedding had been treated with carbaryl. Samples (10 ml each) were kept refrigerated (~4°C) then shipped overnight by the supplier once all the samples had been collected.

For three populations (Harlan, Arundel, and Marcia), three replicates consisting of 10 mixed-stage bed bugs were fed on each sample of chicken blood using the Hemotek system with Parafilm M (American National Can, Menasha, WI) as the membrane. Hence, a total of 120 bugs was tested from each population. Each replicate of bugs was allowed to feed for 20 min. then the number of bugs that had fed to repletion was noted. The condition of bugs in each replicate was evaluated at 24 h and 6 d after feeding.

After bioassays were concluded, remaining blood was refrigerated until it would be chemically analyzed using LC/MS/MS to determine the quantity of carbaryl in the blood. Only the 1 d and 6 d blood samples had sufficient volume for analyses.

Results and Discussion

Bed bug feeding response was not significantly influenced by blood sample (F = 1.38; df = 3, 24; p = 0.34); the percentage of bugs that fed to repletion averaged 60.0%, 66.6%, 65.5%, and 71.1% for chicken blood samples drawn at 1, 4, 6, and 13 d, respectively, after carbaryl treatment of chicken pens and bedding. Although these blood samples caused no apparent feeding deterrence, the bugs' feeding response differed among populations (F = 106.78; df = 2, 24; p > 0.001), with Marcia having a consistently lower feeding rate (25.8%) than Harlan and Arundel (89.2% and 82.5%, respectively) (Fig. 1). The reluctance of Marcia to feed demonstrates that some bed bug populations adapt poorly to artificial feeding systems.

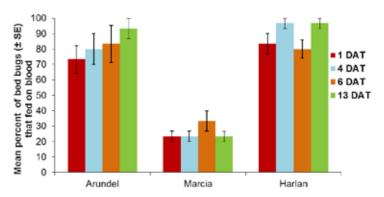


Fig. 1. Mean percent of bed bugs feeding to repletion on blood drawn from chickens at 1, 4, 6, and 13 d after their cages and bedding had been treated with carbaryl.

No mortality was observed at 24 h after feeding for any of the bed bug populations, thus only the percent mortality of fed bugs at 6 d after feeding was compared for both Harlan and Arundel across all four blood samples. Marcia was not included in these analyses because so few bugs had fed. Mortality differed by blood sample (F = 54.6; df = 3, df =

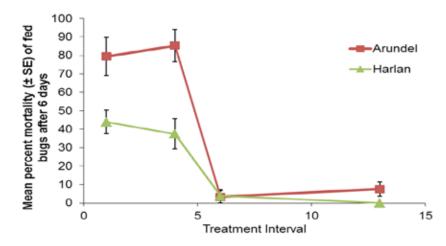


Fig. 2. Mean percentage mortality of bed bugs 6 d after feeding on blood drawn from chickens at 1, 4, 6, and 13 d after their cages and bedding had been treated with carbaryl.

LC/MS/MS analyses indicated chicken blood contained low but detectable levels of carbaryl (0.003 ppm) at 1 DAT, whereas carbaryl was undetectable at 6 DAT. Note, however, that these levels may have been influenced by the lag time between collection and analyses given that some of the chemical may have degraded.

Although none of our laboratory populations was completely eliminated after feeding on the shipment of contaminated blood, our bed bug populations were greatly reduced. We have revised our bed bug feeding protocol to now require an initial assessment of blood quality prior to feeding all of our laboratory populations. Hence, each new lot of blood is fed to three replicates of ≥10 Harlan bed bugs, and these bugs are evaluated for a 4-d period. Blood is to be discarded if these test bugs exhibit intoxication symptoms or die. This further extends the time requirement for laboratory maintenance of bed bugs. Given that Harlan was not as sensitive as Arundel to carbaryl-tainted blood, it may not be the optimal population for testing, but it is the easiest to rear and is a standard laboratory strain.

Since the mass die-off of bed bugs in our laboratory, we have changed suppliers. We recommend that other laboratories thoroughly investigate the animal husbandry practices of blood supply facilities prior to acquiring blood from them. Ask the company to clarify ALL insecticide use; make sure that they do not simply focus on insecticides that are directly applied to test animals. Also, as each lot of blood is acquired, we advise taking the additional precaution of test feeding a small sample of bed bugs several days prior to feeding all laboratory bugs.

Conclusions

This study demonstrates how animal husbandry practices in biological supply facilities can negatively impact bed bug rearing success. In this case, the provider's practice of using carbaryl to dust chicken pens and bedding apparently provided for insecticide-contaminated blood when blood was drawn from chickens within several days after the treatment. In laboratory bioassays, large numbers of bed bugs succumbed after feeding on blood drawn on 1 and 4 DAT whereas very low mortality was evident for bugs that fed 6 and 13 d DAT. Based on our data, bed bugs appear to be very sensitive to low amounts of carbaryl in blood.

Acknowledgements

We thank Harold Harlan for providing our laboratory with bed bugs. We also thank Matt Beal of the Ohio Department of Agriculture for having the blood samples analyzed. This research was supported in part by State and Federal funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University.

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Optimization of an *In Vitro* System for Rearing Bed Bugs with Notes on Their Development on Different Host Bloods

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Recently, the bed bug, *Cimex lectularius L*. (Hemiptera: Cimicidae), has re-emerged as a serious and growing problem, not only in North America, but globally (Boase 2001, Doggett et al. 2004, Potter 2006, Kilpinen 2008). This resurgence has renewed interest in a number of scientific groups that have incorporated bed bugs in their research programs. Despite this interest, scientific output regarding bed bugs seems not be proportional to the demand of knowledge needed for managing infestations. Part of the reason for this is due to the difficulty to produce bed bugs for experimentation. Many researchers have utilized live animals or humans as hosts for rearing bed bug colonies. However, the use of live hosts for feeding bed bugs can be difficult because of regulatory institutional restrictions that safeguard animals and humans, the need of restraint equipment, and the discomfort to the experimental hosts. By developing and using in vitro feeding techniques, it is possible to largely remove the necessity for the use of live experimental hosts and produce large numbers of synchronized individuals for conducting research.

Several authors have used artificial feeding systems for producing bed bugs (Bell and Schaefer 1966, Montes et al. 2002). The system consists of warming up blood contained in a membrane, with a circulating water bath. Earlier works showed, however, that this system did not produce consistently bed bugs. Females fed on citrated rabbit blood mixed with ringer solution produced fertile eggs but females died after a second feeding with the same mixture (Bell and Schaefer 1966). These authors also concluded that blood stored 4 days at 4° C became toxic to bed bugs. In another study, Montes (et al. 2002) claimed that heparinized chicken blood was more suitable than defibrinated lamb blood for maintaining bed bugs. Nevertheless, Montes' study did not present data on fecundity or nymphal development rate, two key parameters for production of bed bugs. Studies that address the effects of quality and quantity of blood ingested by bed bugs on reproductive and development parameters are still few, and also little is known about what is the most suitable blood (s) for bed bug rearing. Here is examined the reproductive potential and development rates of bed bugs fed on chicken, rabbit or cattle blood. Blood was offered either fresh or refrigerated for various times. All experiments were conducted with a membrane-based system similar to the one used by Montes et al. (2002). Insects were obtained from a colony maintained at 26° C, 50% RH, and a photoperiod of 12:12 (L:D) h. The colony was established from a sample collected in a human dwelling in New Jersey City in 2008.

Studies with fresh blood. In a first experiment, bed bug females were offered chicken, rabbit or bovine blood (blood mixed with 3.8% sodium citrate at a 19:1 ratio), and blood ingestion was estimated. Independently of the blood meal source, females ingested the same amount of blood ($F_{(2,50)} = 1.66$; p = 0.19) (Table 1). However, the total number of eggs produced were different between females fed on chicken blood than those fed on rabbit blood (t = 2.95; t = 29.7; t = 20.05) or than those fed on bovine blood (t = -4.62; t = 25.7; t = 20.05) (Table 1). Similar egg production was observed between females fed on rabbit and bovine blood (t = -1.8; t = 32.2; t = 0.18) (Table 1). A higher number of eggs produced by females fed on chicken blood suggest that chickens, a natural host of bed bugs, have a better efficiency of conversion of blood to eggs when compared with non-natural hosts such as rabbits or bovines.

In a second set of experiments, pre-oviposition time, egg production, and nymphal development rate was estimated from individuals fed on 3.8% citrated blood (chicken or rabbit blood) or from individuals fed on defibrinated rabbit blood. Results showed that the preoviposition time (time between feeding and initiation of oviposition) was similar in females fed on chicken, or rabbit blood ($F_{(2,18)} = 0.22$; p = 0.8) (Table 2). Likewise, egg production did not differ among cohorts of females ($F_{(2,21)} = 0.48$; p = 0.6) (Table 2). There was no difference in the mean period length between one developmental stage to another (Table 2) as well as no difference in the mean total length of the nymphal development period in all three the cohorts ($F_{(2,18)} = 2.37$; p = 0.12) (Table 2).

Study with refrigerated blood. Groups of females were offered citrated blood (chicken or rabbit), or defibrinated blood that was fresh, stored at 4°C for 7 or 21 days. While females fed on chicken blood refrigerated for the various times had similar egg production ($F_{(2,62)} = 3.03$; p > 0.05), females fed on citrated ($F_{(2,62)} = 5.04$; p < 0.05) or defibrinated rabbit blood ($F_{(2,62)} = 6.04$; p < 0.05) stored for 21 days produced lower number when compared with the same blood fresh or stored for 7 days (Fig. 1).

Conclusions

- Females fed on chicken blood produced more eggs than females fed on rabbit or bovine blood.
- Bed bugs completed their nymphal development at similar rates when fed on chicken or rabbit blood.
- Females fed on rabbit blood refrigerated for three weeks had lower egg production. Chicken blood refrigerated up to three weeks might be suitable for rearing and maintenance of bed bugs. On-going studies are being conducted to determine whether bed bugs complete development when fed on three-week refrigerated chicken blood.

Acknowledgments

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Table 1. Blood consumption and egg production of bed bugs fed on different blood sources

Blood ¹	Total mean weight gain (mg) ±SEM/female ²	Total mean eggs³ ±SEM/female
Chicken	11.9 (±0.95)a	18.9 (±1.76)a
Rabbit	10.2 (±0.55)a	12.8 (±1.21)b
Bovine	12.0 (±0.86)a	10.1 (±0.95)b

¹ Blood was mixed with 3.8% sodium citrate at a 19:1 ratio

Table 2. Preoviposition time (days± SEM), egg production, and developmental times¹ (days± SEM) of bed bugs fed on various bloods

	Chicken ³	Rabbit ³	Rabbit⁴
Pre-oviposition time	4.7 (a) ±0.21	4.6 (a) ±0.32	4.4 (a) ±0.20
Total mean eggs ²	16.6 (a) ±2.73	13.6 (a) ±1.47	15.8 (a) ±2.36
Egg to 1st	5.2 (a) ±0.17	5.1 (a) ±0.12	5.7 (a) ±0.18
1st to 2nd	5.17 (a) ±0.31	4.8 (a) ±0.35	4.4 (a) ±0.20
2nd to 3rd	3.3 (a) ±0.21	4.1 (a) ±0.12	3.6 (a) ±0.22
3rd to 4th	4.1 (a) ±0.17	4.1 (a) ±0.12	3.5 (a) ±0.20
4th to 5th	5.3 ±0.21	4.5 (a) ±0.19	4.4 (a) ±0.20
Total days			
feeding to 5th	27.8 (a)± 0.54	27.3 (a)± 0.41	26.5 (a)± 0.20

¹ Cohorts of six, eight and seven first-instar nymphs (chicken, citrated rabbit, defibrinated rabbit blood, respectively), were followed until reaching fifth-instar nymphal stage.

Values within rows followed by the same letter are not significant different at P > 0.05.

² Females (n =18) were held individually in 4 ml vials and provided with a strip of cardboard to reach the warm blood and to deposit eggs. Females were fed twice (a week apart) and weighed individually after feedings. A fed male was present in the first 48 h of each feeding time to secure fertilization ³ Total mean eggs produced by females after feedings.

² Total mean egg number of eight females fed twice.

³ Blood mixed with 3.8% sodium citrate at a 19:1 ratio

⁴ Blood mechanically defibrinated

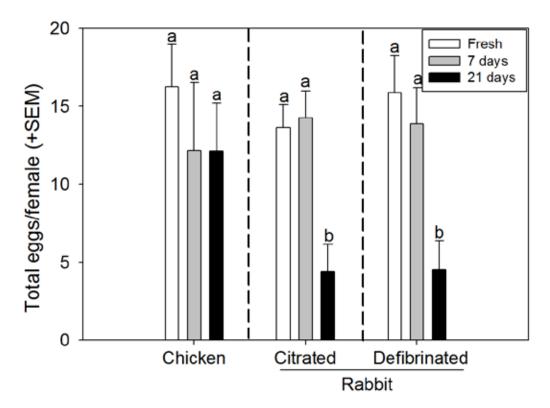


Fig. 1. Egg production of females fed on chicken and rabbit blood stored for various times. Blood was offered fresh, refrigerated for 7 or for 21 days. The bars within type of blood with the same letter are not significantly different from one another (P > 0.05).

Susceptibility of Multiple Field Collected Strains of Bed Bugs, *Cimex lectularius*, to Selected Insecticides

Sumiko R. De La Vega and William A. Donahue, Jr. Sierra Research Laboratories, Modesto, CA

Resistance is the genetically acquired ability of an organism to survive a pesticide application at doses that once killed most individuals of the same species. This has become increasingly widespread amongst populations of bed bugs throughout the United States which has made combating this pest quite difficult. For laboratories and universities rearing bed bugs for testing, it is important to know the resistance levels of each strain of bed bug being reared and used for testing but unfortunately, if the initial numbers of field-collected bed bugs is small, it can take over a year for the colony to reach usable numbers for large-scale testing.

Although a full dose response is helpful in determining lethal dose and baseline resistance information on newly-colonized strains, the objective of this research was to evaluate a discriminating dose test which could be just as helpful in characterization of new colonies without sacrificing a large portion of the population. The initial full dose response tests with permethrin were initiated in 2008 and repeated every two years to evaluate status of the colonies over time. The discriminating dose tests were carried out in 2012 on various strains in colony to selected pyrethroids, a neonicotinoid, and a carbamate.

Materials and Methods

Test substances:

• Permethrin technical, deltamethrin technical, imidacloprid technical, propoxur technical, Permanone 40 – 40% permethrin formulation

Strains tested:

- Harlan/Ft. Dix" susceptible laboratory strain in colony since 1973, obtained 2008
- "Earl" –pyrethroid-susceptible field strain, collected in Modesto, Stanislaus County, CA, 2007
- "Cincinnati" –field strain, collected in Cincinnati, Hamilton County, OH, 2007, pyrethroid- resistant upon initial testing
- "James" pyrethroid-susceptible field strain collected in Modesto, Stanislaus County, CA, 2009
- "Jersey" moderately pyrethroid-resistant field strain collected in Jersey City, Hudson County, NJ, 2010
- "Wolverine" –moderately pyrethroid-resistant field strain collected in Modesto, Stanislaus County, CA 2010
- "Stockton 2" highly pyrethroid-resistant field strain collected in Stockton, San Joaquin County, CA, 2011

6" x 6" glass panels were used as the substrate on which the test substance was applied with three replicates in each test group. 1 mL of the test substance was applied to each panel with a micropipette and evenly distributed by an acid brush, within the wax outline of a 10 cm circle. Panels were allowed to dry and ten (10) adult bed bugs of mixed sex ratio were placed on each treated panel and confined with a 100 x 15 mm Petri dish lid. Mortality data was recorded at 5, 15, 30, 45, 60 min, and 2, 4, 6, 24, 48, 72, 96, and 120 hrs.

The permethrin full dose response test utilized rates of 0.0005%, 0.005%, 0.05% and 0.5% of the test

material and the discriminating dose tests used a common label rate for the insecticide (0.05% was used for all test substances with the exception of propoxur, which was applied at a 0.5% rate).

Results and Discussion

A full dose response test for permethrin was completed in 2008 against the Harlan and Cincinnati bed bug strains which had been recently acquired at that time. The Harlan strain reached 100% mortality by 24 hours at all rates. The maximum percent mortality in the Cincinnati strain was only 20.0% at the highest rate, 0.5%. In 2010, a full dose response for permethrin was carried out again with Harlan and Cincinnati as well as three additional new strains: Earl, James, and Wolverine. Harlan showed the same results as in 2008 with 100% mortality by 24 hours regardless of rate. The Cincinnati strain showed a very slight dose response, reaching 40.0% mortality at 0.5%, the highest rate. Earl was susceptible to permethrin, demonstrating >90.0% mortality at 24 hours at the 0.05% and 0.5% rate and 83.3% 24-hour mortality at the 0.005% rate. The James strain showed a stronger dose response with 66.7% 24-hour mortality at the 0.005% rate, 86.7 at 0.05%, and 93.3% at the 0.5% rate. The Wolverine strain only demonstrated 32.1% 24-hour mortality at the 0.005% rate and 66.7% at the 0.05% rate but reached >90.0% at the 0.5% rate. In 2010, based on these results, the Cincinnati strain was a permethrin-resistant strain while the other strains had lesser degrees of permethrin resistance with the exception of the Earl strain which was permethrin-susceptible.

The full dose response for permethrin was repeated against just two strains, Earl and Cincinnati in 2012. Earl demonstrated nearly the same results as two years prior. However, Cincinnati showed a marked loss of resistance, reaching 95.9% mortality at the 0.5% rate and 70.0% at the 0.05% rate by 24 hours.

Discriminating dose tests in 2012 with permethrin at just one rate, 0.05%, revealed to us the same findings of Earl's continued susceptibility (Figure 1) and Cincinnati's resistance loss (Figure 2) when compared to results at the 0.05% concentration two years prior, but required much fewer bed bugs than a full dose response. This single dose response test was also used to evaluate other selected strains, showing the susceptibility to permethrin of Earl, Harlan, James, and Cincinnati, the moderate resistance of Jersey and Wolverine (<50.0% mortality at 120 hours) and the highly resistant nature of the Stockton 2 strain (3.3% mortality at 24 hours). (Figure 3)

A discriminating dose test with another pyrethroid, deltamethrin (0.05%) was also carried out, eliciting slightly better efficacy against the moderately resistant strains, Jersey and Wolverine (<70.0% mortality at 120 hours). Harlan, Earl, James, and Cincinnati all demonstrated >90.0% mortality at 24 hours. The Stockton 2 strain again showed resistance, resulting in 20.0% mortality at 120 hours. (Figure 4)

Imidacloprid was diluted in acetone to a discriminating dose of 0.05%. All strains were susceptible to Imidacloprid with the exception of Stockton 2. Earl, Harlan, and James all reached 100% mortality and Cincinnati, Wolverine, and Jersey showed >90.0% mortality at 24 hours. Stockton 2 reached 70.0% mortality by 72 hours but showed recovery down to 50.0% by the end of the test (120 hours). (Figure 5)

A discriminating dose of 0.5% was used to test propoxur and rapid knockdown was observed with 100% mortality achieved by just 2 hours in all strains except Stockton 2. Stockton 2 reached only 57.7% mortality by 24 hours. (Figure 6)

Conclusions

With the discriminating dose tests, we were able to evaluate relatively quickly the susceptibility/resistance of many strains to various insecticides without thoroughly taxing the colonies. Monitoring the results of a single dose over time also showed a significant decrease in the resistance level of our

Cincinnati strain to permethrin. Discriminating dose tests, repeated annually or biannually, can be helpful in monitoring any genetic shifts of bed bug populations in colony. The reversion of our Cincinnati strain to a pre-resistant state may also indicate a need to challenge our resistant colonies in order to maintain their resistance levels.

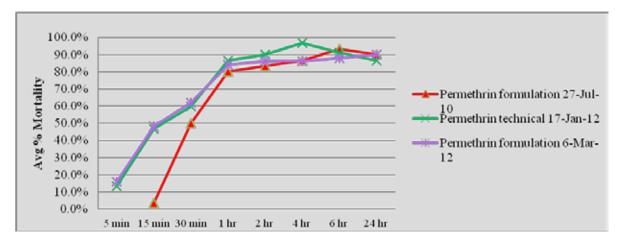


Fig. 1. Mortality of Earl strain bed bugs exposed to 0.05% permethrin applied to glass panels from 2010 – 2012

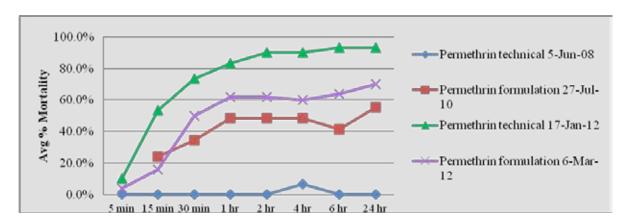


Fig. 2. Mortality of Cincinnati strain bed bugs exposed to 0.05% permethrin on glass panels from 2008 – 2012

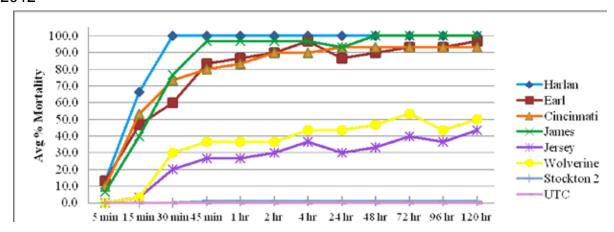


Fig. 3. Mortality of bed bugs exposed to glass panels treated with 0.05% technical permethrin, 2012

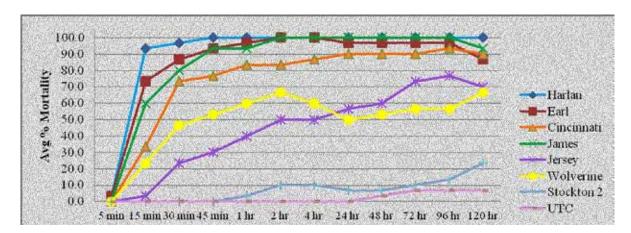


Fig. 4. Mortality of bed bugs exposed to glass panels treated with 0.05% technical deltamethrin, 2012

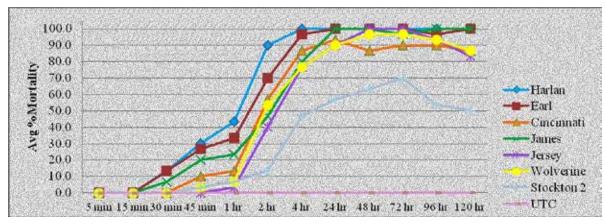


Fig. 5. Mortality of bed bugs exposed to glass panels treated with 0.05% technical imidacloprid, 2012

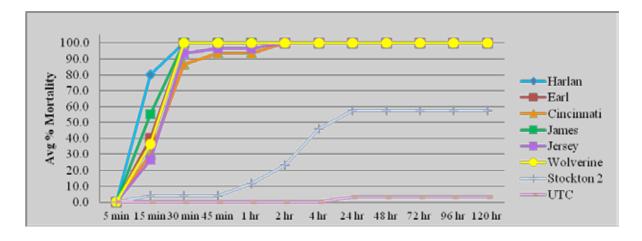


Fig. 6. Mortality of bed bugs exposed to glass panels treated with 0.5% technical propoxur, 2012

Evaluation of the Residual Activity and Repellency of Selected Insecticides Against Bed Bugs, Cimex lectularius

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Bed bugs are nidicolous insects in the family Cimicidae thought to have associated with humans and bats where all three evolved together in caves located in the Middle East. These wingless insects are thigmotactic, preferring rough surfaces such as wood or paper, clustering together in contact with each other where fecal matter, egg shells and exuvia accumulate. Bed bugs will return to these "Brood Centers" after a blood meal, remaining in a quiescent state while digestion takes place, usually about a week. It is believed that hunger is the stimulus for bed bugs to leave the harborage and seek a host for reproductive or molting purposes.

A repellent is a chemical that makes unattractive to arthropods a habitat, food source, or oviposition site ordinarily sought and frequented. One of the objectives of this research was to identify a repellent to be used in a "Push-Pull" control strategy, one which repels and attracts insects in the same environment to enhance control efforts. An active repellent could be used to "flush" insects from their harborage, thereby exposing them to various control measures such as vacuuming, steam treatments, heat treatments or pesticide applications used in an integrated pest management (IPM) control program. Active or passive bed bug traps would play a key role in this "Push-Pull" control strategy as a part of an IPM program. The primary objective was to evaluate the contact activity (mortality, repellency, egg distribution and hatch) of selected strains of bed bugs exposed to treated fabric substrates.

Materials & Methods

Test Chemicals:

- Permethrin Impregnated Fabrics (3) 0.52% a.i.
- Phantom Aerosol Chlorfenapyr 0.5%
- Alpine Aerosol Dinotefuran 0.5%
- Bedlam Aerosol Sumithrin 0.4%, MGK-264 1.6%
- Bed Bug Gel Lactic Acid 20%, Rosemary Oil 1%, Hops Flower Essential Oil 1%

Bed Bug Strains:

- "Harlan" Laboratory Susceptible Strain (Ft. Dix, NJ 1973 SRL 2008) Adults Mixed Sex Ratio
- "Earl" SRL Field Collected (Modesto, CA 2007) Susceptible Strain Adults Mixed Sex Ratio
- "James" SRL Field Collected (Modesto, CA 2009) Strain, 7X Permethrin Resistant Adults Mixed Sex Ratio

Lethal Time (LT) Treatment 1 - Treated and untreated fabrics were cut into circles to fit into individual labeled 10 cm Petri dishes. The cloth swatch "panels" were secured to the bottom half of the Petri dishes with hot glue on the edges to restrict the bed bugs to the top (visible on the lab bench without

disturbing the replicates) of the panels. Approximately ten (10) bed bugs were placed on the top of the panel inside the Petri dish. The bed bugs remained on the panels (continuous forced exposure) for the duration of the bioassay. Control replicates contained untreated black recycled woven poly and "Stripe" Stitch Bond and were set-up exactly the same as the treated replicates. Care was taken to avoid cross contamination during all phases of the bioassay.

Lethal Time (LT) Treatment 2 and Repellency - Treated and untreated fabrics were cut into half circles to fit into individual labeled 10 cm Petri dishes (Fig.1). Each treated half circle was paired with a half circle of untreated "Stripe" Stitch Bond. Untreated controls were half circles of untreated black recycled woven poly and untreated "Stripe" Stitch Bond paired together to form an untreated "panel". The cloth swatch "panels" were secured to the bottom half of the Petri dishes with hot glue on the edges to restrict the bed bugs to the top (visible on the lab bench without disturbing the replicates) of the panels. Approximately ten (10) bed bugs were placed on the top of the panel inside the Petri dish. The bed bugs remained on the panels (continuous exposure) for the duration of the bioassay. Control replicates were treated exactly the same as the treated replicates. Care was taken to avoid cross contamination during all phases of the bioassay.

Efficacy Determinations – Bed bugs were assessed for knockdown and mortality. Knockdown was assessed at 15, 30, 45, 60 minutes, 2 and 4 hours post-exposure of bed bugs. Mortality was assessed by probing for movement at 24, 48, 72, 96 hours and repellency data were taken at 12, 13, 14, 15, 16, 17, 19 and 20 days post-exposure of bed bugs to the "panels". Repellency data included location of eggs, hatch rate of eggs, nymph location and nymph mortality. Laboratory temperature and relative humidity (min/max) were recorded at the time of bed bug exposure through the end of the evaluations.

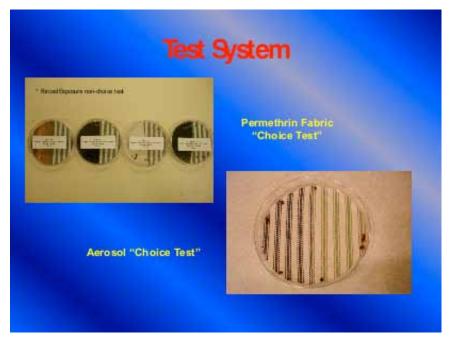


Fig. 1. "Choice Test" configuration for treated and untreated fabrics to determine mortality, spatial repellency and oviposition site repellency of selected compound against several strains of bed bugs.

Results & Discussion

All three experimental permethrin treated fabrics demonstrated 100% mortality of the susceptible strain of bed bugs "Harlan" at 24 hours post-exposure with forced exposures. Experimental perme

thrin treated fabrics demonstrated 50 - 78% mortality of the field collected strain of bed bugs "James" at 24 hours post-exposure with forced exposures (Table 1). Untreated control mortality was zero for the 24-hour evaluation.

Lethal Time (LT) bioassays were conducted to determine not only mortality of bed bugs during forced exposure, but mortality of bed bugs that were allowed to move freely between treated and untreated fabrics. The bed bugs that were allowed to move freely between treated and untreated fabrics showed 24 hour average percent mortality of 64.0, 46.0 and 52.0 for samples 1, 2 and 3 respectively (Table 2). Mortality data were taken daily up to 96 hours, the extended time showed only small increases of mortality averages ranging between 12 and 32%.

The remaining bed bugs were monitored for oviposition, egg hatch and nymph mortality during the 21 day study. Oviposition was predominantly on the untreated side on all three test panels. Sample 1; averaged 3.9% (3 eggs) laid on the treated side compared to 96.1% (73 eggs) laid on the untreated side. Sample 2; averaged 6.3%, (6 eggs) laid on the treated side and 93.7% (90 eggs) laid on the untreated side. Sample 3; averaged 1.6%, (2 eggs), laid on the treated side and 98.4% (124 eggs) laid on the untreated side. This suggests the bed bugs preferred the untreated fabric and avoided the treated side in each bioassay. Untreated controls had 313 eggs laid on the two untreated fabrics (Table 3).

Bed bug hatch rates were comparable between treated and untreated fabrics, and did not show ovicidal activity. Average bed bug hatch rates ranged between 69.8 and 86.6% for the treated sides. Untreated control hatch rates averaged 87.0% for the untreated fabrics. Nymph mortality averaged 19.7%, 34.4% and 19.8% for samples 1, 2 and 3 respectively. Untreated control average nymph mortality was 2.6%. Nymph mortality rates imply that they also avoided the treated fabrics when searching for their first blood meal (Table 3).

Results from the "Choice Test" bioassays with residual aerosol treatments to the mattress ticking showed lower than expected mortality of bed bugs with Sumithrin at 42% and Chlorfenapyr, Dinotefuran and lactic acid + essential oils at 0% (Table 4). Most of the bed bugs settled on the untreated side of the test chamber within 4 hours after exposure, 58-96% remained on the untreated side 96 hours after exposure, 64-100%. Most of the eggs laid were on the untreated side of the test chamber, 69-100%, with very high hatch rates between 96.6 and 100%. Mortality of the hatched nymphs was 0.6, 0.6, 41.7 and 78.0% for lactic acid + essential oils, dinotefuran, sumithrin and chlorfenapyr, respectively.

Conclusions

The results from these evaluations suggest that the methodology for evaluating repellency is reliable and predictive using this small scale bioassay. The bioassay is sensitive in detecting differences in bed bug strains and chemicals (actives & formulations). Demonstration of repellency for both adults and nymphs as well as oviposition sites is an important factor in "Push-Pull" applications, but one needs to understand the dynamics of the ecosystem when including the host and "Brood Centers" (harborage) in human habitats. These types of studies are important in evaluating urban IPM control options based on unique bed bug behavior.

Table 1. Average percent mortality of adult bed bugs (2 Strains) confined to three types of permethrin treated fabrics ("Non-Choice") compared with an untreated control as a continuous forced exposure lethal time (LT) evaluation.

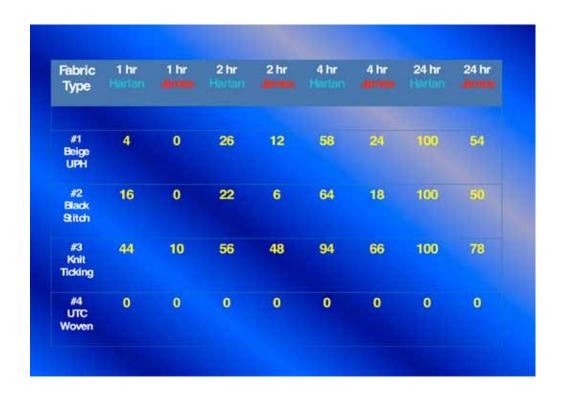


Table 2. Average percent mortality of adult bed bugs (Harlan) confined to three types of permethrin treated fabrics ("Choice Test") compared with an untreated control as a continuous exposure lethal time (LT) evaluation.

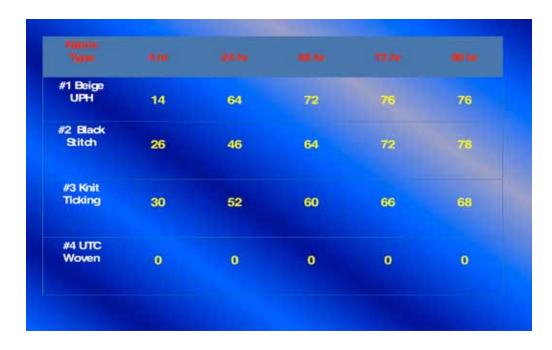


Table 3. Total # of Eggs, average percent hatch and average percent nymph mortality of bed bugs (Harlan) confined to three types of permethrin treated fabrics ("Choice Test") compared with an untreated control as a continuous exposure evaluation.

Fabric Type	Treated Side Total # Eggs	Untreated Side Total # Eggs	Avg.% Hatch	Avg % Nymph Mortality	
#1 Beige UPH	3	73	83.5		
#2 Black Stitch	6	90	86.6	34.4	
#3 Knit Ticking	2	124	69.8	19.8	
#4 UTC Woven	124	189	87.0	2.6	

Table 4. Mortality, distribution, average percent hatch and average percent nymph mortality of bed bugs (Harlan) confined to treated mattress ticking ("Choice Test") compared with an untreated control as a continuous exposure evaluation.

Treatment	Max Adult %Mort 96 hr	% Adults Untreated Side		Avg% Distribution Eggs		Avg% Hatch	Avg% Nymph Mortality
		4 hr			Matemated		
Chlorfenapyr Aerosol	0	96	82	13	87	96.6	78.0
Dinotefuran Aerosol	0	58	74	31	69	98.0	0.6
Sumithrin Aerosol	42	68	64	1	99	97.1	41.7
Lactic Acid + Essential Oils	0	96	100	0	100	100	0.6

SYMPOSIUM: BED BUGS: DISTRIBUTION, SUCCESS STORIES AND OUTREACH

EPA'S Collaborative Efforts to Combat Bed Bugs

Susan Jennings
US Environmental Protection Agency
Office of Pesticide Programs

During this session, Ms. Jennings will discuss the value of collaborating on solutions for the bed bug problem. She will focus on EPA's collaborative approach to bed bugs, which involves a multi-pronged strategy that addresses expediting registrations, improving education/outreach activities and encouraging IPM efforts. Many of EPA's efforts involve collaborating with stakeholders, including the federal partners, which is imperative to developing comprehensive approach to bed bug control. Key EPA activities will be discussed, including the Federal Bed Bug Workgroup, the newly released communications clearinghouse for bed bugs, and the upcoming revisions to the efficacy guideline for bed bugs. Ms. Jennings will also outline EPA's interest in some of the bed products being marketed that are exempt from registration under section 25(b) of FIFRA. EPA is looking at these products and would be interested in analyzing any data for individual products that demonstrates that they are ineffective against bed bugs.

Success Stories in Affordable Multifamily Housing



A.Taisey, M. Maley, P. Coles, and C. Koplinka-Loehr Northeastern IPM Center, Cornell University, Ithaca, NY

The StopPests in Housing program strives to eliminate high-level pest infestations at public housing authorities (PHAs) across the U.S. using integrated pest management (IPM). To meet this goal, staff members at the Northeastern IPM Center at Cornell University utilize a network of researchers, public health specialists, and housing professionals to advise PHAs on how to transition their housing portfolios to IPM. In addition to mass-communications through the website and presentations at housing association conferences, the StopPests team works one-on-one with individual PHAs—coaching them through IPM implementation.

PHAs are most likely to fully adopt IPM authority-wide when a few institutional changes occur:

- Get executive management to back the IPM program.
 See: www.stoppests.org/stoppests/assets/File/Presentation%20for%20EDs%20and%20 Boards.ppt
- Hire a qualified pest management firm or get staff licensed to apply pesticides.
 See: www.stoppests.org/what-is-ipm/using-ipm/pesticide-applications
- Pick a pilot site that has fewer than 200 units and (most importantly) an enthusiastic and supporting property manager. See: www.stoppests.org/ipm-training/the-training-day/information-for-external-organizations-proposing-ipm-to-a-pha
- Integrate IPM specifications into the residential lease and SOPs. See: www.stoppests.org/what-is-ipm/using-ipm

Once the framework and support is in place, a PHA should

- Place monitoring devices in every unit and check them after a week, recording catch numbers. This establishes a baseline of where infestations are or are not present.
 Monitoring continues in all units. See: www.stoppests.org/what-is-ipm/using-ipm/ pesticide-applications/in-house-pest-control
- Train the IPM team so that everyone who lives and works on-site knows their role in pest management. See: www.stoppests.org/ipm-training/training-opportunities
- Allocate time and resources to focus areas. See: www.stoppests.org/what-is-ipm/using-ipm/ focus-units
- Use renovation and move-in as an opportunity to prevent pests.
- Track data from work orders and PMP service reports to identify trends and track efficacy. See: www.stoppests.org/success-stories/evaluate-your-success

More than 30 PHAs have received training and support from the StopPests in Housing program over the last five years. Every site presents unique challenges due to variations in factors like pest pressure, political atmosphere, cultural considerations, and resource limitations. A few examples of success include:

- Staff members committed to meet with residents two times before bed bug treatment to assess needs and make sure homes were prepared to receive effective treatment.
- A PHA staff member works closely with their pest management firm, a cleaning company, the School IPM program, and residents. Having a contractor routinely steam baseboards in the

homes that have a history of bed bugs increases resident participation. Also, an after-school program on IPM resulted in four kids (from different sites) reporting bed bug infestations in their homes. Communications never reached these homes because the parents spoke a little-known dialect. Tracking language helped this PHA predict the social activities of their residents and thus target bed bug education efforts.

• The PHA's Executive Director was so knowledgeable about bed bugs that she was able to stop a negative newspaper article. She educated the reporter who came to her with accusations. Rather than publishing a negative story, the reporter used her as a source of good information.

Read StopPests in Housing case studies at www.stoppests.org/success-stories/case-studies.

A ProActive Approach for Commercial Bed Bug Control

Jason Meyers BASF Corporation

Previous laboratory studies have suggested that preventative control of bed bugs (*Cimex lectularius*) in the field is promising. The principal goal of this project was to reduce establishment of bed bug populations using residual, non-pyrethroid-based insecticides. Here in are presented both lab and field studies to suggest successful prevention was likely achieved during a field investigation.

In apartments, most bed bugs are found in and around the bed, with scattered numbers elsewhere (Potter, et al. 2006). A laboratory study demonstrated six month residual control of two pyrethroid resistant bed bug strains using chlorfenapyr (Phantom SC® termiticide-insecticide) on mattress ticking, concrete, and medium density fiberboard (Haynes et al., unpublished). Another laboratory study shows a significant increase in chlorfenapyr (Prescription Treatment® brand Phantom® Pressurized Insecticide) adsorption to the bed bug integument over time (Kells, unpublished). Residual efficacy of dinotefuran and diatomaceous earth (Prescription Treatment® brand Alpine® Dust Insecticide) has shown significant bed bug mortality even after one year (BASF, unpublished).

A Midwestern hotel was identified as a candidate for the program as there were eight (out of 65 rooms) bed bug infestations in the previous six months prior to treatment. The preventative program involved the treatment of primary and secondary areas within each hotel room. These are areas bed bugs are more likely to establish. Primary areas are treated every six months and secondary sites every 12 months using Phantom PI and Alpine Dust. Inspections were completed prior to treatment and every 3 months for one year. Inspections revealed only one 4th or 5th instar nymph during the six month inspection at the base of a bed frame. Additionally, the hotel had no complaints of ants, roaches, or beetles, which had been a significant issue for the business.

The goal of the proactive approach is to reach a balance of financial capability of the property owner/manager and the Pest Management Professional's ability to sell a preventative service. This program will successfully reduce the incidence of bed bug establishment, hotel room downtime, customer complaints, and litigation. This program is an untapped niche in the pest control market, creating an opportunity for premium service and expanding business opportunities.

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Efficacy of Phantom SC Termiticide-Insecticide & Prescription Treatment Brand Uld Hydropy 300 on Field-Collected and Lab-Reared Bed Bug (Cimex lectularius L.) Populations

Robert W. Davis¹ and Cole Younger²
¹BASF Corporation; ²Stillmeadow, Inc.

Bed bug (*Cimex lectularius L*.) infestations have become more widespread across the United States and many parts of the world. Phantom SC Termiticide-Insecticide has been an important tool for use by pest professionals for bed bug control. Phantom is a pyrroll chemistry that exhibits a mode of action within the mitochondria of the target pest (Hollingworth and Gadelhak 1998). It is not a nerve toxin. It is a slower acting, non-repellent that can provide curative efficacy as well as protection from post treatment establishment of bed bug populations. Phantom can provide control of both pyrethroid resistant and susceptible bed bug populations (Romero et al. 2010). Formulation changes can impact speed of control. Topical bed bug treatments with Prescription Treatment® brand Phantom Pressurized Insecticide (0.5%) provided an LT_{50} of 1.5 days. Comparatively, Phantom SC treatments (0.5%0 provided an LT_{50} of 5.6 days (Romero et al 2010). The purpose of this research was to evaluate the potential decreasing time of control by tank mixing Phantom SC with synergized pyrethrins. Differences in speed of control were also noted between field collected and lab reared populations.

Treatments in this study included Phantom SC (0.5%), Phantom SC (0.5%) & Prescription Treatment® brand ULD® Hydropy-300® (0.25%), Phantom SC (0.5%) & ULD Hydropy (0.5%), Phantom SC (0.5%) & Prescription Treatment® brand Microcare® 3% CS (0.1%) and Suspend® SC (0.06%). All treatments were applied topically to adult mixed-sex bed bugs. Each bed bug was dosed with selected treatment and observed for up to 12 DAT. There were 3 reps of 10 bed bugs each. Both lab reared (susceptible) & field collected populations were tested. There were 5 treatments and untreated controls. Each was applied to the bed bugs by label directions. Observations for signs of toxicity, moribund and mortality were made at 1 and 4 HAT, and 1-12 DAT.

Lab reared bed bug populations exhibited $LT_{50\text{'s}}$ of 275 and 54 hours for the untreated controls and Phantom SC (0.5%), respectively. The Phantom SC (0.5%) and ULD Hydropy-300 (0.25%), Phantom SC (0.5%) and ULD Hydropy-300 (0.5%), Phantom SC (0.5%) and Microcare 3% CS (0.1%), and Suspend SC treatments all exhibited $LT_{50\text{'s}}$ of less than 1 hour. However, for field collected bed bug populations, $LT_{50\text{'s}}$ of 2378, 77, 57 and 70 hours were observed for the untreated controls, Phantom SC (0.5%), Phantom SC (0.5%) and Microcare 3% CS, and Suspend SC treatments, respectively. The Phantom SC (0.5%) and ULD Hydropy-300 (0.25%) and Phantom SC (0.5%) and ULD Hydropy-300 (0.5%) treatments exhibited $LT_{50\text{'s}}$ of 2.5 hours and less than 1 hour.

These data show that there can be differences in speed of kill between lab reared and field collected populations, with field populations taking longer for mortality. This may be a function of pyrethroid resistance mechanisms and/or overall fitness of bed bug populations. These are important considerations when evaluating efficacy studies. The addition of the synergized pyrethrins (ULD Hydropy-300 (0.25% & 0.5%)) to Phantom SC (0.5%) provided for very fast speed of kill for both lab reared (< 1 hour LT50) as well as field collected (2.5 and < 1 hour LT50, respectively) bed bug populations. This can be an important tool to allow Pest Professionals to provide fast curative control of existing bed bug populations with the ULD Hydropy-300 treatment and support longer term efficacy with the concurrent Phantom SC treatment within a tank mixed application.

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SYMPOSIUM: BED BUG MANAGEMENT PRACTICES

Canine Scent Detection for Bed Bugs: Effectiveness and Pitfalls

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Bed bugs are currently recognized as the most challenging urban pest with increasing occurrence in the U.S. Although they are not known to transmit human diseases, bed bugs severely reduce quality of life by causing discomfort, anxiety, sleeplessness, and ostracism (Hwang et al. 2005, Russell et al. 2012). The control cost for an apartment can be anywhere from a few hundred to > 1,000 dollars. The economic loss for hotel owners, furniture rental companies, rental home owners is often much higher due to loss of business and lawsuits (Potter 2006). Bed bug control service is now offered by most companies in the U.S. and becomes a major revenue source to many pest control companies. A recent survey indicates 92% of the firms surveyed reported increased bed bug cases over the last year (Potter et al 2011).

Low level detection of bed bugs is essential for early identification of infestations, efficient and economical eradication of infestations, and reducing the spread of bed bugs within housing communities and into society. Canine scent detection has the potential for efficient detection of low level bed bug activity and provides immediate results, a combination of benefits not shared by other detection tools or methods, In addition to these benefits, canine scent detection is very well suited for large scale inspections and inspection of non-traditional areas (i.e. retail stores, theaters, office buildings, schools, etc.) where other detection methods are often not effective or economically impractical. Potter et al (2011) reported 15% of companies surveyed use trained dogs for detecting bed bugs. In addition to assisting in the detection of bed bug activity and supporting bed bug management detection and control programs, canine scent detection also provides another avenue for revenue for pest control companies.

Although canines are capable of effective detection of bed bugs (Pfiester et al. 2008), no standard methods for training and evaluating dogs exist for bed bug detection after they have been purchased from the original trainer. Canine scent detection firms often state or imply accuracy rates 95-98% on their websites and printed materials. Such accuracy rates are not supported by the results of our field evaluations of canine scent detection teams in multi-family housing units. Our preliminary investigations of seven canine detection firms, in naturally infested apartments revealed detection rates between 10% and 83% with only two teams achieving detection rates greater than 56%. Subsequent inspections with 4 dog/handler teams were evaluated at a different multifamily housing complex with similar results (detection rates 15% - 77%).

"False positive" rates (alerts when live bed bugs are not present) are more difficult to evaluate due to the possibility that dogs alerted on bugs that could not be detected through other means. As a result, the argument can be made that it is impossible to be 100% certain that a dog has alerted falsely in natural field conditions. For the sake of being conservative, rather than calculate false alert rates we

can instead refer to them as unconfirmed alerts. In our field evaluations with 11 different dog/handler teams unconfirmed alerts ranged from 0% to 57% with median value of 14%. Over the next twelve months after canine inspection, bed bugs were discovered in only one apartment where unconfirmed alerts occurred, strongly suggesting that the unconfirmed alerts were in fact false positives. False indications during bed bug inspections have serious economic and legal implications and can result in the unwarranted application of pesticides.

Rarely do the results of controlled experiments translate to what occurs under field conditions, thus it is not surprising that detection rates below 95-85% were realized during our field evaluation. Further evaluations in controlled environments showed the dog teams could perform extremely well but varied significantly between the time of the day and the evaluated environment (Cooper and Wang, unpublished data). Detection firms appeared genuinely surprised by the performance of their scent detection teams in our field studies, indicating that the results did not correlate to what they perceive the accuracy of their team(s) to be during field inspections. The significant discrepancy between perceived and actual performance reveals a need for establishing better methods for training and evaluating canine scent detection teams on an ongoing basis.

Based upon the field performance of the bed bug scent detection teams evaluated there is an urgent need for additional research to: 1) identify aspects of maintenance training that promote detection failure in naturally infested conditions; 2) identify aspects of maintenance training that promote false alert rates in naturally infested conditions; 3) identify ongoing training methods necessary to reduce the discrepancy between training and field performance. Without such research, the canine scent detection service could be in danger of losing public trust; result in unnecessary treatment costs, missed detections, and possible lawsuits.

Acknowledgements

The authors would like to thank Boyd Gonnerman and Marcus Kwasek for their assistance during the field inspections. Financial contributions from Copesan Pest Services, New Jersey Pest Management Association, Associated Pest Services and GIE Media (Pest Control Magazine) helped support this research. A special thank you is also extended to all of the canine scent detection firms that willingly participated and cooperated in this study.

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Using Bed Bug Monitors to Maximize Effectiveness of Bed Bug Management Programs

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Adopting an effective monitoring tool and method were proven to be extremely beneficial to bed bug management programs. Monitors help detect bed bugs early, guide bed bug treatments, and determine whether bed bugs are successfully eliminated. Among the two dozens of bed bug monitoring tools available, only a few of them are effective. Pitfall-type monitors placed under furniture legs provided much more accurate estimation of bed bug infestations levels, bed bug distribution patterns in the infested apartments, and whether bed bugs were still present after treatment (Wang et al. 2009a, b; Wang and Cooper 2012). The effectiveness of bed bug monitors is affected by the monitor type, number and method of placement, and presence/absence of hosts. Passive monitors (those without attractants) are most effective in occupied rooms. An active monitor is most effective in non-occupied rooms because presence of hosts creates competition with the monitor. Passive monitors are more affordable and convenient than active monitors, but they require longer time to obtain results. Only a small proportion of bed bugs are trapped each day. Therefore, it is not realistic to use monitors to trap out all bed bugs in a room. Nevertheless, placing pitfall traps under furniture legs alone were able to significantly reduce bed bug numbers.

Bed bugs frequently appear in non-sleeping areas regardless of the infestation levels. Their hiding places are often related to structural features or layout of the furniture. It is beneficial to place passive monitors off the sleeping areas such as perimeters and corners of each room including closets, bathrooms, and kitchen to maximize the probability of detecting bed bugs and accurately determine the bed bug distribution patterns. Bed bugs primarily rely on carbon dioxide to locate a host. Their host searching behavior is often random and does not necessarily follow the CO₂ gradient in the environment. Combining CO₂ and chemical attractants improves trap efficacy. Placing and examining monitors regularly during a bed bug treatment program should be considered as a standard procedure to minimize unnecessary pesticide applications, improve the treatment effectiveness, and save cost.

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Active Monitoring of Bed Bugs in Occupied Apartments

Susan C. Jones and Joshua L. Bryant Department of Entomology, Ohio State University, Columbus, OH

The overall effectiveness of a near-final prototype of the Verifi™ Bed Bug Detector was demonstrated in occupied apartments in Columbus, OH. The success rate of the prototype Verifi detector (82.4%) compared favorably with do-it-yourself dry ice traps (82.4%) and canine-handler teams (80.0%). In this trial with the prototype Verifi detector, 10 of 17 rooms were confirmed to have bed bugs within 24 h; bed bugs were captured from one additional room at 48 h (11/17 rooms) and from three additional rooms at 7 d (14/17 rooms). Furthermore, subsequent examinations of the same detector during the 7-d period often indicated that additional bed bugs were captured over time. All stages of bed bugs were captured using the detector, with the majority contained in the pitfall and a few on the harborage. The overall functionality of the prototype was confirmed, but study results revealed the need for slight design changes, which were incorporated into the commercialized Verifi detector. No significant difference in bed bug captures was evident for detector placements on/near the bed, frequently used upholstered furniture, or baseboards that were in close proximity to the bed or upholstered furniture. We recommend these as prime placement sites for the commercialized Verifi detector. Our study indicates that the Verifi bed bug detector is an effective active monitoring device.

Introduction

Early detection of bed bugs (*Cimex spp.*) is critical in order to quickly implement measures to eliminate these cryptic insects, which can be very difficult to locate in low-level infestations. Since the resurgence of bed bugs (*Cimex lectularius*) in the U.S., which began in the late 1990s, various detection methods have been developed. Devices can be generally categorized as passive or active bed bug detectors or monitors. Passive detectors rely on bed bugs encountering the devices through the insect's typical search behavior, activity patterns, or random movement, and pitfall traps and glue boards are included in this category. Active detectors attract bed bugs from a distance using one or more biologically-based components that center on cues provided by the human host or by the bugs themselves; they typically incorporate heat, carbon dioxide (CO₂), and chemical attractants such as pheromones and/or kairomones.

In the case of canine detection, the dogs use scent cues provided by live bed bugs and viable eggs. Canine-handler teams have gained notoriety for quickly surveying the premises for low-level bed bug infestations, although their high cost prohibits wide-spread use. In a controlled experiment in hotel rooms, Pfiester et al. (2008) demonstrated that well-trained canine/handler teams had a very high success rate (98%) locating live bed bugs. However, the ability of canine-handler teams to accurately locate bed bugs in real world conditions can vary substantially among teams, with some having very low success rates (pers. comm., R. Cooper and C. Wang).

Active detectors typically are much more effective than passive detectors in capturing bed bugs. However, many commercially available detectors are quite costly. A do-it- yourself (DIY) trap that uses dry ice as a source of CO₂ (Wang 2010) is much more affordable than commercially available devices, but inconvenience and safety concerns due to the dry ice are among the factors limiting the wide-spread use of this trap. Furthermore, the trap operates only during a single night's deployment and therefore is impractical for a long-term monitoring program. Nonetheless, these dry ice traps have been demonstrated to quickly attract bugs in the lab as well as in apartments, even with low-level infestations (Wang et al. 2009a,b; 2011).

Launched by FMC Professional Solutions in October 2011, the VerifiTM Bed Bug Detector is the first device to offer active bed bug detection for up to 90 d (http://www.fmcprosolutions.com/BedBugs/Home.aspx). The Verifi detector measures 5 x 4 x 3 in. (ht x width x depth), and its various components are shown in Fig. 1. Each detector contains a CO₂ booster pack (Fig. 1A) and a lure (Fig. 1B) that emits two distinct attractants—a kairomone that attracts host-seeking bed bugs and a pheromone that attracts aggregating bed bugs. The CO₂ pack utilizes a chemical reaction to mimic a breathing host for ~24 h whereas the two attractants are emitted for up to 90 d, at which time these latter components can be replaced for on-going detection. The CO₂ cartridge can be replaced at any time. The multiple attraction mechanism combining CO₂, a pheromone, and a kairomone is designed to bring bed bugs into an open receptacle (pitfall) (Fig. 1C) or onto a harborage (Fig. 1D) that both can be quickly examined. The device can be affixed in place with its adhesive backing (Fig. 1E).

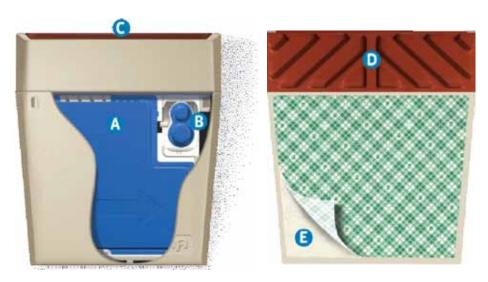


Fig. 1. Front and back views of the Verifi detector showing various components: A) CO₂ booster pack, B) lure with pheromone and kairomone chambers, C) pitfall, D) harborage, and E) adhesive.

In the current study, the effectiveness of three active monitoring methods, canine-handler teams, DIY dry ice traps, and near-final prototype Verifi Bed Bug Detectors were assessed sequentially within the same occupied apartment units.

Materials and Methods

Three approaches to active monitoring of bed bugs were evaluated in a 13-story high-rise apartment building in Columbus, OH, during a 10-day period from late June into early July 2011. Currently occupied one-bedroom apartments were selected from a list of those with suspected bed bug activity as provided by the apartment management. Twenty rooms (bedroom or living room) with suspected bed bug activity were used in the current study.

Residents first were briefly interviewed as to their history of bed bug bites and/or sightings and the primary sites in their apartment where they rested and slept. The latter answers were used to determine placement of detectors and traps so that all were in close proximity to frequently used furniture. A site diagram was sketched for each room indicating room dimensions and furniture placement, with subsequent entries indicating the location of the single DIY trap per room, as specified by use directions (Wang 2010), and three prototype Verifi detectors per room.

Canine-handler teams were used in 10 rooms at the start of the study and in the remaining 10 rooms

at the end of the study. Neither dry ice traps nor prototype detectors were in rooms when the teams did their inspections. Canine-handler teams from a single company were employed, with two dogs and the same handler. For each time period, the handler went through all 10 rooms with the first dog, then through all rooms again with the second dog. Any alert (a characteristic change in canine behavior in response to an odor, as interpreted by the handler) was noted by the handler, and the location of each alert was recorded on the site diagram by OSU personnel.

Immediately after the initial inspection by the canine-handler team and 1 d prior to placing prototype Verifi detectors, a DIY dry ice trap was installed for 24 h in each of 10 rooms. DIY traps were similarly installed in the remaining 10 rooms immediately after removing the prototype detectors. The single DIY dry ice trap per room was positioned on the floor next to the bed or frequently used upholstered furniture. All dry ice traps were examined at 24 h and the number and stage of bed bugs in or near the trap were assessed, then the trap was removed.

Prototype Verifi detectors then were installed in 20 rooms, with 3 detectors per room. The detectors were placed on the bed, frequently used upholstered furniture, or baseboards in close proximity to the bed or upholstered furniture. The number and stage of bed bugs in the pitfall, on the harborage, and on/near the detector were assessed on-site at 24 h, 48 h, and 7 d, with any captured bed bugs left in place. After the final inspection, detectors were returned to the lab and dismantled for final bed bugs counts that included any inside the detectors.

Results and Discussion

Results for the prototype Verifi detector compared favorably with two other active monitoring approaches—canine-handler teams and DIY dry ice traps. As shown in Table 1, the dog-handler team made apparently erroneous calls in four rooms, including three false positives (incorrect indication of bed bugs when bugs were not present) and one false negative (incorrect indication of no bed bugs when bugs were present), whereas three false negatives were documented for the DIY dry ice traps as well as for the Verifi detectors.

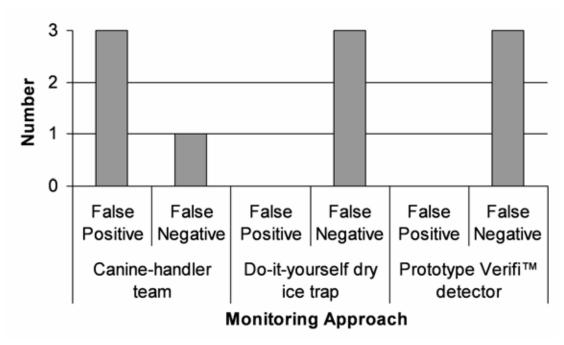


Fig. 2. Overall number of errors with three active bed bug monitoring approaches evaluated in occupied rooms in a high-rise apartment building in Columbus, OH.

Canine-handler Team Performance: Dogs alerted in 19 of 20 rooms, but these results include three false positives and one false negative resulting in an overall success rate of 80.0%. The two dogs had variable success rates in accurately identifying bed bugs, with the first dog making four errors and the second dog making five errors.

For the three rooms that were designated as false positives, no bugs were captured in either the Verifi detectors or dry ice traps, and an in-depth follow-up visual inspection for ~1 h by a 4-person team revealed no evidence of live bed bugs. It is possible that we overlooked a low-level infestation, but this is unlikely given our thorough inspection. The false negative occurred when the dogs failed to alert in one room where bed bugs were captured in the two monitoring devices.

Active Monitoring Devices

Overall, 3 of the 20 rooms with suspected bed bug activity showed no evidence of any bed bugs, resulting in a total of 17 bed bug positive rooms for the two active monitoring devices evaluated in this study.

DIY Dry Ice Trap Performance: The DIY traps had an 82.4% success rate, with 14 of 17 rooms confirmed to have bed bugs. An average of 6.7 bed bugs was caught per dry ice trap in bed bug positive rooms.

Prototype Verifi Bed Bug Detector Performance: The prototype Verifi detector had an 82.4% success rate, with 14 of 17 rooms confirmed to have bed bugs within a 7-d period. All stages of bed bugs were captured. Within 24 h, 10 rooms were confirmed to have bed bugs. At 48 h, bed bugs were captured from one additional room (11/17 rooms). At 7 d, bed bugs were captured from three additional rooms (14/17 rooms). Furthermore, subsequent examinations of the same detector during the 7-d period often indicated that additional bed bugs were captured over time.

Numbers of captured bed bugs ranged from 1 to 282 in prototype Verifi detectors that had been in place for 7 d in occupied rooms. An overall average of 36.9 bed bugs was captured during a 7-d period in the 14 rooms. In 7 rooms, ≤10 bed bugs were captured (avg. = 4.4 bed bugs).

No significant difference among detector placement was evident based on overlap of the 95% confidence intervals for the binomial proportions for successful captures of bed bugs. Success rates averaged 61.1% for placement on/near the bed, 65.0% for upholstered furniture, and 50.0 % for baseboards. Based on our study results, these should be prime placement sites for the commercialized Verifi detector.

In our study, the majority of bed bugs were contained in the pitfall of prototype Verifi detectors, but a few were found on the harborage. However, an issue of concern was that some captured bed bugs were escaping. For example, numerous first and second instar nymphs were observed inside the vent holes in the pitfall and many subsequently made their way into the interior of detectors. Furthermore, there also was strong field evidence that older stages occasionally escaped from apparently undisturbed detectors. Laboratory tests confirmed that some bed bugs were able to escape from the pitfall of prototype detectors. The commercialized Verifi detector incorporated our recommendations to (1) decrease the diameter of vent holes in the pitfall to prevent entry of first and second instar nymphs, and (2) manufacture a slicker pitfall surface so that bugs could not escape once they were captured. With its modified pitfall, the commercial version of Verifi is expected to have improved performance.

In our study, we also noted that some bugs were displaced from the pitfall when prototype detectors were positioned between the cushions of upholstered furniture, presumably due to air currents and other disturbances. Hence, directions for the commercialized Verifi detector indicate that these devices should be affixed to hard surfaces. When removing the prototype detectors, we noted that the adhesive backing sometimes damaged paint and drywall, and this warning is included in instructions with the commercialized Verifi detector.

The commercialized Verifi detector also incorporated our recommendations to roughen the texture of the outer device so that bed bugs could more readily navigate the surface, and to narrow the spaces between the paperboard chevrons of the harborage so as to create more suitable resting sites for these thigmotactic insects. The commercialized Verifi detector is priced much lower than larger active detectors, and it is a safe device that can be left in place and routinely serviced (90-d intervals), hence providing a useful addition to our arsenal of tools to help combat bed bugs.

Conclusions

The overall functionality and effectiveness of the prototype Verifi detector was demonstrated in this field study. Furthermore, the success rate of prototype Verifi detectors compared favorably with DIY dry ice traps and canine-handler teams.

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Challenges in Initiating Community-Based IPM (Bed Bugs)

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The annual increase in bed bug infestations within multi-unit housing facilities has become a financial nightmare for the housing industry. In 2011, the National Apartment Association surveyed its members only to find that bed bugs out-ranked all other issues (e.g. property taxes; utility billing, code enforcement and landlord/tenant issues) as being "extremely important". The reason that bed bugs are so high on the list of concerns is not because of the stigma associated with this pest, but rather the financial impacts that bed bugs are having on the operating budgets of multi-unit facilities.

Privately owned facilities that serve middle to upper income clients have seen their profitability decrease significantly due to the costs of bed bug remediation. Chemical and or heat remediation can typically cost between \$800 and \$1500 per unit. In many cases, the entire treatment, at full-price, will be applied a second or third time if a single bug is found after the initial treatment (interpretation of the 30 warranty can vary from company to company). An additional problem is that bed bugs have the ability to spread from one unit to another via common wall voids. Because of this ability to move through the walls, pest management professionals often recommend that some, or all units (as many as 8) adjacent to the infested unit be treated. While the default treatment of adjacent units may have merit in many cases, it adds considerably to the overall cost and may not always be necessary. In those communities that serve low income clients, that are at high risk of re-infestation, the costs of bed bug remediation are not sustainable.

In 2011, HUD publicized the "Guidelines for Bed Bug Control" which stated that all bed bug treatment would be at the owner's expense. It was also recommended that bed bug infestations should be treated within 5 days of bed bugs being identified. These guidelines have created difficulties across the nation in HUD facilities as naïve housing managers have relied on treatment recommendations from pest management companies that may or may not be bed bug remediation experts. For example, a HUD facility in Louisiana spent \$60,000 within 40 days to heat treat 5 apartment units (and adjacent units), and fumigate the occupants belongings with Vikane in outdoor chambers. Why move out the belongings when the units were going to be heat treated? After the heat and fumigation, two to five additional bed bugs were found. The pest management company then recommended that the entire building be tented and fumigated for \$230,000. In a Section 8 facility in Harrisonburg, VA, the management was being told by their heat treatment technician that seeing one or two live bugs running around after the treatment was "ok", and that the bugs would die later. There is no data to support this statement. Not surprisingly, several of the heat treated units were treated multiple times at the resident's expense. In both cases, the management company felts pressure to do whatever the pest control company recommended out fear of bed bug spread, and/or the 5 day recommendation from the HUD guidelines.

Because the long-term costs of bed bug remediation are not sustainable in multi-unit facilities there is a need for a more comprehensive approach to bed bug prevention and management. An Integrated Pest Management program based on education for housing personnel and residents, as well as prevention and monitoring, is the most practical way of keeping costs manageable. However, there are some challenges that are unique to bed bugs that make the implementation of an IPM program difficult. The first is that bed bug IPM in an apartment building necessitates a community approach,

with all residents and managers cooperating. However, residents are often embarrassed about bed bugs and do not want to report them to the management. Second, apartment and facilities managers are often afraid of bed bugs and refuse to enter an infested unit to make any assessment regarding the severity of the infestation. The third challenge is that IPM requires more a hands-on involvement in the pest management process than the managers are ready to give. Many managers feel that bed bugs control is not part of their job description.

Regardless of the challenges to community-wide IPM, such a program will shortly become an economic necessity. So the question is how does a housing community implement a bed bug IPM program? Since 2011, the Virginia Tech Dodson Urban Pest Management Laboratory has been evaluating different components of a bed bug prevention protocol in the field. Our intention was to develop a protocol that could be implemented by apartment managers and residents to prevent bed bugs from being brought into facilities, and to stop their spread if an infestation did develop.

The protocol consists of the following elements:

- Bed bug training for residents and staff
- Monitoring (passive monitors; pitfall)
- Vacuuming (before and after application of residual dust)
- Application of diatomaceous earth (at carpet tack strip; under baseboards; behind faceplates; perimeter of drop ceilings)
- Portable heat box for treating furniture and personal items
- Mattress encasements (for those residents who have confirmed bed bugs)

As of July 2012, our laboratory has held 5 bed bug training programs for HUD facilities staff, women's shelter volunteers, and elderly/disabled apartment residents. Surveys of residents taken before and after training have indicated that their ability to identify bed bug evidence had increased. Most were aware of looking for live bugs or experiencing bites, but few recognized fecal spots or cast skins as a signs of a bed bug infestation prior to the training. The training program also greatly increased the knowledge of the facilities managers regarding the usefulness of heat treating incoming residents' belongings, using monitoring devices in adjacent units, and applying diatomaceous earth as a means of preventing bed bug spread.

Pitfall type monitoring devices are useful both in units that have history of bed bug infestation and those that are adjacent to units that have been infested. Monitoring previously treated units gives some measure of the treatment efficacy over time. The monitors also alert managers to the potential reintroduction of bed bugs from an outside source (possibly frequented by the resident). Monitors placed in units adjacent to infested units will reduce the necessity of treating those units when no bed bug evidence is found. The monitors should left in place for 2-3 weeks to determine if the adjacent units actually have bed bugs or not.

We have found that the utility of the vacuum in this protocol is: 1. To remove old bed bug evidence, making the bed bug inspection easier and more accurate. 2. To remove debris of all kinds from along the baseboard area around the room perimeter. Debris removal makes the application of diatomaceous earth easier and more precise under the baseboards or at the carpet tack strip. 3. The vacuum is also used to remove any over application of diatomaceous earth that might later be picked up in air currents or misidentified by the resident as a "toxic" dust.

Diatomaceous earth is applied with a pressurized CO₂ duster to the entire perimeter of the apartment unit, either under the baseboards, or at the carpet tack strip. Faceplates are removed and dusted behind with either the CO₂ duster or a bulb duster. The perimeter of drop ceilings and any voids running vertically between floors are also dusted with the diatomaceous earth. Having the windows open during the application greatly reduces dust in the air.

The utility of a portable heat chamber is that new residents can have all of their personal items and furniture heat treated for bed bugs prior to moving into their unit. The University of Florida has developed a portable heat box that can be assembled to heat treat furniture and personal items, then disassembled and taken to another location (http://www.youtube.com/watch?v=W0CGXbZYmCA). Members of our laboratory built the heat chamber at a local women's shelter and had the shelter manager treat the chamber full of furniture and personal belongings. Sentinel bed bugs that had been hidden deep inside items placed in the chamber and were killed successfully as the result of the treatment.

Mattress encasements are arguably the only luxury item in this protocol. Because they neither prevent or remediate bed bugs, their utility is limited to trapping any bugs that are on the mattress surface or in the box springs. However, the trapping of bed bugs inside the box spring does make any future bed bug treatment easier in that the box springs does not have to be inspected. The encasement also prevents a new bed from becoming contaminated when it is moved into an infested unit.

As of June 2012, our laboratory has treated 125 apartment units using the protocol described above. We have learned that light-weight power dusters may be fine for treating carpet tacking, but cannot cope with vinyl baseboards. A heavy duty duster with a brass wand is absolutely necessary for dragging under hundreds of baseboards. We also learned that manages may be very excited about bed bug prevention but facilities personnel are somewhat reluctant to take on bed bug work themselves, and need constant encouragement and or chiding (big surprise). Finally, residents who were initially very apprehensive (and vocal) about our use of the dust (most have breathing problems) and the invasion of their apartments, did not mid the treatment once it began. Many stated on the survey that the treatment did not bother them, or that they found the treatment interesting.

Will this protocol prevent bed bug spread and lower the overall treatment costs for apartment facilities? This we do not yet know. Our laboratory will be monitoring several facilities over the next year to determine if this protocol can lower overall bed bug remediation costs. However, the sole practice of monitoring of adjacent units (as opposed to the default treatment of all 8 adjacent units) will go a long way to keep treatment costs down.

SYMPOSIUM: GREEN CHEMISTRY FOR URBAN PEST MANAGEMENT

Using Green Insecticides to Control Ants, Wasps, Spiders, and Termites

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Because of potential acute or chronic toxicological problems, insecticide application is generally considered as one of the last steps of an IPM program. For many pests, insecticide is used in the last resort if other measures fail to completely eliminate the target pest, which is often the case. Issues involving insecticide include actual and perceived toxicity to humans, pets and the environment. Green chemistry usually refers to natural products such as plant-derived essential oils, but some synthetic insecticides have similarly good toxicological and environmental profiles. Some natural products are active against insects and exempt from federal registration but synthetic insecticides must be registered. A select group of what may be considered synthetic green chemistries has recently been developed and registered to manage urban insect pests. In addition, we found that some pesticides used in the urban environment are effective at significantly lower than maximum label rates and are rendered 'essentially green' by applying them at the lowest effective rate, what we term the minimum effective dose (MED). Depending on application strategy, the MED of many natural insecticides we have examined require rates of 2% or higher to manage or eliminate urban pests whereby the MED of some synthetic pesticides is < 1/1000 or 1/10,000 that dose. Since dose is an important element, the safety and likelihood of contamination of some synthetic insecticides applied at MED is not measurably greater than that for some natural products applied at higher rates.

Ants. Because biologically significant concentrations of pyrethroids, questionable quantities of fipronil and several other insecticides have been consistently detected in mandated monitoring of streams, watershed, and other waterways throughout California, we undertook a state-supported cooperative project with PMPs to reduce pyrethroid use by at least 50% for urban pest ant control. In most states, including California, most of the insecticide to control urban pest ants is sprayed around the perimeter of homes. According to California Pesticide Use Reports, thousands of Kg of Al of some insecticides are applied each year. Not available for use by homeowners, significant quantities of fipronil were detected in most runoff water samples. Coupled with the official use reports, this suggests that much of the pesticide in runoff water results from commercial application, primarily to control the Argentine ant, *Linepithema humile*, around urban structures.

Using self-designed ant IPM programs, six PMP companies reduced pyrethroid use > 50% at a total of more than 500 homes while minimizing callbacks and maintaining high levels of customer satisfaction as to the level of control provided. The primary criterion for success was customer satisfaction. The PMPs assumed poor control would be reflected in complaints, callbacks, and cancelled accounts. The companies intentionally applied less pyrethroid on the IPM route, used fipronil judiciously, and along with some other environmentally friendly pesticides primarily used EcoSMART Technologies,' commercial sprays and powders containing rosemary, peppermint, and wintergreen oil (EcoExempt IC2) and 2-phenethyl propionate, pyrethrins, and thyme (EcoPCO WPX).

The PMP companies used route records and return card and telephone surveys to compare the results obtained on traditional and IPM routes that they continued simultaneously for the summer months. There was no significant difference between the perceived level of satisfaction of customers on the traditional or IPM routes, the number of complaints or callbacks, or the number of cancellations. Reducing the amount of pyrethroid applied did not adversely affect perceived control.

We demonstrated that direct spray containing only essential oils could, in fact, be effective against *L. humile* and other species. It had been suspected that the effects against ants observed in our previous trials with PMP cooperators was attributable to the overwhelming influence of sprayed fipronil. In a pilot field trial on Santa Cruz Island, CA we observed the effect 7 days post-spray of directly spraying a commercial essential oil product (IC2, EcoSMART Technologies, Inc) into the entrances of active colonies of *L. humile* and harvester ants, *Pogonomyrmex sp.* The purpose of the trial was to determine if the effectiveness of baiting would be improved if the ant population in an area to be baited was first reduced by eliminating all colonies located in a detailed search. We sprayed about 0.5 liters of IC2 into every ant nest entrance we could find in a 1.5 hectare site. Because of special environmental sensitivities, we were not permitted to use any synthetic pesticide. Red food dye was added to the aqueous IC2 spray to mark where the spray was applied. The dyed sprayed left a mark on the soil at the nest entrances that lasted >2 weeks.

The IC2 had a dramatic effect. All of the nests sprayed with IC2 were eliminated within 7 days, probably sooner. We left the island hours after treatment and did not return for a week. We unearthed each nest to confirm it was no longer active. We eliminated 12 *L. humile* and 3 *Pogonomyrmex* colonies, all of the colonies treated. Although previous studies indicated limited residual effect, this study showed that direct application of an essential oil commercial product was effective against ants.

Wasps. The activity of essential oil spray against yellow jackets was determined during the ant study on Santa Cruz Island described above. Subterranean colonies of the western yellow jacket, *Vespula pensylvanica*, fortuitously discovered as we searched for ant nests were treated with ca. 0.75 L of IC2 sprayed directly into the nest entrances. Three nests were discovered, the return rate of foraging wasps from the nests ranging from 15 to 45-min at 1400h. All foraging activity ceased by 7 days post-spray. The nests were unearthed and found to contain no live adults or brood. Although previous studies indicated that essential -oils are highly repellent and are therefore likely unsuitable as bait ingredients, direct spray of essential oil kills many species of hymenoptera within minutes or hours rather than days. As we theorized with ants, activity of the treated yellow jacket nests likely declined < 7 days.

Spiders. The marble cellar spider, *Holocnemus pluchei*, is a common pest in southern California. Although they do not bite, this spider and several closely related species often build to nuisance pest status under eaves, in the corner of windows, and nearly any undisturbed area around buildings. Hundreds are sometimes found on a single home. PMPs devote a great deal of effort and resources to control this pest, usually treating with residual pesticide. Synthetic insecticides labeled for spider control and commercial essential oil products containing rosemary were tested against certain species of spiders.

Spiders for the test were collected from buildings on the U. C. Riverside campus. They were collected by teasing them into 4-oz (118.3 ml) transparent styrene cups. Individual spiders were kept in the cups, covered with paper toweling, and provisioned with a 6.4 by 2.5-cm piece of bond paper on which they could rest. The spiders were fed stunned German cockroaches, *Blattella germanica*,

weekly.

Spiders were treated by spraying them with serial dilutions of aqueous test material from ca. 38 cm (15 in) with 2 pumps of liquid from a half-full 0.5 L trigger sprayer equipped with a fan nozzle. Treated spiders were noticeably wetted. The spiders were then immediately teased into a clean 118.3-ml styrene cup provisioned with a fresh strip of bond paper. Care was taken to not transfer excess liquid from the treatment cup into the holding cup. Tap water was used as a control. Treated spiders were observed every few minutes for an hour, then periodically for up to 48 h post-spray. The spiders were considered dead or moribund if they were motionless when probed or paralyzed and could not remain upright within 2 min of being turned over.

Most insecticides labeled for use against spiders were highly effective against cellar spiders at 1/20 to 1/60 their maximum label use rate. This activity at reduced rates is shown in Fig. 1 and Fig. 2. The number in parentheses is the maximum label rate and the asterisk indicates the concentration at which 100% kill was achieved. For most insecticides, even its maximum use rate is very low, often in the parts per million range. As indicated, the effective rate was considerably lower than the maximum label rate. We call this lower rate the Minimum Effective Rate (MER), and suggests this rate be used as a more environmentally friendly rate for controlling cellar spiders. The MER of 6 insecticides we tested was significantly lower than their maximum label rates. Rosemary, 2% was also effective, especially if a small amount of pyrethrins was added. These and preliminary studies we made indicate that the MER for the black widow spider, *Latrodectus hesperus*, and the brown recluse spider, *Loxosceles reclusa*, is similarly lower than maximum label rate.

The MER calls into question whether conventional insecticides applied at dramatically lower rates should be considered "essentially" green. Much of the justification for using so-called green insecticides around homes comes from mandated water runoff analyses throughout California and elsewhere where pyrethroid insecticides and others have been detected in nearly every water sample collected. Much of the detected insecticide is a result of applying maximum rates. The effectiveness of MER suggests that biologically relevant concentrations of some insecticides in water runoff may be reduced below relevant levels by applying these insecticides at their MER. This MER is what we call the "essentially green" level. It may be accomplished with low volume direct spray of the lowest effective concentration. For example, the equivalent AI contained in 10 L of spray applied at the maximum label rate would be theoretically reduced to 500 ml at 1/20 MER and to just 167 ml at 1/60 MER. The cumulative effect at reducing runoff of selected AIs could be significant.

Termites. Altriset termiticide (DuPont) may be considered a green chemistry. A synthetic insecticide, Altriset is the only termiticide that has no signal word on its label and requires no protective equipment be worn for mixing or application. It has an excellent toxicological and environmental profile, as well as being used in a manner consistent with minimizing runoff and contamination of any kind. It is toxicologically as 'green' as most registered essential oils used for insect control, perhaps more so.

Under the auspices of the Environmental Protection Agency, we undertook a mulit-year study to determine the efficacy of Altriset against the western subterranean termite, *Reticulitermes hesperus*, a widespread destructive termite in California. Ten homes documented by us to be infested with active infestations of *R. hesperus* were thoroughly treated with 0.06% Altriset by PMPs. Both the PMPs we inspected each home before treatment. Under our direction, the PMPs used their own equipment for treatment after we calibrated it. We were present at the time of applications. Altriset was applied at the prospective label rate of 10 gallons of finish liquid per 10 linear feet of trench or drilled length

completely around each structure. Where accessible, additional Altriset was applied at that rate at or near areas indoors where any live termites were found. We noted exactly where and how much Altriset was applied. We thoroughly inspected each home at intervals afterwards. The location of every point of infestation or activity was We were present at the time of applications and we assured recorded for each inspection.

Single application of Altriset completely around the 10 homes infested with western subterranean termites, and a few specific applications where termites were found indoors, completely eliminated the infestations. In one instance a follow-up treatment to a hidden area where termites were later found remedied the situation. The hidden area was along a crack in the foundation of a remodeled garage. The crack and termites were hidden under installed carpet. Once discovered, the carpet was pulled back and Altriset was applied in drill holes adjacent to the crack. Subsequently, no live termites were found there.

Based on post-treatment inspections, the Altriset treatment provided 100% control of western subterranean termites for >4 years, the length of the experiment. The PMPs involved and the homeowners were pleased with the level of control obtained.

Summary. Contrary to common misconception, direct sprays of certain essential oils are lethal to insects. Several oils and blends are very active, resulting in knockdown or death of insects within minutes. Yet, depending on the oil involved, their agonistic or antagonistic effects on specific insect hormones and nerves effects insects. They are considered 'green' or relatively safe because they have little or no known effect on mammals and other vertebrates. Because of repellency and avoidance, topically applied green chemistries are usually more effective than are residues. Such chemistry is particularly effective against sedentary insects or insects or spiders than can not avoid the spray. Some effective synthetic chemistries may be considered to be 'essentially' green vis-à-vis good activity at especially low rates of application. This 'essentially' green concept can be demonstrated by the low MER of many insecticides against spiders. Synthetic insecticides such as Altriset may be considered an effective urban green chemistry for insect control.

Fig. 1. Mortality of cellar spiders, *Holocnemus pluchei*, sprayed directly with dilutions of Termidor (fipronil).

Material	Label Rate (%)	% Dead 4h	% Dead 2d	% Dead 7d
Termidor	Max (0.06)	0.0	66.6	100
(9.1% SC)	1/2	0.0	66.7	100
	1/10 (0.006)*	0.0	66.7	100
	1/20	0.0	0.0	83.3
	1/40	0.0	0.0	66.7
	1/80	0.0	0.0	66.7
	1/100	0.0	0.0	66.7
Untreated	-	0.0	0.0	0.0

Each with 3-6 replicates H. pluchei

Asterisk indicates lowest rate at which 100% mortality was achieved.

Fig. 2. Mortality of cellar spiders after being directly sprayed with dilutions of bifenthrin or beta-cyfluthrin

Material	Label Rate (%)	% Dead 4h	% Dead 2 d	% Dead 7d
Talstar SC	1/10 (0.006)	100		
(bifenthrin)	1/60	100*		
	1/100	33.3	33.3	33.3
Tempo Ultra SC	1/2 (0.025)	100		
(β-cyfluthrin)	1/20	100*		
	1/60	33.3	33.3	33.3
	1/100	0.0	0.0	0.0
Untreated	-	0.0	0.0	0.0

Each with 3 replicates H. pluchei

Asterisk indicates lowest rate at which 100% mortality was achieved.

Research in Urban Pest Control Using Natural Products

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Natural products are chemical compounds or substances produced by living organisms. Examples include various alkaloids, phytosterols, polyphenols, antimicrobials, venoms, and other toxins. Essential oils are one category of natural products. These are generally hydrophobic liquids containing volatile aroma compounds derived from plants. These oils are "essential" in the sense that they carry the distinct scent or essence of the plants that produce them. For example, the odor of eucalyptus oil reminds one of eucalyptus trees. Essential oils are typically obtained by steam distillation, cold pressure expression, or solvent extraction. The essential oil extracts usually contain a blend of many individual compounds.

Historically, natural products, including essential oils, have been used as perfumes and other fragrances, in aromatherapy, as antiseptics and local anesthetics, as mild topical expectorants and decongestants, as diuretics, and as insect repellents and insecticides. A number of natural products have received exemption from the traditional United States insecticide registration process under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). These so-called minimum risk pesticides are exempted by the FIFRA 25(b) rule. In addition to pesticides, there is a list of inert ingredients eligible for use in FIFRA 25(b) pesticide products. The 25(b) insecticides are shown in Table 1. Note that a number of the exempted active ingredients can be complex mixtures of compounds (e.g., cedar oil, geranium oil, mint oil, and thyme oil) whereas other exempted active ingredients are very specific (e.g., eugenol, sodium chloride, and zinc metal strips). The chemical composition of mixtures may be affected by plant species, cultivar, plant age, growing conditions, etc. For example in a recent study by Singh et al. in 2005, six cultivars of menthol mint, Mentha arvensis L., were examined for the content of essential oils. Menthol was the primary constituent ranging from 77.5 to 89.3% of the essential oils, menthone ranged between 0.3 and 7.9% and isomenthone ranged between 3.7 and 6.1%. A total of 67 compounds were isolated from the six cultivars. Interestingly, not all of the compounds were found in all of the cultivars and the composition of the essential oils was different for each of the six cultivars.

Not only does the composition of essential oils differ with different species and different cultivars of plants but essential oil composition may change in storage over time. In some recent fumigation studies in our laboratory we found that fresh limonene was toxic to red imported fire ants, *Solenopsis invicta* Buren, however, following two years in storage under laboratory conditions the same test material had lost significant toxicity. Analysis of the limonene (Fig. 1) revealed that oxidation occurred even when the limonene was stored in its amber glass container at laboratory conditions and under a laboratory hood. Stability can therefore become an issue with essential oils. The addition of antioxidants, or storage under controlled temperature conditions, might be required to prolong the stability of the active ingredient.

Our recent studies have focused on the toxicity of 12 different essential oils ranging from trans-cinnamaldehyde and citronellic acid to alpha- and beta-pinene and linalool. We have examined the fumigation toxicity of these essential oils against several representative urban insect pests. These pests include the German cockroach, *Blattella germanica* (L.) (adult males), the warehouse beetle, *Trogoderma variabile* (Ballion) (adults and larvae), and the red imported fire ant (adult workers). We used a simple fumigation technique to determine the toxicity of the essential oils. We confined groups of 6 to

10 insects in 0.95 liter glass jars, applied different volumes (1-500 μ l) of technical essential oil to the lids of these jars and then sealed the jars. The jars containing the insects and essential oils were then incubated for 24 hours at 25°C. We scored mortality 24 hours after exposure and used probit analysis (SAS Institute 2010) to estimate LC $_{50}$ values in units of μ l of essential oil per liter of air.

Menthone, linalool, and carvacrol were exceedingly toxic to red imported fire ants with LC_{50} values of <0.08 µl per liter of air. Citronellic acid, geraniol, and thymol were much less toxic with LC_{50} values of greater than 500 µL per liter of air. For adult male German cockroaches, menthone, carvacrol, and the pinenes were also quite toxic and in this case thymol was also a toxic essential oil. As with red imported fire ants, citronellic acid and geraniol were relatively non-toxic. For adult warehouse beetles carvacrol, cineole, menthone, and trans- cinnamaldehyde were significantly toxic. However unlike adult male German cockroaches, thymol was not toxic. No essential oil was significantly toxic to larval warehouse beetles in our fumigation studies.

We also found that mixtures of essential oils may have unexpected and unusual results. For example, mixtures of eugenol and trans- cinnamaldehyde showed antagonistic responses. Mixtures of cineole and menthone were antagonistic at a ratio of 20 to 80%, additive when mixed 50 to 50%, but synergistic when mixed at 80% cineole and 20% menthone. Other examples of synergism include mixtures of limonene and menthone (at 80 to 20%), and cineole and limonene (at 50 to 50%).

In conclusion, many essential oils are blends of active and inactive compounds. The chemical composition of these blends may vary with plant species, cultivar, and growing conditions. Even pure single compounds may have stability issues that affect toxicity.

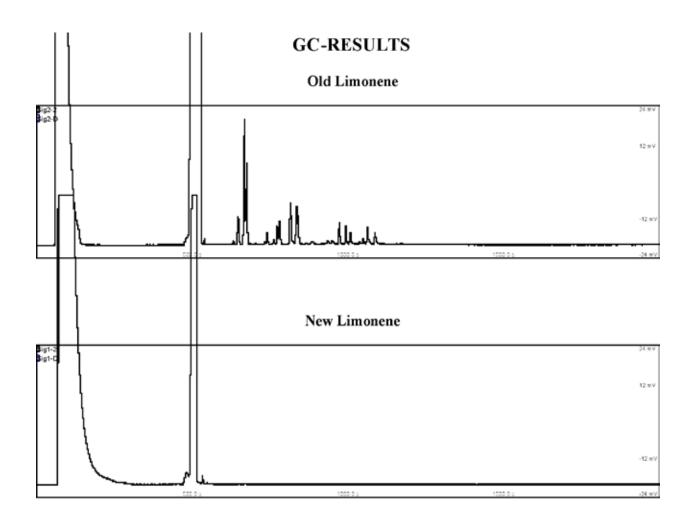
Fumigation toxicity of essential oils varies with the target pest species and developmental stage making generalizations about the toxicity of specific essential oils difficult. Mixtures of essential oils may have additive, synergistic, or antagonistic effects on toxicity. Clearly further research focused on the range of toxicity and synergistic effects of essential oils needs to be conducted.

Table 1. Active ingredients exempted under 25(b) of the Federal Insecticide, Fungicide, and Rodenticide Act. (http://www.epa.gov/oppbppd1/biopesticides/regtools/25b_list.htm)

Linseed oil
Malic acid
Mint and mint oil
Peppermint and peppermint oil*
2-Phenethyl propionate
(2-phenylethyl propionate)
Potassium sorbate*
Putrescent whole egg solids
Rosemary and rosemary oil*
Sesame (includes ground sesame plant)
and sesame oil*
Sodium chloride (common salt) *
Sodium lauryl sulfate
Soybean oil
Thyme and thyme oil*
White pepper
Zinc metal strips (consisting solely of zinc
metal and impurities)

* indicates exempt active ingredients that are also exempt from pesticide residue tolerance requirements.

Fig. 1. Gas chromatograph results showing difference in composition of "old" and "new" limonene samples. Note that the "old" limonene was stored in an amber glass bottle at laboratory conditions under a hood for approximately 2 years. Thanks to Esther Ngumbi and Henry Fadamiro for providing the GC results.



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Change in Pesticides Since 1990: Comparing Pesticide Actives, Toxicity, and Inerts from 1990, 2000, and 2010

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This review looks at the change in pounds of active and inert ingredients found on the MSDS and change in toxicity based on the product's signal word. Using purchase records for 1990, 2000, and 2010, all purchased products were converted to pounds, then divided by the number of applicators the company had at the end of each year. To get an accurate list of actives, inerts and signal words, old label books were consulted and a search using the internet was done looking for labels and MSDS sheets valid for the year the product was purchased.

CHANGES IN ACTIVE INGREDIENTS: SEE TABLE 1. The most likely reason for this 70.8% 1990 through 2010 decrease in active ingredients are:

1990-2000- A 59.5% decrease in pounds of active per year per applicator

Change from Organophosphates (OP) to Pyrethroids. Using in-house mix/usage charts I found Pyrethroids were mixed/used at about 25% the same amount of active for an OP.

Fleas were King in 1990. In the 1980s and early 1990s, fleas were king. By the mid 1990s flea control products in pet collars had greatly reduced flea populations. The reason this makes a difference? A typical flea service includes treating all the carpet inside and most of the grounds outside. Compared to the typical ant treatment of treating cracks, a flea treatment uses a lot more chemical. Unfortunately it's not until 2002 that we have any data on treatments by pest so this statement on fleas is based on personal knowledge of company operations at the time and with helping technicians in the field with pest concerns. Other, but indirect, evidence includes: From 1991 through 1995, fleas were covered in 6 different trainings at our service centers, no other pest was covered more than twice.

	Active Ingredients							
	1990 Lbs/appl/yr	2000 Lbs/appl/yr	2010 Lbs/appl/yr	90-00 % change	00-10 % change	90-10 % change		
Actives	139.59	56.49	40.70	-59.5%	-27.9%	70.8%		
Actives w/o Exempt	139.59	56.49	33.87	-59.5%	-40.0%	75.7%		
Appl	397	541	552					

2000-2010- A 27.9% decrease in pounds of active per year per applicator

Targeted application of pesticides. The mid-late 1990s was a time when Western and many other pest control companies moved to more targeted applications concentrating on cracks and other SIPM techniques, for example we discontinued routine spraying of eaves, instead using a brush (Webster) to remove spiders and their webs. During this time we repeatedly covered with our technicians the waste of chemical applied in the open where the pests are not found and where sun rays degrade residuals. Products applied in cracks would contact pests and hold up until the next service.

Starting with 2002 we can determine the number of services per applicator. Using 2002 the number of services divided by the total pounds of actives, we can extrapolate back to 2000 to come up with 0.024222 lbs of active per service. In 2010 this number is 0.016797 lbs of active per service, or from 2000 through 2010, a 30.65% decrease in pounds of active per service. If we remove the pounds of actives of exempt products, the decrease is 42.3%. Our trainings on concentrating applications based on the biology of the target pest was having an effect on amounts of product applied.

The use of Termidor for ant control. Our major pest in 2002 was and still is today ants, and it was common for Pyrethroids applied outside to "push" ants inside resulting in several inside treatments. We think Termidor's (Fipronil) December 2002 registration in California for ant control was largely responsible for the decrease in ant callbacks. Technician after technician told us the difference Termidor was making in their control of ants. Before Termidor, not only did we have more ant callbacks, each callback generally included both an out and inside treatment and often, after removing the wall plates, a dusting of walls.

CHANGES IN TOXICITY- ACTIVES. In 1990, 66.5% of the actives came from a product with a Danger or Warning signal word. In 2000, this percentage dropped to 8.2% and in 2010 to 1.5%. Overall from 1990 through 2010, the pounds per year per applicator of actives from Danger and Warning labeled products decreased by 99.3%. The change from organophosphates and the closely related carbamates, to the less acutely toxic Pyrethroids accounts for a large part of decrease in Danger and Warning labeled products. Other decreases came from customers (Schools, etc.) asking for caution only products.

CHANGES IN INERT INGREDIENTS. Did inerts go through a change similar to actives? Yes, the inerts had an overall decrease of 79.6%, going from 235.73 Lbs/App in 1990 to 48.15 in 2010. If we remove the inerts from EPA exempt products, the overall (1990-2010) decrease is 86.9%. It makes sense that if fewer containers of actives were used, less inerts would be used. But which inerts changed and why? In 1990 solvents were 87.9% of all inerts, in 2010 solvents (minus food-grade solvents) were 44.9% of inerts. Why the big change in solvents.

A number of factors pushed the industry away from products with large amounts of nonfood-grade solvents:

1) concerns about odor/toxicity. In 1990, odor was still part of many of our services. It seems odd using today's low/no odor products, but it was not long ago that customers expected an odor with their pest control service, but in the late 80's, odor for many customers became associated with toxicity and pest control companies started asking for lower odor products. Catalyst (propetamphos) was a lower-odor Safrotin and Dursban LO (chlorpyrifos) was a lower-odor Dursban 2E/4E. But what someone in the industry might consider low odor, does not mean the public will agree. Even these "low odor" products left a noticeable post-application solvent odor that some customers felt was not acceptable. As Pyrethroids came to be used in the industry, manufacturers formulated the products

not as emulsifiable concentrates (high in solvents) but, as wettable powders, flowables, microencapsulations, etc. Products low in solvents and the odors caused by them.

2) increased use of roach baits. The availability of effective roach baits led to a decrease in residual aerosols. In 1990 we purchased 24,616.33 pounds of residual aerosols, most for roach control. In the mid to late 1990s very effective roach baits came onto the market and in 2010 the pounds of residual aerosols dropped to 3,174.64 pounds.

BIORATIONAL PESTICIDES

EPA defines biorational pesticides as "inherently different from conventional pesticides, with different mode of actions and lower risk of adverse effect from its use". EPA definition is inclusive of a) biopesticide b) reduced risk Pesticide and c) minimum risk pesticides. It does not address the formulation types directly. Overall, characteristic of Biorational pesticides include:

- Different mode of action than conventional pesticides (Organophosphates, Carbamate, Pyrethroids).
- Low or no adverse risk to non-target organism including humans, wildlife, and environment.
- · Less persistence or rapid degradation in environment
- · Specific target pest.
- · Low use rate.
- Works well with IPM program.
- · Reduces the reliance on conventional pesticides.

Although pyrethrins applied into a wall void fits a biorational approach, pyrethrins space treated (fogged) into the air does not. Since we have no way of telling from raw numbers if the product was used in a wall or in the air, any pyrethrin product that also could be used to space treat was excluded in our biorational numbers. In 2010, the two most commonly used products were exempt plant oil products.

BIORATIONAL ACTIVES: AS A PERCENTAGE OF ALL ACTIVES

1990 14.79% 2000 48.91% 2010 65.07%

REDUCED IMPACT PEST MANAGEMENT. Several years ago we started testing plant oils (EcoSmart products) on a few residents. Each year we added accounts until we had several thousand accounts being treated on the outside mostly with plant oils. In 2010 we started switching all our offices over to what we call Reduced Impact Pest Management. Depending on pest pressure, our primary products are "green" and low impact; Essentria G, Essentria IC3, EcoPCO WP X, insect baits, Microcare, etc. and depending on ant pressure, a possible Termidor treatment in April or May. In the summer months, the green products are still used, but they may need to be supplemented with a second Termidor treatment, using Premise 2, and/or the application of pyrethroids. This approach is how we were able to go from 48.91% BioRational Actives in 2000 to 65.07% in 2010.

Trial of a Minimum-Risk Botanical Compound To Control the Vector Tick of Lyme Disease

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Concerns about the spread of the deer tick and tick-borne diseases, coupled with innovations in chemical control strategies, led us to test the efficacy of a rosemary oil-based botanical insecticide, Eco-Exempt® IC2, against all stages of the deer tick in southern Maine. We recorded the abundance of nymphal and adult I. *scapularis* ticks before and after applications of IC2, bifenthrin, and water sprayed by licensed applicators during *I. scapularis*' seasonal peaks. We also examined the effects of IC2 and bifenthrin on non-target insects, such as soil dwelling arthropods and pollinators, via pitfall trap and plot count surveys.

Materials & Methods

Impact on Ticks -- In 2009, we established fifteen 70 x 70 m spray grids within oak forest stands in the Cape Elizabeth, ME (43°34'N, 70°13'W). For both the nymphal and adult experiments, three grids were designated IC2 grids and three bifenthrin grids (total 12 grids). Three additional grids sprayed with water served as reference grids for both experiments. We randomly assigned spray treatments to grids then collected ticks by flagging vegetation. Ticks were sampled at weekly intervals before and after the spray treatments to account for tick seasonality as well as treatment effect. The nymphal and adult sprays were timed to coincide with the peak summer and fall seasons of nymphs (June 15) and adults (Oct. 19), respectively. IC2 (4oz./gal), was applied by high-pressure spray at 4 gals./1000 sq. ft. and bifenthrin (0.06% as SPECKoZ®) at 1 oz./1000 sq. ft.

Non-Targets -- Coinciding with summer spray treatments, we assessed the impact of the two acaricides on abundance of ground-dwelling arthropods caught in pitfall traps in 70 x 70m spray grids. We also assessed treatment impact on abundance of bee pollinators (such as *Andrena* and *Bombus*), as well as non-bee pollinators (such as long-horned flower beetles, *Typocerus*), and total insects visiting flowering plants and their nests in the 70 x 70 m spray grids. For the soil arthropods we deployed 1 pair of pitfall traps in each of the five 10 m² plots in each nymphal experiment spray grid. Traps were filled with 30 ml 70% ethanol and collected after 24-30 hours with sampling approximately on the same schedule as nymphal tick sampling in 2009.

Results

Ticks: Summer application

- Nymphs: Pre-spray IC2, bifenthrin, and reference nymph counts were greater than post-spray counts. Model fit was adequate (Pearson 2/DF=1.1, residuals approximately 2 distributed and 95% <3.8). On all post-spray sampling dates out to 1yr, nymph counts on IC2 and bifenthrin plots were equivalent, and lower than on reference plots. Larvae: There were fewer post-spray larvae in both years on sprayed plots (reference > IC2 = bifenthrin) on sampling dates during the larval seasons of 2009 and 2010.
- Adults: There were fewer post-spray adults in both years on sprayed plots (reference > IC2 =

bifenthrin) on sampling dates during the adult tick seasons of 2009 and 2010, this effect was still evident at 1.25yr post-spray (October 18th, 2010).

Autumn Application

- Adults: Relative to reference plots, ticks were substantially reduced on plots treated with IC2 and bifenthrin (treatment × period interaction P < 0.0001). Model fit was adequate (Pearson 2/DF=1.0, residuals approximately 2 distributed and 95% <3.8). On pre-spray plots, adult counts among treated and reference plots did not differ. One week post-spray adult counts on IC2 and bifenthrin plots were zero and remained zero or close to zero through the spring of 2010. One year later, adult counts on IC2 plots were still lower than on reference plots but higher than on bifenthrin plots. After the adult spray application on October 21, 2009, we also sampled for nymphs (June 1 and July 15, 2010) and larvae (August 27, 2010) in the adult spray plots.
- For both dates, mean nymphs/ m^2 in IC2 and bifenthrin plots were zero and significantly less (P < 0.05) than in reference plots, 0.1 (SE=0.07) and 0.1 nymphs/ m^2 (SE=0.03) for June 1 and July 15, respectively. On August 27th mean larvae/ m^2 in IC2 (0.15 SE=0.08) and bifenthrin plots (0.03 (SE=0.03)) were equal and significantly less (P < 0.05) than in reference plots (4.1 larvae/ m^2 SE=1.79).

Non-Targets: **Pitfall trap arthropods (70 x 70 m grids)** -- The orders Coleoptera, Hymenoptera, and Collembola were selected for analysis and represented 12%, 5%, 42% respectively, of all arthropods collected in this study.

- There was a significant treatment × date interaction for Coleoptera (P = 0.02). Model fit was adequate (Pearson 2/DF=1.0, residuals approximately 2 distributed and 95% <3.8). Pre-spray, there were no differences among treatments. One week post-spray, a decline in abundance on all plots is evidence of a regional temporal decline, but in both IC2- and bifenthrin-treated plots, relatively greater declines indicated both acaricides caused a significant reduction in beetle abundance (treatment × date interaction P < 0.05 July 5th vs. 12th). After July 12th (3 weeks post-spray), only bifenthrin demonstrated a significant reduction in abundance from pre-spray levels. In September and October abundances across all treatments declined and no significant differences were observed among treatments. Most Coleopterans were distributed among the families Carabidae (42%), Staphylinidae (20%), Nitidulidae (12%), and Scarabaeidae (10%).
- A similar treatment × date interaction (P < 0.0001) was observed for the Hymenoptera as seen in the Coleoptera; pre-spray, there were no differences among treatments but post-spray treatment effects were evident. Model fit was adequate (Pearson 2/DF=1.0, residuals approximately 2 distributed and 95% <3.8). One week post-spray, IC2 and bifenthrin both demonstrated fewer Hymenoptera (treatment × date interaction P < 0.05 July 5th vs. 12th). At week 3 (July 26th), both IC2 and bifenthrin resulted in a significant reduction in total abundance of Hymenoptera relative to the reference treatment, but numbers recovered in IC2 plots by the end of August. Fewer Hymenoptera were seen in bifenthrin plots throughout the season until October. The two most abundant families for this order were the Formicidae (66%) and Platygastridae (13%).
- For Collembola there was a significant treatment \times date interaction (P = 0.006) attributable to the acaricidal treatments one-week post-spray, but beyond this week neither IC2 nor bifenthrin had a negative impact on abundance in this order. Model fit was adequate (Pearson 2/DF=1.2, residuals approximately 2 distributed and 95% <3.8).

Pollinator and pollinator nest count/survival (70 x 70 m grids) -- On the nymphal spray grids, there were no significant differences among the grids for the number of nests produced (n = 3 nests per treatment, Kruskal-Wallis P = 0.16). Bee/non-bee pollinator/total insect abundances on 70 x 70m spray grids were difficult to interpret given weather-driven sporadic sampling.

Pollinator counts on 2 x 2m plots -- There were no significant treatment × date interactions for bee pollinators, non-bee pollinators, and total insects (all P > 0.05, model fit adequate (Pearson 2/DF 1.0-1.2, residuals approximately 2 distributed and 95% <3.8). Only date was significant in all three models, meaning neither IC2 nor bifenthrin had a negative impact on these non-target arthropods. Date differences were driven by greater abundances in August 2010; summer 2010 was drier and sunnier than summer of 2009.

Phytotoxicity -- Phytotoxic effects of IC2 application were noted on trees and shrubs (Prunus, Vaccinium, Quercus, Acer, and, Rubus, Picea spp), ferns and mosses (Sphagnum, Pteridium, Osmunda, and Onoiclea spp), and herbs and forbs (Trientis, Seline, and Aralia spp). Effects were not quantified but, by visual inspection the following year, did not appear long-lasting.

Conclusions

Safeguarding human health and the environment -- Eco-Exempt® IC2, a minimal risk botanical product containing rosemary oil, is as effective for months after application as the synthetic pyrethroid bifenthrin in controlling nymphs and adults of *Ixodes scapularis*. In addition, the compound, when applied pre-eclosion, also appears to inhibit larval emergence. While IC2 may negatively impact three orders of non-target insects (Coleoptera, Hymenoptera, Collembola), this effect was less than that of the synthetic pesticide, and populations were greatly restored within one month of application. Neither synthetic nor botanical compounds were shown to harm pollinators visiting flowering plants. One negative impact of IC2 as applied by high-pressure sprayer is a degree of phytotoxicity, which, while evident post-spray, appears not to be permanently harmful to vegetation. To minimize the number of comparisons to be made, no adjuvant for IC2 was used in our study; its addition, as recommended by the manufacturer, might allow reduction in IC2 concentration to a level that should also reduce phytotoxicity without loss of effectiveness.

Environmental impact -- Recognizing the public's concern about the potential damaging effects of synthetic pesticides on non-target species, the environment, and human health, there has been a surge of interest and research in the development of effective, botanically-derived, minimal risk acaricides, particularly as a component of a multi-faceted IPM approach involving habitat modifications and management of white tailed deer, the tick's primary reproductive-stage host. As even more effective botanically-derived acaricides are discovered, years may take place before they are commercially developed and marketed. The research reported here demonstrates that an effective, minimal risk tick control product is already available.

Economic Benefits -- It appears that the major challenge to the botanical acaricide industry is not so much improvement of effectiveness as it is to reduction of cost. For example, the price of sufficient IC2 product to high-pressure spray one acre would be roughly \$490, while that of an equivalent amount of bifenthrin would be ~\$27. While this differential would be less important on small peridomestic plots, considering the total costs of application, it rapidly becomes more significant as treated areas increase. A mitigating factor that has yet to be explored for botanicals, however, is the possibility that, as reported for synthetics, a single application to eradicate adults in the fall may sufficiently reduce ticks year-round, particularly if combined with other components of an integrated tick management program. Other benefits of IC2 application affect the balance of environmental protection and costs. Being a food grade compound, there are few concerns about the return of pastured animals to grazing after application.

Implementation of IPM -- We believe that this field trial and the one that preceded it confirm the acaricidal efficacy of this rosemary-containing compound – and that this effectiveness invites further

studies to determine the most appropriate times (or time) for its application relative to the phenologies of the nymphal and adult stages of *I. scapularis*. This knowledge will guide just how its use can be most effectively and economically incorporated into an integrated tick management plan for use on residential properties, farms, recreational areas, public properties, schools, and outdoor industrial sites.

A complete report of this project is available at http://projects.ipmcenters.org/Northeastern/Funded-Projects/ViewProject.cfm?projectID=1428180

Synergistic Insecticidal Action of Plant Essential Oil Mixtures

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There is renewed interest in evaluating properties and value of plant extracts in pesticide formulations. Plant essential oils are comprised of a complex blend of terpenes and their derivatives. These plant terpenes have been shown to elicit adverse effects in arthropod pests in the form of repellency and/or toxicity. The specific properties of an extract and the contribution from its terpene compounds are important for product development, performance standards, sourcing of quality ingredients, supply and production. Mode of action studies involving terpenes identify structure activity relationships which occur at selected receptor sites in insects, and the resulting potency of such terpenes and synergistic properties therein. These mode of action studies have demonstrated targeted, safe action of plant terpenes at the octopamine receptor site in invertebrates, and high throughput octopaminergic screening systems have been developed to further evaluate this site of action. Alternative modes of action and effects on signal transduction pathways have also been investigated to better understand the specific contribution of these plant terpenes. These data have been correlated by repellency and toxicity trials in various laboratory settings. Synergistic action of plant terpenes has also been demonstrated with conventional pesticide chemistries utilizing distinct modes of action. A thorough understanding of plant essential oils and their constituents will assist in future development of plant based pesticides that are useful and effective in society.

Push and Pull Strategy in Control of Filth Flies in Urban Settings

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Stable flies (*Stomoxys calcitrans*) and house flies (*Musca dometica*) are two of the most serious pests commonly found in agricultural and urban settings. Although stable flies are primarily around livestock animals, they sometimes observed to attack pet animals and humans in rural and recreation areas. Their feeding on livestock animals can led to increased disease incidence, reproductive failure and reduction of meat and milk yields, with estimated economic loss up to 2 billions of dollars in beef and dairy industry. The housefly is a well-known cosmopolitan pest of both agricultural and urban environments. Houseflies cause annoyance to humans and animals and vector many medical and veterinary pathogenic organisms. The use of insecticides become a common control method, but, both flies have developed resistance to several insecticide classes. The current presentation reports our recent discoveries on the identification of novel botanical-based fly repellents, oviposition deterrents and larvicidal activities, as well as some potential food-grade fly attractants. I will also discuss the future development of Push-Pull strategy using these potential attractants and the behavioral inhibitants/repellents in fly management.

The present study reports the discovery of several natural substances associated volatile compounds that may be used as chemical cues for adult flies for host and oviposition location. Among them, 1-octen-3-ol, phenol and cresol and sulfide-related compounds elicited significant EAG responses from both species, and being further shown with strong behavioral responses. Both liquid and granules of the repellent formulations have been developed, and tested with significant repellency and deterrence against both adult and immature of both fly species.

Plant Essential Oil Properties and Applications in Low-Impact and Pest Management

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The Georgia Structural Pest Control Commission defines *Green Pest Management* as "a type of pest control service that employs an Integrated Pest Management (IPM) approach while utilizing fewer of the earth's resources as part of a larger effort to reduce human impacts on the environment" (agr. georgia.gov). Product choice in the offering of "green" pest management services by pest management companies are mainly represented by those containing plant essential oils. Pest management companies committed to "green" services have seen, in some cases, double digit growth in these offerings over the past 5-7 years. It is worth noting that "green" services are offered almost exclusively to residential customers for the control of household pests. The use of essential oil-based products is much more limited in commercial pest control.

Consumers of residential pest control services find "green" pest management services attractive. Results of a 2007 Harris Interactive poll on "green" pest services found that:

- 59% indicated that they would pay a premium for "green" pest services in particular.
- The top three characteristics of "green" pest services were, in descending order of importance to homeowners: less toxic, biodegradable, and all natural ingredients.
- Women were more likely than men to value "green" qualities and to be willing to pay more for such products and services.
- Men and women were equally-likely to seek out "green" products or services, but men were more likely to select "green" services for the benefit of the environment, while women were more likely to do so for their family.
- Women were also more likely than men to believe in, seek out, and pay a premium for "green" pest services.

Research on plant essential oils has revealed some general characteristics of these chemicals for use in urban pest management:

- Contact Toxicity: Low to Moderate in comparison to traditional actives.
- Topical Toxicity: Low to Moderate in comparison to traditional actives.
- Fumigant Toxicity: Moderate to High in comparison to traditional actives.
- Repellency/Deterrency: High in comparison to traditional actives.
- Volatility: High in comparison to traditional actives.

In consideration of the use of essential oils for the management of ants in residential accounts: Even if essential oils had toxicity profiles comparable to traditional actives, their repellency & short residual (volatility) would render them less effective in comparison to actives that are not repellent (repellency trumps contact toxicity) and that have a longer residual life. As direct-spray topicals, essential oils clearly show some toxicity to some urban pests.

Question: How do we explain the growth in residential pest control services that are based on a set of active ingredients that show little contact toxicity and are highly repellent and ephemeral (volatile)?

Answer: In part, I believe the answer to this question can be best explained by the Placebo Effect. A *Placebo* is "a pharmacologically inactive substance (or procedure) that can have a therapeutic effect if administered to a patient *who believes that he or she is receiving an effective treatment.* A Placebo Effect is not something that occurs naturally. It must be manufactured in the sense that it occurs only in the presence of therapeutic intent (or the perception of such intent)" (Bausell 2007).

The response to my Placebo argument is likely dependent upon your point of view. Given the general characteristics of plant essential oils (outlined above) many researchers, quantitative by nature because of their training, find it difficult to explain the use of essential oils in pest management programs, especially in comparison to the benchmark activity of more traditional active ingredients. Their thought process is, in part, geared towards explaining the efficacy of pest management programs in light of mortality-induced reductions in pest numbers---i.e., a researcher's perception is that a customer of a pest control service can only be happy if there are no pests, and the chemical tools needed to rid a site of pests must eliminate (kill) it. The point of view of the practitioner, however, is somewhat more flexible. The practitioner's perception of success is a gratified customer. As outlined above, and described in Bausell (2007), it may be that customers of "green" services are, in part, reacting to a Placebo treatment. But, does it matter? Ultimately, does it matter whether we explain the growth in "green" services as the result of (a) product effectiveness or (b) Placebo? At the end of the day, the residential customer is happy.

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SYMPOSIUM: VALID AND PUTATIVE <u>HUMAN SKIN INFESTATIONS</u>

Delusory Parasitosis: A Veterinary Perspective

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We cannot, as vets, ask a cat if she is itchy; we depend upon our own objective findings as well as the history dictated to us by the owner. If the skin appears inflamed, hyper pigmented, or we visualize the parasites in question – we know something is truly agitating the animal. If none of these symptoms are present, we must trust that the history presented to us is the truth and that the client has the animal's best interests at heart. They just want them to feel better and return to their normal lives.

This system breaks down when a client suffers from delusory parasitosis by proxy. Delusory parasitosis is the belief that one's own body or environment is infested with 'tiny critters' of countless descriptors. Some sufferers may even tear their skin open, hoping to find one of the offenders to show their doctors. An indication by proxy causes these delusions to fall upon a pet or even a child. The sufferer goes to extreme lengths, seemingly selflessly, to provide the animal with any and every treatment available to stop the infestation. The client also collects numerous samples of anything from skin scrapings to dust particles found in the household for the vet to examine microscopically, convinced they've captured the imaginary culprit. When no treatment can be found to get rid of the infestation, some owners may switch vets, or turn to other specialists. Others, in their frustration, schedule the healthy animal for euthanasia. This more often occurs during odd hours at a nearby emergency clinic, with a doctor unfamiliar with the animal's extended healthy history. They choose a vet that is unlikely to talk them out of their decision; they want the infestation out of their house, out of their lives for good. Unfortunately, a mental disease cannot be cured by simply removing a fixation. Sufferers of DP will find something new to fixate on or they will simply believe that the infestation has moved off of their pet and into their house, onto themselves.

As briefly mentioned before, some of the trademark symptoms of a sufferer of DP by proxy are relentless pursuit of an infestation that should have been taken care of by initial treatments. The client returns again and again with their pet, insisting that more and more tests be done, more treatments be administered, no matter how extreme. They spend endless hours on the internet researching their bug of choice and discover more imaginative ways to capture the creatures. They present their vet with jars of water, Petri dishes (jar lids) of water, scrapings of skin, dust particles from the house, and strands of string they may find on the animal or near the animal – all for microscopic examination. Blood work is sent out, specialists are enlisted, even those of other fields: parasitologists, public health administrators, even entomologists. The 'bugs' seen by the owner may be described in numerous ways, some of the most common are: skin-colored or black bugs, worms infesting the bowel or any orifice, and strands or fibers infesting the skin of the animal. Without fail they are always called "tiny" or "barely visible" which makes it all the more rational that the parasite is so difficult to isolate and capture. Frequently, they too are affected by the 'bug' that is causing their pet to itch and show their lesions to the vet hoping it will help with diagnostics. Lesions are typically found in places easy to reach, like the limbs, upper back, and trunk regions.

A colleague once said to me of DP, "these cases literally drain the life out of you," which couldn't be more true. The sufferer is dissatisfied that the infestation isn't going away, often getting extremely angry with their vet that they aren't smart enough to figure out a proper diagnosis. The pet may be made increasingly anxious by increasing trips to the vet and may start to actually develop dry and itchy skin as a result of over-bathing by the owner. Finally, the vet is left constantly clamoring to treat an invisible, asymptomatic, disease, dedicating more and more of her time to the unsolvable case. The reality is that a veterinarian isn't a human doctor. Although we are certainly taught how to maintain a good relationship with our clients, how to deal with distraught owners, and how to provide comfort to the grieving, we are not taught to evaluate the psyche of our clients. In cases of animal cruelty, the owner becomes suspect relatively quickly; in cases of delusion, the owner appears to be deeply concerned over the plight of their animal. Why should we suspect them of anything more than caring? Sufferers of DP appear innocent enough until the fourth, fifth, sixth visits, when the case has already progressed too far. Increasing awareness of the disease in veterinary practices may allow practitioners to recognize these symptoms early in their clients and avoid putting the animal through unnecessary treatments. In an effort to increase understanding of the disease we will review several case studies submitted to us by local veterinarians. We created a questionnaire with as little bias as possible and directed it by fax to over 400 clinics through Georgia and Alabama. Even with the promise of a pizza party to the first three responders, we received all of three responses. One response was simply a no; they had not had any experiences with sufferers of delusional parasitosis. The other two are laid out below along with our own experience at Hollyberry Animal Hospital.

The first two cases were presented by Dr. Mary Schick of Atlanta Animal Allergy and Dermatology, the first of which was a case of mistaken diagnosis. By increasing awareness of a mental disease. there's always the problem that the client that really does have a mysterious infestation, may be accused of simply suffering from delusions. This is one such case. A 51-year old woman presented to the veterinary dermatologist with lesions all over her neck and chest. She was wealthy and married to a high profile man of the community. Her husband was entirely asymptomatic, which is what drove her previous vet to send her to a psychologist. She and her husband both shared a bed with their two decadently long-haired white cats. The woman described them as frequently being 'draped across their shoulders and necks' during the night. After treating the woman for Morgellon's disease for two years and seeing no decrease in the lesions around her neck, the psychologist referred the woman to Dr. Schick. Both woman and cats had endured two years of pruritic skin lesions. Skin scrapings came up negative and a skin biopsy was ordered. Finally the parasite in question was revealed: Cheyletus eruditus was found burrowing pseudo-tunnels through the keratin layers of skin. Client and cats were treated with lime sulfur baths for 8 weeks and the infestation resolved. This case is a perfect example of what can occur when every sign would point towards delusion and as a result a real disease was left untreated for years.

Case two is a very typical case of delusional parasitosis, carrying with it many of the trade-marks that are associated with the disease. A 40-year old, unmarried, RN presented to the dermatologist with her beloved dog. She was in the midst of a very nasty divorce; stressful or traumatic life events are often a trigger for delusional parasitosis, and most sufferers are middle-aged single women. When she walked into the office, her appearance made the majority of Dr. Schick's staff wonder if the woman was using her RN status to self medicate. "All of us thought – drug abuse" says Dr. Schick. The woman presented her dog to the doctor and launched into the story of her divorce and that ever since the whole process had begun, she'd allowed the dog to sleep in her bed with her to give her comfort and solace. Showing the doctor her own lesions along her arms and legs, she described the tiny 'fibers' that must be passing from the dog's skin into the sheets of her bed and affecting her. She described them as wriggling into her skin, and burying themselves deep inside, although she did admit

that they were inanimate. She brought with her several zip-locked bags of the so-called 'fibers' which appeared simply to be bits of lint and strands from fabrics in her bedroom. She described the dog as suffering greatly from the strands and that he was endlessly itchy. Upon examination, Dr. Schick found no evidence of inflamed skin, no lesions present, no fibers obviously present, and no redness in the ears or allergy symptoms. Skin scrapings were negative. The dog appeared overall to be in near perfect health. Oftentimes, skin scrapings may come up negative even though a parasite is present so the woman was offered 8 weeks of lime sulfur baths for herself and her dog. The dog remained asymptomatic and healthy and the woman continued to suffer from itchy lesions caused by an infestation of 'fibers'. She continued to bring in bag after bag of fibers and the staff at the clinic began to identify her lesions as closely related to those resulting from amphetamine and cocaine abuse. When it was suggested that she seek the advice of a psychologist, the woman vanished. This, unfortunately, is typical. Forty percent of all delusional parasitosis cases not only do not resolve but worsen, even with psychiatric assistance.

Another case was presented by the North Fulton Animal Emergency Clinic, although there is very little information about this case because the woman also suffered from paranoia and a paralyzing phobia of men. The staff of the clinic was made up of mostly men and the woman refused to come inside with men present. When a woman staff member was enlisted to go outside and talk with the woman, she described tiny, infinitely small brown bugs crawling in her dog's fur and on his skin. The tech examined the dog and found none. The dog's skin and fur also appeared healthy and unaffected. The woman told them that she could only come at night because there were fewer men outside at night and that the bugs came out during the night. The bugs made her and her dog both very itchy. The woman was asked to enter the building so that additional testing might be performed on the dog. She refused because there were men in the building. She came back several times over the next month hoping to find a night when no men were present. Unfortunately for her, there was always at least one man on shift that night and so the woman never entered the building and eventually stopped coming.

The final case to be presented is a case first dealt with by myself and secondly passed on to Dr. Nancy Hinkle, who is still dealing with it to this day. A mid-aged, single, woman presented at Hollyberry Animal Hospital in early 2011 ago with her parrot. The parrot was suffering from a very mild case of feather picking which appeared to be simply a result of seasonal molting, which requires no treatment and should resolve itself in weeks. The woman was distraught over the feather picking and was convinced she'd seen tiny mites in the bird's feathers. These mites must be the source of the agitation and they must be removed so that her bird could have some relief. Although a molting cycle was suspected, and the bird appeared overall healthy, skin and feather scrapings were taken and visualized microscopically. The slides were negative for mites but since such tests cannot be termed absolutely negative, the bird received two treatments of Ivermectin, to be given 10 days apart. Following treatments the bird was re-examined and the owner brought with her several jar lids of water for me to look at under the microscope, insisting that they contained the mites. She confided that she believed they only came out at night and that she could see them floating around her house when she shined a flashlight into the darkness. The appearance of the parrot had improved slightly and feather picking seemed to have decreased as would be expected with a seasonal molting cycle. Unfortunately, the owner was so consumed with capturing the source of her bird's 'problem' that she seemed unable to realize that whatever problem there may have been was steadily resolving itself. This behavior continued on for months, her bringing in more and more samples, many of them being tiny fabric strands that she'd captured floating around her home, and I continued to humor her by allowing her to use my microscope. This 'problem' that wasn't actually a problem was taking up so much time, time that should have been devoted to other animals with legitimate problems. Exas-

perated, I turned to my husband, who works in the VRZB branch of the CDC. He introduced me to Morgellon's disease and told me that they received many calls from people reporting odd infestations on a weekly basis and offered me the number of an associate in the special pathogens branch of CDC. This pathologist urged me to understand and sympathize with the client. Her delusions were very real to her, she wasn't trying to waste my time or be a nuisance. She felt that we were resolving this problem together. This brought me closer to understanding what a unique position a veterinarian is in as opposed to a medical doctor, a therapist, and even friends and family of the sufferer. Unlike the others, we are not judging the health of the owner, we are judging the health and improvement of the animal. This places the sufferer beside the vet, side-by-side we are working together to figure out what their pet needs – we are cohorts on the cusp of figuring out a mysterious disease. We are the professionals that initially trust them without question. This level of trust is of utmost importance to the owner of the animal, they need to feel that they are getting closer to answering the mystery of the disease. Although, when the disease is a product of delusions, we can never answer that question to their satisfaction.

The special pathogens associate then referred me to the local UGA Cooperative Extension agent for additional help. This agent informed me with a sigh that they had already been on this case for quite some time and they had offered the woman every explanation available and she had not been satisfied. Ultimately, I was referred to Dr. Nancy Hinkle and she took on the case. The owner had declined an initial blood wellness profile on her parrot and was still convinced that the pathogen could be found via microscopy. Because she was taking so much of my time from other patients, I was forced to begin charging her for use of the microscope. This put a stop to the influx of samples and she directed all of her questions solely to Dr. Hinkle. At present she has her own microscope with a video camera hooked up to it. She documents the movement and reactions of dust strands upon applications of water and hydrogen peroxide treatments. She films what would most accurately be described as Brownian motion and truly believes that the 'jumps' and flicks of the strands must mean that they're alive and burrowing into the skin of her bird and herself.

There is little a veterinarian can do to convince a sufferer of DP that there are no bugs on their animal and that they need to have themselves evaluated by a mental health professional. Showing numerous negative results and enacting treatments that would have resolved a problem, if there was one. doesn't take the itch out of their skin. The only thing we can do, as vets, is raise awareness of the existence of this disease, the causes of the disease, and keep the safety of the animal in mind. As a cohort of the sufferer, working alongside them to help treat their ailing animal, there is a level of trust that might be taken advantage of. There's a real possibility that we may be the part of the professional community that has the best chance to reach out to that sixty percent that can experience successful remission of the disease. By simply going over the treatments done, the negative results, and the reality that all that could have been done, has been done, we may be able to reach out to them and convince them that there's a possibility of mental illness. This doesn't make them bad people or irresponsible pet owners; they're compassionate and concerned which is what every vet appreciates from their clients. This honest approach may help prevent euthanasia of the healthy pet and give the owner the peace they've been seeking. Some will never experience that peace but if we can bring it to some, we should do everything we can to recognize the disease and proceed cautiously and patiently.

Procedures and Guidelines for Handling Extension Clientele with Mystery Bug Infestations

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In the United States, insect diagnostics is still provided by most state extension services as a free service. Many diagnostic samples received by extension entomologists from the public are accompanied by descriptions of attacks or irritation caused by something presumed to be an insect or mite. Subsequent examination of these samples often reveals no evidence of biting insects or arthropods. Such samples can be classified as "mystery bug" infestations. Mystery bug cases commonly involve samples of clothing, lint and debris from around a home, or samples taken from on or in a client's body. The time required to assist with mystery bug cases can be substantial, taking away from other extension duties and often resulting in no satisfactory solution for either the sample submitter or the extension specialist. This paper quantifies some of the experiences of extension entomologists with mystery bugs in Texas, and suggests some rules to facilitate efficient handling of mystery bug cases.

Taxonomy of Mystery Bug Cases

For practical purposes, mystery bug cases can be classified into one of three broad categories: (1) cryptic arthropod infestations (actual pest infestations), (2) illusions of parasitosis (non-pest explanations unrelated to mental illness), and (3) delusions of parasitosis (non-pest explanations related to mental illness). The role of an extension entomologist is to accurately identify and suggest solutions for arthropod infestations, hence only mystery bug cases that fall into the category of cryptic arthropod infestations will be legitimately an entomologist's responsibility. Nevertheless, it is critical for the extension entomologist to be aware of the existence of, and distinctions between, these three categories.

Cryptic arthropod infestations include insect ectoparasites of humans and pets such as head lice and bed bugs, as well as bird and rodent mites, chiggers, fleas and ticks. These pests can be difficult to detect and diagnose; but in most cases a trained pest management specialist, or even a homeowner, should eventually be able to locate and collect samples containing one of these pests. Cryptic pest samples are usually obtained from pest management professionals, samples sent by a client, or from sticky cards placed in the home or office. Diagnosis of microscopic human- or pet-transmitted mites, such as scabies and ear mites (Alexander 1984), is generally beyond the expertise of the entomologist. Such pests must be diagnosed and treated by a medical doctor or veterinarian.

The term *illusions of parasitosis* was coined by Waldron (1972) to describe a real physical or medical condition, or an emotional state, that misleads a person into thinking that he or she has an insect infestation. Causes of illusions of parasitosis include certain medical conditions (e.g., carcinoma, diabetes, and hyperthyroidism), side effects of prescription or non-prescription drugs, alcoholism or drug abuse, environmental irritants or allergens, the power of suggestion acting within a group, or difficulty shaking the sense that a previous (real) arthropod infestation is not resolved (Hinkle 2000). Even though illusions of parasitosis includes certain psychological phenomena, such as the power of suggestion, it should not be considered a form of mental illness, and normally can be resolved by reason or the assurances of a professional that an actual infestation is no longer present. Although the extension entomologist may provide a useful service by confirming that no legitimate pest concern exists, finding the solution to a case of illusions of parasitosis is frequently a job for a medical or environmental health professional.

When an actual arthropod infestation cannot be detected, and physical, simple psychological or medical causes for biting or crawling sensations have been ruled out, mental illness must be considered. *Delusions of parasitosis*, also known as Ekbom's syndrome (Ekbom 1938), is a very specific type somatic delusion in which the victim falsely believes that insects or other arthropods are crawling on, biting, burrowing into the skin, or otherwise invading the body (Hinkle 2000). Because a delusion is a false belief that cannot be corrected by logic or reason, advice offered by an entomologist assuring a delusional client that he or she has no real insect problem will be rejected. Nevertheless, delusional patients will often return repeatedly to extension staff with samples, certain that the latest sample holds positive proof that their infestation is real. It should be noted that delusions of parasitosis may be secondary to other psychiatric conditions, such as schizophrenia, obsessive compulsive disorder, depressive disorders, etc. (Freudenmann and Lepping 2009). For the purposes of the practicing entomologist, it seems sufficient to classify all such disorders under the heading of delusions of parasitosis.

Survey of Texas Entomology Extension Staff Regarding Mystery Bugs

In Texas, diagnostic samples are commonly sent to offices of over 30 extension and research entomologists, some of whom work in large metropolitan areas, and some of whom are assigned to rural communities. In May 2012 I conducted an informal online survey (http://www.surveymonkey.com) of Texas AgriLife Extension Service entomologists in Texas concerning their experiences with mystery bug cases. Invitations were sent to 36 current, or recently retired, extension entomologists (including the author). The sample set included on-campus faculty with medical veterinary or urban entomology assignments (2), extension entomology specialists (13), extension program specialists with urban-oriented assignments (4), and county-level, entomology (agricultural) extension agents (15). Of the 34 invitations sent out, I received 24 responses (70.6% response rate).

All of the extension entomologists surveyed were familiar with, but not necessarily comfortable handling, mystery bug cases. When asked about their familiarity with the condition of delusory parasitosis, 58.3% were "very familiar" and 37.5 were "mostly familiar". Only one respondent was "slightly familiar" with the condition. When asked about their comfort level to professionally handle calls and samples from delusional clients, 26.1% said they were "mostly uncomfortable". Fifty-two percent were "very" or "mostly" comfortable.

Prevalence of illusions and delusions of parasitosis in the general population is not well appreciated by the public or extension administrators. When entomologists were asked how often they determined that samples or inquiries from the public were determined to be "unrelated to an actual insect or arthropod problem", the most common response was "7-12 times a year" (39.1%) followed by "1-6 times a year" (30.4%) and "less than one a year" (13%). Two respondents said they received samples more than once a month, and two respondents had never received such an inquiry. When asked how often they received samples that they believed were likely related to someone suffering from delusional parasitosis, 39.1% responded "1-6 times a year", 30.4 responded "less than once a year", 8.7% responded either "more than once a month", "7-12 times per year", or "never". One person responded that they didn't know. Based on these responses, it is likely that 100-200 mystery bug samples, or more, are submitted each year to Texas extension entomologists. Of these perhaps half or more were suspected of being delusional in nature.

Having a medical professional to whom clients can be referred can be a valuable asset to extension entomologists. Only 9.1% respondents, however, knew of a medical professional to whom they were comfortable referring clients (n=22). When asked what resources would be most helpful to help them work with samples and questions concerning purported cryptic insect and mite problems, the most common resource mentioned was a "list of medical professionals willing to work with mystery

bug cases" (16/19 respondents), followed by "resources for clients on diagnosing bug bites" (12/19 respondents), "references for self on biting insects/delusions" (8/19 respondents), "training for self on how to recognize/respond to mental health issues" (8/19 respondents), "resources for family members" (7/19 respondents). More "laboratory/diagnostic training for self on mites and biting insects" was last, checked by only 4/19 respondents.

Guidelines for Handling Mystery Bug Cases

Because of the potential for mystery bug cases to take an inordinate amount of an extension entomologist's time, it is essential to develop efficient and practical procedures for handling calls and samples. I have formulated the following rules for handling mystery bug cases over a period of 20 years, and offer them as suggestions to other entomologists:

Develop a professional, "efficient" response. Because of time constraints, when possible, avoid direct contact with clientele submitting mystery bug samples. I have found email or telephone to be the preferable mode of communication with sample submitters. While this rule should be flexible, the entomologist must understand that if a client is truly delusional, no amount of face-to-face time will convince the client that insects or mites are not the problem. Our office uses the same standardized form for all insect and mystery bug samples: http://citybugs.tamu.edu/files/2011/10/Entomology-Plant-Pathology-Form-2011.pdf. Clients who contact our office ahead of a visit are encouraged to download a copy of the identification form from our extension website (for mailed-in samples) or told that they will fill out a form and leave their sample at the receptionist's desk.

Do not accept medical samples. Medical samples include bodily fluids, fecal material, blood, skin scrapings, clothing, etc. Most extension offices are not equipped to safely handle medical samples, including samples sent from hospitals. All extension support staff should be aware of this rule, and it must be strictly followed and emphasized at the reception desk, over the phone and on websites.

Limit the kind and number of samples that will be accepted. Vacuum cleaner bag contents, containers with more than approximately 100 ml of liquid, or large artifacts that cannot be handled or stored conveniently, should generally not be accepted. When pre-screening clientele we emphasize the need for a discrete (insect-only) sample. Environmental samples, such as sweepings from floors or windows, require an inordinate amount of time to screen, and are rarely productive. We also limit clients to five bags or containers. Guidelines used by our office can be downloaded at http://citybugs.tamu.edu/id-help/sample-guidelines/

Be ready to provide good resources relevant to mystery bug cases. Our office uses a fact sheet designed specifically to answer the most common questions received by people submitting mystery bug samples. We use the Diagnosing Mysterious Bug Bites factsheet (http://citybugs.tamu.edu/factsheets/biting-stinging/others/Ent-3006).

Listen, but set limits on calls. Clientele with mystery bug cases deserve as much respect and time as anyone else. Many people have been dismissed by other professionals as delusional or irrational, sometimes for good reason. Others, however, have a story to tell. The entomologist should learn to listen, but also to take charge of conversations by asking critical questions such as: "How long has the problem being going on?" "Do the 'suspected bites' occur in one particular place [in the home]?" "Have you noticed any signs of birds or rodents, like mice, in the home recently?" "Do you have pets?" "Has a pest control professional been able to inspect your home, or look at any samples?" [if so, "What was their assessment?"] "Have you been using pesticides?" "Are others in the home also complaining of similar biting sensations?" "Have you seen a doctor?" "Have you asked your doctor whether your symptoms could be related to any medication you might be taking?" "How can I be of

assistance to you today?" The last question can be useful when clients seem to want to talk, but the conversation is becoming one-sided, or irrelevant to the expertise of the entomologist.

Remember your limits. Entomologists should not attempt to make final diagnoses, especially when actual insect or mite problems have been ruled out. Distinguishing between illusions and delusions of parasitosis is a job for a medical professional. When illogical descriptions of insect or mite infestations are supplied by a client, they should be corrected if possible; however, when a diagnostic sample cannot be produced, the entomologist is under no obligation to continue involvement.

Involve doctors and family members when possible and appropriate. Not all non-arthropod, mystery bug cases are cases of mental illness. However when mental illness is involved, there is generally little an entomologist can say or do to convince the client to seek appropriate help from a mental health professional. A delusional client, however, may be willing to visit a more traditional medical health professional. If so, providing a business card and an invitation to the doctor to call and discuss any insect-related questions may be productive. Family members may also be confused about a loved one's "bug" problem, and can benefit and receive insight about alternative explanations from an entomologist. The emphasis should be on providing information about all potentially relevant alternative explanations rather than diagnosing a client as either illusional or delusional.

Conclusion

Cases of mysterious bites or crawling insects are commonly encountered by Texas AgriLife Extension Service entomologists, can require an inordinate amount of the entomologist's time, and are frequently difficult (or impossible) to resolve to everyone's satisfaction. Extension and public health entomologists should understand the taxonomy of mystery bug cases and become proficient in reaching a professional assessment about whether a mystery bug case is likely to be arthropod-related or not. Finding a balance between setting an appropriate professional "distance" and acting on a sincere desire to help illusional and delusional clientele, requires both experience and judgment. Better communication and cooperation between entomologists and the medical and mental health community will be essential for progress to be made in many mystery bug cases involving non-arthropod causes.

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Diagnostic Quagmires: Miscellanea and Debris Misconstrued as Ectoparasites and Ectoparasites Mistaken to be Miscellanea

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Diverse arthropods cause direct harm to human health from their bites and stings, by contact with their exudates, secretions, and urticating hairs, and as a result of them infesting the skin or viscera (Pollack 2010). Those who suffer from real as well as imagined infestations frequently find themselves in a diagnostic abyss of sorts. Despite training to consider a wide spectrum of potential diagnoses, clinicians tend to be bereft of entomological expertise. Accordingly, they frequently err when they attempt to identify an arthropod they encounter on or in a patient, or to ascribe blame for a bite-like lesion. Conversely, entomologists may have relevant taxonomic skills, but should not examine patients or render diagnoses. Absent a clinically skilled entomologist, or some level of collaboration between practitioners in the two disciplines, pandemonium reigns supreme. A few examples are presented herein to illustrate certain such problems and to serve as the bases for strategies meant to reduce diagnostic errors.

Perceptions, sensations and lesions associated with real and presumed infestations: "What bit me"?

Health care professionals, entomologists and pest control workers all too frequently receive inquires and samples from persons convinced that they have been bitten or infested by an insect, tick or other parasite. In many cases, the lesions or sensations derive from a bona fide parasite, but in others the cause of the irritation is elusive or imagined.

Some bites and infestations go unnoticed, whereas others may cause immediate injuries, sensations and infections far out of proportion to the diminutive size of the arthropod. Bites by the aptly named "no-see-ums" (ceratopogonid flies), by some kinds of thrips (Thysanoptera) and certain kinds of blood feeding mites are just a few examples of tiny creatures causing major distress. Being that the villains are often not seen, or are overlooked when they bite, it is not surprising that some sufferers believe their eyes are playing tricks on them. Upon hearing such descriptions of the complaints, family members, acquaintances and physicians may understandably conclude that the patient might benefit from psychological assessment and treatment.

Many bite-like sensations and physical injuries, however, are not so readily attributed to an arthropod, whether it is seen or not. Often, the complainant wrongly ascribes a sensation or physical lesion with the first creature that s/he observes, even if that creature is physically incapable of causing such an injury – or is not even an animate object! Coincidence, rather than causation, serves as the proverbial red herring leading to many incorrect conclusions. In the absence of an obvious and bona fide villain, many suffers are said to suffer from delusions or illusions of parasitism, or are labeled with yet other monikers that can be similarly confusing and frustrating to the patient as well as to his/her family and physician. My colleagues presenting in this symposium have offered insight on those conditions, so I shall not dwell on what others have so aptly offered.

Discussions of ectoparasitic arthropods tend to follow either a taxonomic orientation or one based upon an organ-specific site of injury. It can be instructive, instead, to apply a different ecological approach, one that considers the duration of residence by the infesting arthropod (Pollack and Marcus,

2005). The attacks by certain ectoparasitic arthropods, such as biting flies, bed bugs, fleas, soft ticks and biting mites, are just fleeting. They make contact and leave in just minutes, and their effects may not even be noted until some time, thereafter. Furthermore, there is a broad array of other arthropods that may cause irritation by their defensive bites and stings, with urticating / vesicating hairs and exudates, and as a result of their misdirected feeding attempts. Whereas the contact with these arthropods may be momentary, the injuries and allergic reactions may persist for hours, days or weeks, thereafter. Yet others, such as bot fly larvae and hard ticks, are transient residents that remain for days or weeks. Next along the continuum are the chronic residents, such as lice and scabies mites, that cannot survive long off of a person. The most chronic and persistent villains, however, seem to be those that exist solely in the minds of the beholders. Shaking off a phantom is a most difficult endeavor, and sadly, such efforts frequently fail.

Brief encounters (measured in minutes) of the biting kind: Most entomologists have likely lost count of the number of fear-laden reports they've fielded of 'giant mosquitoes' (crane flies) that have invaded dwellings. Fortunately, those creatures cause threats only of the imagined kind. Similarly, most of our colleagues likely have received their share of questions and reports of 'bites' or lesions assumed to result from the attacks of diverse biting flies, fleas, ticks and spiders. Whereas a lesion might have been caused by such an insult, efforts to identify the villain, based solely upon the appearance of the lesion, is a task fraught with error and risk. Only qualified physicians should examine a skin lesion and render a medical conclusion. Entomologists, even those who feed arthropods upon themselves, should resist the urge to examine the lesions of others and to utter conclusions. Whenever possible, the biting or infesting arthropod should be captured (physically or digitally) to serve as the objective basis for ascribing blame.

Diverse creatures and other objects are frequently misconstrued to be bed bugs. Indeed, nearly 90% of the many thousands of samples, submitted to my lab (by homeowners, landlords and health professionals), as presumed bed bugs were so misidentified. Of the majority that are not bed bugs, some are the closely related bat and bird bugs (other kinds of cimicids). The bulk of the remainder is comprised of cockroaches, diverse kinds of beetles, flies, silverfish, a broad array of other arthropods (some which do bite), and a cornucopia of fabric lint 'pills' and other debris and detritus. Such errors frequently have caused the deceived to: dispose of possessions that have fiscal or sentimental value, abandon their dwellings, misapply pesticides, isolate themselves from their families and acquaintances, and instigate legal challenges. Even if the observed pests were bona fide bed bugs, many of these steps would be unjustified.

Various ornithonyssid and dermanyssid mites derive nourishment by blood feeding upon their preferred rodent and avian hosts. The populations of these mites can reach truly impressive levels in dwellings adorned with nests of their natural hosts, and the mites may massively invade the human living spaces should their preferred hosts die or leave. Despite being poor hosts for these mites, people are frequently bitten. These creatures are tiny and are easily overlooked by those without excellent visual acuity, even when many thousands of the mites are wandering on walls and furniture, or while they are actually biting a person. Some sufferers complain incessantly and soon become social pariahs, or worse, they may bathe their homes and bodies in pesticides in efforts to treat the unseen creatures. Confirming the identity of these beasts (if present) is critical, as it will help guide the way to a rational, quick and effective intervention. Other sufferers merely believe they are endowed with biting mites, thanks to exaggerated media reports and mischaracterizations on numerous amateurish web sites. These, too, lead to considerable apprehension, mistaken diagnoses and improper treatments. Many submitted 'biting mites' are actually dust mites or other kinds of non-biting mites. Such mysteries can be readily solved, and the consequent errors averted, by capturing a few of the villains,

and then having these identified by personnel who have relevant expertise.

Transient (those that visit a person for days or weeks) residents: Diverse hard (ixodid) ticks are the most frequently encountered transient arthropod parasites. Hard ticks attach superficially for just a few days or for more than a week, depending upon the species and developmental stage of the tick. Promptly finding and removing them can dramatically reduce risk of many kinds of tick-borne infections. Once removed, the ticks should be retained and their identity confirmed – ideally rapidly - by those with sufficient expertise. Findings (species, stage of development and estimated feeding duration) can be of great value to the patient as well as his/her physician in evaluating medical management options (e.g. presumptive treatment vs. awaiting the appearance of symptoms or the results of infection assays). These assessments can be made quickly and effectively on the basis of an examination of the tick or a good digital image, thereof.

Travelers as well as relatively sedentary persons occasionally may suffer from myiasis, the infestation by bot flies. Depending upon the species, bot flies oviposit on or in a wound, on the hair or skin of animals, on clothing, on soil or vegetation, or even directly to the body of a mosquito or other arthropod. In response to direct skin contact, the fly larvae then hatch and quickly invade the skin. The irritation caused by the fly larva frequently causes an infested person to seek medical evaluation and treatment. Often, the lesion is misconstrued to be a pimple, puncture wound or a skin infection, and a topical antibiotic applied. Occluding the posterior spiracles of the larvae with such a preparation has caused many a sufferer (and clinician) to be amazed and horrified as the larvae then 'back out' of the skin and drop to the examination table. Yet other kinds of flies may infest deeper tissues and present a significant medical condition (Finlay et al. 1999). Confirming the precise identity of the larva is a critical step in appropriate medical management decisions.

Chronic (those that cannot live without us) residents: In North America and elsewhere a proportion of human beings may host any of three kinds of human lice. These include head lice, body lice and pubic (crab) lice. Head lice are most prevalent on children in the kindergarten through 4th grade age levels, and even amongst them, the point prevalence averages just 1%. Body lice almost exclusively infest a small proportion of adults who are indigent. Pubic lice are generally restricted to those who are sexually active. Human lice nearly top the list of those human parasites that are frequently misidentified, leading to rampant misdiagnoses and mismanagement.

As part of an effort to assess the acumen of diverse diagnosticians, I tabulated data accompanying more than one thousand submissions of objects presumed to represent a head louse or head louse egg (Pollack et al. 2000). Whereas school nurses were the most adept at correctly interpreting evidence of extant (active) and extinct head louse infestations (specificity = 70%), fewer than half (32%) overall) of the louse-related objects they submitted were recently viable. Hatched and dead eggs were frequently mistaken to be a sign of an extant infestation. Hence, most of the children they had labeled as being 'lousy' had been misdiagnosed, and these children were subsequently mismanaged (treated when unnecessary and excluded from school without justification). Children and adults suffered misdiagnoses even more frequently when the diagnostic task was performed by teachers, relatives, barbers/beauticians, physicians and when they self-diagnosed their own malady. Overall, fewer than half (46%) of submissions labeled 'lice' actually contained a louse or louse egg. Nearly as many (43%) were composed of debris. About 10% contained arthropods other than lice (e.g. ants, beetles, flies, thrips, ticks, springtails), and nearly 1% of the 'objects' were simply sections of knotted hair. Although other arthropods were most frequently mistaken to be lice, bona fide lice were occasionally misconstrued to be ticks and other pests. Diagnostic errors (relating to head lice) are the rule, not the exception. Indeed, one could flip a coin as the sole diagnostic criterion and be right more often than how this task is performed in our schools. Accordingly, the identification of infesting arthropods should

rely upon personnel with relevant entomological expertise.

Scabies is a condition caused by the infestation by the mite *Sarcoptes scabiei*. In North America, it is most prevalent amongst the indigent, but it can affect persons of any age or economic class. Their secretions and excretions are immunogenic and frequently elicit an allergic response that causes considerable itching by the infested person. Because of their small size, their location in the skin and the difficulty in sampling and visualizing them, these mites present a challenge to the clinician and patient. The majority of scabies diagnoses in North America are presumptive; that is, they are rendered on the basis of the symptoms, and treatment is generally prescribed without demonstrating the mite, itself. This phenomenon results from the general lack of formal training in parasitology that students receive in our medical schools. Indeed, of the most recent one hundred scabies diagnoses that I have been asked to consult upon, just two satisfied the objective criterion of demonstrating the causative parasite. This is yet another example of a quagmire that leads to frequent misdiagnoses and unnecessary or incorrect presumptive treatment.

A glaring disconnect is apparent between clinicians and those personnel with entomological and parasitological expertise. Precious few clinicians (including dermatologists and psychiatrists) have entomological training, and few entomologists are licensed to practice medicine. Hence, collaboration between medical/veterinary and entomological practitioners should be encouraged to more effectively and quickly evaluate and resolve presumed infestations. Traditionally, academic, extension and museum workers based at land grant universities have served to identify diverse arthropods of medical relevance. Increasingly, these centers have suffered profound fiscal shortages as well as regulatory obstacles and liability concerns. Hence, relatively few entomologists can – or will – accept clinical samples. Of those that do, the turnaround time rarely is sufficient to facilitate assistance with medical decisions and management.

The Laboratory of Public Health Entomology at the Harvard School of Public Health provided rapid identification services for many decades to clinicians and to the general public. A reorganization of that institution in 2010 caused this service to relocate to a new independent venture. IdentifyUS LLC (https://identify.us.com) provides rapid, independent, confidential and expert evaluations of physical specimens as well as of digital images. Reports with the assessments are almost invariably sent within hours of when the specimen was received. An online secure portal facilitates uploading of images and data as well as delivery of results, and this often is accomplished within just minutes of image submission. The service focuses mainly upon medically relevant arthropods, but a cadre of taxonomists, epidemiologists and ecologists are available to offer guidance on diverse pests to clinical practitioners, pest control professionals, property owners and tenants, state and municipal officials and others. This service satisfies the need by clients to receive expert assessments and guidance within clinically relevant intervals, and is fully consistent with a much-overlooked basic tenet of integrated pest management – to confirm the identity of a presumed pest before embarking upon an intervention.

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Working With Pest Management Professionals and Delusory Parasitosis Cases

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Pest management professionals (PMPs) are often the first contacts by people experiencing an unidentified biting pest problem. It is important for PMPs to recognize and address these situations in ways that may not appease the customer, but may potentially reduce further harm to the DP sufferer who may already be misapplying pesticides and other chemicals in futile attempts to resolve the problem. This paper quantifies some of the experiences of pest management professionals in North Carolina and reinforces the need to maintain professional approaches to these cases.

Unidentified Biting Pests

The sensation of something biting or crawling on the skin can have many causes (Hinkle 2000, 2010). Some of the cases may be actual parasitic arthropods including but not limited to mites (scabies, bird and rat mites), lice, fleas, and bed bugs. In other cases, the individual may be suffering from Illusions of Parasitosis as coined by Waldron (1972) in which an individual mistakenly thinks that the sensations they are experiencing are caused by a parasitic arthropod. Other physical or mental factors including diseases, chemical irritants/allergen, side effects of medications, or emotional trauma may trigger these sensations. The term Delusory Parasitosis or Delusions of Parasitosis, or Ekbom's Syndrome (Ekbom 1938) is used in the medical profession to describe patients who are cannot be dissuaded from their belief that they are infested with an arthropod pest in spite of evidence to the contrary (Hinkle 2000).

Whether a person experiences a problem due to a real arthropod pest or illusions/delusions of parasitosis, the pest management professional (PMP) is often the first (or second) point of contact for these individuals (Hinkle 2000). Physicians examining patients that are experiencing pruritis or dermatitis may attribute the problem to insects and recommend the services of a pest control company (Hinkle 2000). St. Aubin (1981) stressed the importance for PMPs to identify a pest before taking further remedial action.

Survey of North Carolina Pest Management Professionals

In April 2012, I conducted an informal online survey (http://www.surveymonkey.com) of pest management professionals (PMPs) who are members of the North Carolina Pest Management Association. A total of 52 members responded to the survey. The NCPMA has 208 member companies with over 1800 contacts. The low response rate can be attributed to many factors and cannot be used as a measure of the relative extent of DP encounters across the pest control industry in North Carolina. The data are likely skewed towards PMPs more familiar with the problem of DP. I did not try to correlate locale (area of North Carolina) with the responses by individuals. For the sake of simplicity in reporting results, I will use to "DP" as referring to calls/situations that involve any contact that started as an unidentified biting "pest" (i.e., excluding situations where actual flea or bed bug specimens were found). In some cases, actual pests may have been identified subsequently, but most of the situations referenced were for biting pest situations that were never resolved. In this survey, 40% of the respondents said that they received 1-5 calls annually; 33% reported receiving 6-10 DP contacts and 15% had more than 20 calls annually. In terms of the demographics of these cases, 85% of respondents said that the majority of their callers were female and over 40 years of age (Figure 1) which fits previously reported trends summarized by Hinkle (2000, 2010).

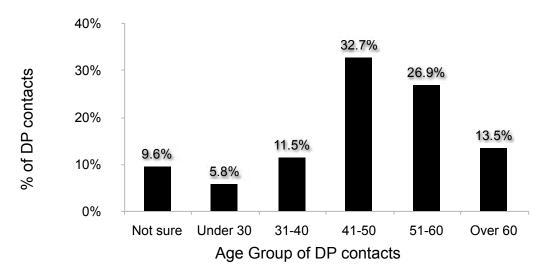


Fig. 1. Age Distribution of DP contacts

Fleas and bed bugs were the most common pests that DP contacts mentioned as the likely cause of their problem. Lice, scabies, mites (i.e., "other mites" aside from scabies), and thrips were also mentioned. Twenty-three percent of the respondents had DP contacts that mentioned Morgellons (Pearson et al. 2012) as a possible cause while 93% of respondents had DP contacts who stated that they did not know what might be causing their problem.

When asked what pests (excluding bed bugs) had been found as causal agents of the DP contact, 94% reported that they most frequently found no evidence of a pest. Fleas and mites were among the most common actual pests found. However, accurate identification of parasitic mites including scabies, bird and rat mites is difficult even for trained entomologists and more so for the PMP with minimal identification skills (Alexander 1984) and so the results reported here are considered suspect without knowing the credibility of the actual determiner. Twenty-four percent of respondents reported finding Morgellons which have at this time no medical/scientific credibility as an actual parasite (Pearson et al. 2012). Carpet beetles were unintentionally omitted from the survey in spite of their documented importance as a dermal allergen (Jacobs 2010).

Goddard (1995) and others have reported that DP sufferers often resort to excessive and inappropriate use of pesticides. As shown in Figure 2, 83% of the respondents said that their DP contacts reported the use of total release aerosols which are often perceived as a simple and effective way to control pests indoors. A combined total of 56% of respondents had DP contacts that applied pesticides off-label, i.e., applying registered pesticides that were labeled for outdoor use only or applying agricultural pesticides or products that were no longer registered for use. Forty percent of DP contacts had used "natural products", which could include essential oils and plant extracts, because of their perceived lack of toxicity to humans and pets.

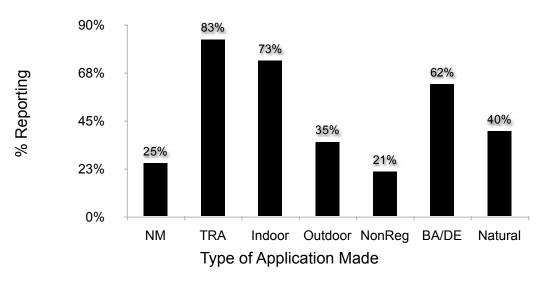
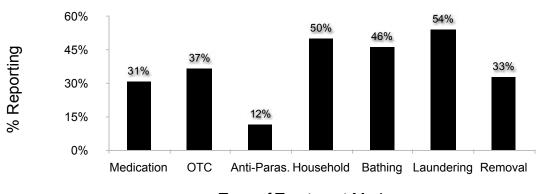


Fig. 2. Percentage of Customers Reporting Applications of the Following Control Measures Inside Their Homes (NM – No control mentioned, TRA=Total Release Aerosol, Indoor – A pesticide labeled for indoor use, Outdoor – A pesticide labeled for outdoor residential use only, NonReg – Other types of pesticide not labeled for residential use, BA/DE – Boric acid or Diatomaceous earth; Natural - A product such as essential oils, tea tree extract, etc.

Survey data on various other non-pesticide control measures employed by DP sufferers corresponded to those summarized by Hinkle (2000, 2010). Forty-six of respondents had DP contacts report frequent (i.e., more than once daily) bathing/showering and more than 50% reported daily laundering of clothing and bed linen. Half of respondents had DP contacts that reported applying household chemicals (such as bleach and ammonia) to themselves. Medications were commonly used by DP contacts: 31% reported individuals that had used prescription scabicides; 37% had individual who reported using over-the-counter medications; and 12% reported customers that had used veterinary anti-parasite medications. Thirty-three percent of respondents reported DP contacts that had attempted to physically remove "parasites" from their body.

Dealing with DP cases can be expensive for both the sufferer, who may spend hundreds or thousands of dollars with chemical treatments on their own or by hiring pet control companies. In this survey, 50% of the respondents stated that they never perform chemical treatments before confirming that a pest is present. Thirty-eight



Type of Treatment Made

Fig. 3. Percentage of Customers Reporting Applications of the Following Control Measures to Themselves (Medication – A prescription scabicide/pediculicide, OTC – Over-the-counter scabicides/pediculicides, Anti-Paras – A veterinary anti-parasitic medication, Household – A household product such as bleach, Bathing – Multiple daily baths/showers, Laundering – Multiple daily laundering of clothing/bed linen, Removal – Physical removal of objects from skin.

percent reported that they rarely (<25% of DP contacts) apply a pesticide in these situations. One respondent mentioned that he was more likely to do apply a pesticide for a routine customer (e.g., a weekly rental management). DP cases can also an economic the PMP (particularly for PMPs that do not charge for an initial visit when no chemical treatment is performed during that visit. Eighty-seven percent of respondents reported that they conduct at least a visual inspection of the premises in most cases (6% reported never doing a visual inspection in a DP contact) and 58% reported placing monitors and 67% collected (or accepted) samples. The number of visits made for individual DP cases is shown in Table 1. The majority of PMPs made 2-3 visits. In some cases, the second visit may be a "courtesy" visit but more often the return visit was to check monitors placed during the initial visit and/ or conduct another inspection or evaluation particularly in situations where pesticide treatments were performed on a previous visit. Some PMPs state (in the comments section) that they provide more visits to current service customers.

Table 1. Typical number of trips to customer's residence before discontinuing service if no pest is identified.

Number of service visits	% Reporting
One	27%
Two	35%
Three	33%
Four or more	5%

Hinkle (2000) emphasized the importance of encouraging DP sufferers to seek the help of a physician through whom they might obtain needed medical assistance. Seventy-one percent of survey respondents rarely (23%) or never (48%) mentioned Delusory Parasitosis to the customer. Those PMPs that do mention are more likely to have attended seminars at professional meetings. Comments made by PMPs in this survey included "We're not doctors". Several respondents noted that when no evidence or a pest is found, they do encourage the customer or encourage the customer's spouse (or another family member) to contact a physician or public health specialist for further assistance.

Conclusion

Many PMPs address DP cases annually and a few have set policies on handling these situations. It is important for PMPs to approach these situations on a case-by-by cases basis. First, they must confirm whether or not there is an actual arthropod pest causing the problem. Second, they need to ask questions about what pesticides and "other" chemicals" have been applied by the customer before considering any applications. Most of the insecticides used by the general public and PMPs are pyrethroids which are more likely to cause paresthesia or dermal irritation (Knox et al. 1984) from overexposure. PMP management needs to train their technicians to refrain from placating the DP customer by applying a pesticide when no evidence of a pest is found and then to take the lead in guiding the customer by recommending that they seek medical assistance with their problem.

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Costs and Consequences of Ekbom Syndrome (Delusory Parasitosis)

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Individuals suffering from Ekbom Syndrome (ES) lose a lot; they lose sleep, they lose comfort, they lose their friends, they lose their families. Frequently they lose their jobs, often leading to financial losses that result in loss of the home, so they end up living in a motel – or living out of their vehicle. They lose normality. And in some cases they lose their lives, pushed to suicide by desperation (a word heard frequently from this group).

Ekbom Syndrome is the psychological condition in which the individual considers his body to be infested by invisible bugs. According to sufferers' descriptions, "it's not a bad itchy feeling, it's a small bugs crawling on me feeling." "I'm feeling exactly what you describe, the invisible bugs all over my body."

Because nothing captures these losses like the words of Ekbom sufferers themselves, copied below are postings from various websites dealing with "invisible bugs." (These are copied directly, including typographical errors.)

Financial Losses

Quantifying the economic impact of this condition on a family is difficult because so many aspects of life are affected by ES. In accounts of ES sufferers found on various websites, only a small percentage address expenditures related to their condition. We compiled 50 ES accounts that did address the financial costs, and the most common estimate (27 of 38) was, "we have spent thousands of dollars" treating for this pest. Comments included, "I spent \$1,000 in three weeks," "We've spent almost \$3,000 in 4 months," and "We spent over \$13,590 in 10 years (including burning everything in the house)."

When costs were specified, they included "thirteen visits to four different vets, spent thousands trying to kill the mites," and "... well over \$4,000 at the vet's in four months." "I spent in excess of \$5,000 trying to figure out what was crawling on me." "So far, in 7 months, my bill is up to \$3,000."

In describing environmental suppression attempts, posters said they "...paid \$1,000 to the exterminator," and "In four years I spent over \$4,000 on pest control." On average, these people contract with a new pest control company every three to six months. Likewise, "I had my house tented to the tune of about \$2400," and "we have spent over \$10,000 in a year in exterminators, thrown out furniture, supplies, and taking clothes to the laundry every day." "I have had five different pest control companies treat my home; however none of them were successful."

It should be noted that none of the accounts included in this survey had succeeded in eliminating the 'infestation.' For example, "We spent almost \$30-40,000 to get rid of these invisible bugs, but still no luck for over a year now."

Because these individuals consider both their bodies and their environment to be infested, they spend money in personal and home eradication attempts. Typically they have consulted several physicians and purchased prescription medications (e.g. permethrin cream, Lindane shampoo, Flagyl, parasiti-

cides, and antibiotics). They have concocted all sorts of home remedies, ordering exotic ingredients to use in treating their skin (or to take internally). They have purchased devices like ozone generators, ultrasonic pest repellers, specialized vacuum cleaners with HEPA filters, and parasite "zappers." Some have bought steam-cleaners, which they use to apply pesticides to their carpets and furniture.

Several websites now market directly to this segment, turning up in searches for "skin parasites," "invisible mites," "invisible bugs," and such terms. That's why products from KleenGreen, KleenFree, Natural Ginesis, CedarCide, AllStop, Arbonne, EcoLiving Friendly and such sites repeatedly show up in discussions of products ES sufferers have tried.

Pharmacy expenditures can be significant, as was demonstrated by a recent case. Based on the pharmacy records she provided, over a period of nine weeks six different physicians prescribed her 39 drugs including 7 different antibiotics (23 separate bottles), 6 of a parasiticide (ivermectin), 4 pain blockers, 3 corticosteroids, 2 antihistamines, and 1 anxiolytic. The pharmacy bill for these medications alone was \$776.99 over this 67-day period. Additionally the individual recounted significant health problems, including a serious automobile accident twenty years previously, resulting in injuries for which she had been taking methadone for two decades.

Lose Belongings

Once sufferers discover that pesticides and cleaning do not work, they typically start ridding the household of belongings that they perceive to be infested. "We ripped out the carpet in our bedroom and put in laminate," reported "J" on the bird mites forum. Similarly, "We threw away our couch this week and ripped out our downstairs carpet and are trying to find a time to rip out the upstairs." Another victim, Becky, moved out of her Austin, Texas, home and into a trailer hoping to escape the bugs that torment her. "We ripped out our carpet and burned our carpet and furniture and moved out into our R-V and they were still on me." Similar reports include the following:

"We got rid of our cloth furniture and carpeting too, which cut down on the areas that they could easily infest, but the worst part is our bodies."

"I have spent a ton of money on DIY remedies, and taking out all the carpeting in our house and replacing with wood floors."

"I have moved three times and they follow me, I have thrown all my furniture and most of my clothes away because they are on them and won't die."

"I threw out everything I owed and I moved at least 5 times trying to get away from this stuff, which also cost me thousands of dollars."

Lose Relationships

As their "infestation" consumes all their time and attention, ES sufferers talk about nothing but the bugs, which eventually results in alienating their friends and family who grow tired of hearing about the bugs. Frequently sufferers are so concerned about transmitting their bugs to other people that they isolate themselves, refusing to allow friends or family to visit. Typical comments are along the lines of, "I feel so alienated and lonely I don't socialize anymore and just do not know how much longer I can go on," and "I feel so isolated and I have nobody that really understands or cares."

Lose Sanity

While ES sufferers repeatedly assert that they are not crazy, they frequently allude to the feeling that their sanity is slipping away. "i've spent all my savings (im not that old so it wasnt a lot), i have had no family/social life for over a year. i cant work. i cant survive. i need answers, before its too late."

"I am at my wits end. It is like i am having nightmares while I am awake."

"I'm loosing my mind and life."

"i have been called crazy by my family, every doctor i have seen thought the same thing. The crazy things we are doing. People in our life think we are crazy."

"I don't know if I'm dying or losing my mind, or both."

"I am out of money and half crazy."

"i feel so alone and crazy. I'm very sleep deprived."

"I have gotten a new car but it is now infested and have switched offices twice. I have probably blown my professional reputation of 18 years by spraying, vacuuming, etc. in the office. The smell of the sprays offends my co-workers. I now just keep quiet and suffer because people do not understand and I do not want them to think that I am insane."

"My family is starting to think I'm losing my mind, and the doctor couldn't tell what my bites were from. I miss my Grandchildren, but wouldn't want them or anyone to go through this. My Grand kids mean everything to me. I'd rather die than to have them end up thinking the Grandma that used to be so much fun turned into a crazy old lady who didn't want to see them because of bugs on her skin that no one could see!"

Lose Their Lives

While there are few documented cases of ES sufferers committing suicide, it must be remembered that most entomologists who deal with ES callers are unable to follow up with these individuals and frequently lose contact with them, so the ultimate outcome is rarely known. In on-line forums, there are certainly plenty of sufferers talking about suicide, as illustrated by the comments below.

"I had the horrible sleep interferring sensation of creepy tiny bugs (invisible) crawling in my hair, neck and arms and found this forum while desperately looking for some ideas of the cause and cure. I thought I was going crazy. It is like a curse on me and no way to break it unless I end my own life, which could happen because I cannot live with these things forever."

"Can someone please help me before I go crazy or suicide? I totally understand the feeling of suicide."

"I tried everything to get rid of this devil. After six months of this hell, I was willing to try anything, because yes, I was close to suicide. My quality of life was zero and I was at a point where I had nothing to live for. I lost my job, lost my apartment, lost my girlfriend, and I was financially destroyed due to this beast- you know the drill- Doctors, motels, pesticides, all of it. Like others, they were in my car and at my place of employment. They also seemed to be preferential, in that there was something about me that they liked, but other people seemed not to be bothered by them, even if they had

physical contact with me like shaking hands. This is to say the least, mind-boggling. Doctors are clueless and suggested I was on drugs, which I am sure you know is insulting. Their ignorance is equally disgusting. My frustration and inability to communicate my condition with others had me on the verge of insanity."

"I'm not entirely sure when I became a host, its been within the past 2-3 years at an old home I shared with a roommate. At this time I'm unable to take advantage of all of the suggestions as I am unable to afford extra showers and/or items that cost any money at all. I take lots of sleeping medication at night to simply sleep through the night. However, one day I only hope to never wake up at all. I'm totally unable to find any purpose. I just went to the doctor yesterday only to find out that he thought I was on drugs and recommended I be on anti-depressants and seek mental help. I've spent all my money on all kinds of different strategies, my problem is that I have no regular home now... If anyone has any suggestions that could assist my sanity I'd appreciate your help. I'm almost to a point of suicide. I can't take the itch anymore. I can't function. My friends don't believe me. My family doesn't believe. I don't know what to do."

"I moved but have brought my hell with me. My car is infested. I had the Bugman come out and spray with bifenthrin and then fogged (I think with pyrethrin) ... I got in my car 72 hours later and felt crawlies. I vacuumed my car with an industrial vacuum but that night I got mauled. They are in my windpipe and eyes and ears and nose. I feel like I am the host. Moving has not helped. I am so depressed I feel suicidal... I have no one to help me. I don't even drive my car and I am losing my life. I don't know how much longer I can live like this."

Lose a Normal Life

These people exhibit a variety of unusual behaviors. They shave their scalp. Often sufferers hole up in their homes for months at a time, refusing to see friends and family, with polyethylene sheets covering doors and windows. After completely emptying the home of furnishings, they sleep on air mattresses covered with plastic shower curtains.

"The next night when i got home and got in bed I felt tingles, like bugs jumping on my ankles and calves. I freaked out because they started biting me and crawling allover. Eversince I cannot see what is eating me alive but nothing I do gets rid of them. I have taken bleach baths, Epson salt baths, cedar oil and tea tree oil baths, ammonia baths, used permethrin cream multiple times, changed apartment, changed car, threw out all my belongings, they are still on me." Another noted, "i have survived the last year and i am despersate to LIVE again."

Lose Pets

People frequently assume that they acquired their infestation from an animal, such as a pet, or conversely that the parasite transferred from them to their pet. For instance, "I gave this to my cat who is equally tortured now." Another reported, "November past year I woke up with tons of bites on my back, derm and I thought it was coming from my dog who used to sleep with me. Dog was allergic to any medicine to cure him. So I did one permethirn and gave away my dog." Euthanasia is sought as a solution for the infestation, "I had to put my poor dog down after 2 years of him going through this nightmare; I hate the thought that I was the one who brought these things home to him."

Conclusion

A little experience with ES sufferers illustrates how much it is costing them; each is losing his economic stability, his home and possessions, his family and friends, and his sanity and normal life. Ekbom Syndrome steals its victims' lives and affects everyone around them, co-workers, friends and family. The consequences of this condition are far-reaching, and the costs cannot be fully estimated.

Publications dealing with Ekbom Syndrome, including reviews of recommended therapies, can be found at Hinkle, N.C. 2011. Ekbom Syndrome: A Delusional Condition of "Bugs in the Skin". Current Psychiatry Reports 13(3): 178-186. http://www.ent.uga.edu/pubs/EkbomCurrPsychiatryRpts.pdf

SYMPOSIUM: ANTHROPOD ASSOCIATED ALLERGY

Overview: Allergy to Arthropod Allergens/Bites/Secretions/Venoms

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Arthropods contribute to human misery in many ways. In agriculture, they destroy food-crops, affect livestock, and cause substantial damage to stored grains. In urban/rural settings, they discomfort human lives by sharing homes, food and clothing. They also transmit pathogens of human diseases. Moreover, some of these arthropods trigger allergic reaction in humans when they produce allergens, inject toxic venoms, and secrete inflammatory chemicals and saliva. The American academy of allergy classifies allergic reactions in three categories: local/low, moderate and severe. The symptoms of local allergic reaction include: swelling, urticarial, welts, itching malaise and anxiety. The moderate allergic reaction elicits swelling, sneezing, dizziness, chest pains, abdominal pains, nauseas and vomiting. The severe reaction results into marked weakness, confusion, blood pressure change. shock, collapse and unconsciousness. Allergies are common to special secretions produced by cockroaches, certain larvae, and blister beetles. Salivary secretions of biting insects (e.g., mosquitoes, flies, lice, fleas, kissing bugs and chiggers) produce allergic reaction in certain individuals. Allergy is also instigated from accidental ingestion/inhalation of insect remains such as decaying body parts, empty egg cases, casted skins and hairs/scales. Bites from brown recluse, black widow and hobo spiders are known to induce severe reactions alerting for immediate medical treatments. Other arthropods responsible for allergy include: chiggers, itch and mange mites, fire ants, puss caterpillars and brown tail moth larvae. Although a vast majority of people have suffered from allergy to arthropods, research in this area is at its lowest due to lack of funding.

Allergic Reactions to the Asian Needle Ant, Pachycondyla chinensis (Emery)

Patricia Zungoli and Eric Benson
Entomology
School of Agricultural, Forest and Environmental Sciences
Clemson University

The Asian needle ant, *Pachycondyla chinensis* (Emery) is a ponerine species endemic to parts of Asia; now generally found extensively in China, Japan, Korea, Vietnam with isolated collections reported widely in other geographic regions. It was first documented in the United States in 1932 in Georgia, North Carolina, and Virginia (M.R. Smith, 1934). In 2004, the species was reported in South Carolina (Zettler 2004) and likely common, but unidentified as early as 1997 (Nelder et al. 2006). The current distribution includes Alabama, Georgia, North Carolina, South Carolina, Tennessee, Virginia, and Washington, DC (unpublished data), although old records also indicate reports from Florida and New York (Guénard et al. 2009).

Most often, *P. chinensis* is found in damp areas in soil beneath rocks, mulch, leaf debris, and land-scape objects (statues, railroad ties, ornamental stone, etc.) or in stumps and logs also frequently occupied by termites which Asian needle ants readily consume. We have only observed them in habitats in contact with the soil from which they act as generalist scavengers and predators.

Where it occurs, the Asian needle ant is a dominant species. In forest and urban habitats in a study in SC, *P. chinensis* often occurred to the exclusion of most native species, representing approximately 25% of all ants collected (unpublished data). In a NC study, when present, *P. chinensis* was more abundant than all native species combined (Guénard and Dunn 2010).

In addition to its ecological implications, the Asian needle ant also is noted as a potential medical threat because of its potent sting. Unlike the fire ants, Solenopsis spp., the Asian needle ant is not aggressive and only stings when it is trapped between clothing, or between the skin and a substrate. In documented sting cases, victims are usually stung by a single ant, although that single ant may deliver multiple stings (Nelder et al. 2006). Our first knowledge of problems associated with this species came from staff at the Greenville Zoo (Greenville, SC) who reported painful stings from ants while carrying out their work primarily when engaged in outdoor activities (Nelder et al 2006). In a 2005 survey, victims described their sting reactions in which initially they experienced intense pain and itching at the sting site. With some, this escalated to becoming overheated and nauseous and others also experienced a systemic reaction with hives, chest pain, respiratory distress and swelling of the tongue and face leading to anaphylaxis (Nelder et al. 2006). Other reactions were described as 1) an intense pain that faded and returned over several hours, but not always at site of sting, 2) pain and itching persisted 2-3 days, and 3) welts disappeared after 5-7 days. Three instances of anaphylaxis have been reported in North America with no fatalities (Leath et al. 2006, Nelder et al. 2006). However, no record of sting frequency is available for this region, and we speculate that most ant stings in the southeastern United States would be attributed to fire ants rather than Asian needle ants making these data difficult to collect.

In its established range in Korea and Japan, *P. chinensis* is well known to sting humans and induce allergic reactions (Cho Y.-S et al. 2002. Fukuzawa, M., F. 2002). In Korea, Cho and colleagues (2002) reported a 23% sensitization rate to *P. chinensis* in the population they tested with 2.1% show

ing systemic reactions. In its known range in the United Arab Emirates, four fatalities were reported due to the congener, *P. sennaarensis* (Mayr) (Dib et al. 1992). No anaphylaxis has been reported in Iran where incidences of stinging events are abundant, but, Nikbakhtzadeh and colleagues (2009) reported differences in toxin composition for *P. sennaarensis* based on geographic location. The refereed literature have no accounts to date indicating allergic reactions in the United States to any of the other *Pachycondyla spp.* established here including *P. gilva* (Rogers), *P. stigma* (F.), P. harpax (F.), and *P. villosa* (F.), although undocumented accounts of *P. villosa* stings can be found, on the Internet (Anonymous 2012, Wild 2012). Orivel and Dejean (2001) compared the impact of varying concentrations of venom from 12 *Pachycondyla* Smith species during simulated stinging events of *Acheta domesticus* (L.), and speculate that all have venom comprised of various neurotoxins and histolytic compounds.

The mechanism of reaction has been investigated and IgE binding was indicated in patients who had experienced anaphylaxis (Kim et al. 2001). The major allergen associated with *P. chinensis* was identified as 23kDa allergen in antigen 5 family of proteins (Lee et al. 2009). Cross-reactivity with other venoms has been reported (Yun et al. 1999, Kim et al. 2001, Lee et al. 2009). Only limited cross-reactivity is reported with *Solenopsis invicta* Buren, while elevated levels of cross-reactivity were reported for some *Vespula* Thompson and *Polistes* Latreille. This is likely due to the preponderance of antigen 3 in the allergen of the former, and the high level of antigen 5 in the latter two groups.

To understand the true significance of the Asian needle ant as a medical threat more research is warranted to determine if any portion of the undocumented causes of anaphylaxis presented in hospital emergency rooms can be attributed to this species. Coordination with hospitals and allergists could yield a clearer picture.

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Allergy to Fleas / Mites / Ticks

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Some fleas, mites, and ticks bite humans, producing inflammation and itching as host reactions to the substances injected by the arthropods. These hematophagous ectoparasites circumvent host hemostasis by injecting saliva containing substances that counter platelet activation, vasoconstriction, coagulation (clotting), and other defenses (Ribeiro and Francischetti 2003). Tick saliva also has analgesic factors that inhibit histamine and bradykinin, contributing to the painless bite on their natural hosts. These salivary factors are often recognized as antigens by the host immune system, eliciting antibody production and subsequent host responses including inflammation and itching. The degree of reaction is dependent both on the arthropod and on host attributes (individual physiology, atopic predisposition, prior exposure, etc.).

The standard allergic cascade has three main manifestations: itching, swelling, and redness. The IgE-linked antigen activates mast cell degranulation, releasing histamine, prostaglandins, tryptase, heparin, and other bioactive compounds. Histamine is key to many symptoms associated with arthropod bites, as it stimulates surrounding nerves, resulting in itching, produces vasodilation, and increases vascular permeability, causing swelling (edema) and reddening of the area. Heparin similarly affects blood vessels, causing endothelial gaping, which allows fluid leakage and results in tissue swelling. Prostaglandins produce pain. Classically, symptoms progress based on exposure history from essentially asymptomatic when immunologically naïve, to immediate-type hypersensitivity (largely driven by IgE), to delayed-type hypersensitivity (driven by T-cell mediated responses), and sometimes ultimately (with a long history of exposure) to tolerance, in which loss of IgE due to antibody isotype switching to IgG results in loss of mast cell/histamine driven symptoms such as itching and edema. In practice, mixed immediate and delayed hypersensitivity responses are common.

In any allergy situation, individual physiology determines how the individual will respond to a particular allergen. For this reason, it is impossible to diagnose the causative agent of an allergic reaction based on the lesion, signs, or symptoms. An individual's reaction may also change over time as the immune system becomes more or less sensitive to the allergen.

Flea Allergy. Flea salivary secretions have been called the strongest itch-inducing substances known to man. In pets, especially dogs, flea feeding can produce Flea Allergy Dermatitis (FAD), a hypersensitivity condition in which the animal experiences severe and sustained itching, causing it to lick and scratch until the skin is abraded and denuded. FAD frequently leads to secondary infection.

Because fleas are strictly hematophagous, feeding frequently on the same host, they are especially likely to produce allergic reactions. It has been conjectured that the cat flea, *Ctenocephalides felis* (Bouchè), evolved on felines, because cats are less likely to experience FAD than are dogs. This is believed to demonstrate a longer evolutionary history between cats and cat fleas in which the feline immune system coevolved with the flea, moderating effects of flea saliva.

Mite Allergy. Chiggers (also known as redbugs) are the larval stage of mite species in the family Trombiculidae. In the U.S., the most commonly encountered chigger is *Eutrombicula alfreddugesi*. These larvae are light orange and barely large enough to see with the naked eye (ca. 0.25 mm long). Chiggers are typically clumped in distribution, with concentrations found where the eggs were deposited by the female, explaining why individuals typically are attacked by several chiggers at a time, very rarely a single chigger.

Because chiggers live on the ground, they climb on the host's foot and then move up the body, illustrating why the majority of chiggers are attached on the legs and lower body regions. Their upward progress is typically slowed by clothing constriction, thus concentrating chigger bites around the socks and underwear.

Once on the host, the chigger inserts its chelicerae into the skin and secretes digestive enzymes that break down skin cells and form a feeding tube called a stylostome. Chiggers do not burrow into the skin, but host swelling in response to their presence may cause surrounding tissue to bulge around the mite, giving the illusion that the chigger is digging into the dermis.

Depending on the host's sensitization to chigger secretions, itching may begin within minutes of attachment or not commence for 12 to 24 hours. Typically, pruritus persists for a week to 10 days. Treatment is symptomatic (anti-itch creams, diphenhydramine or other antihistamines); there is no remedy because the body's immune system requires several days to break down and eliminate the stylostome and materials left behind by the chigger's feeding.

Humans are unnatural hosts for chiggers, so neither the mite nor the human benefits from the experience. Despite their feeding efforts, chiggers are unable to obtain suitable nutrients from humans, so soon die and fall off, usually within a day or two. Meanwhile, the human immune system is reacting violently to the allergens in chigger saliva. Typically by the time the human starts itching, the mite has already died, so treatment strategies aimed at killing the chigger (e.g., nail polish) are futile and too late. However, because the same nerves that transmit the pruritic sensation also carry other signals, any stimulus (heat, cold, pain, etc.) can override itching and produce temporary relief.

Chigger habitat is where their preferred hosts, small rodents and reptiles, are found, so common sites are weedy areas and bramble patches. Contrary to folklore, chiggers do not inhabit Spanish moss, but are found in the underbrush surrounding trees bearing Spanish moss (Whitaker and Ruckdeschel 2010).

In addition to chiggers, other mites can attempt to feed on people, despite humans not being acceptable hosts. Straw itch mites (*Pyemotes ventricosus* and *P. tritici*) are used in biological control because they parasitize many arthropod pests. They may become quite numerous on insects feeding in hay fields; once harvested in the hay, they become a threat to horses and humans contacting the contaminated hay. Despite transferring to the vertebrates, they cannot survive on them, so soon die. Nevertheless, their feeding attempts leave behind pruritic lesions and can even produce systemic effects (nausea, fever, etc.) in susceptible individuals.

Another mite, *Pyemotes herfsi*, has caused outbreaks in humans in areas where mite numbers have built up as they parasitized Cecidomyidae fly larvae in oak leaf galls. Because these mites are found in trees and fall down onto their human victims below, typical lesions produced are primarily on the upper portion of the body, especially exposed areas of the neck and arms. In areas where these mites are found, people outside during the late summer and fall may acquire large numbers of these mites, resulting in pruritic wheals that persist for a week or more. Again, because these mites are arthropod parasites, not adapted for feeding on vertebrates, humans are dead-end hosts (Broce et al. 2006).

By comparison, scabies mites (*Sarcoptes scabiei*), which are valid human parasitic mites, do not produce symptoms due to their feeding activity, but rather due to the material they leave behind, such as feces, cast skins, and eggs. These highly antigenic substances deposited in skin tunnels stimulate the immune system, resulting in extreme pruritus until the host system has eliminated them.

Scabies mites are the only mites that can successfully feed and reproduce on humans (neglecting Demodex mites, which are considered benign or beneficial commensals). Numerous other mites (*Cheyletiella, Notoedres, Otodectes* and such) that are mammal ectoparasites may occasionally attempt to feed on humans, producing a transient and self-resolving rash, but none of them are able to successfully parasitize humans.

Ticks. Most ticks affecting humans are hard ticks (Ixodidae), so soft ticks will not be included in this discussion. A female tick lays a single batch of eggs, all of which hatch at once producing a mass of tick larvae host-seeking in a single site simultaneously. This explains why individuals typically get several seed ticks (larval ticks) on them, rather than one at a time.

All tick life stages (other than eggs) are parasitic and must feed on a host. Tick larvae, nymphs, and adults feed only on vertebrate blood. Because they fall off the host between meals, each stage of the tick must locate a new host. Typically larvae and nymphs feed on small animals (such as rodents and rabbits), while adults seek out larger mammals (dogs and deer). Most tick species that parasitize humans have a broad host range. In the southeastern U.S., the three species most commonly found feeding on humans are the lonestar tick (*Amblyomma americanum*), the American dog tick (*Dermacentor variabilis*), and the deer tick (*Ixodes scapularis*). The brown dog tick (*Rhipicephalus sanguineus*) almost never attaches to humans.

The tick inserts its hypostome into the skin, using its barbed chelicerae to saw through the skin into vascularized tissue below. The tick then secretes a substance called cement into the surrounding tissue to anchor it in place while feeding. Once the hypostome is securely placed, the tick injects analgesics, anticoagulants, and other substances to facilitate feeding (Francischetti et al. 2010). Typically the tick is attached for ca. 24 hours before it begins actively feeding and alternates injecting saliva with sucking up blood in cycles over the next several days until the tick is fully engorged. At that point, the tick removes its mouthparts, but leaves behind the attachment cement containing immunoreactive polypeptides that remain as potent allergens for weeks (or perhaps months), leaving an indurated area and causing continued pruritus. Anecdotal accounts indicate that the allergenic substances in cement can be "reactivated" by subsequent tick bites, so that itching resumes after presumed healing (similar to the phenomenon described by Goddard et al. 2011 in bed bug exposure). In the Southeastern U.S., the lonestar tick seems to produce the most severe and longest-lasting pruritus of any tick species attacking humans, perhaps because the cement layer almost invariably is retained in the skin upon tick detachment (Needham 1985).

One must keep in mind that the reaction to tick bites also has a role as a protective response, allowing early detection and removal of the tick. For example, Burke et al. (2005) found that a history of itch in response to *Ixodes scapularis* bites was associated with a decreased risk of Lyme disease.

A particularly unusual allergic condition associated with ticks is red meat allergy (Van Nunen et al. 2009). In some susceptible individuals, exposure to tick bite can result in antibodies to galactose-beta 1,3-galactose, conveying cross-resistance to red meat. The resultant reaction, when the sensitized individual consumes beef, pork, or other mammalian meat, can produce severe anaphylaxis, to the point of being life-threatening.

Conclusion

The bites of fleas, mites, and ticks, among other arthropods, can produce host reactions ranging from mild to very severe, depending on the arthropod, the amount of salivary secretions injected, the host, host physiology, host exposure history, and other factors.

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National Conference on Urban Entomology at a glance

For locations, see the detailed program on the following pages

SUNDAY,	MAY 20
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8:00 - 4:00eXtension work group meeting 2:30 - 5:00 Registration and presentation upload 6:00 - 8:00

Welcome reception (free hors d'oeuvres and cash bar)

MONDAY, MAY 21

6:30 - 8:00Breakfast

7:00 - 5:00Registration and presentation upload

7:45 - 10:00Opening session

10:00 - 10:30**BREAK**

10:30 - 12:00Student paper presentations Submitted papers, general

Awards luncheon 12:00 - 1:30

1:30 - 3:00Student paper presentations 1:30 - 3:30Submitted papers, termites

3:30 - 4:00**BREAK**

4:00 - 5:32 **SYMPOSIUM** Bed bugs: distribution, success stories,

and outreach

4:00 - 5:45 Submitted papers, termites 6:00 Dinner on your own

TUESDAY, MAY 22

6:30 - 8:00Breakfast

7:00 - 5:00Registration and presentation upload

8:00 - 9:45Submitted papers, bed bugs 8:00 - 9:30Submitted papers, ants

10:00 - 10:30**BREAK**

10:30 - 12:30**SYMPOSIUM** Green chemistry for urban pest management

SYMPOSIUM Valid and putative skin infestations

12:30 Lunch on your own

2:00 - 3:45**SYMPOSIUM** Caribbean crazy ants

1:45 - 3:40**SYMPOSIUM** Arthropod associated allergy

3:45 - 4:00**BREAK**

4:00 - 4:45SYMPOSIUM Submitted papers, general

EVENING RECEPTION 4:15 - 5:00

6:00 - 9:00Student competition awards presented

WEDNESDAY, MAY 23

7:00 - 8:30Breakfast

7:00 - 10:00Registration and presentation upload

8:30 - 10:30**SYMPOSIUM** Bed bug management practices

8:30 - 10:40**SYMPOSIUM** New technologies for termite management

10:30 - 10:45**BREAK**

10:45 **Business** meeting

11:30 Executive committee business meeting

SUNDAY, MAY 20

8:00 – 4:00 eXtension work group meeting **2:30 – 5:00** Registration and presentation upload

6:00 – 8:00 Welcome reception (free hors d'oeuvres and cash bar)

MONDAY, MAY 21

Breakfast PERIMETER PAVILLION

7:00 – 5:00 Registration and presentation upload — SALON A

SALON BCDE

7:45 Welcome and Orientation

FAITH OI, Conference Chair

University of Florida

BILLY SKAGGS, Chief Operating Officer

Georgia Dept. of Agriculture

VALERA JESSEE, Executive Director Georgia Pest Control Association

Conference Overview

ROGER GOLD

Texas A&M University

DAN SUITER, Local Arrangements Chair

University of Georgia KAREN VAIL, Awards Chair

University of Tennessee

8:15 Distinguished Achievement Award in Urban Entomology

The Arnold Mallis Memorial Award Lecture: Science of -omics in urban pest management

SHRIPAT KAMBLE University of Nebraska

9:15 Student Scholarship Award Presentations

KAREN VAIL

University of Tennessee

9:15 – 9:30 Bachelor of Science Award

Population growth characteristics of incipient colonies of the Eastern subterranean termite, Reticulitermes flavipes (Isoptera: Rhinotermitidae)

MARK A. JANOWIECKI, Susan C. Jones and

Joshua L. Bryant

The Ohio state university

9:30 – 9:45 Master of Science Award

Extraction efficiency of Dermatophagoides pteronyssinus from

several substrates using two techniques ASHLEY E. RODEN and Brian T. Forschler

University of Georgia

9:45 – 10:00 Ph.D. Award

Effect of high temperatures on residual insecticides

used for bed bug treatments

MARGIE P. LEHNERT, Eric P. Benson and

Patricia A. Zungoli Clemson University

10:00 - 10:30 BREAK

SALON BC STUDENT PAPER PRESENTATIONS

1	1/	Λd	era	ŧΛ	re

Tracie Jenkins, University of Georgia Grzegorz Buczkowski, Purdue University

10:30 – 10:40 Subterranean termites (Isoptera: Rhinotermitidae)

of Alabama: a new identification tool using the

worker, soldier, and imago castes

CHARLES D. STEPHEN, Xing P. Hu and Charles H. Ray

Auburn University

10:40 – 10:50 Social disaster in termites

ZNAR BARWARY and Xing Ping Hu

Auburn University

10:50 – 11:00 Comparison of food preference in *Reticulitermes*

using choice and no choice bioassay designGRETCHEN PERKINS and Brian T. Forschler

University of Georgia

11:00 – 11:10 Genetic engineering of hindgut bacteria from Formosan subterranean

termites, Coptotermes formosanus Shiraki, to serve as

"Trojan Horse" for termite control

CHINMAY TIKHE, Claudia Husseneder and Jennifer Delatte

Louisiana State University

11:10 – 11:20 Evaluation of three termite bait materials:

Pine wood, cardboard and corn cob CAI WANG and Gregg Henderson

Louisiana State University

11:20 – 11:30 Population genetics and breeding structure of subterranean termites

(Reticulitermes flavipes) from infested urban structures in Nebraska

ABDUL HAFIZ AB MAJID, Shripat T. Kamble and Nick Miller

University of Nebraska

11:30 – 11:40 Biology and management of the dark rover ant

(Hymenoptera: Formicidae)

T. CHRIS KEEFER and Roger E. Gold

Texas A&M University

11:40 – 11:50 Instar determination of the Dubia cockroach (*Blaptica dubia*):

A maximum likelihood approach

HAO WU, Arthur Appel and Xing Ping Hu

Auburn University

SALON DE SUBMITTED PAPERS - GENERAL

Moderators:

Joe DeMark, Dow Agrosciences Ronda Hamm, Dow Agrosciences

Center for Disease Control and Prevention's "Biology and control of vectors and public health pests: the importance of integrated pest management": A curriculum for public environmental health specialists

MARC L. LAME and Michael E. Herring

Indiana University

Centers for Disease Control and Prevention

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10:30 - 10:40

10:45 - 11:00	Factors affecting cockroach control in low-income housing: Bait efficacy studies
	DINI M. MILLER, T. C. McCoy and M. Stedfast Virginia Tech
11:00 – 11:15	Discovery and development of a new bait for silverfish control STEVEN R. SIMS, Arthur G. Appel and Marla J. Eva BASF
	Auburn University
11:15 – 11:30	Fifty years of innovation for structural fumigation with sulfuryl fluoride (Vikane® gas fumigant) ELLEN THOMS
	Dow Agrosciences
11:30 - 11:45	Megacopta cribraria in North America: The rapid dispersal of a
	species complex from Asia TRACIE M. JENKINS and Tyler D. Eaton
	University of Georgia
11:45 – 12:00	Method for evaluating residual toxicity of new insecticides in vitro
11000	against life stages of the cat flea, Ctenocephalides felis
	COLE YOUNGER
	Stillmeadow Inc.
12:00 – 1:30	Awards luncheon — PERIMETER PAVILLION
	CALONIDO
	SALON BC
	STUDENT PAPER PRESENTATIONS
	Moderator:
1.20 1.40	Tracie Jenkins, University of Georgia
1:30 – 1:40	Optimizing pest management curricula for use in K-12 classrooms MAKENA MASON, Maria Aihara-Sasaki and Kenneth J. Grace University of Hawaii
1:40 - 1:50	Pyrethroid-resistant bed bugs Cimex lectularius L.: Characterization
	of the cuticle using molecular, SEM, and GC-MS methods
	REINA KOGANEMARU, Dini M. Miller and Zach N. Adelman
	Virginia Tech
1:50 - 2:00	Effects of various blood alcohol concentrations on bed bugs (Cimex lectularius)
	RALPH NARAIN, Shripat T. Kamble and Nicholas Miller
	University of Nebraska
2:00 - 2:10	Metabolic rate and water loss of the common bed bug, Cimex lectularius
	BRITTANY DELONG, Dini Miller and Don Mullins
	Virginia Tech
2:10 - 2:20	Bed bug (Cimex lectularius L.) survivorship at various temperatures
	MOLLY L. STEDFAST and Dini M. Miller
2:20 - 2:30	Virginia Tech Ruining your picnic: Prevalence of pest ants in urban parks in Tucson, AZ
2:20 - 2:30	JAVIER MIGUELENA and Paul B. Baker
	University of Arizona
2:30 - 2:40	Tracking Argentine ant foragers using the sandwich ELISA test
	JINBO SONG, Eric Benson, Patricia Zungoli,
	Simon Scott and Patrick Gerard
	Clemson University

2:40 – 2:50	Fact or fiction: Evaluating claims of repellency of various plants to Argentine ants JACOB HOLLOWAY, Daniel Suiter, Brian Forschler and Wayne Gardner University of Georgia
2:50 – 3:00	Efficacy of professional-use insecticides against the invasive "kudzu bug" <i>Megacopta cribraria</i> NICHOLAS SEITER, Eric Benson, Patricia Zungoli, Jeremy Greene and Francis Reay-Jones Clemson University
	SALON DE SUBMITTED PAPERS - TERMITES
	Moderators:
	Clay Scherer, DuPont Professional Products
1:30 – 1:45	Raj Saran, DuPont Professional Products Toxicity and horizontal transfer of a dry formulation of fipronil (Termidor® Dry) against Formosan subterranean termites BAL K. GAUTAM and Gregg Henderson Louisiana State University
1:45 – 2:00	Field and laboratory evaluations of Altriset® in southern Arizona PAUL B. BAKER
2:00 – 2:15	University of Arizona Performance of Recruit® HD in field trials against Heterotermes aureus (Snyder) in Arizona JOE DeMARK, Mike Lees and Joe Eger
	Dow Agrosciences
2:15 – 2:30	Performance of Altriset™ (chlorantraniliprole) termiticide against Formosan subterranean termites, <i>Coptotermes</i>
	formosanus Shiraki, in laboratory feeding cessation and collateral transfer trials ROBERT T. PUCKETT, T. Chris Keefer and Roger E. Gold
2:30 – 2:45	Texas A&M University Altriset™ (chlorantraniliprole) termiticide performance against Formosan subterranean termites, Coptotermes formosanus
	Shiraki, in field trials through 3-years ROGER E. GOLD, T. Chris Keefer, and Robert T. Puckett Texas A&M University
2:45 – 3:00	Distribution of pyrethroid termiticides in 'ABC' gravel BRAD M. KARD, George J. Tompkins, George L. Rotramel and Leo A. Renello Oklahoma State University
	Pest Management Industry Consultant Rotramel Technical Services Arizona Chemical Group, Inc.
3:00 – 3:15	Long term residual performance of Altriset® termiticide in pre-construction and post-construction applications CLAY W. SCHERER, Nicola T. Gallagher and Mark A. Coffelt DuPont Professional Products

3:15 - 3:30Evaluation of two ready-to-use Termidor® formulations and Premise® Foam for the control of the drywood termite Incisitermes snyderi (Kalotermitidae) using localized treatments to naturally infested lumber ROBERT HICKMAN and Brian T. Forschler **BASF** Corporation University of Georgia 3:30 - 4:00**BREAK** SALON BC **SYMPOSIUM** Bed bugs: Distribution, success stories, and outreach **Organizer and Moderator:** Kaci Buhl, National Pesticide Information Center, Oregon State University 4:00 - 4:18 EPA's collaborative efforts to combat bed bugs SUSAN JENNINGS US EPA, Office Of Pesticide Programs 4:18 - 4:36 Success stories in low-income multifamily housing ALLISON TAISEY Cornell University, Northeastern IPM Center 4:36 - 4:54 Bed bug distribution and pesticide incidents in the United States KACI BUHL Oregon State University 4:54 - 5:14 A ProActive approach for commercial bed bug control JASON MEYERS **BASF Pest Control Solutions** 5:14 - 5:32 Efficacy of Phantom® SC termiticide-insecticide & Prescription Treatment® brand ULD® HydroPy-300® on field collected and lab reared bed bug (Cimex lectularius) populations ROBERT W. DAVIS and Cole Younger **BASF Pest Control Solutions** Stillmeadow Inc. SALON DE SUBMITTED PAPERS – TERMITES **Moderators:** Jason Meyers, BASF Pest Control Solutions Kyle Jordan, BASF Pest Control Solutions Molecular evolution of Reticulitermes from the Southeastern USA 4:00 - 4:15SU YEE LIM and Brian T. Forschler University of Georgia 4:15 - 4:30Nasutitermes corninger: Large-scale foraging arena and spot treatment with Termidor® Dry **RUDOLPH SCHEFFRAHN** University of Florida

4:30 – 4:45	Separating individual and group behaviors in termite bioassay design BRIAN T. FORSCHLER
4:45 – 5:00	University of Georgia Dispersal dynamics of the exotic arboreal termite Nasutitermes corniger SEEMANTI CHAKRABARTI and Rudolf H. Scheffrahn University of Florida
5:00 – 5:15	Using the Advance® Termite Baiting System to monitor termite populations and provide community-wide structural protection Faith Oi and KYLE JORDAN University of Florida BASF Pest Control Solutions
5:15 – 5:30	Colony effects of Termidor Dry: Results from tunnel exposure test XING PING HU and Jordan (Zhonglin) Yuan Auburn University
5:30 – 5:45	Evaluation of Novaluron for use as a termite bait active KENNETH S. BROWN, Ed Vargo, Claudia Riegel, Roger Gold, E. Freytag, T. Chris Keefer and J. H. Cink BASF Pest Control Solutions North Carolina State University City Of New Orleans Mosquito And Termite Control Board Texas A&M University
6:00	Dinner (on your own)
6:30 - 8:00 7:00 - 5:00	TUESDAY, MAY 22 Breakfast — PERIMETER PAVILLION Registration and presentation upload — SALON A
	SALON BC SUBMITTED PAPERS - BED BUGS Moderator:
8:00 – 8:15	Changlu Wang, Rutgers University A novel method for artificially feeding bed bugs, Cimex lectularius L. (Hemiptera: Cimicidae) Eva Chin-Heady, RONDA L. HAMM, Joe J. DeMark, Steve Nolting, Gary Bennett and Kurt Saltzmann Dow Agrosciences LLC, Indianapolis Dow Agrosciences LLC, Fayetteville
8:15 – 8:30	Purdue University, West Lafayette Exploring a novel detection technique for the common bed bug DONG-HWAN CHOE
8:30 – 8:45	University of California-Riverside Laboratory and field evaluation of factors affecting bed bug monitors NARINDERPAL SINGH, Changlu Wang and Richard Cooper
8:45 – 9:00	Rutgers University Lessons learned in bed bug rearing JOSHUA L. BRYANT and Susan C. Jones The Ohio State University
	J

	anorial comoronica on criban Emaniciogy
9:00 – 9:15	Optimization of an in vitro system for rearing bed bugs with notes on their development on different host bloods ALVARO ROMERO, Richard G. Santangelo and Coby Schal New Mexico State University North Carolina State University
9:15 - 9:30	Susceptibility of multiple field collected strains of bed bugs, Cimex lectularius, to selected insecticides SUMIKO DE LA VEGA and William A. Donahue, Jr. Sierra Research Laboratories
9:30 – 9:45	Evaluation of the residual activity and repellency of selected insecticides against bed bugs, <i>Cimex lectularius</i> WILLIAM A. DONAHUE, JR. and Sumiko De La Vega Sierra Research Laboratories
	SALON DE SUBMITTED PAPERS – ANTS AND TERMITE
	Moderator: Robert Hickman, BASF Pest Control Solutions
8:00 - 8:15	Impact of systemic thiamethoxam on Argentine ant foraging behavior in aphid-infested pepper plants DANIEL R. SUITER, Ronald D. Oetting, Mercedes D. Guerra and Monica M. Townsend University Of Georgia
8:15 – 8:30	Taking the fire out of the ant: Using a cold storage trailer to treat ant-infested soils KAREN M. VAIL, Jennifer Chandler, Jeremy Shoop, Anne-Marie Callcott, Kevin Hoyt and Richard Evans University of Tennessee City-State LLC USDA-APHIS-PPQ UT Forest Resouces Research & Education Center
8:30 – 8:45	DuPont Arilon®: A new residual insecticide formulation with higher durability and flexibility RAJ SARAN, Sara Kudlie, Clay Scherer, Nicky Gallagher and Mark Coffelt DuPont Professional Products
8:45 – 9:00	Evaluation of ArilonTM and AdvionTM for the control of ants and other perimeter pest species NICOLA T. GALLAGHER, Clay W. Scherer, Raj K. Saran and Mark A. Coffelt DuPont Professional Products

9:00 – 9:15	Argentine ant management on Santa Cruz Island CHRISTINA L. BOSER, Kathryn R. Faulkner, Coleen Cory, Lotus A. Vermeer, John M. Randall and Scott A. Morrison The Nature Conservancy The National Park Service
9:15 – 9:30	Ants up north: Survey of urban ants with pest management personnel LAUREL D. HANSEN Spokane Falls Community College
10:00 - 10:30	BREAK
	SALON BC SYMPOSIUM
	Green chemistry for urban pest management Organizers and Moderators:
	Joel Coats, Iowa State University
	Don Reierson, University of California – Riverside
	David Murphy, EcoSMART Technologies, Inc.
	Gretchen Paluch, EcoSMART Technologies, Inc.
10:30 – 10:45	Natural insecticides for control of ants, spiders, and wasps DON REIERSON
10.17.11.00	University Of California – Riverside
10:45 – 11:00	Research in urban pest control using natural products
	ARTHUR G. APPEL, Steven R. Sims and Marla J. Eva Auburn University
	BASF
11:00 - 11:15	Change in pesticides since 1990: Comparing pesticide actives,
	toxicity, and inerts from 1990, 2000, and 2010
	KEITH WILLINGHAM
	Western Exterminator
11:15 – 11:30	Trial of a minimum-risk botanical compound to control the
	vector tick of Lyme disease
	CHARLES LUBELCZYK
11 20 11 15	Maine Medical Center Research Institute
11:30 – 11:45	Synergistic insecticidal activity of plant essential oil mixtures STEVE BESSETTE and Gretchen Paluch
	EcoSMART Technologies, Inc.
11:45 – 12:00	Push and pull strategy in control of filth flies in urban settings JUNWEI JERRY ZHU
	USDA-ARS
12:00 – 12:15	Plant essential oil properties and applications in low-impact ant
	pest management
	DANIEL SUITER
	University of Georgia

SALON DE SYMPOSIUM

	Valid and putative human skin infestations
	Organizers and Moderators:
	Nancy Hinkle, University of Georgia
	Michael Merchant, Texas AgriLife Extension Service
10:30 - 10:35	Introduction
	NANCY C. HINKLE
	University of Georgia
10:35 - 10:55	Contagion or the veterinary X-Files? A vet's perspective on Ekbom
	Syndrome
	FRAN NICHOLSON
	Hollyberry Animal Hospital
10:55 - 11:20	Procedures and guidelines for handling extension clientele with
	mystery bug infestations
	MICHAEL E. MERCHANT
	Texas Agrilife Extension Service
11:20 - 11:45	Diagnostic quagmires: Miscellanea and debris misconstrued as
	ectoparasites and ectoparasites mistaken to be miscellanea
	RICHARD J. POLLACK
	IdentifyUs LLC
11:45 – 12:10	Working with pest management professionals and delusory
	parasitosis cases
	MICHAEL WALDVOGEL
	North Carolina State University
12:10 – 12:25	Risks and consequences of Ekbom Syndrome treatments:
	Medical-veterinary and urban entomology
	NANCY C. HINKLE
	University of Georgia
12:25 – 12:30	Questions and answers
	MICHAEL E. MERCHANT
	Texas Agrilife Extension Service
12:30	Lunch on your own
	SALON BC

SALON BC SYMPOSIUM

Caribbean crazy ants
Organizer and Moderator:
Dawn Calibeo, University of Florida

2:00 – 2:05

Introduction

DAWN CALIBEO

University of Florida

2:05 – 2:20 Morphological and molecular diagnostic identification of *Nylanderia*

sp. nr. pubens JASON MEYERS

BASF

2:20 – 2:35 Combination baiting approach cuts field populations of *Nylanderia*

pubens in half

JOHN H. PAIGE, Faith Oi and Dawn Calibeo

	D E' 410 '
	Bayer Environmental Science
2:35 - 2:50	University of Florida Biological aspects of Nylanderia pubens in Florida
2:35 – 2:50	SHWETA SHARMA, Eileen Buss, Steven Valles and David Oi
	USDA-ARS-CMAVE
2:50 - 3:05	Realized natality of Nylanderia sp. nr. pubens under
2100 0100	laboratory conditions
	DANNY McDONALD and Roger Gold
	Texas A&M University
3:05 – 3:20	Field biology and nutritional studies for Caribbean crazy ants
	JODI SCOTT, Stephanie Larrick, Roberto Pereira and Phil Koehler
	University of Florida
3:20 – 3:35	Caribbean crazy ant IPM strategies
	DAWN CALIBEO and Faith Oi
	University of Florida
3:35 – 3:45	Discussion/Questions
	DAWN CALIBEO
	University of Florida
	SALON DE
	SYMPOSIUM
	STMI OSIOM
	Arthropod associated allergy
	Organizer and Moderator:
	Shripat Kamble, University of Nebraska
1:45 - 2:05	Allergy to arthropod allergens/bites/secretions/venoms
	SHRIPAT T. KAMBLE
	University of Nebraska
2:05 - 2:25	Cockroach allergens
	COBY SCHAL
	North Carolina State University
2:25 – 2:45	Fire ant allergy
	ROBERT VANDER MEER
	USDA-ARS-CMAVE
2:45 – 3:05	Asian needle ant allergies
	PATRICIA ZUNGOLI and Eric Benson
2.05 2.20	Clemson University
3:05 – 3:20	Allergy to fleas/mites/ticks
	NANCY HINKLE and Don Champagne
3.20 3.40	University of Georgia
3:20 – 3:40	Skin reaction to bed bug bites
3:45 – 4:00	RONALD HARRISON, Orkin LLC BREAK
5:45 - 4:00	DKEAR

SALON BC SUBMITTED PAPERS – GENERAL

	Moderator:
	Dawn Calibeo, University of Florida
4:00 - 4:15	Novel approach for educating public about structural fumigation
	Robert I. Krieger, BARB A. NEAD-NYLANDER and Ellen M. Thoms
	University Of California-Riverside
	Dow Agrosciences
4:15 – 4:30	Recent trends in urban spiders in Georgia, including an update on the
	brown recluse spider, Loxosceles reclusa Gertsch and Mulaik
	LISA M. AMES

4:30 - 4:45

4:00 - 4:15

4:15 - 4:30

6:00 - 9:00

7:00 - 8:30

7:00 - 5:00

University of Georgia
Efficacy of Metarhizium anisopliae (Metschnikoff) Sorokin
(Hypocreales: Clavicipitaceae) and diatomaceous earth against
Periplaneta americana L. (Dictyoptera: Blattidae)
WAQAS WAKIL, Muhammad Asim and Muhammad Yasin
University of Agriculture, Faisalabad, Pakistan

SALON DE SUBMITTED PAPERS – GENERAL

Moderator:

Kyle Jordan, BASF Pest Control Solutions

NPMA perspectives on bed bug best management practices JIM FREDERICKS

National Pest Management Association

Assessing bed bug (Cimex lectularius) control programs using mock environments

KYLE JORDAN and J. Lawrence BASF Pest Control Solutions Eurofins Agroscience Services

Evening reception and tour of Orkin training facility (Buses leaving Marriott for Orkin at 5:00, 5:10 and 5:15 pm.)

WEDNESDAY, MAY 23

Breakfast — PERIMETER PAVILLION
Registration and presentation upload — SALON A

SALON BC SYMPOSIUM

Bed bug management practices Organizer and Moderator: *Changlu Wang, Rutgers University*

8:30 - 8:45	Canine scent detection: Their effectiveness and pitfalls
	RICHARD COOPER
	Rutgers University
8:45 - 9:00	Using bed bug monitors to maximize effectiveness of bed bug
	management programs
	CHANGLU WANG
	Rutgers University
9:00 – 9:15	Heat treatments and other non-chemical tools
	STEPHEN KELLS
	University of Minnesota
9:15 – 9:30	Bed bug insecticides: Hunt for a hammer
	MICHAEL POTTER
	University of Kentucky
9:30 – 9:45	Active monitoring of bed bugs in occupied apartments
	SUSAN JONES
	The Ohio State University
9:45 – 10:00	Community-based IPM
	DINI MILLER
	Virginia Tech
10:00 - 10:15	Bed bug management in hotels
	RON HARRISON
	Orkin LLC
10:15 – 10:30	NPMA perspectives on best bed bug management policies
	JAMES FREDERICKS
	National Pest Management Association
	CALONIDE
	SALON DE
	SYMPOSIUM
	New technologies for termite management
	Organizer and Moderator:
	Raj Saran, DuPont Professional Products
8:30 - 8:40	Introduction
	RAJ SARAN
	Dupont Professional Products
8:40 - 9:00	Development and commercialization of Recruit® HD, the first
	durable bait for subterranean termites
	(Isoptera: Rhinotermitidae)
	JOE E. EGER, Joe J. DeMark, Michelle S. Smith and Ronda L. Hamm
	Dow Agrosciences
9:00 - 9:20	Performance of Recruit HD® versus Coptotermes formosanus
	Shiraki in trials conducted by the City of New Orleans Mosquito
	and Termite Control Board
	CLAUDIA RIEGEL and Barry Yokum
	City Of New Orleans Mosquito And Termite Control Board
9:20 - 9:40	Termidor High Efficiency termiticide copack with Termidor
	HE technology: An overview of BASF's new termiticide
	KYLE JORDAN, Robert Davis, Robert Hickman,
	Tom Nishimura, Jason Meyers and William Kolbe

BASF Pest Control Solutions

9:40 – 10:00 Performance of Termidor® HE termiticide against subterranean

termites in laboratory collateral transfer trials and field applications $% \left(1\right) =\left(1\right) \left(1\right) \left$

T. CHRIS KEEFER, Robert T. Puckett, R. W. Davis, Ken S. Brown,

and Roger E. Gold Texas A&M University

10:00 – 10:20 Altriset®: A new termiticide with unique post-exposure effects,

delayed toxicity and favorable environmental profile

RAJ SARAN, Sara Kudlie, Nicky Gallagher, Clay Scherer and

Mark Coffelt

Dupont Professional Products

10:20 – 10:40 Delayed impacts of Altriset® termiticide on Eastern subterranean

termite colonies

SUSAN C. JONES and Joshua L. Bryant

The Ohio State University

10:30 - 10:45 BREAK

SALON BC

10:45 Business meeting

11:30 Executive committee business meeting

2012 National Conference on Urban Entomology Planning Committee

Faith Oi (University of Florida), Conference Chair Grzegorz Buczkowski (Purdue University), Program Dan Suiter (University of Georgia), Proceedings Dan Suiter (University of Georgia), Local Arrangements Tracie Jenkins (University of Georgia), Local Arrangements Kyle Jordan (BASF), Local Arrangements Dini Miller (Virginia Tech), Secretary Roger Gold (Texas A&M University), Treasurer Laura Nelson (Texas A&M University), Assistant to Roger Gold Dan Suiter (University of Georgia), Sponsorship Shripat Kamble (University of Nebraska), Sponsorship Gary Bennett (Purdue University), Sponsorship Raj Saran (DuPont), Sponsorship Karen Vail (University of Tennessee), Awards Raj Saran (DuPont), Awards

2014 National Conference on Urban Entomology Planning Committee

Faith Oi (University of Florida), Conference Co-Chair Grzegorz Buczkowski (Purdue University), Conference Co-Chair Bob Kopanic (S.C. Johnson and Son), Program Co-Chair Dini Miller (Virginia Tech), Program Co-Chair Karen Vail (University of Tennessee), Awards Co-Chair Raj Saran (DuPont), Awards Co-Chair Roger Gold (Texas A&M University), Treasurer (2014 is last year) Laura Nelson (Texas A&M University), Assistant to Roger Gold (2014 is last year)
Gold and Nelson (Texas A&M University), Local Arrangements Dan Suiter (University of Georgia), Sponsorship Chair Dan Suiter (University of Georgia), Local Arrangements Shripat Kamble (University of Nebraska), Sponsorship Gary Bennett (Purdue University), Sponsorship Kyle Jordan (BASF), Secretary and Proceedings Co-Chair

Jason Meyers (BASF), Proceedings Co-Chair

Recipients of The Distinguished Achievement Award in Urban Entomology

1986	Dr. Walter Ebeling (University of California, Los Angeles)
	Dr. James Grayson (Virginia Polytechnic Institute & State University)
1988	Dr. John V. Osmun (Purdue University)
	Dr. Eugene Wood (University of Maryland)
1990	Dr. Francis W. Lechleitner (Colorado State University)
1992	Dr. Charles G. Wright (North Carolina State University)
1994	Dr. Roger D. Akre (Washington State University)
	Dr. Harry B. Moore (North Carolina State University)
	Dr. Mary H. Ross (Virginia Polytechnic Institute & State University)
1996	Dr. Donald G. Cochran (Virginia Polytechnic Institute & State University)
1998	Dr. Gary W. Bennett (Purdue University)
2000	Dr. Michael K. Rust (University of California, Riverside)
2004	Dr. Roger E. Gold (Texas A&M University)
2006	Dr. Coby Schal (North Carolina State University)
2008	Dr. Nan-Yao Su (University of Florida)
2010	Dr. Donald A. Reierson (University of California, Riverside)
2012	Dr. Shripat T. Kamble (University of Nebraska, Lincoln, NE)

NCUE Conference Chairs

1986	Patricia A. Zungoli (Clemson University)
1988	William H. Robinson (Virginia Polytechnic Institute & State University)
1990	Michael K. Rust (University of California, Riverside)
1992	Gary W. Bennett (Purdue University)
1994	Roger E. Gold (Texas A&M University)
	Judy K. Bertholf (DowElanco)
1996	Donald A. Reierson (University of California, Riverside)
1998	Brian T. Forschler (University of Georgia)
	Shripat T. Kamble (University of Nebraska)
2000	Shripat T. Kamble (University of Nebraska)
2004	Daniel R. Suiter (University of Georgia)
2006	Dini M. Miller (Virginia Tech University)
	Bob Kopanic (S.C. Johnson and Son)
2008	Richard Houseman (University of Missouri)
	Bob Cartwright (Syngenta)
2010	Karen Vail (University of Tennessee)
2012	Faith Oi (University of Florida)

BYLAWS NATIONAL CONFERENCE ON URBAN ENTOMOLOGY

ARTICLE I- NAME

The name of this organization is the National Conference on Urban Entomology.

ARTICLE II-BACKGROUND

In the spring of 1985, individuals representing urban entomology and the pest control industry came together to organize a national conference to be held biennial. The mission of these conferences was to open channels of communication and information between scientists in industry, academia, and government, and to foster interest and research in the general area of urban and structural entomology. The primary scope of the National Conference is to emphasize innovations and research on household and structural insect pests. It is the intent; however, to provide flexibility to include peripheral topics that pertain to the general discipline of urban entomology. It is anticipated that the scope of the conference could change through time, but the emphasis would be to provide an opportunity for urban entomologist to meet on a regular basis. It is not anticipated that any specific memberships would be required or expected, but that the cost associated with the conference would be met through registration fees and contributions. In the event that funds become available through donations or from the sale of conference proceedings, that these resources will be spent to meet expenses, to pay the expenses for invited speakers, and to provide scholarships to qualified students working in urban entomology. It is the intent of this organization to be non-profit, with financial resources provided to the Conference to be used entirely in support of quality programming and the support of scholarships.

ARTICLE III-OBJECTIVES

The objectives of this organization are:

- 1. To promote the interest of urban and structural entomology.
- 2. To provide a forum for the presentation of research, teaching and extension programsrelated to urban and structural entomology.
- 3. To prepare a written/electronic proceedings of all invited and accepted papers given orprepared at the biennial meeting.
- 4. To promote scholarship and the exchange of ideas among urban entomologists.
- 5. As funds are available, scholarships will be awarded to students pursuing scholastic degrees in urban entomology. Three levels of scholarships will be offered: the first level is for Bachelor students; the second level is for Masters students; and the third level is for Ph.D. candidates. These students must register for, and attend, the conference and present the paper in order to receive funding. These scholarships will be awarded based solely on the merits of the candidates, and the progress that they have made towards completion of their research and scholastic degrees.
 - The student will receive funding only if they are currently enrolled in a university at the time that the conference is held.
- 6. There may also be first, second, and third place recipients of an onsite student competition for students who are currently involved in their undergraduate or graduate programs. These students can compete for scholarship funds; however, if any student has already been awarded a scholarship for the current meeting, and wishes to participate in this onsite competition, their presentation must be completely separate, and they must be properly registered in advance for this competition.

ARTICLE IV-JURISDICTION

The jurisdiction of this conference is limited to events held within the United States of America; however, we will be supportive of international urban entomology conferences as they are organized and held.

ARTICLE V-MEMBERSHIP

There are no membership requirements associated with this organization except for the payment of registration fees which go to offset the cost of holding the conference, preparation/printing of proceedings and the offering of scholarships. All persons with an interest in urban entomology are invited to attend the conferences and associated events.

ARTICLE VI-OFFICERS

Leadership for the Conference will be provided by the Chair of the Conference Committee. The Executive committee will be composed primarily of representatives from academia, industry and government. There will be seven officers of the Executive Committee and will include the following:

Chair of the Conference Committee

Chair of the Program Committee

Chair of the Awards Committee

Secretary to the Conference

Treasurer to the Conference

Chair of the Sponsorship Committee

Chair of the Local Arrangements Committee

The Chair of the Conference Committee will preside at all Committee meetings, and will be the Executive Officer for the organization, and will preside at meetings. In the absence of the Chair of the Conference Committee, the Chair of the Program Committee may preside. The voting members for executive decisions for the conference will be by a majority vote of a quorum which is here defined as at least five officers.

The duties of the officers are as follows:

Chair of the Conference Committee: To provide overall leadership for the Conference, to establish ad hoc committees as needed, and to solicit nominations for new officers as needed.

Chair of the Program Committee: To coordinate the conference in terms of arranging for invited speakers and scientific presentations as well as oversee the printing of announcements, programs and proceedings.

Chair For Awards: To oversee and administer the Mallis Award, scholarships and other honors or awards as approved by the executive committee.

Secretary: To take notes and provide minutes of meetings.

Treasurer: Provide documentation of expenditures, and the collection and disbursement of funds. To act on behalf of the executive committee in making arrangements with hotels, convention centers and other facilities in which conferences are held.

Chair For Sponsorship: This committee will be involved in fund raising and in seeking sponsorship for various aspects of the conference. It will also contact contributors and potential contributors to seek donations and support for the conference and associated events. It is anticipated that the committee will be composed of at least one member representing academia, and one member representing industry.

Chair For Local Arrangements: To gather information on behalf of the executive committee for hotels, convention centers and other facilities in which the conference is to be held. To arrange for audio/visual equipment, and to oversee the general physical arrangements for the conference.

ARTICLE VII-TERMS OF OFFICE & SUCCESSION OF OFFICERS:

Officers may serve for a maximum of four conference terms (8 years); however, if no new nominations are received, the officers may continue until such time as replacements are identified and installed.

The Awards Chair is the last position to be served, and may be relieved from NCUE officer duties unless asked or willing to serve NCUE in another capacity.

The Conference Chair may serve for one conference after which time they will become the Chair of the Awards Committee.

The Program Chair may serve for one conference term after which time they will become the Conference Chair.

The Secretary may serve for one conference term, after which time they will become the Program Chair.

The Chair for Local Arrangements should change with each conference unless the meetings are held in the same location.

The Chair the Sponsorship Committee (to include both an academic and industry representative) will serve for two conferences.

The Treasurer will serve for two conference cycles, unless reappointed by the Executive Committee.

ARTICLE VIII-NOMINATION OF OFFICERS

Nominations for any of the chair positions may come from any individual, committee, or subcommittee, but must be forwarded to the Chair of the Conference before the final business meeting of each conference. It is further anticipated that individuals may be asked to have their names put into nomination by the Chair of the Conference. In the event that there are no nominations, the existing Chair may remain in office with a majority vote of the Executive Committee for the conference. It is clearly the intent of these provisions that as many new people be included as officers of this organization as is possible, and no one shall be excluded from consideration.

ARTICLE IX-MEETINGS

Conferences of the National Conference on Urban Entomology will be held every two years. Meetings of the officers of this organization will meet at least annually either in direct meetings or by conference calls in order to plan the upcoming conference, and to conduct the business of the organization.

ARTICLE X-FINANCIAL RESPONSIBILITIES

All financial resources of the Conference will be held in a bank under an account named, "National Conference on Urban Entomology", and may be subjected to annual audits. Expenditures may be made in support of the conference, for scholarships and other reasonable costs; however, funds may not be used to pay officers', or their staff's salaries, or for

officers' travel expenses. In the event that this organization is disbanded, all remaining funds are to be donated to the Endowment Fund of the Entomological Society of America.

ARTICLE XI-FISCAL YEAR

The fiscal year will run from January 1 through December 31 of each year.

ARTICLE XII-AMENDMENTS

The bylaws for this organization may be amended by a two-thirds affirmative vote of the attendees at the business meeting, provided that the proposed amendments are available for review at least 48 hours in advance of the voting.

ARTICLE XIII-INDEMNIFICATION

The National Conference on Urban Entomology shall indemnify any person who is or was a party, or is or was threatened to be made a party to any threatened, pending or completed action, suit or proceeding, whether civil, criminal, administrative or investigative by reason of the fact that such person is or was an officer of the Committee, or a member of any subcommittee or task force, against expenses, judgments, awards, fines, penalties, and amount paid in settlement actually and reasonably incurred by such persons in connection with such action, suit or proceeding: (I) except with respect to matters as to which it is adjudged in any such suit, action or proceeding that such person is liable to the organization by reason of the fact that such person has been found guilty of the commission of a crime or of gross negligence in the performance of their duties, it being understood that termination of any action, suit or proceeding by judgment, order, settlement, conviction or upon a plea of nolo contendere or its equivalent (whether or not after trial) shall not, of itself, create a presumption or be deemed an adjudication that such person is liable to the organization by reason of the commission of a crime or gross negligence in the performance of their duties; and (II) provided that such person shall have given the organization prompt notice of the threatening or commencement (as appropriate) of any such action, suit or proceeding. Upon notice from any such indemnified person that there is threatened or has been commenced any such action, suit or proceeding, the organization: (a) shall defend such indemnified person through counsel selected by and paid for by the organization and reasonably acceptable to such indemnified person which counsel shall assume control of the defense; and (b) shall reimburse such indemnity in advance of the final disposition of any such action, suit or proceeding, provided that the indemnified person shall agree to repay the organization all amounts so reimbursed, if a court of competent jurisdiction finally determines that such indemnified persons liable to the organization by reason of the fact that such indemnified person has been found guilty of the commission of a crime or of gross negligence in the performance of their duties. The foregoing provision shall be in addition to any and all rights which the persons specified above may otherwise have at any time to indemnification from and/or reimbursement by the organization.

Modified: 5/19/10-passed

2012 NCUE Closing Business Meeting Minutes May 23, 2012

Atlanta, GA

Sponsorship registration \$26750 + sponsorship \$34700 = \$61450 total

Carry-forward funds \$45000 (practically break-even)

Proceedings contents must be submitted to Dan by July 9.

San Antonio, May 18-21, 2014

Officers for 2014:

Conference chairs: Faith Oi, Grzesiek Buczkowski

Program: Robert Koranic, Dini Miller

Awards: Karen Vail, Raj Saran

Secretary: Kyle Jordan Treasurer: Roger Gold Sponsorship: Dan Suiter

Local Arrangements: Roger Gold

Future of NCUE

ESA assumption of NCUE

Opinions range from negative to 'maybe'

Coby indicated that the conference is run by conference managers paid by the hotel, not ESA, which may be beneficial, Roger indicated that NCUE was informed that we would be directly billed for hours put in by conference planners

NCUE would be under the governance of the ESA governing board

Austin pointed out that having larger numbers might help sustain the conference, perhaps on a trial basis as a tag-on to the annual ESA meeting

Ellen Thoms recommended an association that specializes in infrastructure (like GPCA) - might be impossible to extract from ESA once involved

Kaci Buhl mentioned working with a university event planner (Illinois put together a national IPM conference) Ted Granovsky added that funds are not easily made available to MUVE, encouraged branching out to more med/vet folks

Bob Davis suggested the meeting would get lost during the annual ESA meeting but may work with branch meetings as part of ESA

Solicit input from folks who are not present at the business meeting (NCUE email list)

Faith encouraged forming an ad hoc committee to analyze options for next steps/plans ... Survey will be put online once a study committee does some more research

Informal poll taken to determine whether NCUE remains independent - independent carries

Someone needs to volunteer to run the organization or need to hire a manager

Ellen encouraged the decision be made before the 2014 meeting so the new person could gain hands-on experi-

ence

2016 location options: W Yellowstone, Albuquerque (Bob Davis), Portland (Kaci Buhl), Jackson Hole, Seattle (Bill

Donahue)

Clay recommended joining forces with the Fire Ant conference since there is a lot of overlap

Meeting adjourned

Executive session

Support for the meeting in 2014

Do not allow moderator to deliver first paper (have a separate moderator & timer)

Some complaints about some people doing too many talks

Blocks of time for concurrent sessions need to be the same (not 15 min talks at the same time as 20 min talks)

Backup audio

Student reception for co-mingling of demographic

Thompson, Derrig & Craig, P.C.

CERTIFIED PUBLIC ACCOUNTANTS

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Andrea Derrig, CPA

Peggy Adcock, CPA Sandy Beavers, CPA Alline Briers, CPA Gay Vick Craig, CPA Kay Dobbins, CPA Emily Hogan, CPA Lyn Kuciemba, CPA James Larkin, CPA Alice Monroe, CPA Marian Rose Varisco, CPA

April 18, 2012

National Conference of Urban Entomology Board of Directors c/o Texas A&M University Center for Urban and Structural Entomology 2143 TAMU College Station, TX 77843-2143

Dear Board of Directors,

The organization's average annual gross receipts for the three-year period of 2009, 2010, and 2011 are \$21,472. Therefore a Form 990 is not required. A Form 990-N (the e-Postcard) has been electronically filed with the IRS for the 2011 tax year to notify the IRS that the organization's average annual gross receipts are under the \$50,000 threshold.

Sincerely,

Dillard Leverkuhn, CPA

List of Attendees 2012 National Conference on Urban Entomology May 16-19, 2010 Atlanta, Georgia

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