

# INTEGRATED PEST MANAGEMENT TACTICS IN THE SUBURBAN LANDSCAPE

by

CHRISTOPHER MICHAEL SCOCCO

(Under the Direction of Daniel R. Suiter)

## ABSTRACT

Human structures may be occupied, at least temporarily, by insects and other arthropods. Some are perennial pests, while others occasionally invade structures. A field study in 2008 – 2009 determined that occasional invader species in natural and artificially-created harborages adjacent to buildings were primarily isopods (Malacostraca: Isopoda) (37%), spiders (Arachnida: Araneae) (28%), earwigs (Insecta: Dermaptera) (13%), and adult crickets (Insecta: Orthoptera) (8%). Pitfall trapping demonstrated seasonal differences in activity of occasional invaders with the number of harborages observed. In laboratory assays, five plant-extracted essential oils (spearmint, peppermint, wintergreen, cinnamon and clove) were repellent to Argentine ant, *Linepithema humile* (Mayr), workers at concentrations from 0.10 to 10% (v/v) at 0 and 7 days after application. Essential oils are viable alternatives to conventional chemical repellents of the Argentine ant and occasional invader species.

INDEX WORDS: integrated pest management, IPM, occasional invader pests, essential oils, urban pest management, Argentine ants, *Linepithema humile*

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## DEDICATION

Erika: thank you for always encouraging me and reinforcing to me that I could do this. Without your everyday help and guidance, this degree would have been out of my reach. Even when this seemed unattainable at times, you were willing to lend a hand for my benefit without thinking. Not only are you my sister, but you are one of the best friends that someone could ever have and I dedicate this work to you.

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## TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS .....	v
LIST OF TABLES .....	vii
LIST OF FIGURES .....	viii
CHAPTER	
1 A REVIEW OF THE SCIENTIFIC LITERATURE ASSOCIATED WITH THE MANAGEMENT OF OCCASIONAL INVADER PEST ARTHROPOD SPECIES .....	1
2 NONCHEMICAL CONTROL METHODS FOR OCCASIONAL INVADERS IN THE SUBURBAN LANDSCAPE.....	23
3 THE REPELLENT EFFECTS OF FIVE PLANT-EXTRACTED ESSENTIAL OILS AGAINST THE ARGENTINE ANT, <i>LINEPITHEMA HUMILE</i> (MAYR) .....	68
4 CONCLUSION.....	94

## LIST OF TABLES

	Page
Table 1: Mean ( $\pm$ S.E.) number of indicator species trapped in plots containing artificial harborage.....	45
Table 2: Mean ( $\pm$ S.E.) number of indicator species trapped in plots containing natural harborage.....	46
Table 3: Mean ( $\pm$ S.E.) dry weight (biomass) (in g) comparison of indicator species trapped in plots containing artificial and natural harborage .....	47
Table 4: Essential oil comparisons by individual oil ages .....	92
Table 5: One way ANOVA (PROC GLM) for individual oil rates and ages .....	93



## LIST OF FIGURES

	Page
Figure 1: Composition and structure of the three-cup pitfall trap. ....	48
Figure 2: Mean ( $\pm$ S.E.) number of occasional invader indicator taxa trapped in artificial harborage trapping sites (UGA Griffin Campus site) in response to harborage density with control = 0, low = 1, and high = 3 harborages .....	50
Figure 3: Mean ( $\pm$ S.E.) number of occasional invader indicator taxa trapped in natural harborage trapping sites (Griffin Housing Authority site) in response to harborage presence or absence for all trapping dates and species .....	52
Figure 4: Overall trap catch of <i>Harpalus</i> spp. beetles captured by date .....	54
Figure 5: Overall trap catch of adult crickets captured by date .....	56
Figure 6: Overall trap catch of nymphal crickets captured by date .....	58
Figure 7: Overall trap catch of other carabid beetles captured by date .....	60
Figure 8: Overall trap catch of earwigs captured by date .....	62
Figure 9: Overall trap catch of isopods captured by date .....	64
Figure 10: Overall trap catch of spiders captured by date .....	66
Figure 11: Response of Argentine ant workers to harborages treated with various chemicals (one of five essential oils, Cinnamite, or hexane) and aged for 0 or 7 days.....	91

## CHAPTER 1

# A REVIEW OF THE SCIENTIFIC LITERATURE ASSOCIATED WITH THE MANAGEMENT OF OCCASIONAL INVADER PEST ARTHROPOD SPECIES

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**Abstract** Human dwellings, institutions and other structures are frequently occupied, at least temporarily, by insects and other arthropods. Some are perennial or severe pests in these environs, while others only occasionally invade structures from neighboring areas of surrounding landscapes usually in response to environmental conditions. Research studies directed to the biology, ecology and management of these occasional pest invaders are available but have, to date, not been assimilated into an organized review of the existing literature on the subject. This review attempts to organize that existing literature based upon potential groups of occasional invaders, know biological information on selected pest groups that contributes to their pest status and management, and management tactics for these pests.

**Key Words** urban pest management, integrated pest management, IPM, occasional invader species, structural and household entomology, literature review

## Introduction

Occasional, or accidental, invaders are described as any infrequently observed insects or arthropods that may enter a structure in a wandering phase of their lives. Occasional invaders are oftentimes considered pests because they may enter structures when environmental conditions inside the structure rather than outside the structure are more conducive to their survival. Unlike the domesticated German cockroach, *Blattella germanica* L., which lives and reproduces inside of structures, occasional invaders do not complete their life cycles inside structures and are generally considered to be beneficial insects and arthropods when found in their natural habitats. However, when occasional invaders move into a structure, they immediately become anthropomorphic pests because they are unwanted in a home, institution or structure.

Management of occasional invader pests is primarily by chemical control methods. Traditionally, perimeter sprays are used to form a barrier around a structure to deter occasional invaders from entering. While there are no specific data on insecticide use for these pests, the U.S. Environmental Protection Agency (U.S. EPA) (2004) estimates that 41% (\$1.29 billion) of insecticides sold in the United States in 2001 were in the home and garden market sector, which was the only sector with an increase in the amount of insecticides sold year-to-year. This indicates that homeowners and professionals continue to use insecticides rather than ecological, mechanical and other tactics for pests in the landscape.

Reliance upon conventional chemical insecticides has likely slowed the development of basic biological and ecological knowledge of occasional invaders as well as the development of methods other than insecticides for their management. Non-chemical methods, such as sealing potential entry points or removing exterior harborages, have promise in a comprehensive management approach. Efficacious alternatives to conventional insecticides should be explored.

Development of IPM programs, rather than solely relying upon insecticidal control, can reduce the potential for insecticide impacts on nontarget species, humans, and companion animals while reducing adverse environmental impacts.

Development of integrated pest management programs for insects and other arthropods that occasionally invade human domiciles, institutions and structures has been limited by lack of basic biological and ecological knowledge of these invader species, reliance on conventional chemical insecticides for their control and lack of efficacy data for alternative practices and tactics. The review of scientific literature presented herein is aimed at assimilating and organizing existing research literature pertaining to occasional invader pest species and their management. The review is organized into sections addressing definitions and background, occasional invaders and current management approaches.

## **Background**

Bennett et al. (1977) defined an occasional invader pest as any insect or other arthropod that wanders inside a dwelling at some period during its life, but does not complete its entire life cycle there. According to Appel (2003), control of occasional invaders is difficult because they are seldom observed in sufficient numbers to study their general ecology and biology. This factor is complicated by inadequate knowledge for successfully establishing and maintaining laboratory colonies for basic studies of these pests. Thus, development of IPM programs for their management has been hindered (Appel 2003).

According to the United States Environmental Protection Agency (US EPA), IPM is an approach to pest management that uses a combination of biologically-based practices that are efficacious, economically-feasible and environmentally-friendly. By using the most up-to-date

information, including life cycles, environmental interactions, and available control methods, the pest populations can be managed economically, with little risk to humans or the environment (US EPA 2009). Many variations of this definition are used today. Some are exclusive for specific commodities or industries (e.g., structural/household IPM), while others have broad applicability and are not exclusively aimed at only one segment of pest management. The U. S. Department of Agriculture (USDA) appreciates that multiple approaches to IPM are being taken and that the objectives of specific IPM programs are modified to meet the specific needs of each targeted area, commodity or pest management group (Fernandez-Cornejo and Jans 1999).

Prior to the advent of IPM, pesticide usage was the primary approach to control pests and, because of misuse, undue human exposure and insecticide resistance, pest resurgence, pest outbreaks and environmental pollution occurred (Ehler 2006). However, as Ehler (2006) notes, when IPM programs are tailored to specific pests and are used correctly, even with the sensible use of chemical control methods, they are generally effective in permanently reducing pest populations.

### **Occasional Invaders**

Shetlar (2002) found a diverse group of potential occasional invaders in the urban landscape, some being long-term pests and others being only temporary. Occasional invaders may include predators, scavengers or detritivores (e.g., organisms that feed on decaying detritus). Shetlar (2002) noted understanding the ecology and behavior of insects and other arthropods in landscapes can help predict possible invasion of structures. Predatory insects and other arthropods, including ground beetles (Coleoptera: Carabidae), earwigs (Dermaptera), spiders (Araneae) and centipedes (Chilopoda), are considered beneficial in the urban landscape but can become pests when they invade a structure. Ground beetles and earwigs are especially

dependent on high moisture levels in the landscape. Optimal moisture levels often occur in mulched areas. This promotes reproduction and provides an optimal habitat for landscape pests and may provide prey for potential predators (Shetlar 2002).

Earwigs and ground beetles are common occasional invaders. Both often reproduce and live in close proximity to structures. Earwigs (usually *Forficula* spp.) are nocturnal predators of insects and other arthropods (UMaine 2009). As daybreak approaches, they search for shelter to harbor until the next night. These harborage sites include rocks and stones, mulch, wood piles, around plants or in other moist, dark places. They overwinter as adults after ovipositing eggs in burrows, constructed underneath stones, mulch or large pieces of wood and other debris in the landscape (Lamb and Wellington 1975). Ground beetle (*Megacephala* and *Harpalus* spp.) adults are also hunting predators that routinely forage in the landscape. Both earwigs and ground beetles can enter structures through openings, i.e., cracks and crevices, in the foundations or walls of structures. They usually invade structures in response to either hot and dry weather conditions or the onset of winter (Shetlar 2002). The probability of these occasional invaders accidentally entering a building is increased when they are in close proximity to structures.

Many genera of spiders also occur in the urban landscape. Like ground beetles and earwigs, spiders are predaceous and generally considered to be beneficial (Turnbull 1973). Many hunting spider groups, such as jumping spiders, ambush or stalk their prey either in foliage, on flowers or on the ground. They will sometimes enter structures when searching for prey (Turnbull 1973, Shetlar 2002). Because they are active hunters and not web builders, they require appropriate nesting sites in which to place their egg sacs. They often do so under mulch, in wood piles, or under stones that provide appropriate shelter (Shetlar 2002). These spiders are characteristically univoltine and males and females actively search for mates in the latter part of

summer into early fall. They can accidentally enter structures during this period of wandering for mates (Shetlar 2002).

Field and house crickets (*Gryllus* and *Acheta* spp.) are one of the most common occasional invader pests (ISU 2005). Crickets spend the majority of their life cycles outside structures and enter them accidentally (Bennett et al. 1997, ISU 2005), most likely due to unsuitable weather conditions (e.g., cooling temperatures toward the end of summer or extremely dry conditions). Because crickets overwinter in the egg stage, nymphal crickets begin to emerge in spring or early summer and feed on an assortment of plant materials (ISU 2005). During the summer, adults mate and lay eggs to complete the cycle. In the fall, cold temperatures kill the remaining adult crickets (ISU 2005). It has been suggested that to manage cricket populations in the urban landscape, vegetation and debris located around structures should be removed because these materials can serve as harborage for all life stages of these species. Similarly, sealing cracks, crevices and other openings in structures will mitigate cricket entry into the structures. Lastly, chemical control approaches can be used if other management methods have apparently failed (ISU 2005).

Scorpions (Arachnida: Scorpiones) are also occasional invader pests. McIntyre (1999) reported that the majority of scorpions encountered by humans were found in homes. Knowledge of scorpion ecology and biology is lacking, especially in urban landscapes and human structures. However, McIntyre (1999) postulated that environmental factors, (e.g., irrigated lawns, woodpiles, palm trees, and periodic soil disturbances) enhance populations of scorpions in landscapes. She further suggested that encounters with scorpions in homes can be reduced using mechanical control methods, such as exclusion.



Detritovore and scavenger arthropods are also a part of the suburban landscape. These arthropods feed on decaying plant matter, fungi and other detritus. They are usually considered to be beneficial because they may specialize on plant materials or fungi and also provide the predaceous arthropods with a food source (Shetlar 2002).

Isopods (Isopoda) are generally not considered to be pests in the landscape, but can become pests if they invade structures. In general, isopod populations can increase via harborage sites with excessive mulch or pine straw in the landscape (Jordan and Jones 2007). Such mulches are used to retain soil moisture for landscape plantings and, thus, provide optimal conditions for isopods and other soil-dwelling detritivores.

Millipedes (Diplopoda) are also scavengers and are generally considered to be beneficial, as they feed on decaying materials and fungi. They become nuisance pests when they invade structures. According to Shetlar (2002), millipedes, along with other detritivores and scavengers, are part of a larger food web and can potentially be a food source for predatory arthropods in the landscape. However, like other occasional invaders, if environmental conditions become unstable, these arthropods will move inside of homes and institutions if proper exclusion measures are not taken.

Some cockroaches, such as the smokybrown cockroach, *Periplaneta fuliginosa* (Serville), are not domesticated insect pests. These cockroaches are scavengers and prefer to live outdoors rather than inside of a structure, harboring in mulch or pine straw which provides high humidity and sufficient darkness during the daytime. However, smokybrown cockroaches, which are highly susceptible to desiccation, live in close proximity to structures and may occasionally invade homes or establishments when exterior temperatures and moisture levels become less favorable for their survival. These cockroaches are peridomestic pests and are usually found on

patios, decks, in animal dishes and on walls outside of homes. They sometimes enter structures in the autumn or winter through cracks or crevices and can occasionally live in wall voids or attics (Appel and Smith 2002). Control of smokybrown cockroaches has historically relied almost exclusively on perimeter sprays of insecticides around structures. However, an IPM program for smokybrown cockroach control has been developed by Smith et al. (1995) and directly compared to a traditional perimeter spray. In this comparison, 80% less active ingredient of the insecticide was used and cockroach population reduction was faster and persisted longer with the IPM program than when only a perimeter spray was used.

The Argentine ant, *Linepithema humile* (Mayr), while not a typical occasional invader, resides in urban and suburban landscapes and frequently invades homes and structures. According to Shetlar (2002), ants are opportunistic in urban or suburban landscapes. Some Argentine ants feed only on sugar, while other prey on insects. However, when in a disturbed habitat such as a landscape around a structure, ants have abundant nesting sites and food sources. Mulches, wood and stones provide the required shelter for ants for nesting, while garbage cans and kitchens may provide additional food resources.

The Argentine ant was first recognized in New Orleans by Edward Foster in 1891. It was likely introduced into Louisiana through shipments from South America (Newell and Barber 1913). Native to northern Argentina, southern Brazil, Uruguay and Paraguay (Suarez et al. 2001, Tsutsui et al. 2001), it was first described as *Iridomyrmex humilis* by Australian myrmecologist Gustav Mayr when he collected worker ants in Buenos Aires, Argentina in 1866, giving rise to the name “Argentine ant” (Foster 1908). It is a cosmopolitan pest (Suarez et al. 2001) and in the U.S., it is found in agricultural and urban areas in at least 21 states, including the southwestern and southern U.S. and Hawaii, due to human-related dispersals (Newell and Barber 1913,

Holway 1999, Suarez et al. 2001). When foraging, workers will invade urban structures in large numbers in response to temperature extremes, moisture gradients or resource availability (Klotz et al. 1995, Vega and Rust 2001). The Argentine ant is a tramp ant species that adapts easily to disturbed, or urban, environments and prefers habitats that are in close proximity to humans (Vega and Rust 2001).

Argentine ants are a highly successful invasive species. They are successfully established throughout the U.S. largely because they are a tramp ant species and have taken advantage of human activities for dispersal. After becoming established in an area, Argentine ants generally out-compete native ants for food and territorial resources (Knight and Rust 1990, Suarez et al. 2001, Vega and Rust 2001). Factors attributed to their rapid spread and extensive range outside of its native range include multiple queens (polygyny) (Vargo and Fletcher 1989), multiple colonies (polydomy), little-to-no intraspecific aggression (unicoloniality) (Holway et al. 1998), and using reproductive budding (sociotomy) to form new colonies (Passera 1994). They also have a relative lack of native natural enemies and competitors (Kabashima et al. 2007), and the large size of their colonies compared to those of native ants provides a numerical advantage (Vega and Rust 2001).

In a pest ant survey by Knight and Rust (1990), Argentine ants were the most frequently encountered ant by pest management professionals in California and were also the most difficult ant species to control. Likewise, in a year-long study of pest ants in San Diego, CA, by Field et al. (2007), Argentine ants also were the most frequently encountered, comprising 85% of the ants sampled.

Klotz et al. (2007) evaluated six insecticide treatments for Argentine ant control in heavily-infested urban areas over an 8 wk period. Perimeter sprays, gel baits, granular baits, spot

treatments and combinations of treatments were used, with a fipronil perimeter spray being the most effective. It reduced ant activity around homes by 93% in 1 wk and 81% 8 wk post-treatment (Klotz et al. 2007). Control of Argentine ants in urban settings is difficult and requires continual use of the newest chemical formulations for these ants because, despite the variety of chemicals and formulations that have been available for Argentine ant control, no single chemical tactic has provided repeated, substantial control of these ants (Klotz et al. 2002, Rust et al. 2003).

### **Management Tactics**

Management of occasional invader pests currently includes use of conventional chemical insecticides both inside and outside of the structure, mechanical methods (primarily exclusion tactics), and ecological methods (primarily habitat modification). To date, insecticidal sprays directed to the perimeter of structures to repel, kill or prevent occasional invaders from occupying areas adjacent to structures have been the usual stand-alone control methods for these pests. And, in fact, homeowners and other dwellers do not become overly concerned or alarmed with occasional invader pests until the insects and other arthropods have entered the structure to become nuisances or health concerns.

Wood (1986) suggested that several nonchemical approaches, primarily landscape plant management, could be used to reduce the potential of occasional invaders entering homes and structures. He observed that certain landscape plants provide shelter, food, reproductive sites and other resources and, when placed too close to a building or its foundation, can provide easy access from these harborages to the structure. He also noted that organic mulches attract millipedes, crickets and flies while providing potential harborage areas for cockroaches in warmer climates. He suggested planting shrubbery and other landscape plants at least 0.91 m (3

ft) from the building foundation to eliminate plant-to-building contact, and installing plastic weed barriers, doorsweeps or thresholds, and caulking around doors and windows and other openings to exclude occasional invaders from structures. Inside of the structures, he suggested the elimination of potential harborage sites, management of moisture problems and sanitation to eliminate shelter, food and other resources for pest populations. He further recommended that conventional chemical insecticides be used on an as-needed basis reporting that chemical insecticides were more effective in eliminating occasional invader pest problems when they are integrated with nonchemical ecological management tactics than when used alone.

Smith et al. (1995a) used a landscape model to estimate the abundance of smokybrown cockroaches. After correlating landscape and home characteristics with cockroach abundance, they used the model to implement an IPM program to control cockroach populations using sanitation techniques, landscape modification and the judicious use of chemical insecticides, including perimeter sprays and spot treatments (Smith et al. 1995b). Implementation included ecological modification or management (i.e., dethatching and mowing lawns, scheduling irrigation, removing potential cockroach harborages, removing potential food resources), as well as mechanical tactics (i.e., sealing cracks and crevices in exterior walls, using screening where applicable) as exclusion measures. They reported that they used 80% less chemical insecticide active ingredient than perimeter sprays with this approach than with an approach using perimeter sprays only (Smith et al. 1995b).

Even though chemical control in an IPM program may be beneficial, the consequences of pesticide misuse are a growing concern. The use of conventional chemical insecticides in these settings poses several problems including health and environmental concerns often linked to misuse (Curtis et al. 1990). Furthermore, consumer, regulatory and marketing concerns have

resulted in the restriction of use or even removal of several efficacious products from the market. Identification and development of acceptable replacement that retain the characteristics of originally labeled insecticides but lacking negative health and environmental impacts has proven difficult (Elzinga 2004) and, thus, limits the number of efficacious insecticides available for these uses.

Plant-derived chemistries appear to have potential as viable alternatives for conventional chemical insecticides and as additions to the arsenal of options for management of occasional invaders pests. Botanical insecticides (i.e., rotenone, pyrethrum, nicotine) derived from *Derris*, *Chrysanthemum* and *Nicotiana* pre-dated the development and introduction of the synthetic chemical insecticides (Curtis et al. 1990). Rotenone, however, was found to have a higher mammalian toxicity than many synthetic insecticides (Curtis et al. 1990), demonstrating that not all naturally-derived products are safer than their laboratory-produced counterparts. Nicotine and pyrethrum remained in many markets after the large-scale introduction of synthetic insecticides. Pyrethrum provided the framework for development of the synthetic pyrethrins (Curtis et al. 1990, Elzinga 2004).

Plant-derived essential oils have obvious advantages over conventional insecticides. Essential oils are generally well received because of their extended use in Eastern medicine, pharmaceuticals, herbal mixtures and as natural flavorings. Overall, the essential oils possess low mammalian toxicity, making them a favorable alternative to synthetically-derived insecticides (Rajendran and Sriranjini 2008).

Essential oils have repellent, fumigant and other toxic properties against insects and other arthropods including stored product pests (Hori 2003), medically-important pests (Rutledge et al. 1983, Erler et al. 2006), plant-feeding pests (Koschier et al. 2007) and urban or structural pests

(Meissner and Silverman 2001, 2003, Peterson et al. 2002, Appel et al. 2004, Tunaz et al. 2009). Tunaz et al. (2009) found that, of nine essential oils tested, the oil of garlic (*Allium sativum* L.) and a monoterpenoid (allyl isothiocyanate) derived from cruciferous plants had toxic effects as a fumigant against German cockroaches, *Blattella germanica* L., nymphs 24 and 48 h after application. Exposure to garlic oil resulted in 33.3% mortality of nymphs at 24 and 48 h after exposure, and exposure to the allyl isothiocyanate caused 100% mortality within 24 h. These results show that these two chemicals are time-dependent and concentration-dependent, with mortality increasing with concentration (Tunaz et al. 2009).

Peterson et al. (2002) reported that German cockroaches are repelled by catnip oil (*Nepeta cataria* L.) and proposed that the oil be used as a barrier treatment to prevent the cockroaches from entering specific areas. They reasoned that, because the essential oils are volatile, their toxicity to target insects might be used over larger areas than those chemical which must be contacted by the insects.

Erlar et al. (2006) showed that five essential oils repelled *Culex pipiens* L. adults. In Y-tube olfactometer laboratory bioassays, only two essential oils (mint and laurel) were repellent to female *C. pipiens*; the effect was concentration-dependent. Anise, basil and eucalyptus oils were also repellent, but were time-dependent. The oils that were time-dependent showed the most repellency against *C. pipiens* females. Basil and eucalyptus repelled 100% of the females at 10 µL concentration level (Erlar et al. 2006).

Koschier et al. (2007) investigated the post-landing behavior of western flower thrips, *Frankliniella occidentalis* Pergande, when exposed to salicylaldehyde and methyl salicylate, which are secondary defensive compounds of plants. Their data, derived from choice tests using cucumber and bean leaves treated with the plant compounds at multiple concentrations,

suggested that both methyl salicylate and salicylaldehyde are potential repellents to western flower thrips. The thrips left the treated leaf surfaces much sooner than in the control and, when given a choice, the thrips avoided contact with the treated leaves (Koschier et al. 2007). After a 5 h period, no preference in landing behavior could be detected on the salicylaldehyde treated plant material. However, after a 5 h period in the methyl salicylate treated plant material, the thrips were clearly avoiding the treated leaves. They suggested that using plant compounds to control plant-feeding pests may be helpful against herbivory, although the volatilization effect of the chemicals needs to be studied further.

Hori (2003) used an olfactometer assay to show that almost all 57 essential oils tested were repellent against the stored-product pest cigarette beetle, *Lasioderma serricornis* (F.). Effects were concentration-dependent, with concentrations  $\geq 1 \mu\text{L}$  or higher being repellent to the beetles. Some of the oils, including savory, thyme, peppermint and cassia, attracted the beetle at lower concentrations, while higher concentrations were highly repellent. Shiso oil, a common Japanese herb, was a strong repellent even when used in the presence of the odor of cured tobacco. Hori (2003) suggested that the activity of the essential oils against the cigarette beetle was dependent on the dosage, as indicated by the attractant and repellent effects of the same oils at different concentrations. He concluded that shiso oil was best for repelling cigarette beetles infesting stored products. Shiso oil on and around packaging can reduce beetle entry because of its repellent effects, even in the presence of other food scents (Hori 2003).

Two similar repellency studies by Meissner and Silverman (2001, 2003) investigated the effects of aromatic cedar, *Juniperus virginiana* L., mulch on Argentine ant, *L. humile*, foraging activity and nest establishment. When a choice of mulch was offered, worker ants always chose the non-cedar varieties. When cedar mulch was the only choice provided, the ants did not



construct or move nests into the mulch. Their data demonstrated a high mortality of Argentine ants when exposed to the aromatic cedar mulch, leading them to conclude that aromatic cedar mulch could potentially deter ants from nesting in outdoor landscapes and around structures (Meissner and Silverman 2001). They further suggested that aromatic cedar mulch may reduce Argentine ant numbers in well-defined areas, such as planters and around buildings. Replacing other nesting media types, such as pine straw or non-cedar mulches, with aromatic cedar mulch may help keep Argentine ants from crossing substrates and potentially stop nest movement or foraging activities (Meissner and Silverman 2003).

Appel et al. (2004) showed that peanut shell granules containing 2% mint oil from *Mentha arvensis* L., repelled red imported fire ant, *Solenopsis invicta* Buren, workers in laboratory choice tests. They further showed that as little as 15 min of exposure to the mint granules doubled worker mortality in comparison to untreated controls. They suggested that mint oil may have residual effects when placed in fire ant mounds and, although mound fumigation is not generally used for fire ant management, the oil-impregnated granules may offer a residual effect in treated mounds (Appel et al. 2004).

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CHAPTER 2

SEASONAL OCCURRENCE AND RESPONSE OF OCCASIONAL INVADER PESTS TO  
ARTIFICIAL AND NATURAL HARBORAGES IN THE SUBURBAN LANDSCAPE

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**Abstract** Field studies were performed for 1 yr to examine the impacts of harborage on occasional invader insect and arthropod pests, as well as to catalog seasonal activity of indicator species trapped. Through destructive pitfall trapping, indicator species were collected and analyzed. After counting individual insects and arthropods, biomass dry weights were recorded and analyzed and used as another method of measuring trap catch. In general, harborage did not significantly affect trap catch or biomass dry weights throughout the study, contrary to data from other studies. However, seasonal activity of occasional invader pests in the study estimated sample densities among different indicator species trapped which may be useful for further preventative measures for controlling these landscape pests.

**Key Words** occasional invaders, integrated pest management, harborage, seasonal abundance

## Introduction

Occasional invaders are sporadically encountered insects and other arthropods that may enter structures at different life stages or due to environmental cues, such as excessive moisture, extreme temperatures or unsuitable humidity levels (Appel 2003). Occasional invaders may be found in a structure at some point in their life, but do not complete their life cycle there (Bennett et al. 1997). Because they are infrequently encountered, their overall biology, life cycle and general ecology are not completely understood (Mallis 1990, Bennett et al. 1997, Appel 2003).

Ebeling (1978) suggested that urbanization affects the habitat of certain insects and is a direct result of human behavior that creates new or modifies existing habitats. Fleet et al. (1978) suggested that reduction in indoor populations of smokybrown cockroaches, *Periplaneta fuliginosa* (Serville), required the management of outdoor populations. In particular, they recommend that all harborage sites within 30 m of any building should be eliminated (Fleet et al. 1978).

According to Appel and Smith (1996), smokybrown cockroaches can be controlled through various nonchemical control methods. In their study, similar to studies by Fleet et al. (1978) and Smith et al. (1995), habitat was modified or eliminated after identifying the host's harborage site(s), resulting in a decrease in cockroach populations. Robinson (1999) highlighted the importance of utilizing effective nonchemical control methods in the urban environment, suggesting that ineffective nonchemical control methods can result in increased chemical use, and possible misuse, by the consumer. He also noted the importance of nonchemical techniques in slowing the development of insecticide resistance in urban pest populations (Robinson 1999).

Since the original concept of integrated pest management (IPM) was outlined by Stern et al. (1959), implementation has not been fully accepted in the urban and suburban environment (National Research Council 1996, Ehler and Bottrell 2000). According to Appel (1997), the control of domestic and peridomestic pests depends almost entirely on the use of chemical control tactics. While chemical control may be necessary, it should not be the sole means of insect or arthropod control.

According to the U. S. Environmental Protection Agency (U.S. EPA), 41% (\$1.29 billion) of insecticides sold in the United States in 2001 were in the home and garden market sector, 42% in agriculture, and 17% in industrial/government sales. Notably, insecticide sales increased from 5.44 million kg (12 million lb) of active ingredient in 1992 to 7.71 million kg (17 million lb) in 2001 (U.S. EPA 2004). These numbers most likely reflect the use of insecticides without full consideration of an IPM plan. The home and garden market sector is the only one to have experienced an increase in chemical usage, while insecticide use in agricultural and industrial/government usage dropped from 1992 until 2001 (U.S. EPA 2004).

Because of heightened concerns regarding problems associated with conventional pesticide usage (e.g., impacts on non-targets, hazards to humans and the environment, and development of insecticide resistance), alternative pest management measures in the urban environment are currently being utilized for the control of occasional invaders (Ehler 2006). However, a general lack of understanding of the overall biology and ecology of occasional invaders has hindered attempts at their control (Bennett et al. 1997, Appel 2003). Frankie and Ehler (1978) stated that there is not enough ecological evidence to support certain distributions and abundances of insects in urban environments, meaning that certain insects should not technically be thriving in certain urban environments given the conditions. Dreistadt et al. (1990)

suggested that certain plant combinations in urban areas allow insects and arthropods to become abnormally numerous. There remain many unknown biological and ecological factors that need to be examined in order to successfully manage occasional invaders, including preferred habitats, life cycles, population dynamics, pesticide efficacy and behavioral response studies (Frankie and Ehler 1978, Dreistadt et al. 1990, Bennett et al. 1997, Appel 2003). The objective of this study was to correlate harborage density (or presence) with the occasional invader sample size. At two separate sites (one with artificial harborage, the other with natural harborage), pitfall traps were installed every 2 to 3 wk for 1 yr, and the number of ground beetles, earwigs, spiders, isopods and crickets (adults and nymphs) trapped was recorded.

### **Materials & Methods**

**UGA Griffin Campus Test Site.** In this experiment, we evaluated the impact of artificial harborage (plywood placed flat on the ground) on crawling arthropod trap catch on the UGA Griffin Campus (GPS: 33.265858, -84.284990). Our expectation was that we would see higher pitfall trap catch in areas where more artificial harborages were present than in plots where less or no artificial harborages were present. We evaluated one treatment (harborage) at three levels: zero artificial harborages per 4.6 m plot (control), one artificial harborage per 4.6 m plot (low) and three artificial harborages per 4.6 m plot (high).

Harborage was simulated by using a pre-cut, 30 cm x 60 cm x 1.4 cm pieces of untreated, outdoor plywood placed flat on the ground with the long side against the building foundation. Each plot was separated by metal garden dividers, driven into the ground perpendicular to the building wall and flush against it. There were  $n = 6$  plots per treatment level. Consideration for site selected included limited standing or puddling water problems, availability to rainwater,

shallow building foundations, ease of pitfall trap installation and little to no interference by humans or other animals.

The response variable in this experiment was the number of crawling insects and arthropods (crickets, earwigs, isopods, spiders, *Harpalus* spp. beetles and other carabid beetles), hereafter referred to as indicator species, caught in pitfall traps. To trap indicator species, three pitfall traps, similar in design to Northup and Crawford (1991), were installed in each plot at approximately 1.52 m intervals, beginning at 0.92 m from either end of the plot.

**Griffin Housing Authority Test Site.** In this experiment, we evaluated the impact of existing, natural harborage on crawling arthropod trap catch at the Griffin Housing Authority (GPS: 33.242612, -84.266167). In this case, we were evaluating the presence or absence of natural harborage on trap catch, again by monitoring indicator species catch in pitfall traps. Seven apartment buildings, managed by the Griffin Housing Authority (Griffin, GA), were chosen for use in this study. Six of the buildings were the same size (30.31 x 8.23 m) and layout, while one building was approximately half of the size (16.15 x 8.23 m). The foundation landscape in front of each building consisted of plants, bushes, flowers, trees, mulches, leaf litter and grasses. The back of each building contained mostly dirt and grass along the building perimeter. The front of each building was considered to be abundant in natural harborage (approximately 137.5 m<sup>2</sup> of potential harborage among all buildings), while the back of the building was considered a harborage-free environment.

Each apartment building had four pitfall traps installed on the front side of the building and four installed on the back side of the building, with the exception of the small apartment building, which only had two traps installed on the front and two installed on the back. Pitfall traps were installed as close to the foundation wall as possible. This distance between traps was

not standardized, however, because of the variability among some buildings. For example, traps were placed behind flowers or bushes, or close to accumulated leaf litter. This allowed for small variations in trap placement, but overall, each trap in the front and back of the buildings corresponded with every apartment in the building (e.g., a trap was installed in the front and back of every apartment in a building; multiple traps were not installed in front or in back of the same apartment).

**Pitfall Traps.** Pitfall traps design was similar to that of Northup and Crawford (1991). Briefly, traps consisted of a 118 ml Kendall® Precision™ screw-cap specimen cup placed inside of a 473 ml, plastic Solo® cup. A Solo® Cozy™ cup was installed as a funnel and was placed on top of the specimen cup and inside the 473 ml plastic cup (Figure 1). The specimen cups were filled with 59 ml of a 25% propylene glycol solution. Propylene glycol is preferred over ethylene glycol because it has similar preservative properties but is non-toxic and does not need to be handled as an environmentally hazardous waste (Porter 2005, Thomas 2008a,b). Propylene glycol also does not have a sweet taste or smell like ethylene glycol, which can be attractive to dogs, cats and other wild animals and is highly poisonous. A few drops of liquid detergent was added to break the surface tension, allowing arthropods or insects falling into the solution to sink directly to the bottom of the specimen cup.

At both sites, a pre-cut 15.24 cm long by 7.62 cm diam piece of white polyvinyl chloride (PVC) pipe was used to keep the shape of the hole in the ground while the pitfall traps were not in use. Loose dirt and clay from the original hole, as well as added QUIKRETE® Premium Play Sand® (No. 1113) (Quikrete, Atlanta, GA) was used to fill in around the pipe so that it fit tightly within the hole. When traps were not in use, the PVC pipe was deliberately left to project out of

the ground 3 to 5 cm to prevent crawling insects and other arthropods from inadvertently falling into the hole.

**Indicator Species.** Earwigs (Insecta: Dermaptera), adult and nymphal crickets (Orthoptera: Gryllidae), isopods (Malacostraca: Isopoda), spiders (Arachnida: Araneae), and *Harpalus* spp. beetles, as well as other ground beetles, including tiger beetles (Coleoptera: Carabidae), were used as indicator species to monitor occasional invader activity related to harborage. These insects and other arthropods showed activity before the initial trapping and, therefore, could be used as indicators of occasional invaders in the landscape.

**Experimental Protocol.** Pitfall traps were readied in the lab prior to installation in the field. Traps were installed in the afternoon every 2 to 3 wk from July, 2008 to June, 2009 (artificial harborage site) or November, 2008 to September, 2009 (natural harborage site) and left for 48 hrs before being retrieved. Traps were collected, returned to the lab, and all insects and other arthropods removed, then washed and stored in 75% ethyl alcohol. When indicator species were trapped, their numbers were recorded.

In addition to trap catch, biomass dry weight was used as another response variable to estimate pitfall trap catch. To obtain dry weights, cups containing the processed specimens for an individual trapping date were placed in an oven (Model # PR305215G) (Thermo Fischer Scientific, Waltham, MA) for 2 d at 60°C. In a prior study, placement of 10 crickets stored in a propylene glycol solution for 3 d then held in 75% ethyl alcohol for about one wk eliminated all excess moisture after approximately 2 d at 60°C in the oven. After 3 and 5 d, the difference in dry weight biomass of 10 crickets was negligible (<0.0001 g). Once the processed specimens were completely dry, each cup, which represented overall catch from an individual trap, was emptied into a tared weigh boat (#02-203-501) (Thermo Fisher Scientific, Waltham, MA).

Individual cup dry weights were recorded, and specimens were placed back into their specimen cups and stored. This was repeated until all specimens from one trapping date were processed.

**Statistical Analysis.** The number of indicator species per date, per trap (trap catch) and dry weight per cup, per date (biomass) were the response variables in our experiments. Trap catch was square-root transformed, and the transformed variable analyzed by one-way ANOVA (PROC GLM) (Sclotzhauer and Littell 1987, 1997, SAS Institute 2008). Data were first analyzed, by site (natural or artificial harborage), regardless of season or indicator species. For the artificial harborage site, harborage quantity was the independent variable, while in the analysis at the natural harborage site, building site (front = harborage, back = no harborage) was the independent variable. In both cases, the dependent variable was the square-root transformed variable. Data were analyzed by site and by indicator species regardless of season. No seasonal analyses were performed. In all tests, means were separated with Tukey's Studentized Range Test, then back-transformed (SAS Institute 2008). Dry weights were also analyzed by one-way ANOVA (PROC GLM) (Sclotzhauer and Littell 1987, 1997, SAS Institute 2008) for both artificial and natural harborage trapping sites regardless of season, and means separated with Tukey's Studentized Range Test (SAS Institute 2008).

## **Results**

**Artificial Harborage Site (UGA Griffin Campus).** Trap catch in plots containing three artificial harborages (high density) was significantly higher than trap catch in plots containing just one harborage (low density). Pitfall catch of indicator species in plots without a harborage (control) was not significantly different from plots containing one or three harborages (Figure 2).



When analyzed by species, adult crickets, nymphal crickets and other carabid beetles showed significant differences in trap catch. Adult cricket trap catch was significantly higher ( $P = 0.0210$ ) in plots containing three harborages than in plots containing just one harborage, while neither was different from the control with no harborages (Table 1). Nymphal cricket trap catch was significantly larger ( $P = 0.0096$ ) when three artificial harborages were present than when no harborages were present, while neither was different from trap catch in plots containing one harborage (Table 1). Other carabid beetles, including tiger beetles, showed a significant difference ( $P = 0.0380$ ) in trap catch when no harborages were present than when only one harborage was present (Table 1), with no difference when three harborages were present. Trap catch among plots with zero, one and three artificial harborages was not significantly different for earwigs, *Harpalus* spp. beetles, isopods and spiders (Table 1).

**Natural Harborage.** Regardless of trapping date or arthropod species, trap catch was not significantly different between the front (harborage present) and back (harborage absent) of buildings at the site with natural harborages (Figure 3). When analyzed by species, only trap catch of isopods was significantly different ( $P = 0.0031$ ) when harborage was present and absent (Table 2). Trap catch of a combination of *Harpalus* spp. beetles and crickets (adults and nymphs) was not significantly different when harborage was absent and present (Figure 13). Trap catch among plots where harborage was present and absent was not significantly different for adult crickets, nymphal crickets, earwigs, *Harpalus* spp. beetles, other carabid beetles and spiders (Table 2).

**Seasonal Trap Catch Activity.** Seasonal pitfall trap catch from both trapping sites was totaled and graphed. In terms of the total number of each indicator species trapped in comparison to of the total number of arthropods trapped, terrestrial isopods (37%) and spiders (28%)

represented 65% of the total number of arthropods trapped. Earwigs (14%), *Harpalus* spp. (9%), nymphal crickets (6%), other carabids (4%) and adult crickets (2%) comprised the remaining 35% of trap catch.

For certain arthropods, trap catch was associated with known factors of their ecology and biology. For example, *Harpalus* spp. beetles are multivoltine insects with population activity from spring until fall (Kirk 1973). In our data, *Harpalus* spp. beetle trap catch peaked throughout the spring, summer and fall (Figure 4). Other indicator species, such as crickets, exhibited a relatively stable trap catch throughout the year-long study (Figures 5 – 10).

**Biomass.** Regardless of trapping date or species trapped, the dry weight (per trap) of insects and other arthropods were not significantly different at either the artificial or natural harborage sites (Table 3). At the site containing zero, one or three harborages per 4.6 m, mean dry weight was not significant ( $P = 0.2372$ ) (Table 3). At the natural harborage site, mean dry weights between traps placed among vegetation was not significantly different ( $P = 0.0764$ ) from the dry weights of arthropods caught by traps placed in areas where little harborage was present (Table 3).

## Discussion

In this study, the purposeful introduction of artificial harborage positively influenced trap catch. Specifically, the amount of harborage per unit area influenced trap catch of crickets (adult and nymphs) and other carabid beetles, including tiger beetles. Alternatively, natural harborage (plants, leaves, mulch, bushes, etc) did not appear to influence trap catch. When analyzed by indicator species, only isopod trap catch was significantly influenced by the presence or absence of harborage.

A seasonal activity of indicator species was clearly evident for all species trapped. In light of our trap catches, proper actions, such as sanitation and mechanical control, should be taken to reduce the numbers of unwanted occasional invaders in the landscape. Trap catch activity also highlighted population peaks and valleys with most trap catch occurring during the warmer parts of the year. Because all of the arthropods had disconnected population increases and decreases throughout the study, preventative management tactics should be applied before the arthropod(s) become major pests.

Most of the occasional invaders in this study are nocturnal, and either predaceous or detritivorous. Isopods, for example, are crustaceans adapted to living on land. They are nocturnal creatures that live in clutches, usually underneath the soil surface or under harborage (Paris and Pitelka 1962, Raham 1986) and are usually not considered pests in the urban or suburban landscape. In general, harborage sites, such as areas with excessive mulch or pine straw, can increase isopod populations in the urban landscape (Jordan and Jones 2007).

Terrestrial isopods are prone to quick desiccation when humidity is low (Raham 1986). This makes their quick moving ability to find appropriate conditions critical to their survival. However, it may be this constant movement for more suitable conditions that increased their overall trap catch as indicated in Figure 9.

As with almost all terrestrial arthropods, water loss is critical. Most terrestrial isopods have a negative phototaxis, a positive orthokinesis to high humidity levels, and a positive thermotaxis (Raham 1986). According to Raham (1986), these behaviors might be stimulated by chemicals, vibrations, day length, behavior of other organisms, and their own movement patterns. Isopods have evolved a behavioral tactic that allows them to be successful in their own soil niche. They will move from place to place, conglobulate (i.e., roll into a ball) if able,

aggregate, make abdominal movements, and burrow, most likely due to their positive thigmotaxis and positive orthokinesis (Raham 1986).

Hunting and ground spiders are distributed throughout many different niches and microhabitats in North America. In a study by Dondale (1977), the distribution of nine species of hunting spiders was determined. Seven of the nine species of ground spiders had annual developmental stages, while the other two species had a biennial (two-year) developmental period (Dondale 1977). He noted that temperature was likely a factor in the development of the spiders, which might help explain the various population peaks seen throughout the year in the spiders trapped in our study.

The large trap catch of spiders in this study is likely a reflection of their hunting behavior, which entails constant movement. Because these spiders do not build webs to capture prey, but rather hunt for it, they are more mobile and, therefore, more likely to be found in pitfall traps. Unlike isopods, which live in clutches at or under the soil surface, spiders are not soil-dwelling arthropods. When reproducing, female spiders oftentimes seek out spaces, such as woodpiles, as shelter from inclement environmental conditions and/or predation (Dondale 1977). Otherwise, hunting spiders do not actively seek out harborages.

The harborage preference and seasonal separation of hunting spiders was also variable, according to Dondale (1977), which may also be another variable in the population peaks throughout our study. Dondale (1977) predicted that the separation throughout the season between different spider species allow for a greater diversity in a habitat, without forcing a niche overlap or selection pressures to occur on one or more species of spider.

*Harpalus* spp. beetles, notably *H. pennsylvanicus*, are nocturnal, predaceous ground-dwelling beetles in the family Carabidae. Kirk (1973) found that during the daytime hours, adult

beetles stayed in burrows or under harborage unless humidity was high, soil moisture was high, and the temperature was cool. *Harpalus* spp. beetles will sometimes stay under harborage or in soil burrows at nighttime if the relative humidity is not high enough to prevent desiccation. He noted that overwintering adult beetles started to emerge from the soil in early June and peaked in mid-July. After the first peak in adult emergence, the second generation of adults that overwintered as larvae started to emerge and peaked in early September (Kirk 1973).

Because the investigation performed by Kirk (1973) was in the northern plains of the United States, the temperature gradient between the southeastern United States and the northern plains may be a factor in adult emergence found in our study. In the trap catch abundance (Figure 4), *Harpalus* spp. beetles started emerging from the soil in late-May to early-June and peaked around mid- to late-June. Our findings were similar to the findings of Kirk (1973). However, dissimilar to Kirk's (1973) findings, the second generation of *Harpalus* spp. beetles started to emerge in late-August, peaked around mid-September, with catches occurring as late as October.

Other ground beetles, including tiger beetles (mostly *Megacephala carolina*), have also been studied. Tiger beetles are highly dependent on abiotic functions to regulate their body temperature (Pearson 1988). It is during this time that they may seek out microclimates that include shaded areas or damp areas, and they may sometimes burrow into the soil (Pearson 1988).

Tiger beetles are diurnal or nocturnal, depending on the species, and are active predators as both adults and larvae. They are particularly sensitive to changing environmental factors such as temperature, moisture, and daylight, and may harbor under foliage or leaf litter to reduce desiccation and/or reduce internal body temperature (Pearson and Voegler 2001). They also noted that because tiger beetles are highly sensitive to environmental changes, they may leave

their current habitat very quickly if conditions change, especially because they are good fliers and fast runners. These insects are generally not considered to be occasional invader pests, however, with their particular responses to environmental conditions, they may wander into a structure or find a suitable condition around a structure, making them a potential pest.

Earwigs are nocturnal insects that possess positive thigmotaxis (Lamb 1975). They seek out dark, dry and cool crevices in soil or under harborage during the daytime (Burnip et al. 2002). Earwigs overwinter as adults after constructing burrows containing the eggs, usually underneath stones, mulch or large pieces of wood and other debris in the landscape (Lamb and Wellington 1975). Lamb (1975) utilized this biological and ecological information to develop earwig trapping methods within the landscape.

According to Burnip et al. (2002), earwigs have two life stages. The “nesting stage” occurs in the spring, where the adults that have overwintered show parental care for the brood and nymphs in their subterranean nests (Burnip et al. 2002). In the “free-foraging stage”, nymphal earwigs that have undergone their first molt will leave the nest and remain away from it, relying on shelter to be available to them (Burnip et al. 2002). They also noted that in both phases, adults and nymphs leave the nest and forage; however, in the “nesting phase”, earwigs always return to the nest; whereas, in the “free-foraging phase”, they depend on shelter being accessible while away from the nest (Burnip et al. 2002).

The “free-foraging phase” that earwigs are subjected to may increase their total pitfall catch around structures. However, the “free-foraging phase” is dependent on the “nesting phase” ending. Because earwigs overwinter as adults (Lamb and Wellington 1975), the “nesting stage” usually occurs in the spring when temperatures begin to rise. However, shifts in average daily temperatures would most likely affect when the “nesting phase” begins. For example, the

average daily temperature during the spring in the South is more than likely higher than the average temperature in the North. With that in mind, the transition from “nesting phase” to “free-foraging phase” is most likely suspended in states with cooler average temperatures because of environmental cues, which may also translate into shorter “free-foraging phases” because of the earlier onset of winter in northern states. Because of these environmental factors, it would seem that earwig populations may be more abundant in warmer climates than in cooler climates and that more earwigs would be in the “free-foraging phase” because of the warmer temperatures. This could potentially translate into more earwigs becoming occasional invader pests in the urban landscape because of their high mobility and extended departures from the nest.

In the investigation reported herein both nymphal and adult cricket populations were relatively steady throughout the year. In January and February, adult crickets were not trapped. They likely expired due to cold temperatures, but were encountered from March until December in relatively constant, although low, numbers (Figure 5). In April, nymphal crickets began emerging according to our trap catch data and, although trap catch numbers began to decline in October, nymphal crickets remained active until December (Figure 6).

According to Bennett et al. (1997), crickets spend the majority of their lifetime outdoors and only enter structures accidentally, most likely due to inadequate environmental conditions, such as low humidity or extreme high or low temperatures. Crickets overwinter in the egg stage (ISU 2005), with nymphal crickets beginning to emerge in spring or early summer. They feed on a variety of plant materials. Because these creatures are sensitive to temperature and humidity, as are most insects and related arthropods, debris, such as leaf litter, mulches and plant materials can serve as harborages. This provides shelter to the insect during daytime hours, when

temperatures are high, and may provide an added benefit of increased humidity to the insect because the harborage sites can retain added moisture (Scocco, pers. obs.).

Our study may not have played as important of a role, as others have suggested, in determining a community's insect or arthropod population. In prior literature, such as studies performed by Kirk (1973), Burnip et al. (2002) and Jordan and Jones (2007), many occasional invaders repeatedly rely on harborage sites for shelter during the daytime hours when temperatures are too high or when conditions are too dry. If steps are taken in the urban or suburban landscape, such as using exclusion, sanitation and the judicious use of insecticides, the numbers of occasional invader pests can be reduced. Occasional invaders mistakenly enter structures looking for more conducive conditions. As noted before, most of these occasional invaders are actually beneficial to the homeowner's landscape by being predaceous on landscape pests such as grubs, aphids or other plant-feeding pests.

Occasional invader insects and arthropods only tend to become a pest once the threshold of a structure is broken. However, using nonchemical control methods, such as sealing cracks and crevices, using doorsweeps and weather stripping, sealing windows and screens, and checking and eliminating other potential methods of entry can help reduce their occurrence inside structures.

However, because these insects are infrequently encountered, according to Appel (2003), the overall information on biology, ecology, behavior and other life history aspects are lacking. Laboratory rearing of occasional invaders is more difficult than other model insects and arthropods, such as *Drosophila melanogaster* Meigen, because they are uncommonly encountered and information on their biology is deficient. Further research on the biology and ecology of these arthropods will help to control them in the landscape. In order to fully



understand the characteristics of occasional invader pests, further investigation must be conducted on the natural habitats of the creatures to facilitate proper control measures.

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**Table 1. Mean ( $\pm$  S.E.) number of indicator species trapped in plots containing artificial harborage.**

Indicator Species	Artificial Harborage Rate			Statistics <sup>a</sup>	
	Control (No Harborage)	Low (One Harborage)	High (Three Harborages)	F-value	P-value
Adult Crickets	0.04843 $\pm$ 0.01ab	0.02579 $\pm$ 0.01b	0.08169 $\pm$ 0.01a	3.88	0.0210
Nymphal Crickets	0.09972 $\pm$ 0.02b	0.16332 $\pm$ 0.03ab	0.28169 $\pm$ 0.05a	4.67	0.0096
Earwigs	0.22222 $\pm$ 0.05a	0.18052 $\pm$ 0.02a	0.21690 $\pm$ 0.03a	0.64	0.5253
<i>Harpalus</i> spp. Beetles	0.11396 $\pm$ 0.01a	0.07736 $\pm$ 0.01a	0.10141 $\pm$ 0.01a	0.95	0.3890
Other Carabid Beetles	0.14530 $\pm$ 0.02a	0.07450 $\pm$ 0.01b	0.09296 $\pm$ 0.02ab	3.28	0.0380
Isopods	0.04843 $\pm$ 0.01a	0.03725 $\pm$ 0.01a	0.06197 $\pm$ 0.01a	0.90	0.4083
Spiders	0.66097 $\pm$ 0.06a	0.60458 $\pm$ 0.05a	0.64507 $\pm$ 0.05a	0.27	0.7650

<sup>a</sup>Degrees of Freedom = 1,1054 for all treatments (n = 1055). Means within a row followed by the same letter are not significantly different. For each ANOVA, means were separated with Tukey's Studentized Range Test (SAS Institute 2008).

**Table 2. Mean ( $\pm$  S.E.) number of indicator species trapped in plots containing natural harborage.**

Indicator Species	Natural Harborage Rate		Statistics <sup>a</sup>	
	Front (Harborage Present)	Back (Harborage Absent)	F-value	P-value
Adult Crickets	0.0275 $\pm$ 0.01a	0.0193 $\pm$ 0.01a	0.60	0.4382
Nymphal Crickets	0.0806 $\pm$ 0.02a	0.1043 $\pm$ 0.02a	0.62	0.4310
Earwigs	0.4951 $\pm$ 0.05a	0.4672 $\pm$ 0.05a	0.14	0.7069
<i>Harpalus</i> spp. Beetles	0.3183 $\pm$ 0.05a	0.3533 $\pm$ 0.04a	0.34	0.5579
Other Carabid Beetles	0.0786 $\pm$ 0.01a	0.1293 $\pm$ 0.02a	2.86	0.0912
Isopods	2.3694 $\pm$ 0.35a	1.2375 $\pm$ 0.16b	8.80	0.0031
Spiders	0.6464 $\pm$ 0.21a	0.8514 $\pm$ 0.31a	0.29	0.5905

<sup>a</sup>Degrees of Freedom = 1,1026 for all treatments (n = 1027). Means within a row followed by the same letter are not significantly different. For each ANOVA, means were separated with Tukey's Studentized Range Test (SAS Institute 2008).

**Table 3. Mean ( $\pm$  S.E.) dry weight (biomass) (in g) comparison of indicator species trapped in plots containing artificial and natural harborages.**

	Artificial Harborage Rate (Biomass)			Statistics <sup>a</sup>	
	Control (No Harborage)	Low (One Harborage)	High (Three Harborages)	F-value	P-value
<b>Dry Weight (<math>\pm</math> S.E.) Per Cup</b>	0.02688 $\pm$ 0.01a	0.02158 $\pm$ 0.01a	0.02854 $\pm$ 0.01a	1.44	0.2372

	Natural Harborage Rate (Biomass)		Statistics <sup>a</sup>	
	Front (Harborage Present)	Back (Harborage Absent)	F-value	P-value
<b>Dry Weight (<math>\pm</math> S.E.) Per Cup</b>	0.07835 $\pm$ 0.01a	0.06334 $\pm$ 0.01a	3.15	0.0764

<sup>a</sup>Degrees of Freedom = 1,1004 for artificial harborage treatments (n = 1005) and Degrees of Freedom = 1,980 for natural harborage treatments (n = 981). Means within a row followed by the same letter are not significantly different. For each ANOVA, means were separated with Tukey's Studentized Range Test (SAS Institute 2008).



**Figure 1. Composition and structure of the three-cup pitfall trap.**

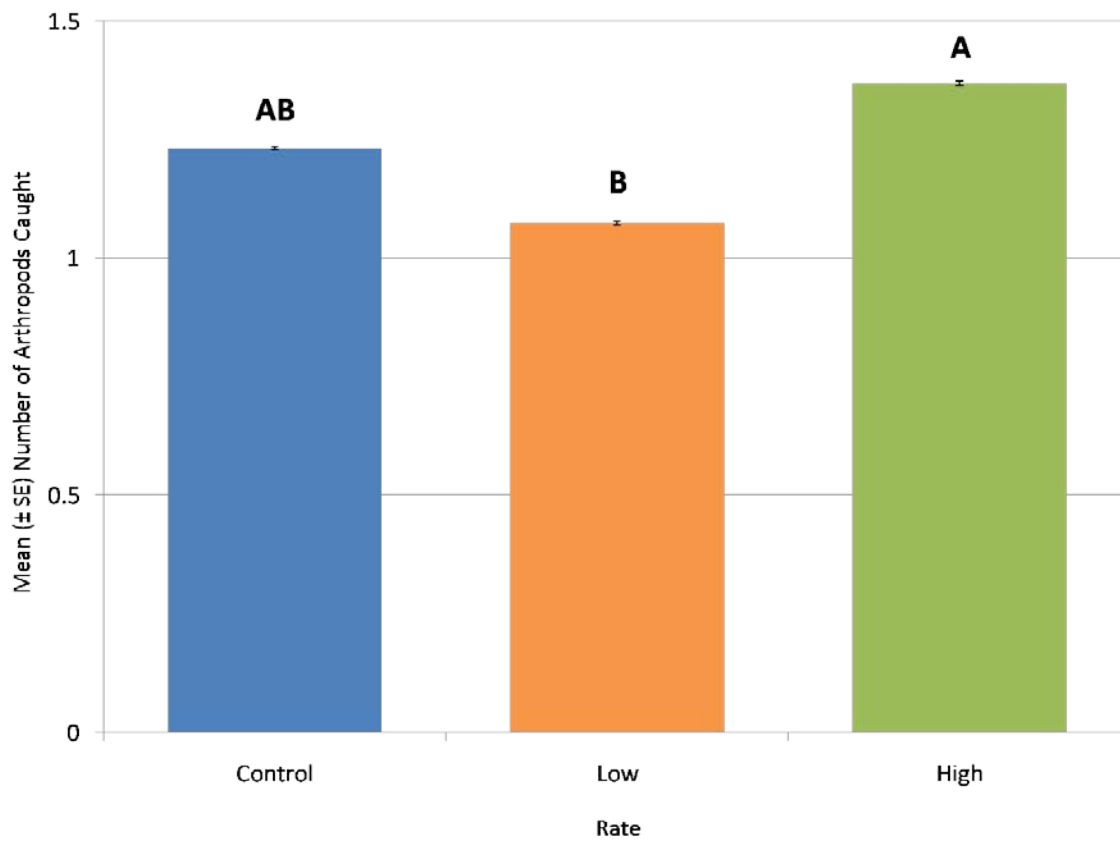


Solo® Cozy™ cup used  
as funnels

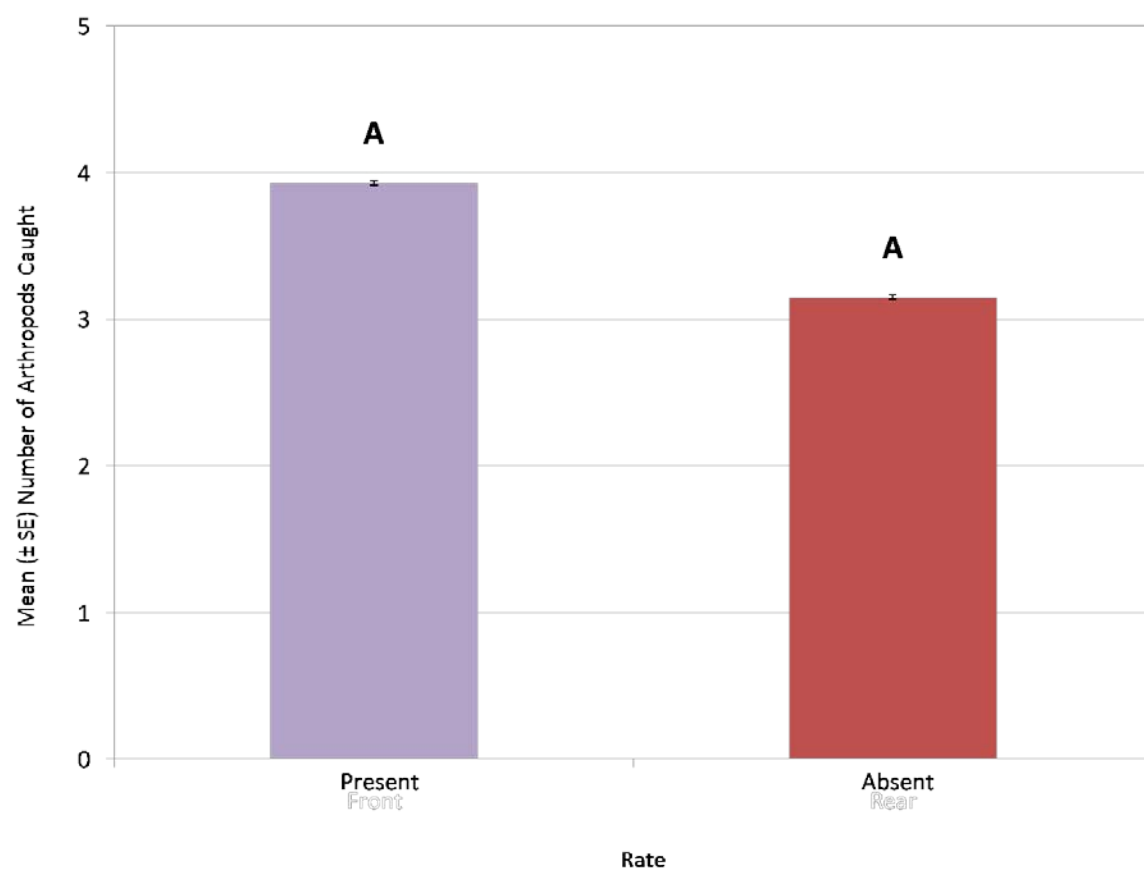
SoloGrips® cup used as  
trap outer shell

Kendall® Precision™  
screw-cap specimen  
cup used to hold  
preservative and  
arthropods

**Figure 2. Mean ( $\pm$  S.E.) number of occasional invader indicator taxa trapped in artificial harborage trapping sites (UGA Griffin Campus site) in response to harborage density with control = 0, low = 1, and high = 3 harborages. Bars representing means that have the same letter are not significantly different (One-Way ANOVA:  $P = 0.0474$ ,  $F = 3.06$ , d.f. = 1, 1295,  $n = 1296$ ). Means were separated with Tukey's Studentized Range Test (Sclotzhauer and Littell 1987, 1997, SAS Institute 2008).**

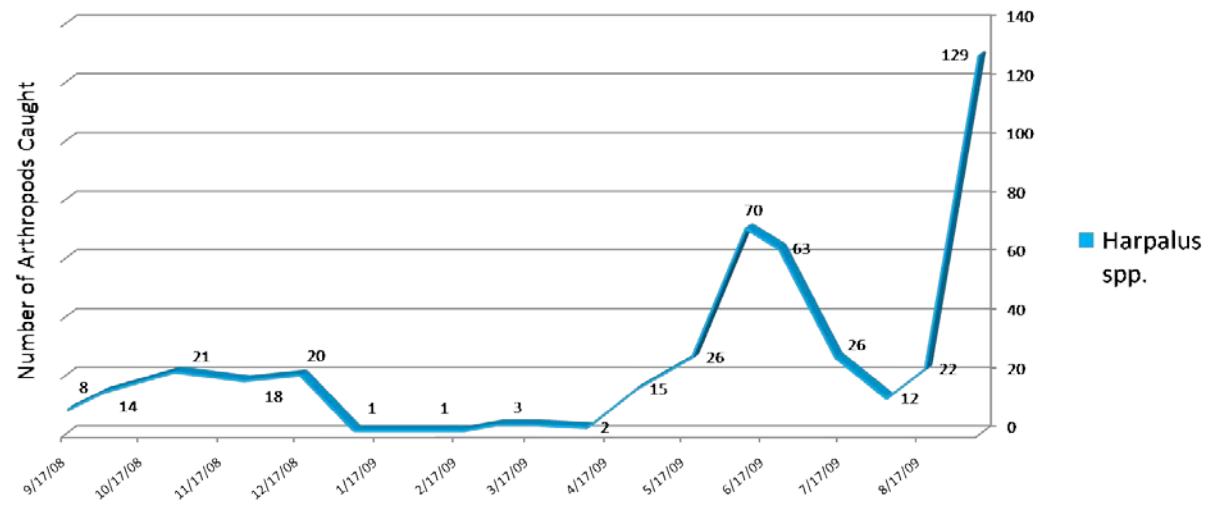


**Figure 3. Mean ( $\pm$  S.E.) number of occasional invader indicator taxa trapped in natural harborage trapping sites (Griffin Housing Authority site) in response to harborage presence or absence for all trapping dates and species. Bars representing means that have the same letter are not significantly different (One-Way ANOVA:  $P = 0.1583$ ,  $F = 1.99$ , d.f. = 1, 1039,  $n = 1040$ ). Means were separated with Tukey's Studentized Range Test (Schlotzhauer and Littell 1987, 1997, SAS Institute 2008).**



**Figure 4. Overall trap catch of *Harpalus* spp. beetles from 2008 to 2009.**

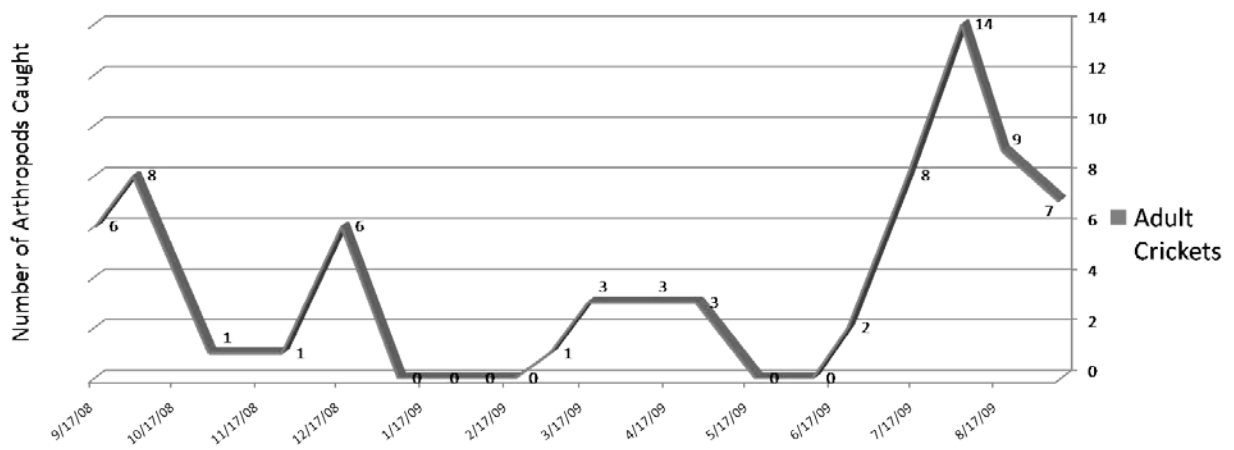
Harpalus spp. Caught Per Trapping Date





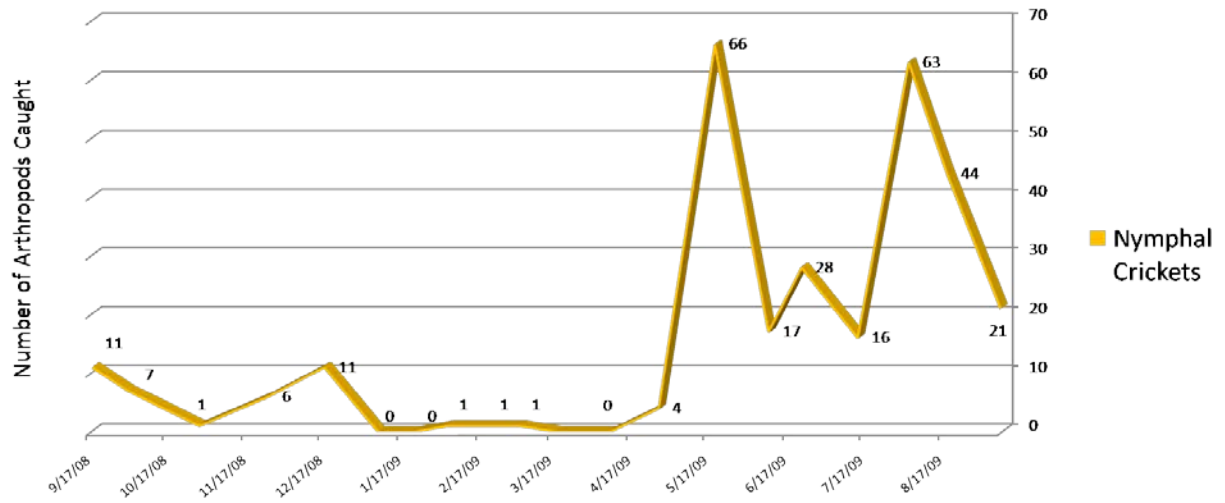
**Figure 5. Overall trap catch of adult crickets from 2008 to 2009.**

**Adult Crickets Caught Per Trapping Date**



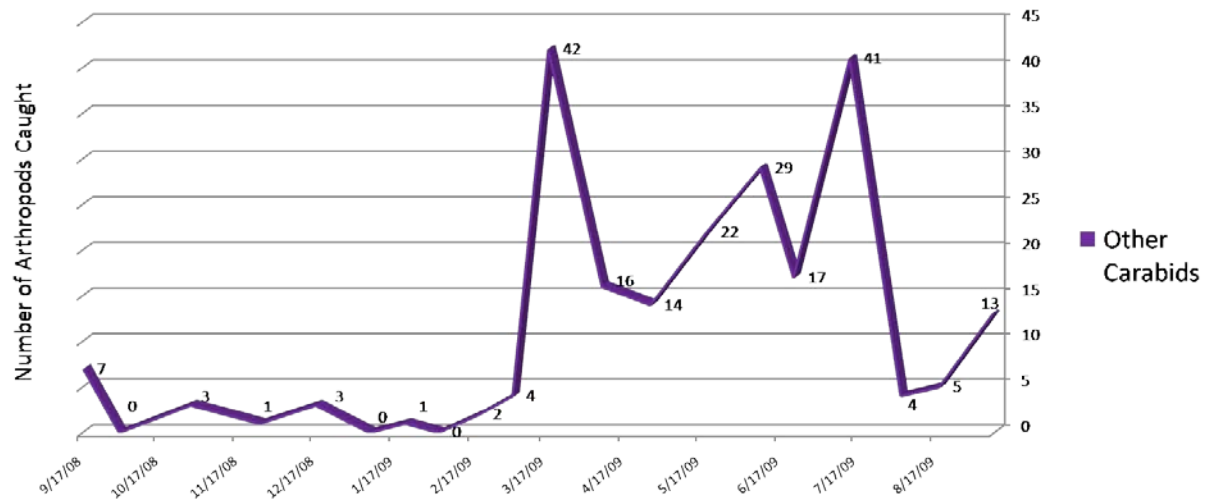
**Figure 6. Overall trap catch of nymphal crickets from 2008 to 2009.**

**Nymphal Crickets Caught Per Trapping Date**



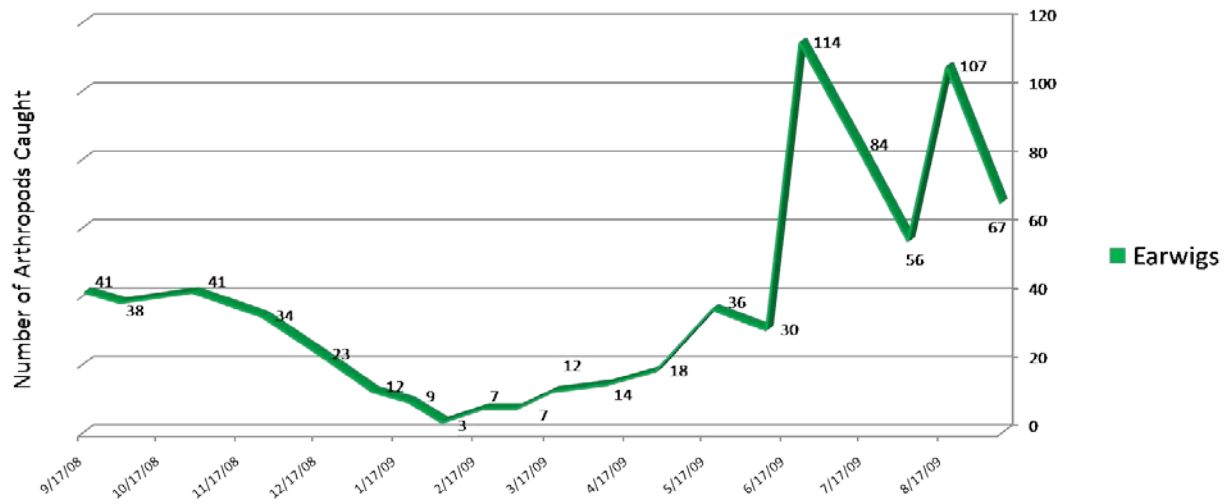
**Figure 7. Overall trap catch of other carabid beetles from 2008 to 2009.**

### Other Carabids Caught Per Trapping Date



**Figure 8. Overall trap catch of earwigs from 2008 to 2009.**

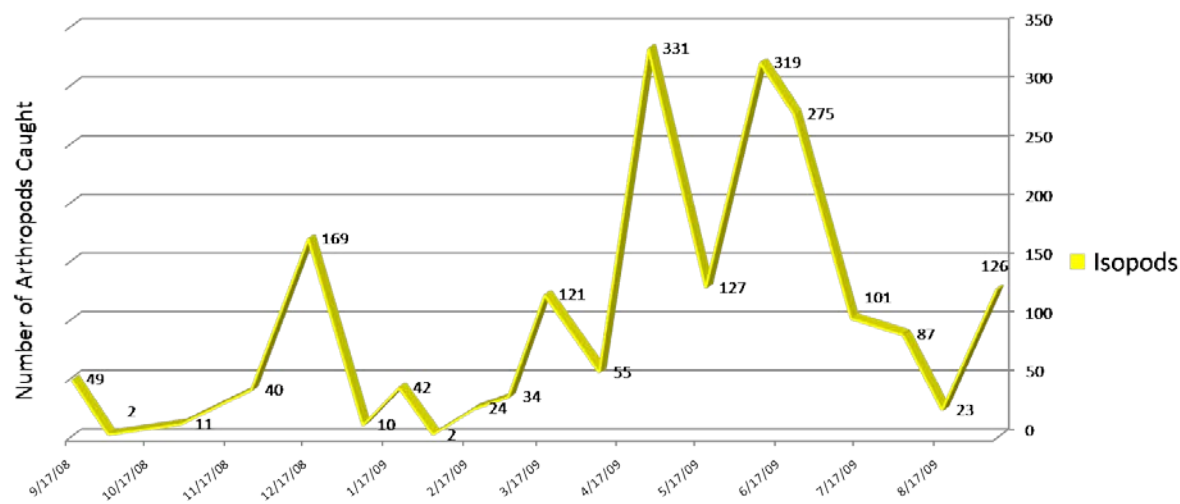
Earwigs Caught Per Trapping Date





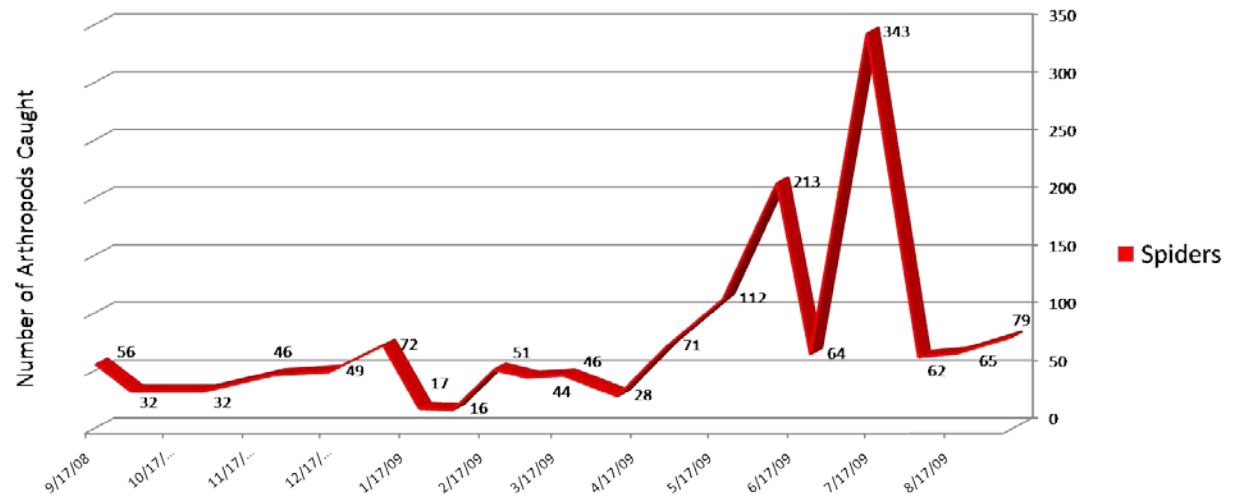
**Figure 9. Overall trap catch of isopods from 2008 to 2009.**

Isopods Caught Per Trapping Date



**Figure 10. Overall trap catch of spiders from 2008 to 2009.**

Spiders Caught Per Trapping Date



## CHAPTER 3

### THE REPELLENT EFFECTS OF FIVE PLANT-EXTRACTED ESSENTIAL OILS AGAINST THE ARGENTINE ANT, *LINEPITHEMA HUMILE* (MAYR)

Scocco, Christopher M. and Daniel R. Suiter. To be submitted to the Journal of Economic  
Entomology

**Abstract** Laboratory bioassays were performed to test the repellent properties of five plant-extracted essential oils against the Argentine ant, *Linepithema humile* (Mayr). Three concentrations (0.10%, 1%, and 10% in n-hexane) of technical grade peppermint, spearmint, wintergreen, cinnamon and clove oils, as well as a negative control [n-hexane] and positive control [Cinnamite] (Mycotech Corporation, Butte, MT) were evaluated in choice tests to evaluate repellency against Argentine ant workers by counting the number of ants entering a preferred harborage that was aged for 0 or 7 d. Means showed significant ( $P < 0.05$ ) differences in the 0.10% treatment, but not in the 1% and 10% treatments. All essential oils in the 0.10% treatment showed significantly different numbers of ants entering the dishes at 0 and 7 d post-treatment. Only the 1% wintergreen and the 10% spearmint oils showed significant differences in the numbers of ants entering the dishes 0 and 7 d post-treatment. Although the repellent activity of the oils at differing concentrations and ages were variable, plant-extracted essential oils may be an ecologically friendly alternative to traditional pesticides when applicable.

**Key Words** choice box bioassay, *Linepithema humile*, essential oils, repellency

## Introduction

The Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), is an invasive, cosmopolitan species that was introduced into the United States around 1891 via the port of New Orleans, LA (Foster 1908, Newell and Barber 1913, Suarez et al. 2001). In the United States, the Argentine ant is a major pest in urban, agricultural and natural environments due to its unicolonial structure which allows the formation of supercolonies with multiple shared nests (Newell and Barber 1913, Suarez et al. 1999, Tsutsui et al. 2000, Vega and Rust 2001, Tsutsui and Suarez 2003). Because these ants were introduced and have lost genetic diversity, they lack intraspecific aggression, allowing populations to increase rapidly (Newell and Barber 1913, Holway et al. 1998, Holway 1999, Human and Gordon 1999, Tsutsui et al. 2001, Suarez et al. 2002). This lack of aggression provides a competitive advantage to *L. humile* when competing against native ants, other introduced ant species (Kabashima et al. 2007), and other insect and arthropod species (Holway et al. 1998, Holway 1999, Tsutsui and Suarez 2003). Other factors in the success of *L. humile* include sociotony (sexual reproduction by division or budding), polydomy (individuals belonging to one colony often occupy multiple neighboring nests), polygyny (multiple nesting queens, which lay eggs throughout the year), lack of natural enemies, and human dispersal, mostly through commerce and various other business operations (Holway et al. 1998, Holway and Suarez 1999, Holway and Case 2000, Vega and Rust 2001, Tsutsui and Suarez 2003).

According to Suarez et al. (2001), *L. humile* is distributed throughout the southeastern United States and in most of California and, because it is a human commensal species, it is more successful in disturbed environments, including urban habitats (McGlynn 1999). They move inside structures in search of food and/or water, as well as relief from extreme temperatures

(Vega and Rust 2001, Schilman et al. 2007). Argentine ants may also become a problem in landscapes and gardens of homeowners because of their ability to tend and defend honeydew-producing hemipteran pest species (Klotz et al. 1995, McGlynn 1999, Vega and Rust 2001, Choe and Rust 2006).

Control of Argentine ants has typically relied on chemicals, more specifically slow-acting baits and perimeter sprays (Vega and Rust 2001). Klotz et al. (2007) found that slow-acting fipronil sprays reduced ant activity by 90% in an 8 wk period; however, using a perimeter spray of fipronil and a perimeter-broadcast of bifenthrin granules achieved the greatest reduction of ant activity around structures. Rust et al. (2003) proposed that the broader the range of concentrations in insecticidal baits, the more effective the control because of delayed toxicity. However, they also noted that finding suitable bait bases and active ingredients that provide delayed toxicity are the most difficult obstacles to overcome when formulating effective Argentine ant baits.

Klotz et al. (1995) suggested that the lack of information on Argentine ant biology has contributed to the failure of most traditional chemistries to successfully control Argentine ants. Soeprono and Rust (2004a) compared different active ingredients in treated materials. In a similar study, the rate of toxicity with different active ingredients suggested slower-acting sprays were more effective at killing a greater proportion of a colony (Soeprono and Rust 2004b). However, it was not necessary for all ants to directly contact the insecticide to cause mortality, but rather contact foraging workers that had been briefly exposed to the treatment (Soeprono and Rust 2004a,b). Because of this horizontal transfer of insecticide among ants, the chemicals may be transferred passively (through a substrate), through touch (antennal recognition or grooming), or when the dead ants are carried away by other workers (Soeprono and Rust 2004a).



In a similar study, Wiltz et al. (2009) treated a pine mulch substrate with either bifenthrin, chlorfenapyr, thiamethoxam or fipronil to assess the contact toxicity and rate of toxicity to Argentine ants. Fipronil, chlorfenapyr and thiamethoxam demonstrated a delayed toxicity that was sufficiently long enough to allow the treated foraging workers to return to the nest (Wiltz et al. 2009). They suggested that delayed toxicity is important because it allowed ants contaminated with the insecticide to return to the nest to recruit other workers to a food/water source or to simply contaminate nestmates.

While synthetic chemicals are widely used for insect and arthropod control, plant-derived essential oils were used before the advent and use of synthetic insecticides (Curtis et al. 1990). Some essential oils are effective at repelling, deterring, and/or killing a variety of arthropods, such as ants (Meissner and Silverman 2001, Appel et al. 2004, Wiltz et al. 2007), cockroaches (Appel et al. 2001, Peterson et al. 2002), termites (Peterson et al. 2004, Cheng et al. 2007), silverfish (Wang et al. 2006), beetles (Hori 2003), thrips (Koschier et al. 2007), ticks (Jaenson et al. 2006), whiteflies (Zhang et al. 2004) and mosquitoes (Rutledge et al. 1983, Omolo et al. 2004, Yang and Ma 2005, Amer and Mehlhorn 2006, Chang et al. 2006). Essential oils are extracted from plants, typically by steam distillation (Peterson et al. 2002). The use of essential oils in pest control products provides an alternative to traditional pesticide chemistries while retaining the ideal characteristics of repellency, deterency, and/or toxicity against insect and other arthropod pests (Isman 2000, Cheng et al. 2007, Wiltz et al. 2007).

Nerio et al. (2010) reviewed studies of essential oils used as repellents against insects and arthropods and concluded that essential oils have a great potential to be used as repellents. However, the mode of repellency (through volatilization) also decreases the time of deterrence against the insects or arthropods. Even so, they recommended further experimentation aimed at

prolonging the repellency of essential oils and that essential oils may eventually replace the traditional synthetic insecticides that are widely used today.

According to Curtis et al. (1990), a large number of plants that yield essential oils are known to be feeding deterrents to insects and other arthropods. Some of these plant-extracted oils have been the starting point for some commercially-produced repellents. The long-term repellent characteristics of essential oils have been challenged by some (Buescher et al. 1982, Rutledge et al. 1983, Nerio et al. 2010), who reported efficacy when freshly applied, but reduced effectiveness as the oils began to age. Thus, the objective of this study was to evaluate the repellent effects of freshly-applied and aged essential oils on Argentine ants, using a choice-based assay similar to that of Ebeling et al. (1966).

### **Materials and Methods**

**Ants.** Argentine ants used in this bioassay were collected from a field site on the University of Georgia Griffin Campus (N 33° 15.973', W 84° 17.336'). When collected, ants (including brood and queens) and accompanying soil, leaf litter, and other debris were placed in a plastic tub (model 400-5N,  $\approx 57 \times 45 \times 13$  cm, Del-Tec/Panel Controls Corporation, Greenville, SC). The tub was prepared in advance of ant collection by coating the inside walls with Fluon™ (Northern Products Inc., Woonsocket, RI) to prevent ant escape. In the lab, ants were provided at least five dead, adult crickets and four cotton balls soaked with 25% (w/v) sugar water as ample food and water sources. In order to prevent ants from desiccating,  $\approx 250$  ml of water was used to wet the leaf litter and debris daily. Ants were held at ambient humidity (55%-60% as determined by a sling psychrometer) and temperature (20°C - 23°C) in the laboratory. Ants were allowed 24 h to acclimate to laboratory conditions before being used in an assay.

Worker ants used in the bioassay were collected from laboratory colonies by allowing several ants to climb onto a small paintbrush. The ants were then gently tapped into a clear, 30 ml, plastic diet cup (Jet Plastica Industries Inc., Hatfield, PA) with inner surfaces coated with Fluon to prevent escape, and then transferred to the Fluon-coated boxes.

**Ant Harborages.** Harborages were prepared using polystyrene culture dishes (35 x 10 mm; Corning Inc., Corning, NY ) and Castone™ (model #99044, Dentsply International Inc., York, PA) powder, a high strength, water-adsorbent dental stone. Castone powder was mixed with water at a ratio of 30 g to 10 ml, after which, approximately 11 g of the mixture was placed into a polystyrene culture dish, so that each dish was approximately half-filled with Castone. Before the Castone hardened, dishes were gently and repeatedly tapped on a horizontal surface in order to remove any air bubbles and to evenly disperse the material to create a surface that filled the entire dish. Newly-prepared dishes were placed in an oven (40°C) and dried for at least 1 d before use. After drying, two holes (1.5875 mm diam and 180° apart) were drilled through the side of the dish, just above the surface of the Castone, to provide entrance and exit holes for the ants. A third hole was drilled in the center of the accompanying lid of each dish. All dishes and lids were cleaned to remove excess plastic and Castone dust.

**Test Treatments.** Seventeen treatment combinations (five oils at three concentrations each plus two controls) were evaluated for their repellency to Argentine ants. Spearmint, wintergreen, peppermint, cinnamon, and clove oils were acquired from Polarome International (Jersey City, NJ). With the exception of spearmint (60% purity), all oils were technical grade (100% pure). Each oil was serially diluted in n-hexane to produce 5 mls of 10%, 1% and 0.10% (v/v) solutions. These solutions were sealed in vials with Teflon tape, capped, and refrigerated (3-5°C) to prevent hexane evaporation. Two additional treatments (n-hexane alone [negative

control] and 1% Cinnamite™ (cinnamaldehyde) suspension in water [positive control] [Mycotech Corporation, Butte, MT]) were prepared and used as controls.

**Bioassay.** For each of the 17 treatment combinations, 0.50 ml of test solution was applied to the dental stone surface in each of two dishes (34 total dishes treated). One dish from each treatment was designated for use 2 h after treatment (Day 0). The second dish was designated for a 7 d residual assay and remained on the laboratory workbench for 168 h (Day 7) without lids prior to use in assays. The “Day 0” dishes remained on the laboratory workbench for 2 h to allow the hexane to evaporate. Prior to each assay, 250 µl of water was added to the dental stone in each dish for moisture, because Argentine ants are highly susceptible to desiccation (Walters and Mackay 2003, Schilman et al. 2007). Immediately after the water had absorbed into the dental stone dishes, lids were placed atop each dish and dishes were placed individually into Fluon-coated, clear, plastic boxes (19 x 14 x 9.5 cm; Tri-State Plastics, Dixon, KY). Twenty-five Argentine ant workers were placed on the bottom of each box and had the choice of either entering the covered dish or remaining outside of the dishes. The number of ants that were in the dish within each box was recorded after 2 h. Each of the 17 treatment combinations at Day 0 and Day 7 were replicated 20 times with four replicates initiated each week over a 12 wk period from July to October, 2008.

**Statistical Analysis.** The number of ants enumerated within each dish (alive plus dead) served as the response variable. Data were analyzed using a two-way ANOVA (PROC GLM) (Schothauer and Littell 1987, 1997, SAS Institute 2008) with treatment and oil age as variables. Because the interaction between these variables was significant ( $F = 6.0$ ; d.f. = 16, 19;  $P < 0.0001$ ), the difference in the number of ants between the two oil ages (Day 0 and Day 7) was analyzed further for each of the 17 treatment combinations and means were separated with

Tukey's Studentized Range Test (SAS Institute 2008). The number of ants entering dishes was further analyzed using a one-way ANOVA (PROC GLM) at each treatment combination of age (Day 0 and Day 7) and oil concentration (0.10%, 1%, and 10%). These means also were separated with Tukey's Studentized Range Test (SAS Institute 2008) (Figure 11).

## Results

**Fresh Deposits.** On Day 0, a mean ( $\pm$  S.E.) number of  $20.6 \pm 1.0$  Argentine ant workers entered the harborages treated with hexane alone. This number was significantly ( $P \geq 0.05$ ) greater than the numbers of ants entering the harborages treated with Cinnamite or any of the three concentrations of peppermint, wintergreen, spearmint, cinnamon or clove essential oils (Tables 4, 5, Figure 11). The mean ( $\pm$  S.E.) number of worker ants recovered from the harborages treated with Cinnamite (1%) was  $1.0 \pm 0.3$  which was significantly ( $P \geq 0.05$ ) lower than the number of ants recovered in nest dishes treated with a 0.10% concentration of all five essential oils and the harborages treated with a 10% concentration of either spearmint or peppermint. The number of worker ants recovered in the Cinnamite treatment did not differ significantly from those recovered from the nest dishes treated with a 1% concentration of all five essential oils or with a 10% concentration of clove, wintergreen or cinnamon essential oils.

**One-Week-Old Deposits.** For those treatments that were aged for seven d prior to placement with worker ants, the mean ( $\pm$  S.E.) number of ants entering the harborages treated with hexane alone was  $20.7 \pm 0.9$ . This number was significantly ( $P \geq 0.05$ ) greater than the numbers of ants entering the harborages seven d after treatment with 1% Cinnamite, all three concentrations of spearmint oil, and the 1% and 10% concentrations of peppermint, wintergreen, cinnamon and clove oils (Tables 4, 5, Figure 11). The numbers of ants entering the harborages

treated with 0.10% of either peppermint, wintergreen, cinnamon or clove were not statistically different from the number entering the hexane-treated harborage.

The mean ( $\pm$  S.E.) number of ants entering the harborage treated with Cinnamite 7 d earlier was  $10.2 \pm 0.6$  which was significantly ( $P \geq 0.05$ ) lower than only the treatment with hexane and the treatment with 0.1% peppermint (Tables 4, 5, Figure 11). Numbers of ants entering the Cinnamite-treated harborages were significantly ( $P \geq 0.05$ ) higher than numbers of worker ants recovered from harborages treated 7 d earlier with either 1% cinnamon, 1% clove or all five oils at the 10% concentration.

**Individual Oil Age Comparison.** In comparing the effects oil age (Day 0 vs. Day 7) within each treatment combination, statistically significant ( $P \geq 0.05$ ) increases in numbers of ants entering the harborages were observed for Day 7 over Day 0 for the five essential oils at 0.10% and for 1% Cinnamite and 1% wintergreen (Table 4). The numbers of worker ants entering the spearmint-treated harborages were significantly lower at Day 7 in comparison to Day 0, while all other treatment combinations were not statistically different between Day 0 and Day 7 (Table 4).

## Discussion

In this bioassay, repellency was indicated by the number of Argentine worker ants entering the treated harborages, with lower numbers indicating avoidance of the harborage by the worker ants and, thus, relative repellency of the active ingredient. The largest number of ants recovered (approx. 82%) was from the hexane-treated harborages, which served as a negative control in these assays. There also was no difference in the number of ants recovered from the hexane treatment after 2 h of aging vs. 168 h of aging (Table 4), thus, confirming complete hexane evaporation from the Castone surface within the initial 2 h period.

Cinnamite, on the other hand, was intended to be a positive control in these assays, and was highly repellent to Argentine ants when the workers were placed with the harborages treated only 2 h earlier. However, significantly greater numbers of ants entered the treated harborages 168 h after treatment (approx. 41%) in comparison to only 4% entering the harborages only 2 h after treatment (Table 4). Cinnamite clearly lost repellency within seven d of application. Wiltz et al. (2007) also reported that Cinnamite (1.5% concentration) deterred starved Argentine ant workers from crossing treated areas to access food within 24 h of application, but they did not assess residual activity.

Repellency and residual activity of the five essential oils were concentration dependent. At 0.10%, the numbers of ants entering the harborages treated with the oils 2 h earlier were significantly less than numbers of ants entering the hexane-treated harborages, but these numbers were also greater than the numbers of ants recovered from the Cinnamite-treated harborages (Figure 11). The percentages of ants recovered from the harborages were 82.4% for hexane (negative control), 58% for peppermint, 38.8% for cinnamon, 35.2% for clove, 34.8% for wintergreen, 25.2% for spearmint, and 4% for Cinnamite (positive control). At the higher concentrations, the percentages of total ants recovered from the harborages treated with the essential oils ranged from 8.8% to 14.4% at the 1% concentration and from 9.2% to 20% at the 10% concentration, while the percentage recovered from the hexane-treated harborages was 82.4%.

At 0.10%, all five essential oils tested exhibited significant declines in repellency within seven d of application to the harborages (Tables 4, 5). At 7 d after application, only the spearmint oil treatment had significantly fewer ants entering the treated harborage than entered the hexane-treated control (Figure 11). At 1%, only wintergreen oil showed a significant decline in

repellency within 7 d of application (Table 4). However, the numbers of ants entering the harborages treated 7 d earlier with the five oils, including wintergreen, was significantly less than the numbers of ants entering the hexane-treated harborages (Figure 11). There were no reductions in repellency over the seven d period of any of the five essential oils when applied at a 10% concentration to the harborages (Table 4, Figure 11).

These results, although only laboratory-based at this time, indicate the potential of using plant-derived essential oils to repel Argentine ants from harborages. Essential oils have been used since ancient times in many aspects, including pest control. They have excellent repellent, toxic and/or fumigant effects toward insects that are of medical (Buescher et al. 1982, Rutledge et al. 1983, Isman 2000, Omolo et al. 2004, Yang and Ma 2005, Amer and Mehlhorn 2006, Jaenson et al. 2006), agricultural (Curtis et al. 1990, Zhang et al. 2004, Wang et al. 2006, Koschier et al. 2007), stored product (Hori 2003), and structural or urban importance (Appel et al. 2001, Meissner and Silverman 2001, Peterson et al. 2002, Meissner and Silverman 2003, Cheng et al. 2007, Wiltz et al. 2007). Because of the recent ecologically friendly movement away from pesticides to naturally-derived alternatives, essential oils are becoming increasingly popular among consumers who want lower impact substitutes to traditional chemistries, with the same reliability of control provided by chemical insecticides or repellents (Isman 2000).

A number of examples are available for their use against insects that may become occasional invaders. Meissner and Silverman (2001) showed that aromatic cedar, *Juniperus virginiana* L., mulch was more efficient at repelling Argentine ants than cypress mulch or pine straw. After given a choice of cedar or non-cedar mulch types, worker ants chose the non-cedar varieties to construct or move a nest. In a later study, they (Meissner and Silverman 2003) further reported that eliminating other possible landscape media, namely non-cedar mulch varieties, may



negatively impact ant nesting establishment. Their study also demonstrated the ability of cedar mulch to prevent Argentine foraging activity when compared to other mulches or media. Not only does aromatic cedar mulch hinder Argentine ant foraging activity and nest establishment, but it was also toxic to Argentine ants in laboratory bioassays (Meissner and Silverman 2001, 2003).

Appel et al. (2001, 2004) investigated the toxic and repellent effects of corn mint oil against German cockroaches, *B. germanica*, American cockroaches, *Periplaneta americana* L., and red imported fire ants, *S. invicta*. In continuous-exposure tests, both cockroach species were exposed to mint-oil impregnated filter paper for up to 24 h. They found toxicity to be concentration-dependent. At 3%, lethal time was slower ( $LT_{50} = 3,318$  min for *B. germanica*,  $LT_{50} = 469.9$  min for *P. americana*) than at concentrations of 100% ( $LT_{50} = 1$  min for *B. germanica* and *P. americana*) (Appel et al. 2001). When red imported fire ants were topically treated in limited- and continuous-exposure tests, the mint oil granules were toxic to the ants (Appel et al. 2004). Exposure to the mint oil granules was toxic at low rates in the continuous-exposure test, and toxicity was positively correlated with granule quantity. The limited exposure test also showed that as little as a 15-min exposure resulted in 2X mortality in comparison to the untreated control after 24 h (Appel et al. 2004).

In repellency tests, Appel et al. (2001) concluded that mint oil granules were repellent, with 92.3 to 100% repellency of German cockroaches and 100% repellency of American cockroaches in a choice-box test. In contrast, in the untreated (control) choice-box, only 2% of American cockroaches and 13% of German cockroaches were repelled. Mint oil has the ability to repel and kill cockroaches, while leaving little-to-no residue (Appel, unpubl. data), but is also highly susceptible to volatilization in open environments.

Using an arrangement similar to the choice-box bioassay in 2001, Appel et al. (2004) placed 10 fire ant workers in a glass Petri dish with one half of the dish bottom covered with mint oil granules and the other half left uncovered. Results demonstrated that the mint oil granules were repellent to worker ants. They suggested that mint oil may have residual effects on fire ant mounds long after the ants initially leave the treated mounds. Although mound drenching is a labor-intensive and uncommonly used method of ant control, the oil-impregnated granules may offer a long-lasting residual effect in treated mounds (Appel et al. 2004).

More recently, Wiltz et al. (2007) tested basil, citronella, eucalyptus, lemon, peppermint and tea tree essential oils against *S. invicta* and *L. humile* for their deterrent and toxic effects. Similar to the foraging activity study by Meissner and Silverman (2003), the deterrence tests were designed to determine whether either ant species would cross oil-impregnated cardboard barriers in order to acquire a food source. All essential oils tested, except eucalyptus, were deterrent to both ant species.

However, the types of essential oils used as repellents or fumigants may have variable results toward their directed pest species (Rutledge et al. 1983, Amer and Mehlhorn 2006). Rutledge et al. (1983) tested 31 repellents, including permethrin, against five species of *Aedes*, *Anopheles*, and *Culex* mosquitoes. They concluded that in order to test repellent effects against a specific insect, the target species must be used during the testing. Even congeneric species, such as using *B. germanica* in place of *B. asahinai*, cannot give actual responses as to how the repellent would work on the target insect (Rutledge et al. 1983). They also noted that in order to test a repellent for broad-spectrum properties, it is highly recommended to also use a variety of insect species in order to establish which chemicals repel specific insect species.

Before the creation of synthetic pesticides, essential oils were the basis of many commercial repellents and insecticides (Curtis et al. 1990). In order to effectively repel insects, the specific oils used, along with the differing oil concentrations, must be exclusive to the study. The test insects used in assays must also be exclusive to the study. Essential oils are volatile, yet it is this property that gives them repellent and fumigant properties; however, volatilization at a rapid rate will cause the essential oils to lose their repellent or fumigant properties over a short period of time, yielding a reduced amount of efficacy for the oils (Chang et al. 2006).

Regardless of the oil tested in our study, all exhibited volatility when formulated at 0.10%, while concentrations  $\geq 1\%$  demonstrated the ability to repel ants when freshly deposited or after being aged for 7 d. The test results show that the positive control, Cinnamite, was more repellent when freshly applied, but started to lose its repellent nature when aged for 7 d. In order to utilize essential oils as repellents, further investigation is needed to slow the volatilization process and develop and target specific oils for specific insects. However, completely stopping the volatilization process of essential oils would also hinder their repellent properties. In order to facilitate an efficacious and economically-consistent repellent for Argentine ants, and potentially occasional invader pests, the characteristics that make essential oils repellent need to be retained, while controlling the rate of volatilization.

In order to utilize essential oils as repellents against pests, their applicability to other potential pests must also be investigated. Because individual essential oils have different effects on individual specific insects and other arthropods, a considerable range of testing must still be completed in order to assess their repellency or toxicity to potential pests. These studies must also account for realistic uses in homes, institutions and other establishments, studying the rate of

volatilization and the ways to possibly control potential pests without losing efficacy of essential oils rapidly.

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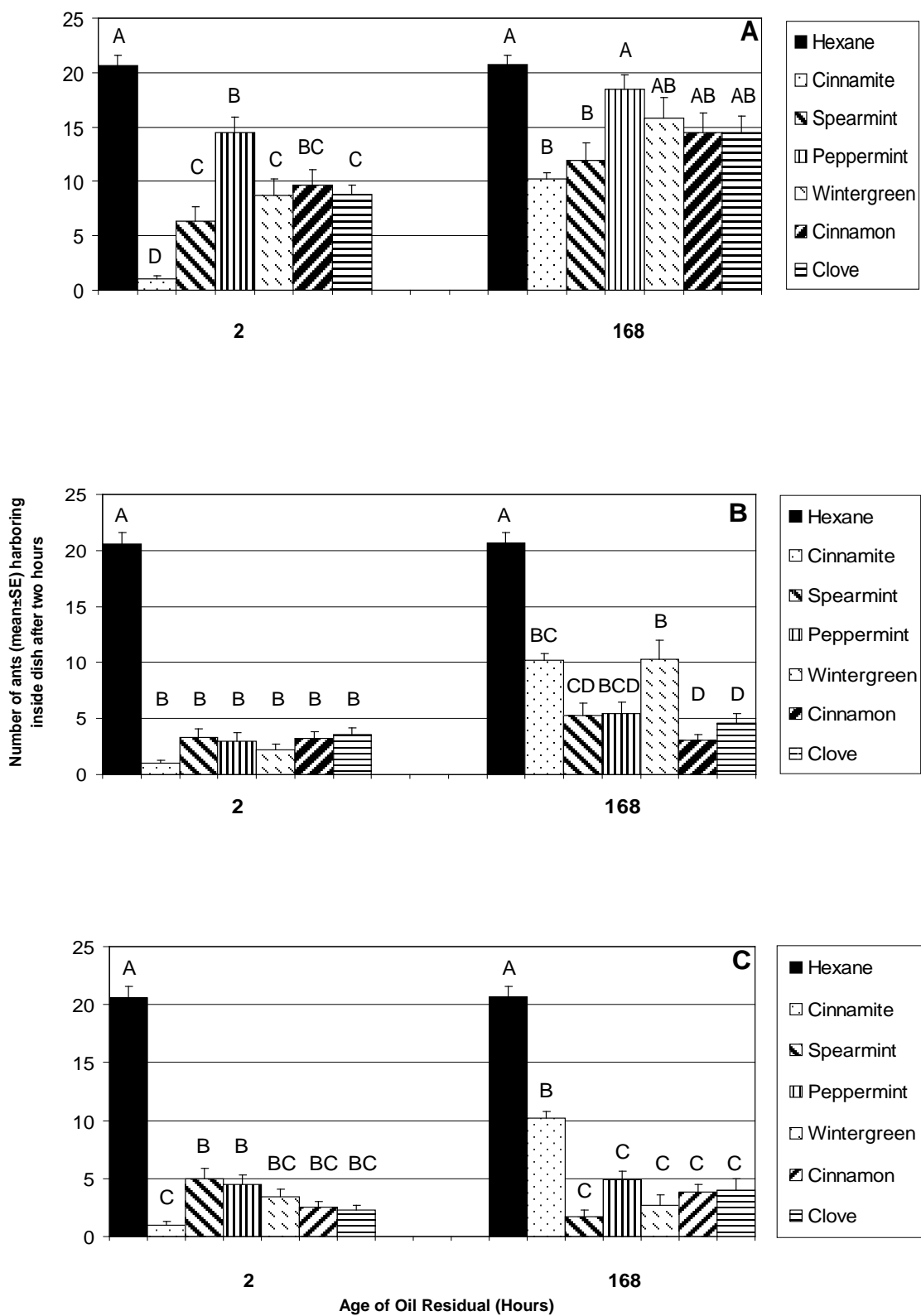
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**Figure 12. Response of Argentine ant workers to harborages treated with various chemicals (one of five essential oils, Cinnamite, or hexane) and aged for 0 or 7 days. Repellency of various essential oils at 0.10% (A), 1% (B), and 10% (C) to Argentine ants (For each graph of residual oil age, means followed by the same letter are not significantly different. For each ANOVA, means were separated with Tukey's Studentized Range Test (Schlotzhauer and Littell 1987, 1997, SAS Institute 2008)).**



**Table 4. Essential oil repellency comparisons by individual oil ages.**

Treatment	Number of ants (mean $\pm$ SE) entering dish at given residual age (hours)		Statistics <sup>a</sup>	
	2	168	F-value	P-value
Hexane	20.6 $\pm$ 1.0a	20.7 $\pm$ 0.9a	0.01	0.9252
1% Cinnamite	1.0 $\pm$ 0.3a	10.2 $\pm$ 0.6b	34.31	<0.0001
0.1% Spearmint	6.3 $\pm$ 1.4a	11.9 $\pm$ 1.6b	6.13	0.0229
0.1% Peppermint	14.5 $\pm$ 1.4a	18.5 $\pm$ 1.3b	8.79	0.0080
0.1% Wintergreen	8.7 $\pm$ 1.5a	15.8 $\pm$ 1.9b	20.13	0.0003
0.1% Cinnamon	9.7 $\pm$ 1.4a	14.5 $\pm$ 1.8b	6.01	0.0241
0.1% Clove	8.8 $\pm$ 0.9a	14.5 $\pm$ 1.5b	15.99	0.0008
1% Spearmint	3.3 $\pm$ 0.8a	5.3 $\pm$ 1.1a	2.00	0.1736
1% Peppermint	3.0 $\pm$ 0.7a	5.4 $\pm$ 1.1a	3.73	0.0685
1% Wintergreen	2.2 $\pm$ 0.5a	10.3 $\pm$ 1.7b	20.85	0.0002
1% Cinnamon	3.2 $\pm$ 0.6a	3.1 $\pm$ 0.5a	0.02	0.8907
1% Clove	3.6 $\pm$ 0.6a	4.6 $\pm$ 0.8a	0.80	0.3835
10% Spearmint	5.0 $\pm$ 0.9a	1.7 $\pm$ 0.6b	12.69	0.0021
10% Peppermint	4.5 $\pm$ 0.8a	4.9 $\pm$ 0.7a	0.09	0.7707
10% Wintergreen	3.4 $\pm$ 0.7a	2.7 $\pm$ 0.9a	0.37	0.5477
10% Cinnamon	2.5 $\pm$ 0.5a	3.8 $\pm$ 0.7a	2.33	0.1438
10% Clove	2.3 $\pm$ 0.4a	4.0 $\pm$ 1.0a	2.55	0.1266

<sup>a</sup>Degrees of Freedom = 1,19 for all treatments. Means within a row followed by the same letter are not significantly different. For each ANOVA, means were separated with Tukey's Studentized Range Test (SAS Institute 2008)

**Table 5. One way ANOVA (PROC GLM) for individual oil rates and ages.**

Oil Rate	Oil Age (in h)	Statistics <sup>a</sup>	
		F-value	P-value
0.10%	2	27.56	<0.0001*
0.10%	168	5.53	<0.0001*
1%	2	108.35	<0.0001*
1%	168	27.20	<0.0001*
10%	2	94.60	<0.0001*
10%	168	48.34	<0.0001*

<sup>a</sup>Degrees of Freedom = 1,6 for all treatments. P-values within each row followed by a “\*” denotes significant differences between essential oils in each ANOVA.

CHAPTER 4

CONCLUSION

The studies described in this thesis have further expanded on naturally-occurring and non-chemical management tactics of potential pests in the suburban landscape. These control strategies were investigated to ultimately be used in part of an integrated pest management program for managing suburban landscape pests. Because plant-derived essential oils are naturally occurring, they are usually widely accepted as an ecologically-friendly alternative to traditional chemical insecticides. The use of physical control, such as harborage removal, is part of an integrated pest management plan that can also reduce or eliminate the amount of chemical insecticides being used around a structure in the suburban landscape.

Previous research demonstrated that plant-derived essential oils are practical in repelling, fumigating or topically killing several types of insect and arthropod pests. Control tactics using essential oils should repel, fumigate or kill by contact application when applied to areas with pest infestations. However, research shows that specific essential oils will only work with specific insects and are generally not a broad-spectrum application. Research also shows that residual activity of essential oils at low ( $<1\%$ ) concentrations is variable with volatilization becoming a factor.

Laboratory bioassays were conducted to assess the repellency and volatilization of plant-derived essential oils. The purpose of these bioassays was to determine if specific essential oils had repellent properties against the Argentine ant, *Linepithema humile* (Mayr). These bioassays also assessed the rate of volatilization of freshly-deposited oils versus aged oils. Data from the assays supported other findings that essential oils can be used in a variety of ways to control pest insects and arthropods, including repellency. Freshly-deposited oils at all concentrations showed significant repellency toward the ants compared to the negative control. However, aged oil deposits at higher concentrations ( $\geq 1\%$ ) showed a significant repellency toward Argentine ants,



while oils of lower concentrations ( $< 1\%$ ) showed little differences in repellency with the positive control, a 1% Cinnamite™ suspension. The data demonstrated the repellent effects of the oils used in the bioassay which could be used by homeowners or commercial applicators to control Argentine ant infestations.

However, data from the bioassay also showed the rate of volatilization of aged oil deposits. The Cinnamite™ positive control showed a significant rate of volatilization after being aged for 1 wk. The essential oils at the 0.10% concentration all had significant volatilization after 1 wk of being aged. The higher concentrations ( $\geq 1\%$ ), however, did not exhibit significant rates of volatilization. This information illustrates the need for essential oil concentrations of at least 1% in order for insect and arthropod pests to properly be repelled, fumigated or killed. However, these details are only relevant to the essential oils and the specific test ants used in this bioassay because individual essential oils are exclusive to the type of insect that they may potentially control.

This information will also be useful in examining the rate of volatilization of essential oils. Currently, essential oils are diluted (v/v) but still used in their raw form for repellency, fumigation or as a contact toxicant. However, volatilization of essential oils is what makes them effective. Further investigation into carrier or fixative materials will be needed to extend the treatment time of the oils without hindering the aromatic properties that make the oils efficacious. This would make essential oil use more economically feasible than the synthetic insecticides still in use today.

Field studies in 2008 – 2009 were performed to evaluate the physical control tactic of harborage removal against occasional invader pests in the suburban landscape. Data from the findings did not correspond to similar studies performed in the urban and suburban landscape demonstrating that harborage removal reduces the occurrence of occasional invader pests in the landscape. Data from this study showed that harborage was generally not significant with the trap catch or biomass of occasional invaders caught. However, the overwhelming majority of supporting literature in this study showed that harborage was significant in insect and arthropod abundance in field study sites.

The seasonal abundance of occasional invaders illustrated their respective estimated sample densities throughout the year-long study. Separate from the harborage amounts used in the study, the number of occasional invader indicator species at each trapping date were recorded and used to assess the samples of occasional invaders in the landscape. This information can be used by homeowners and professionals to understand the occasional invader pests found in the landscape and the time of year that abundances are low or high. Trends throughout the data displayed similar trends established in previous studies, confirming that occasional invaders in the landscape have distinctive and sometimes overlapping densities. Knowledge of potential population trends of occasional invaders can provide further awareness of mechanical control tactics in IPM programs, including sanitation indoors and outdoors and exclusion by minimizing ways of entry into structures. Because occasional invaders are rarely encountered and are difficult to rear in laboratory settings, information of seasonal abundance and sampling densities may also be promising for future studies. These data may potentially offer insight into other biological and ecological characteristics that have not yet been investigated.