REGIONAL VARIATION IN INSECTICIDE SUSCEPTIBILITY IN ODOROUS HOUSE ANTS (*TAPINOMA SESSILE*)

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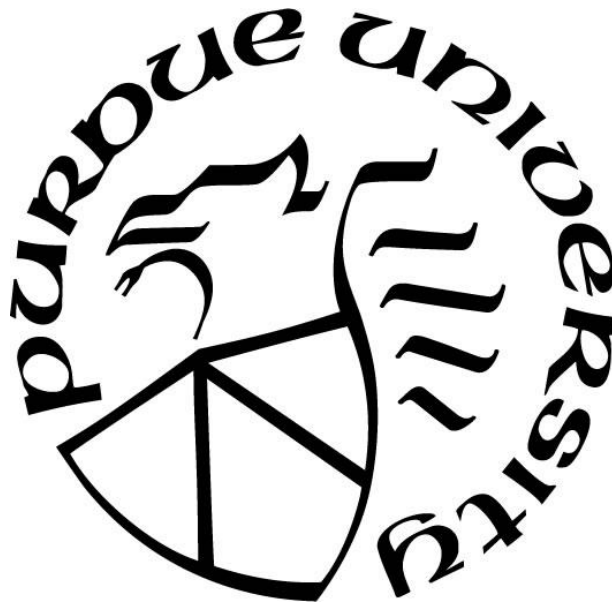**Kaitlyn Brill**

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Dedicated to Kathleen Brill, Dr. C. Tate Holbrook, Dr. David J. Stasek, and Joseph C. Lodmell, who have left positive impacts on my life and have shown me never ending support and encouragement.

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ABSTRACT

Odorous house ants (*Tapinoma sessile*) can be found across all of North America. They are an extreme nuisance pest, as its common name implies. In its natural environment, *T. sessile* is a submissive species, they usually stay in one localized colony and do not compete with other nearby species. They can usually be found in small, single queen colonies in acorns, leaf litter, and many other discrete sites. However, when introduced to urban environments, *T. sessile* flourish. They adapt invasive characteristics and grow from monogynous colonies to enormous polygynous colonies that may contain thousands of queens and millions of workers. They are infamous for invading man-made structures and impeding on both economic and esthetic thresholds. *Tapinoma sessile* are the #1 call-back ant for the pest management industry and are the cause of many control failures and customer dissatisfaction.

Insecticide resistance has been found in many urban pests, including cockroaches and bedbugs. However, no study has been conducted on insecticide resistance with regards to certain social insects such as ants. This may be in part due to their reproductive biology, including the caste system. The goal of the study included in this thesis was to test *T. sessile* colonies in order to assess variation in insecticide susceptibility between colonies. The three main objectives were as follows: 1) to test if there was regional variation in susceptibility between *T. sessile* colonies with regards to three commonly used insecticides for ant control, 2) to compare susceptibility between urban and natural colonies, and 3) to test if there were susceptibility differences between the worker and queen castes.

With regards to both lambda-cyhalothrin and fipronil, there were no significant differences in susceptibilities between natural and urban colonies. However, urban colonies were significantly more tolerant than natural colonies with regards to dinotefuran. Because these results show that urban and natural colonies were not different with regards to lambda-cyhalothrin and fipronil, but there was a significant difference with regards to dinotefuran, this suggests that the local of *T. sessile* colonies is not a reliable predictor on their susceptibility to certain insecticides. However, with regards to comparing castes, queens were significantly more tolerant relative to their worker counterparts across all three insecticides. These results may explain why *T. sessile* are so difficult to control. Further research is needed to build a solid framework for effective odorous house ant control.

CHAPTER 1.   LITERATURE REVIEW

1.1   Introduction and Biology

Odorous house ants*, Tapinoma sessile* (Say), are considered one of the most adaptive and widespread ant species found in North America (Fisher et al. 2007, Buczkowski 2010, Menke et al. 2010). They belong to the subfamily Dolichoderinae and get their common name from the smell they emit when startled or crushed (Smith 1928). They are opportunistic nesters and may choose a wide variety of habitats. In their natural environment, they can be found nesting in acorns, shallow soil, leaf litter, under rocks, and behind tree bark. However, when introduced into urban environments, they can be found in other opportunistic sites including manmade structures (Buczkowski and Bennett 2006, Buczkowski and Bennett 2008a).

1.2   Urbanization and Invasive Characteristics

Rising levels of urbanization inflict negative effects on most ant communities (Salyer et al. 2014). However, many species of ants have adapted to use urbanization to their advantage. *T. sessile* are known to monopolize resources that are left over after urbanization has occurred (Salyer et al. 2014), with little to no competition from other ant species that may have withstood habitat destruction (McGlynn, 1999). Habitat degradation and urbanization promote the evolution of invasive characteristics, such as polydomous and polygynous traits of *T. sessile* that are found near or within urban areas (Buczkowski and Bennett 2008b, Buczkowski and Krushelnycky 2011). Such polydomous and polygynous colonies may contain millions of workers and thousands of queens that inhabit numerous nesting sites (Buczkowski 2010, Menke et al. 2010). Unlike in their natural setting, where *T. sessile* is normally found to be a noncompetitive, monogynous species, urbanized settings allow them to become a dominant nuisance (Buczkowski and Richmond 2012).

Buczkowski and Krushelnycky (2011) demonstrated an example of the adaptability and invasive characteristics of *T. sessile* upon finding a recent invasion in Maui, Hawaii.

1.3   Pest Status and Industry Concerns

T. sessile cause little to no harm to humans because of their lack of a sting, but they are still considered a pest species because of their ability to impede on esthetic and economic thresholds (Lee 2002). Treating for *T. sessile* can often be frustrating as they usually go unnoticed and can easily relocate to new nest sites or create satellite colonies (Layton et al. 2018). They are considered the #1 ranked household pest species and #1 call-back ant among the industry due to numerous control failures (Paysen 2019, Robbins 2015). In a national survey, PMPs reported that *T. sessile* was the most important ant driving sales and the most difficult ant to control (Paysen. 2019). Furthermore, PMPs across the country report control failures in *T. sessile* which decreases profits and increases consumer dissatisfaction. Because of this, different management practices have arisen to combat *T. sessile*. They include the use of specific baits, sprays, and exclusion/prevention techniques. Out of these, the most common management strategy used against pest ants are perimeter treatments with contact residual sprays (Rust et al. 1996, Scharf et al. 2004, Buczkowski et al. 2005, Silverman and Brightwell 2008, Rust and Su 2012). However, there is reason to believe that such perimeter treatments clear out any insect competition that *T. sessile* may face, thus allowing them to become more prevalent in the treated areas (Scharf et al. 2004). Though there is not much solid evidence as to why *T. sessile* has such a rapidly growing pest status, this theory may be a contributing explanation.

1.4   Insecticide Resistance

Insecticide resistance is a major issue for the pest management industry and combating insecticide resistance is a major effort by the pest management industry, the EPA, and chemical manufacturers (Zhu et al. 2016). Insecticide resistance is commonly observed in cockroaches (Fardisi et al. 2019) and bed bugs (Ashbrook et al. 2017) but has never been documented in certain social insects such as ants. Despite the fact that insecticide resistance has never been documented in social insects, PMPs across the country still report control difficulties in ants. Among ants, *T. sessile* are infamous for being very hard to control (Paysen. 2019). This may be in part due to their reproductive biology. Considering the fact that sterile workers are usually the ones exposed to insecticides while outside of the nest, fertile queens rarely come in contact with these toxins, making it plausible that ants may not experience events leading to insecticide resistance. However, in such instances when workers return to their nest, queens may be exposed to insecticides via horizontal transfer (Soeprono and Rust 2004, Buczkowski 2019), making it also plausible that insecticide resistance may come into play among queens and lead to inherited changes among the colony that can impact the colonies sensitivity to the toxins.

1.5   References

Ashbrook A., M. Scharf, G. Bennett, A. Gondhalekar. 2017. Detection of reduced susceptibility to chlorfenapyr- and bifenthrin-containing products in field populations of the bed bug (Hemiptera: Cimicidae). Journal of Economic Entomology 110:1195–1202.

Buczkowski, G. 2010. Extreme life history plasticity and the evolution of invasive characteristics in a native ant. Biological Invasions, 12: 3343-3349.

Buczkowski G. 2019. Trap-treat-release: horizontal transfer of fipronil in field colonies of black carpenter ants, *Camponotus pennsylvanicus*. Pest Management Science, 75: 2195–2201.

Buczkowski, G., G. Bennett. 2006. Dispersed central-place foraging in the polydomous odorous house ant, *Tapinoma sessile* as revealed by a protein maker. Insectes Sociaux, 53: 282–290.

Buczkowski, G., G. Bennett. 2008a. Aggressive interactions between the introduced Argentine ant, *Linepithema humile* and the native odorous house ant, *Tapinoma sessile*. Biological Invasions, 10: 1001–1011.

Buczkowski, G., G. Bennett. 2008b. Seasonal polydomy and unicoloniality in a polygynous population of the odorous house ant, *Tapinoma sessile*. Ecological Entomology, 33: 780-788.

Buczkowski, G., P. Krushelnycky. 2011. The odorous house ant, *Tapinoma sessile* (Hymenoptera: Formicidae), as a new temperate-origin invader. Myrmecological News, 16: 61-66.

Buczkowski, G., D. Richmond. 2012. The effect of urbanization on ant abundance and diversity: A temporal examination of factors affecting biodiversity. PLoS ONE 7.

Buczkowski, G., M. Scharf, C. Ratliff, G. Bennett. 2005. Efficacy of simulated barrier treatments against laboratory colonies of pharaoh ant. Journal of Economic Entomology, 98: 485–492.

Fardisi M., A. Gondhalekar, A. Ashbrook, M. Scharf. 2019. Rapid evolutionary responses to insecticide resistance management interventions by the German cockroach (Blattella germanica L.). Scientific Reports 9.

Fisher, B., S. Cover. 2007. Taxonomic descriptions. Ants of North America: a guide to the genera. University of California Press, 145–146.

Hölldobler, B., E. Wilson. 1990. The ants. Harvard University Press, Cambridge, MA.

Layton, B., J. MacGown. 2018. Control of argentine ants and odorous house ants in the home. Mississippi State University Extension Service, P2407.

Lee, C. 2002. Tropical household ants: pest status, species diversity, foraging behavior, and baiting studies, pp. 3-18. *In* S. Jones, J. Zhai, W. Robinson [eds.], Proceedings of the 4th International Conference on Urban Pests. Charleston, SC, 7-10 July 2002. Pocahantas Press, Blacksburg, VA.

McGlynn, T. 1999. The worldwide transfer of ants: geographical distribution and ecological invasions. Journal of Biogeography, 26: 535-548.

Menke, S., W. Booth, R. Dunn, C. Schal, E. Vargo, J. Silverman. 2010. Is it easy to be urban? Convergent success in urban habitats among lineages of a widespread native ant. PLoS One 5: 2: e9194.

Paysen, E. 2019. 2019 state of the ant control market. Pest Control Technology Magazine, 47: 1–7.

Robbins, S. 2015. Annual Ant Control Issue, Odorous House Ants Simplified. Pest Control Technology Magazine 46.

Rust, M., K. Haagsma, D. Reierson. 1996. Barrier sprays to control Argentine ants (Hymenoptera: Formicidae). Journal of Economic Entomology, 89: 134–137.

Rust, M., and N-Y. Su. 2012. Managing social insects of urban importance. Annual Review of Entomology, 57: 355–375.

Salyer, A., G. Bennett, G. Buczkowski. 2014. Odorous house ants (*Tapinoma sessile*) as back-seat drivers of localized ant decline in urban habitats. PLoS ONE 12: 9: e113878.

Scharf, M., C. Ratliff, and G. Bennett. 2004. Impacts of residual insecticide barriers on perimeter-invading ants, with particular reference to the odorous house ant, Tapinoma sessile. Journal of Economic Entomology, 97: 601–605.

Silverman, J., R. Brightwell. 2008. The Argentine ant: challenges in managing an unicolonial invasive pest. Annual Review of Entomology 53:231–252.

Smith, M. 1928. The biology of Tapinoma sessile Say, an important house-infesting ant. Ann. Entomol. Soc. Am. 21: 307-330.

Soeprono, A., M. Rust. 2004. Effect of horizontal transfer of barrier insecticides to control Argentine ants (Hymenoptera: Formicidae). Journal of Economic Entomology, 97: 1675–1681.

Zhu F., L. Lavine, S. O'Neal, M. Lavine, C. Foss, D. Walsh. 2016. Insecticide Resistance and Management Strategies in Urban Ecosystems. Insects 7:2.

CHAPTER 2. REGIONAL VARIATION IN INSECTICIDE SUSCEPTIBILITY IN ODOROUS HOUSE ANTS (*TAPINOMA SESSILE*)

2.1 Abstract

The odorous house ant *(Tapinoma sessile)* is an adaptive and widespread pest ant species found in North America. Despite the economic and ecological impact of pest ants, effective management still faces many challenges and control failures with liquid spray insecticides in urban and natural environments. In many insects such as bed bugs and cockroaches, chemical control measures have resulted in insecticide resistance. However, in contrast to non-social insects, insecticide resistance has never been documented in social insects. The current study had three main goals. The first objective was to examine regional variation in *T. sessile* insecticide susceptibility to three classes of insecticides commonly used in urban ant control. The second objective was to compare insecticide susceptibility in *T. sessile* colonies collected in natural vs. urban areas. The final objective was to determine if insecticide susceptibility varies in workers vs. queens. A total of 30 *T. sessile* colonies were collected within a 50-mile radius of Purdue University campus, Tippecanoe County, Indiana. Fifteen colonies were collected in natural areas and 15 in urban areas. Insecticide susceptibility of all 30 colonies was tested using residual exposure assays. Urban and natural colonies showed no significant differences in tests with lambda-cyhalothrin and fipronil. In tests with dinotefuran, urban colonies were significantly more tolerant relative to natural colonies. These results suggest that habitat type does not reliably predict susceptibility levels in individual populations. Queens were found to be significantly more tolerant relative to their worker counterparts across all three insecticides. Lower insecticide susceptibility in the queens may explain why *T. sessile* is such a persistent pest in urban environments. This

study's results can in part provide a foundation for the development of more effective and efficient control methods for *T. sessile*.

2.2   Introduction

Ants play critical roles in most ecosystems because they constitute a great part of animal biomass and act as ecosystem engineers (Folgarait 1989). Despite their valuable contributions, many ant species are serious pests in urban, agricultural, and natural environments (McGlynn 1999, Suarez et al. 2010). Globally, the frequency of invasive ants is increasing due to various factors including urbanization (Buczkowski and Richmond 2012), trade (Bertelsmeier et al. 2017), and climate change (Bertelsmeier et al. 2015). Numerous species such as Argentine ants (*Linepithema humile*), red imported fire ants (*Solenopsis invicta*), and big-headed ants (*Pheidole megacephala*) thrive in urban environments, damage agricultural crops, and cause ecological damage by displacing native organisms (Holway et al. 2002). The impact of pest ants is most frequently seen in urban areas where many ants have attained major economic pest status and are serious household, structural, and nuisance pests. For example, 41 species of ants are considered pests in the United States (Hedges 1998).

Pest ants are often numerous, persistent, and difficult to eradicate (Hoffmann et al. 2016). Perimeter treatments with contact residual insecticides is the most common management strategy to reduce the impact of pest ants in urban areas (Rust et al. 1996, Scharf et al. 2004, Buczkowski et al. 2005, Silverman and Brightwell 2008, Rust and Su 2012). Residual spray applications, also known as barrier treatments, are the core of most ant control programs because they are cost-effective and can be applied quickly over large areas using spray equipment. Spray applications typically provide fast knockdown of foraging ants, prevent them from establishing foraging trails across treated surfaces, and provide efficacy for approximately 4-8 weeks (Rust and Knight 1990,

Scharf et al. 2004). Additionally, the efficacy of spray insecticides is greatly increased by insecticide movement from one ant to another by horizontal transfer (Soeprono and Rust 2004, Buczkowski 2019). Despite the tremendous economic and ecological impact of pest ants, effective management still faces many challenges and control failures with liquid spray insecticides in urban (Rust et al. 1996) and natural areas (Silverman and Brightwell 2008, Hoffmann et al. 2016) are well documented. The prevailing treatment strategies and product label rate determinations are not entirely compatible with the biology of many species (Silverman and Brightwell 2008, Buczkowski 2021) for several reasons; 1) cryptic nesting behaviors prohibit the direct treatment of colonies (Rust and Su 2012), 2) colonies are typically very numerous which limits treatment performance and confounds monitoring effects (Hoffmann et al. 2011), 3) large-scale treatments in natural areas with broad-spectrum insecticides are problematic because of non-target effects (Buczkowski and Wossler 2019), and 4) there is large variation in susceptibility to insecticides across species (Buczkowski 2021).

The odorous house ant *(Tapinoma sessile)* is considered one of the most adaptive and widespread ant species found in North America (Fisher et al. 2007, Buczkowski 2010, Menke et al. 2010, Salyer et al. 2014). It has been recognized as an urban pest for nearly 100 years (Smith 1928, Thompson 1990). In natural environments, colonies are small and consist of a few hundred workers and one or a few queens (Buczkowski 2010, Menke et al. 2010). However, in urban environments, colonies tend to act as an invasive species and consist of millions of workers and thousands of queens spread across multiple nesting sites (Buczkowski 2010, Menke et al. 2010). Recently, a non-native, supercolonial population of *T. sessile* was discovered in Hawaii demonstrating its potential global threat (Buczkowski and Krushelnycky 2010). Among ants, *T. sessile* are infamous for being very hard to control (Paysen 2019, Buczkowski 2021). *Tapinoma*

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sessile consistently ranks as the #1 "call-back" ant among pest management professionals (Robbins 2015). A call-back occurs when the applicator is called back because the initial treatment failed necessitating re-application. In a national survey, PMPs reported that *T. sessile* was the most important ant driving sales and the most difficult ant to control (Paysen 2019). Additionally, a recent study evaluated insecticide susceptibility in 12 species of urban pest ants and *T. sessile* were consistently the least susceptible to all insecticides (Buczkowski 2021). Comparatively low susceptibility may be part of the reason why *T. sessile* is so persistent and so difficult to control.

The goals for the current study were tri-fold. The first objective was to examine regional variation in *T. sessile* insecticide susceptibility to three classes of insecticides commonly used in urban ant control. The hypothesis was that *T. sessile* colonies collected in different areas would show significant variation in insecticide susceptibility. The second objective was to compare insecticide susceptibility in *T. sessile* colonies collected in natural vs. urban areas. The hypothesis was that colonies from natural areas would be less tolerant because they are unlikely to have ever been exposed to insecticides whereas colonies from urban areas would be more tolerant because they are frequently exposed to insecticides. The final objective was to determine if insecticide susceptibility varies in workers vs. queens. The hypothesis was that queens would be more tolerant due to their significantly higher body mass. The ultimate goal of this study is to provide a foundation for the development of more effective and efficient control methods for *T. sessile*.

2.3   Materials and Methods

2.3.1   Ant collections

A total of 30 *T. sessile* colonies were collected within a 50 mile radius of Purdue University campus, Tippecanoe County, Indiana (40°25'26.40"N, -86°55'44.40"W). To compare insecticide

susceptibility in natural vs. urban colonies, 15 colonies were collected in natural areas and 15 in urban areas (Buczkowski 2010). Natural areas were large tracts of mixed hardwood forest that contained mature trees and were free of any anthropogenic influence or disturbance. Urban areas were various residential and commercial areas throughout Tippecanoe County. Most urban areas were known to receive insecticides applications with various frequencies ranging from quarterly to annually (Buczkowski 2010, Buczkowski and Richmond 2012). All colonies were brought to the lab and placed in plastic trays coated with Fluon to prevent escapes. The colonies were provided with drinking water and artificial nests consisting of Petri dishes filled with moist plaster. The colonies were maintained on a 20% sucrose solution and artificial diet consisting of essential vitamins, sugars, and proteins (Bhatkar and Whitcomb 1970).

2.3.2   Residual exposure assays

Insecticide susceptibility of all 30 colonies was tested using residual exposure assays. The insecticide chemistries tested were: (1) phenylpyrazole (Termidor SC, BASF Corporation, Research Triangle Park, NC, 9.1% fipronil), (2), pyrethroid (Demand CS, Syngenta Crop Protection Incorporated, Greensboro, NC, 9.7% lambda-cyhalothrin), and (3) neonicotinoid (Alpine WSG, BASF Corporation, Research Triangle Park, NC, 40% dinotefuran). The concentrates were diluted in water according to the label-recommended rates: 0.06% for fipronil, 0.015% for lambda-cyhalothrin, and 0.1% for dinotefuran. For each experimental replicate, 0.42 ml of the dilution was pipetted onto a 10 X 10 cm glazed ceramic tile. Control tiles were left untreated. All tiles were allowed to dry overnight. The environmental conditions in the laboratory were: 28 ± 2ºC, 45 ± 10% relative humidity, and 14:10 L:D cycle. To test the hypothesis that queens are more tolerant to insecticides relative to workers due to their significantly higher body mass, insecticide susceptibility in urban colonies was evaluated in workers (6 replicates of 5 ants

each) and queens (6 replicates of 1 ant each). Control tests for urban colonies consisted of 2 replicates of 5 ants each for workers and 2 replicates of 1 ant each for queens. In contrast to urban colonies which are highly polygyne, natural colonies are typically monogyne (Buczkowski 2010). Therefore, insecticide susceptibility in natural colonies was tested on workers only. For each replicate, the ants were randomly selected from a stock colony using a toothpick and placed in a small holding cup. The inside of the cup was coated with Fluon™ to prevent escapes. When all ants needed for the trial were collected, they were gently tapped out of the cup and inside a plastic ring (9 cm diameter) placed on top of the tile. The inner wall of the ring was coated with Fluon™ to restrict the ants to the treated surface and to prevent escapes. The ants were continuously exposed to the treated tiles. Mortality assessments consisted of the number of ants that were either alive (moving) or dead (no movement when probed). Mortality counts were recorded at different time points depending on the insecticide, with no fewer than 9 time points per insecticide.

2.3.3   Data analysis

The median lethal time (LT_{50}) value was calculated for each replicate colony by using the probit analysis in R (2013). A goodness-of-fit test was performed for each probit model. ANOVA analysis (PROC GLM) was performed in SAS 9.4 (SAS 2008). Analysis of variance (ANOVA, PROC GLM) was performed in SAS 9.4 for each insecticide to examine the distribution of LT_{50} values across the different colonies. The ANOVA test was followed by post-hoc Tukey's HSD tests to separate the means. Pairwise comparisons between workers collected in different habitats (natural vs. urban) and between castes (workers vs. queens) were performed using t-tests in Statistica (2017).

2.4 Results

All three insecticides resulted in 100% mortality of all colonies. However, *T. sessile* showed significant regional variation in insecticide susceptibility (Table 1). Workers collected in natural areas showed significant colony variation when exposed to residues of lambda-cyhalothrin (mean LT_{50} value = 37.2 ± 25.8 minutes, range: 17.7 – 92.1 minutes; ANOVA: F = 16.2, df = 14, P<0.0001) and fipronil (mean LT_{50} value = 259.5 ± 47.0 minutes, range: 143.9 – 318.5 minutes; ANOVA: F = 10.3, df = 14, P<0.0001), but not dinotefuran (mean LT_{50} value = 18.3 ± 6.0 minutes, range: 10.1 – 35.7 minutes; ANOVA: F = 1.4, df = 14, P=0.20). Workers collected in urban areas showed significant colony variation when exposed to all insecticides: lambda-cyhalothrin (mean LT_{50} value = 32.5 ± 8.8 minutes, range: 21.3 – 45.9 minutes; ANOVA: F = 7.3, df = 14, P<0.0001), fipronil (mean LT_{50} value = 217.1 ± 78.5 minutes, range: 106.8 – 333.0 minutes; ANOVA: F = 75.8, df = 14, P<0.0001), and dinotefuran (mean LT_{50} value = 65.9 ± 6.1 minutes, range: 56.5 – 74.5 minutes; ANOVA: F = 2.1, df = 14, P=0.03). Significant colony variation to all insecticides was also observed in queens collected in urban areas: lambda-cyhalothrin (mean LT_{50} value = 78.3 ± 33.0 minutes, range: 31.2 – 138.3 minutes; ANOVA: F = 6.3, df = 12, P<0.0001), fipronil (mean LT_{50} value = 335.9 ± 51.5 minutes, range: 259.1 – 448.6 minutes; ANOVA: F = 37.0, df = 12, P<0.0001), and dinotefuran (mean LT_{50} value = 92.6 ± 33.4 minutes, range: 44.7 – 175.3 minutes; ANOVA: F = 20.3, df = 12, P<0.0001).

Caste had a significant effect on insecticide susceptibility (Figure 1). Workers collected in urban habitats were significantly less tolerant to all insecticides relative to queens collected in urban habitats: lambda-cyhalothrin (mean LT_{50} value for workers = 32.5 minutes vs. 78.3 minutes for queens; t-test: t = -4.4, df = 24, P<0.0001), fipronil (mean LT_{50} value for workers = 259.5 minutes vs. 335.9 minutes for queens; t-test: t = -4.9, df = 24, P<0.0001), and dinotefuran (mean LT_{50} value for workers = 18.3 minutes vs. 92.6 minutes for queens; t-test: t = 2.9, df = 24,

P=0.0008). Habitat type had a significant effect on insecticide susceptibility and workers collected in natural areas were significantly less tolerant relative to workers collected in urban areas when exposed to dinotefuran (mean LT_{50} value for natural workers = 18.3 minutes vs. 65.9 minutes for urban workers; t-test: t = -0.2, df = 28, P<0.0001), but not lambda-cyhalothrin (mean LT_{50} value for natural workers = 37.2 minutes vs. 32.5 minutes for urban workers; t-test: t = 0.7, df = 28, P=0.52) or fipronil (mean LT_{50} value for natural workers = 259.5 minutes vs. 217.1 minutes for urban workers; t-test: t = 1.8, df = 28, P=0.08).

2.5   Discussion

Tapinoma sessile is one of the most widespread pest ants in North America and ranks as the number one household pest species (Paysen, 2019). Field management of *T. sessile* is achieved mainly by using residual spray insecticides applied as perimeter treatments around structures. However, control failures are common and driven by multiple interacting factors. Results of the current study demonstrate significant intraspecific and intercaste variation in insecticide susceptibility within *T. sessile*. Significant variation is insecticide susceptibility was found on a relatively small geographic scale, i.e. colonies collected within a 50 mile radius. It is likely that variation in insecticide susceptibility is even greater across larger geographic scales. Ant management mostly relies on the application of insecticides and understanding the sensitivity of different ant colonies or populations will help in making decisions about appropriate chemical control.

Tapinoma sessile is abundant in a wide range of habitats and has a highly flexible social structure depending on habitat type (Buczkowski 2010, Menke et al. 2010). In natural habitats, colonies are small and typically comprised of a single queen, a single nest, and a few dozen workers. In urban areas, colonies exhibit extreme polygyny and polydomy, and form large

supercolonies containing millions of workers and thousands of queens. The current study tested the hypothesis that colonies from natural areas would be less tolerant to insecticides because they are unlikely to have ever been exposed to insecticides whereas colonies from urban areas would be more tolerant because they are frequently exposed to insecticides. However, no support was found for this hypothesis. Urban and natural colonies showed no significant differences in tests with lambda-cyhalothrin and fipronil. In tests with dinotefuran, urban colonies were significantly more tolerant relative to natural colonies, with mean LT_{50} values of 18 ± 6 and 66 ± 6 minutes, respectively. These results suggest that habitat type and previous exposure to insecticides do not reliably predict susceptibility levels in individual populations. Rather, the results are insecticide and colony specific and may depend on various genetic, biological, and other factors. Investigating these possible factors, or others, could be a worthwhile focus for future research efforts.

The insecticides evaluated in the current study are used widely in the management of various urban pests including ants, bed bugs, cockroaches, and termites. In non-social insects such as bed bugs and cockroaches, the widespread use of chemical control measures has resulted in insecticide resistance. Indeed, high levels of resistance to lambda-cyhalothrin, dinotefuran, and fipronil have been documented in cockroaches (Gonzales-Morales et al. 2021) and bed bugs (Romero et al. 2007, Romero and Anderson 2016). Intraspecific variation in insecticide susceptibility is commonly observed in cockroaches (Fardisi et al. 2017, Fardisi et al. 2019) and bed bugs (Romero and Anderson 2016, Ashbrook et al. 2017, Gonzales-Morales et al. 2021) and is typically related to strain differences where field-collected strains are resistant and laboratory-reared strains are susceptible. Insecticide resistance in bed bugs and cockroaches often reaches extremely high levels and leads to control failures (Romero et al. 2007, Fardisi et al. 2019). In contrast to non-social insects, insecticide resistance has never been documented in social insects.

This is despite widespread, persistent, and repeated use of insecticides for the control of ants, termites, and other social insects such as wasps (Jiang et al. 2014). It is thought that the unique reproductive biology of social insects (i.e. the caste system), is the main factor responsible for the lack of resistance. Individuals exposed to insecticide treatments are typically workers, which are sterile, and therefore do not pass resistance genes to the next generation. However, due to the possibility of horizontal transfer of insecticides to adult queens as well as reproductive brood, it is highly plausible that insecticide resistance may act on the queens and lead to heritable changes in sensitivity of colonies. Intraspecific variation observed in the current study was relatively low.

The hypothesis of proportionality, which states that insecticides affect insects proportionally with their body weight (Robertson and Preisler 1992), was supported in this study. Queens were significantly more tolerant relative to their worker counterparts across all three insecticides. Relative to workers, queens were 2.4 times more tolerant to lambda-cyhalothrin, 5.1 times more tolerant to dinotefuran, and 1.3 times more tolerant to fipronil. Greater insecticide tolerance in the queens may explain why *T. sessile* is such a persistent pest in urban environments. Liquid spray insecticides applied as perimeter treatments around structures create a barrier that targets mostly foraging workers that trail across the treated areas. Unlike workers, queens stay in the nest and are not directly exposed. Some mortality in the queens may occur indirectly through horizontal transfer when workers exposed to insecticide treatments return to the nest and share the insecticide with other members of the colony (Soeprono and Rust 2004, Buczkowski 2019, Buczkowski and Wossler 2019). However, the amount of insecticide transferred may not be high enough to cause mortality. Additionally, urban populations of *T. sessile* are highly polygynous and contain numerous queens (Buczkowski 2010, Menke et al. 2010). Lack of direct exposure

combined with reduced susceptibility and extreme polygyny may allow the queens to survive insecticide treatments and lead to quick regeneration of worker ants.

Tapinoma sessile is unique among pest ants for a number of reasons. It has the widest geographic range and greatest ecological tolerance of any ant in North America (Fisher and Cover 2007). It is highly opportunistic and inhabits a variety of nesting sites in natural and urban areas. In urban areas, *T. sessile* exhibits supercolony behaviors and becomes a dominant invasive pest (Buczkowski and Bennett 2006; Buczkowski and Bennett 2008). It is capable of long-distance jump-dispersal and becoming established in areas previously invaded by other invasive ants (Buczkowski and Krushelnycky 2012). Additionally, *T. sessile* has low susceptibility to insecticides relative to other pest ants. Susceptibility to 3 classes of insecticides was evaluated in 12 species of the most common urban pest ants and *T. sessile* was consistently the least susceptible to all insecticides (Buczkowski 2021). The ability of *T. sessile* to invade new habitats, form large supercolonies, display elevated insecticide tolerance relative to other ants, display elevated insecticide tolerance in queens relative to workers, and display significant variation in insecticide susceptibility across colonies make *T. sessile* a strong contender in global invasions. Future studies should monitor both the pest and invasive status of the species.

2.6   Tables and Figures

Table 1. Mean LT_{50} values (in minutes) and 95% confidence intervals for workers and queens of 15 colonies of odorous house ants collected in natural and urban habitats. LT_{50} means followed by the same letter are not significantly different based on Tukey's HSD test ($P \leq 0.05$).

Table 1. Mean LT_{50} values (in minutes) and 95% confidence intervals for workers and queens of 15 colonies of odorous house ants collected in natural and urban habitats.

| insecticide	colony	Workers (natural)	Workers (urban)	Queens (urban)
λ-cyhalothrin	1	17.7 (16.3 – 19.0) e	39.1 (35.3 – 43.1) ba	not tested
λ-cyhalothrin	2	23.5 (19.5 – 27.5) edc	45.9 (41.3 – 50.9) a	99.4 (76.8 – 125.4) b
λ-cyhalothrin	3	19.5 (14.4 – 24.5) e	25.2 (23.4 – 27.0) bc	62.8 (41.2 – 58.3) c
λ-cyhalothrin	4	20.2 (14.0 – 26.3) ed	44.2 (37.1 – 52.2) a	80.4 (65.0 – 95.0) b
λ-cyhalothrin	5	42.7 (38.8 – 47.2) bdc	23.0 (14.4 – 31.3) bc	not tested
λ-cyhalothrin	6	27.3 (22.9 – 31.8) edc	30.2 (27.3 – 33.2) bac	39.7 (28.9 – 50.0) d
λ-cyhalothrin	7	18.3 (15.1 – 21.3) e	31.7 (26.4 – 37.3) bac	50.1 (39.8 – 59.8) c
λ-cyhalothrin	8	44.3 (39.5 – 49.4) bac	45.9 (41.6 – 50.6) a	123.7 (107.7 – 139.7) ab
λ-cyhalothrin	9	23.5 (18.9 – 28.2) edc	36.1 (32.8 – 39.6) bac	78.4 (59.6 – 97.3) b
λ-cyhalothrin	10	21.9 (19.3 – 24.4) edc	32.5 (29.93 – 35.2) bac	64.5 (42.3 – 85.1) c
λ-cyhalothrin	11	26.5 (22.5 – 30.6) edc	21.5 (19.8 – 23.2) c	110.3 (90.6 –130.9) ab
λ-cyhalothrin	12	92.1 (84.9 – 100.1) a	38.4 (35.9 – 41.1) ba	138.2 (118.5 – 160.5) ab
λ-cyhalothrin	13	91.0 (78.2 – 108.4) a	23.4 (20.9 – 25.9) bc	49.3 (38.4 – 59.6) c
λ-cyhalothrin	14	21.0 (18.8 – 23.2) edc	21.3 (20.0 – 23.0) c	31.2 (24.1 – 39.3) d
λ-cyhalothrin	15	68.0 (59.7 – 78.1) ba	29.3 (26.7 – 31.9) bac	90.0 (76.3 – 103.1) b
dinotefuran	1	22.0 (19.1 – 24.7) a	65.2 (30.6 – 70.1) a	not tested
dinotefuran	2	17.9 (0.4 – 31.9) a	66.8 (62.5 – 71.2) a	87.2 (73.3 – 101.4) bc
dinotefuran	3	14.9 (9.4 – 19.6) a	74.5 (66.8 – 83.2) a	61.6 (52.4 – 71.8) d
dinotefuran	4	10.1 (8.4 – 11.7) a	61.3 (57.6 – 65.3) a	75.2 (59.6 – 93.8) c
dinotefuran	5	20.11 (17.4 – 22.7) a	74.3 (70.1 – 78.6) a	not tested
dinotefuran	6	12.01 (8.1 – 15.5) a	56.5 (53.1 – 60.2) a	71.3 (59.6 – 83.7) d
dinotefuran	7	21.5 (19.0 – 23.8) a	74.3 (69.3 – 79.5) a	44.7 (37.0 – 53.4) e
dinotefuran	8	18.9 (16.3 – 21.4) a	72.8 (67.5 – 78.4) a	81.9 (69.3 – 94.5) bc
dinotefuran	9	15.6 (13.1 – 18.1) a	70.7 (63.4 –78.8) a	127.3 (109.4 – 147.1) b
dinotefuran	10	15.9 (13.3 – 18.5) a	58.7 (54.9 – 62.7) a	72.5 (54.1 – 93.2) d
dinotefuran	11	21.0 (18.3 – 23.6) a	64.92 (60.5 – 69.6) a	175.3 (146.7 – 236.) a
dinotefuran	12	35.7 (32.5 – 39.0) a	60.1 (56.2 – 64.3) a	116.6 (101.5 – 131.1) b
dinotefuran	13	20.4 (14.6 – 26.1) a	60.9 (53.4 – 69.3) a	106.9 (91.4 – 112.2) b
dinotefuran	14	14.0 (11.5 – 16.4) a	62.7 (58.9 – 66.7) a	91.4 (79.5 – 102.2) bc
dinotefuran	15	14.2 (11.8 – 16.6) a	65.3 (60.74 – 70.2) a	92.1 (79.0 – 104.5) bc
fipronil	1	235.6 (223.8 – 247.5) ba	262.5 (253.1 – 272.2) bdc	not tested
fipronil	2	305.3 (261.9 – 376.9) a	275.3 (265.9 – 284.9) bdac	318.4 (285.6 – 351.8) c
fipronil	3	285.1 (274.0 – 296.6) ba	333.0 (323.1 – 342.8) a	378.4 (347.0 – 408.2) b
fipronil	4	279.5 (268.3 – 291.1) ba	244.5 (223.9 – 265.5) dc	362.5 (326.5 – 401.0) b
fipronil	5	143.9 (135.1 – 152.3) c	303.5 (291.9 – 315.9) bac	not tested
fipronil	6	274.8 (263.9 – 286.0) ba	235.9 (224.4 – 247.4) d	318.0 (281.8 – 356.4) c
fipronil	7	314.0 (303.8 – 324.4) a	320.1 (310.0 – 330.2) ba	259.1 (233.8 – 285.2) d
fipronil	8	268.6 (249.2 – 289.4) ba	288.1 (276.1 – 300.8) bdac	382.3 (354.1 – 408.6) b
fipronil	9	238.0 (225.7 – 250.7) ba	167.9 (158.9 – 176.6) fe	372.1 (340.3 – 402.4) b
fipronil	10	300.3 (288.7 – 312.7) a	177.9 (169.7 – 185.8) e	312.5 (278.3 – 343.8) c
fipronil	11	318.5 (295.7 – 348.3) a	139.4 (131.6 – 146.7) fg	332.4 (300.3 – 364.6) bc
fipronil	12	208.0 (183.3 – 231.9) b	140.5 (131.9 – 148.6) fg	277.3 (249.9 – 305.3) d
fipronil	13	259.4 (246.8 – 272.5) ba	106.8 (99.0 – 114.1) h	448.6 (412.8 – 505.9) a
fipronil	14	212.9 (201.5 – 224.0) b	126.0 (117.4 – 134.1) gh	315.6 (284.9 – 346.4) c
fipronil	15	249.3 (237.8 – 260.9) ba	134.4 (126.5 – 141.8) fgh	289.3 (258.7 – 327.4) cd
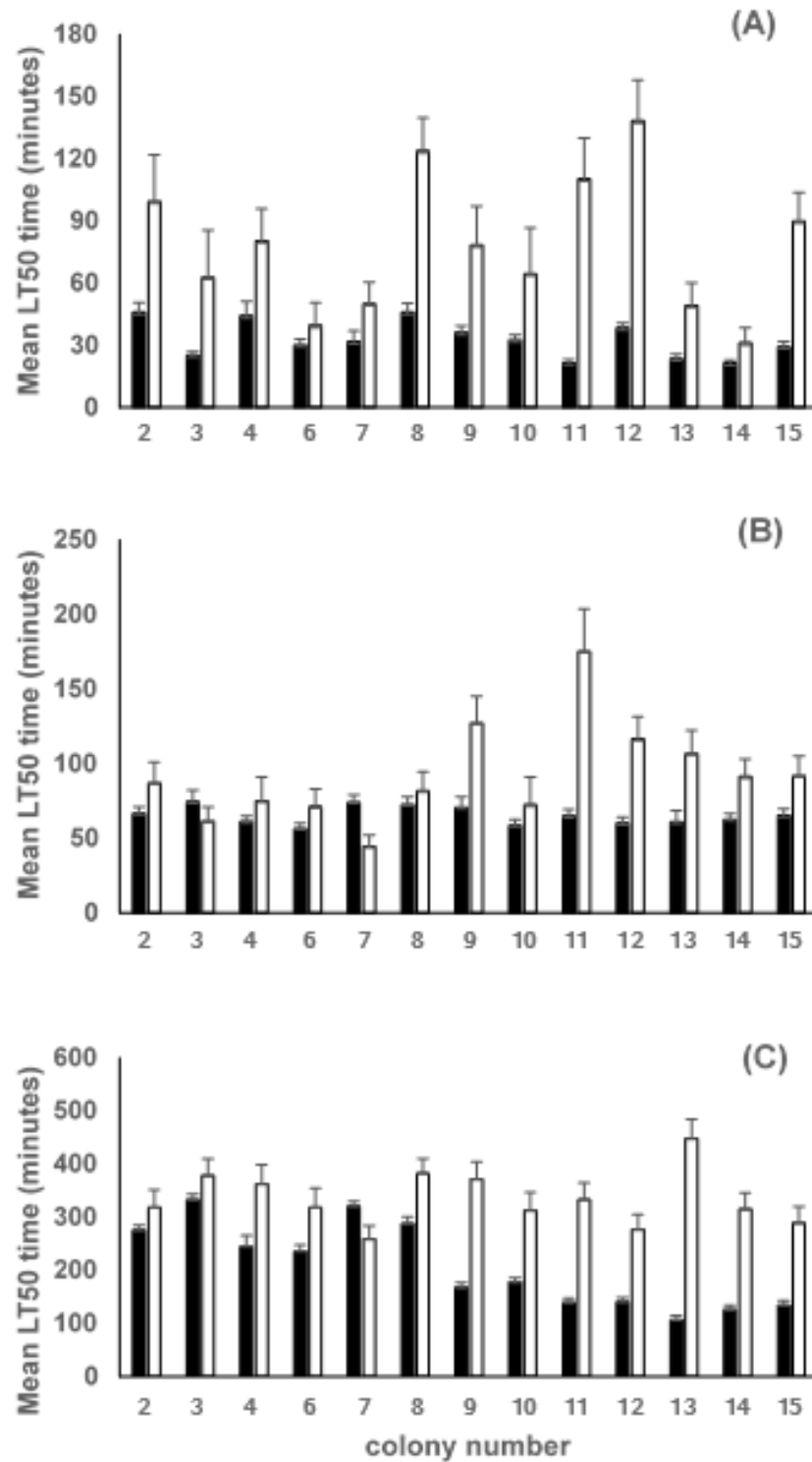
Figure 1. Susceptibility of *T. sessile* workers (black bars) and queens (white bars) collected in urban areas to residual applications of (A) lambda-cyhalothrin, (B) dinotefuran, and (C) fipronil. Bars indicate mean LT_{50} values in minutes and each bar represents a different colony.

2.7 References

Ashbrook A.R., M. E. Scharf, G. W. Bennett, and A. D. Gondhalekar. 2017. Detection of reduced susceptibility to chlorfenapyr- and bifenthrin-containing products in field populations of the bed bug (Hemiptera: Cimicidae). Journal of Economic Entomology, 110: 1195–1202.

Bhatkar A. and W. Whitcomb. 1970. Artificial diet for rearing various species of ants. Florida Entomologist 53: 229–232.

Bertelsmeier C., S. Ollier, A. Liebhold, and L. Keller. 2017 Recent human history governs global ant invasion dynamics. Nat Ecol Evol 1, 0184. https://doi.org/10.1038/s41559-017-0184.

Bertelsmeier C., G. Luque, B. Hoffmann, and F. Courchamp. 2015. Worldwide ant invasions under climate change. Biodiversity Conservation 24: 117–128.

Buczkowski G., M. E. Scharf, C. Ratliff, and G. Bennett. 2005. Efficacy of simulated barrier treatments against laboratory colonies of pharaoh ant. Journal of Economic Entomology, 98: 485–492.

Buczkowski G. 2010. Extreme life history plasticity and the evolution of invasive characteristics in a native ant. Biological Invasions, 12: 3343–3349.

Buczkowski G. and G. Bennett. 2006. Dispered central-place foraging in the polydomous odorous house ant, *Tapinoma sessile* as revealed by a protein marker. Insectes Sociaux, 53: 282–290.

Buczkowski G. and G. Bennett. 2008. Seasonal polydomy and unicoloniality in a polygynous population of the odorous house ant, *Tapinoma sessile*. Ecological Entomology, 33: 780–788.

Buczkowski G. and P. Krushelnycky. 2011. The odorous house ant, *Tapinoma sessile* (Hymenoptera: Formicidae), as a new temperate-origin invader. Myrmecological News, 16: 61–66.

Buczkowski G. 2019. Trap-treat-release: horizontal transfer of fipronil in field colonies of black carpenter ants, *Camponotus pennsylvanicus*. Pest Management Science, 75: 2195–2201.

Buczkowski G. and T. Wossler. 2019. Controlling invasive Argentine ants, *Linepithema humile*, in conservation areas using horizontal insecticide transfer. *Sci Rep* 9, 19495. https://doi.org/10.1038/s41598-019-56189-1

Buczkowski G. 2021. A comparison of insecticide susceptibility levels in twelve species of urban pest ants with special focus on the odorous house ant, *Tapinoma sessile*. Pest Management Science, 77(6): 2948-2954. https://doi.org/10.1002/ps.6331

Buczkowski G. and D. Richmond. 2012. The effect of urbanization on ant abundance and diversity: A temporal examination of factors affecting biodiversity. PLoS ONE 7(8): e41729.

Fardisi M., A. D. Gondhalekar, and M. E. Scharf. 2017. Development of diagnostic insecticide concentrations and assessment of insecticide susceptibility in German cockroach (Dictyopetra: Blattellidae) field strains collected from public housing. Journal of Economic Entomology, 110: 1210–1217.

Fardisi M., A. D. Gondhalekar, A. R. Ashbrook, and M. E. Scharf. 2019. Rapid evolutionary responses to insecticide resistance management interventions by the German cockroach (*Blattella germanica* L.). Scientific Reports, **9**, https://doi.org/10.1038/s41598-019-44296-y

Fisher B. and S. Cover. 2007. Taxonomic descriptions. Ants of North America: a guide to the genera. University of California Press, 145–146.

Folgarait P. 1998. Ant diversity and its relationship to ecosystem functioning: a review. Biodiversity and Conservation, 7: 1221–1244.

Gonzalez-Morales M. A., Z. DeVries, A. Sierras, R. G. Santangelo, M. L. Kakumanu, and C. Schal. 2021. Resistance to fipronil in the common bed bug (Hemiptera: Cimicidae). Journal of Medical Entomology, tjab040, https://doi.org/10.1093/jme/tjab040.

Hedges S. A. 1998. Field guide for the management of structure-infesting ants. 2nd ed. Cleveland, Ohio: Franzak and Foster.

Holway D., L. Lach, A. Suarez, N. Tsutsui, and T. Case. 2002. The causes and consequences of ant invasions. Annual Review of Ecology and Systematics, 33: 181–233.

Hoffmann B., P. Davis, K. Gott, C. Jennings, S. Joe, P. Krushelnycky, R. Miller, G. Webb, and M. Widmer. 2011. Improving ant eradications: details of more successes, a global synthesis and recommendations. Aliens 31: 16–23.

Hoffmann B., G. Luque, C. Bellard, N. Holmes, and C. Donlan. 2016 Improving invasive ant eradication as a conservation tool: a review. Biological Conservation 198: 37–49.

Jiang W., Soeprono A., Rust M.K., and J. Gan. 2014. Ant control efficacy of pyrethroids and fipronil on outdoor concrete surfaces. Pest Management Science, 70: 271–277.

McGlynn T. 1999. The worldwide transfer of ants: geographical distribution and ecological invasions. Journal of Biogeography, 26: 535–548.

Menke S., W. Booth, R. Dunn, C. Schal, E. Vargo, and J. Silverman. 2010. Is it easy to be urban? Convergent success in urban habitats among lineages of a widespread native ant. PLoS One 5: 2: e9194.

Paysen, E. 2019. 2019 state of the ant control market. Pest Control Technology Magazine, 47: 1–7.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Robertson J. and H. Preisler. 1992. Pesticide bioassays with arthropods. CRC Press, Boca Raton, FL.

Robbins S. 2015. Annual Ant Control Issue, Odorous House Ants Simplified. Pest Control Technology Magazine 46.

Romero A., M. F. Potter, D. A. Potter, and K. F. Haynes. 2007. Insecticide resistance in the bed bug: a factor in the pest's sudden resurgence? Journal of Medical Entomology, 44: 175–178.

Romero A., and T. D. Anderson. 2016. High levels of resistance in the common bed bug, *Cimex lectularius* (Hemiptera: Cimicidae), to neonicotinoid insecticides. Journal of Medical Entomology, 53: 727–731.

Rust M.K., K. Haagsma, and D. Reierson. 1996. Barrier sprays to control Argentine ants (Hymenoptera: Formicidae). Journal of Economic Entomology, 89: 134–137.

Rust M. K. and Knight R.L. 1990. Controlling Argentine ants in urban situations, pp. 664–670. *In* R. K. Vander Meer, K. Jaffe, and A. Cedeno [eds.], Applied myrmecology: a world perspective. Westview, Boulder, CO.

Rust M. K., and N-Y. Su. 2012. Managing social insects of urban importance. Annual Review of Entomology, 57: 355–375.

Salyer A., G. Bennett, and G. Buczkowski. 2014. Odorous house ants (*Tapinoma sessile*) as back-seat drivers of localized ant decline in urban habitats. PLoS ONE 12: 9: e113878.

SAS Institute. 2008. SAS/STAT guide for personal computers, version 9.2. SAS Institute, Cary, NC.

Scharf M., C. Ratliff, and G. Bennett. 2004. Impacts of residual insecticide barriers on perimeter-invading ants, with particular reference to the odorous house ant, *Tapinoma sessile*. Journal of Economic Entomology, 97: 601–605.

Silverman J. and R. Brightwell. 2008. The Argentine ant: challenges in managing an unicolonial invasive pest. Annual Review of Entomology 53:231–252.

Smith M. R. 1928. The biology of *Tapinoma sessile* Say, an important house-infesting ant. Annals of the Entomological Society of America, 21:307–330.

Soeprono A. and M. K. Rust. 2004. Effect of horizontal transfer of barrier insecticides to control Argentine ants (Hymenoptera: Formicidae). Journal of Economic Entomology, 97: 1675–1681.

Statistica. 2017. StatSoft, Inc. Tulsa, OK, Version 13.2.

Suarez A., T. McGlynn, and N. Tsutsui. 2010. Biogeographic patterns of the origins and spread of introduced ants. In *Ant Ecology*. Lach, L., Parr, K., and Abbot, K. eds. Chapter 13. Pages 233–244. Oxford University Press.

Thompson R. 1990. Ants that have pest status in the United States, ed. by Vander-Meer, R., Jaffe, K., Cedeno, A. Applied myrmecology: a world perspective. Westview Press, Boulder, CO, pp. 51–67.