

Final Report: Leveraging the chemistry of colony organization for the management of a widespread and expanding urban pest ant

Adrian A. Smith^{*1}, Lawrence M. Hanks¹, and Andrew V. Suarez¹

¹ Department of Entomology, University of Illinois at Urbana-Champaign, Urbana, IL, 61821

*Email: smithaa@illinois.edu Phone: (217) 898 3573

PROJECT SUMMARY

The odorous house ant, *Tapinoma sessile*, is the most common ant in the United States and has been recognized as an urban pest species for more than 100 years. In urban and introduced environments the ant shares many colony characteristics with the global invasive ant species the Argentine ant, *Linepithema humile*, such as colonies that spread across multiple nesting sites, containing thousands of queens and potentially millions of workers. Recently, the invasive-like characteristics of *T. sessile* are beginning to draw attention, with the first report of an established population in a non-native range has come from Hawaii. A new management method being developed for Argentine ants involves utilizing species-specific chemical pheromones to disrupt and manipulate their foraging behavior. Combined with more traditional insecticidal control efforts, the use of pheromones in managing pest ants is a promising and more efficient integrated pest management strategy.

The aim of this research was to provide the foundation for development of pheromone-based management strategies for *T. sessile*. To date, practically no information is available on the pheromones used by this species. We identified the glandular source of the trail and alarm pheromone to be the pygidial gland. However, experiments using pygidial gland extracts did not always induce trail-following behavior. Collectively, our results advance our knowledge of the behavior and chemical ecology of this pest species.

RESULTS SUMMARY

1 - The glandular source of the trail and alarm pheromones of *T. sessile* is likely the same abdominal gland, the pygidial gland.

2 – Deposition of a trail pheromone is not always evident and experimentally inducing trail-following behavior using pygidial gland contents is only somewhat successful.

BACKGROUND

The pest problem

The odorous house ant, *Tapinoma sessile*, has a native geographic range that encompasses most of the United States, making it the most common ant in North America (Fisher and Cover 2007). With the rise of urbanization, this species has been extremely successful at integrating itself into urban environments and has been recognized for nearly one-hundred years as a nation-wide household pest (Smith 1928; Thompson 1990). Outside of the urban environment, colonies are reported to be small (less than 1,000 workers), with a single or a few queens at a nesting site. However, within the urban environment colonies are dramatically different, consisting of potentially millions of workers and thousands of queens spread across

multiple nesting sites (Buczowski 2010; Menke et al. 2010). In fact, the social structure of urban populations of *T. sessile* is quite similar to those of the notorious invasive Argentine ant, *Linepithema humile* (Buczowski and Bennett 2008). Currently, we are just becoming aware of the potential global threat of *T. sessile*, as the first non-native super-colonial population has been reported on Maui, Hawaii (Buczowski and Krushelnycky 2011).

Perimeter treatments with contact residual insecticides and insecticidal baiting are the most common management strategies used on urban pest ants (Klotz et al. 2007; Silverman and Brightwell 2008). Excessive perimeter treatments with widely used insecticides such as Fipronil are known to cause potentially harmful amounts of runoff (Greenberg et al. 2010). Baits are often ineffective as they are overshadowed by abundant natural food sources such as honeydew, extrafloral nectaries, and prey items (Silverman and Brightwell 2008). Integrated pest management strategies call for a more targeted means of control that leverages the biology of the species and more effectively use pesticides when necessary.

Background: management strategies

A common aspect of the biology of both *T. sessile* and the Argentine ant that make both species difficult to control is their use of multiple nesting sites, or polydomy. They exhibit a seasonal expansion of colony size and nesting sites, and populations grow to dominate urban landscapes (Buczowski and Bennett 2008; Heller and Gordon 2006). Colonies undergo fission, splitting-off groups of queens, workers, and brood to expand into areas with favorable microclimates, often associated with food sources (Holway and Case 2000). In response to disturbance, nests are often abandoned and reformed in new locations. When new nests are formed they are usually relocated along an already established pheromone trail (Buczowski and Bennett 2008; Heller and Gordon 2006). The fluidity of nesting sites and the rapid colony expansion abilities of *T. sessile* are demonstrated in a study by Scharf et al (2004) on the effectiveness of insecticidal household perimeter treatments on ant populations. Before treatment and in untreated areas, the authors did not find *T. sessile* in their samples. However, four weeks after the barrier treatment and a reduction in the pre-treatment ant population, *T. sessile* was found in every sample of every treated area. The authors interpreted this as indicative of the “invasive-like characteristics” of *T. sessile* to rapidly recruit nests to vacant resources.

Using pheromones for insect control is a field of research and development that has a rich history of success and implementation (Witzgall et al. 2010). Using pheromones to disrupt and direct the behavior of pest ants is a control strategy that has, relative to other insect groups, received little attention. However, in recent years, pest management techniques aimed at the Argentine ant are beginning to utilize synthetic trail pheromone as a control agent (Tatsuki et al. 2005). The application of synthetic trail pheromone alone to areas infested by the Argentine ant result in a marked decrease in the foraging activity of the ants and their ability to exploit food resources (Nishisue et al. 2010; Suckling et al. 2010). Similar management tactics have been employed on the red imported fire ant, *Solenopsis invicta* (Suckling et al 2010b). When synthetic trail pheromone application is combined with the use of insecticidal baits, the growth of colonies of Argentine ants can be inhibited and populations can be suppressed (Sunamura et al. 2011). Additionally, both alarm pheromones and trail pheromones have been used, with success in several ant species, as an attractant directly applied onto insecticidal baits (Hughes et al 2002; Vander Meer 1996; Greenberg and Klotz 2000).

The most recent combination studies of trail pheromones with insecticidal baits do not explicitly test for using the trail pheromones to direct colonies to bait in a biologically relevant

way. Instead, the work of Sunamura et al. (2011) tests the effect of combining the use of trail pheromone to disrupt foraging efforts with the presence of insecticidal baits. A potentially more powerful integration of pheromones and insecticides that we would use, would involve using the pheromone to reorganize the foraging efforts of the ants to increase the consumption of baits.

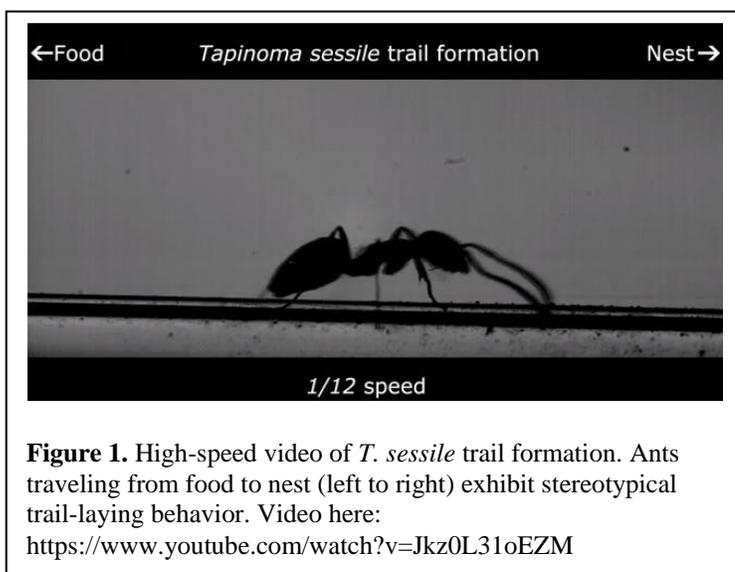
Background: T. sessile

Chemical ecology data on *T. sessile* is almost non-existent. What is known about this species comes from two studies performed over forty years ago. One study suggested that the glandular source of the trail pheromone is the sternal gland, and that two components of the alarm pheromone of the congener *T. nigerrimum* evoke alarm responses (Wilson and Pavan 1959). While the other identified two classes of compounds (iridodials and iridolactones) found in whole body extracts of *T. sessile* workers (Mcgurk et al. 1968). Studies of other *Tapinoma* species indicate that the source of alarm pheromone is the pygidial gland (Hefetz and Lloyd 1983; Tomalski et al. 1987). Only one *Tapinoma* species has had its trail pheromone described. In *T. simrothi* separate compounds in the pygidial gland make up both alarm and trail pheromones (Simon and Hefetz 1991). Recently, another ant species, the Argentine ant *Linepithema humile*, was found to deposit similar pygidial gland compounds (dolichodial and iridomyrmecin) on their trails and the ants follow artificial trails made with these components (Choe et al. 2012). The trail pheromone portion of the gland contents from *T. simrothi* were also shown to be long-lived in the environment, having a biological half-life of up to 19 days; much longer than that of both the Argentine and the fire ant (Simon and Hefetz 1991).

DETAILED RESULTS

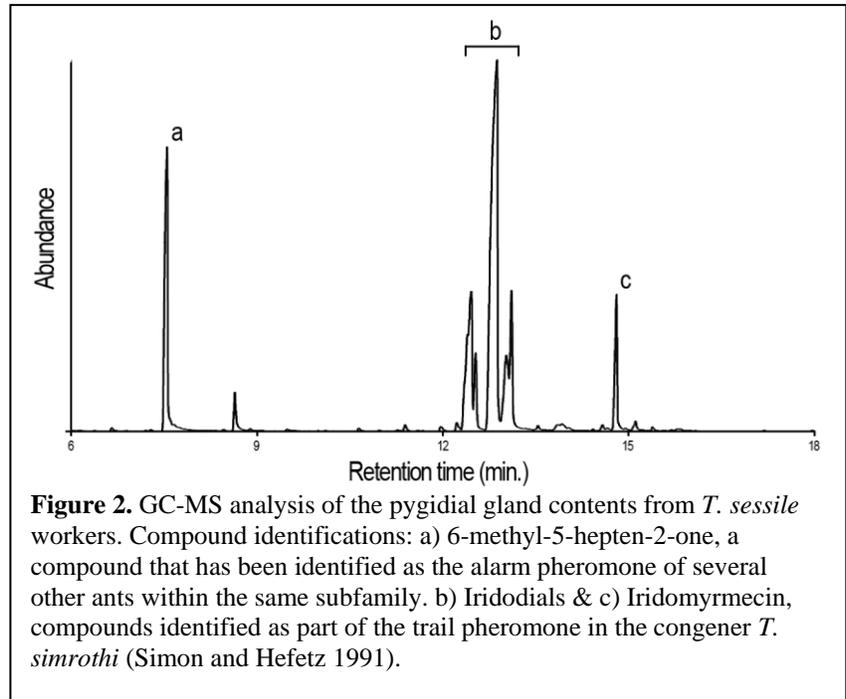
1 - The glandular source of the trail and alarm pheromones of *T. sessile* is likely the same abdominal gland, the pygidial gland.

Dissections of *T. sessile* workers revealed no evidence of a sternal gland. Whole body and abdomen only extractions of *T. sessile* workers yielded no evidence of potential trail-pheromone chemicals not accounted for by what was recovered from pygidial-gland only extractions. This is important because the sternal gland is a known source of trail pheromone for other ants belonging to the same subfamily. These results confirm a histological study on the structure of abdominal glands of another *Tapinoma* species, *T. nigerrimum*. The study showed that the sternal gland is underdeveloped to absent in comparison to other ant genera in the same subfamily (Billen 1986).



High-speed videography of ants returning to the nest from a food source revealed stereotypical chemical trail laying behavior (figure 1). The ants point the tip of their abdomens down towards the substrate, repeatedly dabbing the ground. This behaviour indicates that the trail pheromone is being emitted from the tip of the abdomen. Because ants of this subfamily have their last few abdominal segments very close to each other and all converging on the tip of the abdomen, we are unable to observe the exact anatomical origin of the trail pheromone. The video results do however demonstrate the potential discontinuous nature of the chemical trail as it is deposited.

The pygidial gland of *T. sessile* contains methyl-branched ketone (6-methyl-5-hepten-2-one), iridodials, and iridomyrmecin (Figure 2). 6-methyl-5-hepten-2-one is likely the alarm pheromone for *T. sessile* as it is used for an alarm signal in other species of *Tapinoma* and throughout this subfamily of ants. Iridodials and iridomyrmecin are known to be components of the trail pheromone for the congeneric ant species *T. simrothi*. These compounds have also recently been shown to elicit trail following behavior with the Argentine Ant, *Linepithema humile* (Choe et al. 2012).



2 – Deposition of a trail pheromone is not always evident and experimentally inducing trail-following behavior using pygidial gland contents is only somewhat successful.

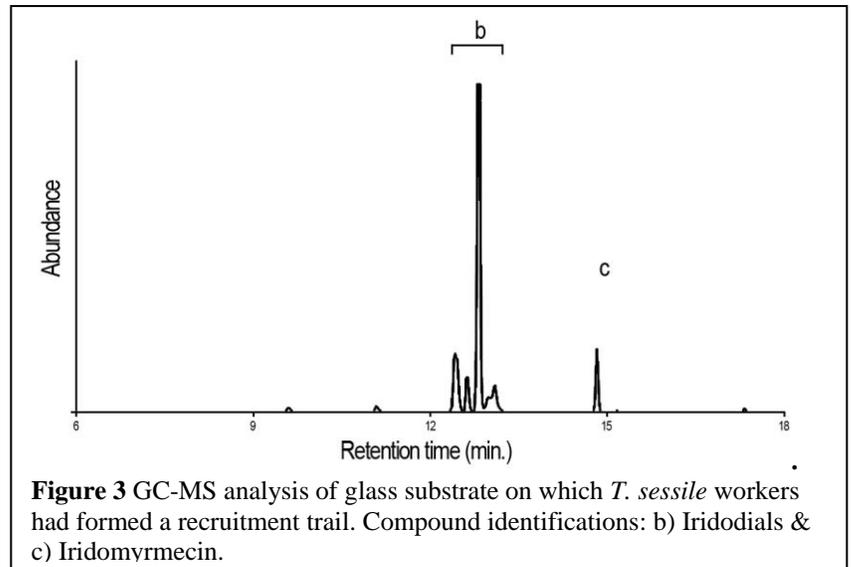
In the lab, colonies of *T. sessile* were starved for 2 days and then presented with a food source elevated above the colony and only accessible via 60cm of chemically clean thin glass tubing. Once the ants found the food, they quickly formed an active trail recruiting nestmates from a foraging arena onto the glass tube and up to the food source. Once a trail had formed the glass tubing was removed and the ants were lighting brushed from the tube. The glass was then immediately washed with hexane solvent to extract any chemical that had been added to the glass. This was also done for a control length of glass that did not lead to the food source and that the ants had not formed a trail on. This method has been used by other researchers to collect and analyse ant pheromone trails (Choe et al. 2012). In 5/18 trials chemicals were found on the recruitment glass that were not recovered from the control (figure 3). In these trails approximately 1 glandular equivalent of iridodials, and iridomyrmecin were recovered after foraging and recruitment had persisted for at least 30 min and after hundreds of returning foragers had passed over. 6-methyl-5-hepten-2-one was the only pygidial gland component not

recovered from the trail; however, it is much more volatile than the other compounds and likely evaporated.

These experiments are largely inconclusive due to the low frequency at which any compounds were recovered from the foraging substrate. This could indicate that the compounds are: 1) airbourne, 2) active and used at a much lower abundance, 3) not capturable with this method, or 4) trail following chemical are not always used by the ants. However, the evidence that was collected

indicates that, if anything, *T. sessile* uses the contents of the pygidial gland for a trail pheromone.

Lastly, pygidial gland contents were extracted from the ants and artificial trails (5) were made by smearing the contents on clean glass tubes and giving the ants a choice of a control (clean) tube and a treated tube. On no occasion did 100% of the ants follow the treated tube. The largest proportion of workers choosing the treated tube was ~70% and the average was 65%. These results are indicative of the pygidial gland having some effect on trail following.



CONCLUSIONS

Although the experimental results of this research were not definitive, this research verified the presence of 6-methyl-5-hepten-2-one as the alarm pheromone in *T. sessile* and provided some experimental evidence that the contents of the pygidial gland are used during foraging trail formation and following.

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