Field Studies of *Wasmannia auropunctata* Alkylpyrazines: Towards Management Applications

by

Elisa J. Troyer[1], Nathan T. Derstine[1], David N. Showalter[1], Eric B. Jang[2] & Matthew S. Siderhurst[1*]

ABSTRACT

Field bioassays with *Wasmannia auropunctata* (Roger) show that the alarm pheromone components 2,5-dimethyl-3-(2-methylbutyl)pyrazine and 3-methyl-2-(2-methylbutyl)pyrazine both attract and arrest ants in a natural environment. Comparisons between lures containing 2,5-dimethyl-3-(2-methylbutyl)pyrazine and 3-methyl-2-(2-methylbutyl)pyrazine singly and in blends (10:1 and 100:1) based on *W. auropunctata* extracts, failed to show differences in the time required to attract a given number of ants. This indicates a lack of synergistic effects between the compounds under these test conditions. A dose response assay with 2,5-dimethyl-3-(2-methylbutyl)pyrazine showed maximal ant response to a 1 mg pheromone lure, a dose which remained attractive for 8 days under field conditions. Several of the field experiments included peanut butter baits, a lure currently used for detection. However, ant counts at peanut butter baits were not greater than at controls suggesting that peanut butter does not produce volatiles that attract ants. With the aim of developing management applications, a series of bioassays were conducted with 2,5-dimethyl-3-(2-methylbutyl)pyrazine in combination with food baits. A separate assay was conducted with Tanglefoot, a sticky catch material. In feeding bioassays, the alarm pheromone decreased consumption of peanut butter and solutions of protein and sugar. Tanglefoot squares failed to catch *W. auropunctata* with any of the lures tested. The field responses of *W. auropunctata* to alarm pheromone lures show a mixed potential for control applications. While the strong attraction and longevity of lures is promising, the inability to increase bait consumption or capture ants with

[1]Department of Chemistry, Eastern Mennonite University, 1200 Park Rd., Harrisonburg, VA 22802
[2]US Pacific Basin Agricultural Research Center, USDA-ARS, P.O. Box 4459, Hilo, HI 96720
[*]To whom correspondence should be addressed.

Tanglefoot presents obstacles to using these alarm pheromone components for ant management.

INTRODUCTION

Wasmannia auropunctata (Roger) is one of the worst invasive pest ants (Lowe *et al.* 2000), with negative impacts on both biodiversity and agriculture in colonized areas (Le Breton *et al.* 2003; Walker 2006; Wetterer & Porter 2003). The ecological impacts of *W. auropunctata* include the displacement of native ants (Le Breton *et al.* 2003; Walker 2006), reduction of invertebrate populations (Ulloa-Chacón *et al.* 1991), and the stressing of vertebrates (Wetterer 1997; Wetterer *et al.* 1999). Although diminutive in size and relatively slow-moving, the venom from their stings can be quite painful and is thought to cause blindness in a number of vertebrates (Wetterer & Porter 2003). Common names for this species, such as "little fire ant" (although it is not a *Solenopsis* sp.) and "electric ant," are derived from this intense sting, and they have become a major deterrent to laborers harvesting infested crops (Conant 2000; Smith 1965). Besides stinging agricultural workers, *W. auropunctata* also impact agriculture by tending homopterans, which cause direct damage to crops and may vector disease (de Souza *et al.* 1998; Spencer 1941).

Although the distribution of *W. auropunctata* is nearly pantropical, with greenhouse infestations reported in temperate areas as far north as Canada and the United Kingdom (Jourdan *et al.* 2002; Wetterer & Porter 2003), its impact is not equal in all invaded areas. *W. auropunctata* has been present in Florida for ~75 years but is not considered a great pest, while the ant has much larger ecological and economic impacts on invaded Pacific islands (Wetterer & Porter 2003).

A variety of *W. auropunctata* control methods have been tried or are currently in use. Chemical poisons used include DDT, malathion, mirex, and the insect growth regulator methoprene (Wetterer & Porter 2003). Williams and Whelan (1992) tested a number of food items and commercial baits both in the lab and the field. The largest numbers of feeding ants were observed on Amdro, Raid Max, and peanut butter baits while Max Force and Logic were not observed to be different from water controls (Williams & Whelan 1992). Amdro has been used in the successful eradications of *W. auropunctata* from Santa Fe Island (Abedrabbo 1994) and Marchena Island (Causton *et al.*

2005) in the Galápagos. However, in both cases the areas inhabited by the ants were relatively small and this eradication method may not be applicable to larger infestations due to the costs involved (Wetterer & Porter 2003). Biological control may be a possibility with at least one currently known parasitoid, *Orasema minutissima* Howard (Mann 1918). Other *Orasema* wasps may be useful for control, but these have not been fully tested (Heraty 1994; Johnson 1988).

One key to controlling the spread of *W. auropunctata* is improved quarantine and rapid eradication of invasive populations (Wetterer & Porter 2003). Detection of *W. auropunctata* commonly utilizes a preferred food item, for example, hot dogs or peanut butter, which is placed on the ground for a period of time and inspected for the presence of ants (Causton *et al.* 2005; Kirschenbaum & Grace 2007). However, food baits are often susceptible to spoilage, may be messy to use, and can be nonspecific in the species of ants they attract. Pheromone lures may alleviate many of these problems in addition to being longer-lasting and easier to use.

Pheromone semiochemicals have not been greatly utilized in ant control. Instead insecticidal baits are among the most common methods used for ant control. However, several studies have explored the possibility of including an attractive pheromone with these baits to increase ant feeding and therefore toxicant consumption (Greenberg & Klotz 2000; Hughes & Goulson 2002; Hughes *et al.* 2002; Robinson & Cherrett 1978; Robinson *et al.* 1982; Vilela & Howse 1988). Most promising has been work conducted with the Argentine ant, *Linepithema humile* (Mayr), and the grass-cutting ants, *Atta bisphaerica* (Forel) and *A. capiguara* (Gonçalves). Addition of the Argentine ant trail pheromone, (*Z*)-9-hexadecenal, to liquid sucrose baits increased bait consumption over untreated controls (Greenberg & Klotz 2000). Similar increased consumption was seen with the addition of grass-cutting ants alarm pheromone compounds to solid baits (Hughes & Goulson 2002; Hughes *et al.* 2002). Another possible pheromone control application is the direct disruption of ant trail following behavior, which would have effects similar to those seen in pheromone mating disruption (Suckling *et al.* 2008). Both Suckling *et al.* (2008) and Tatsuki *et al.* (2005) have shown that application of the Argentine ant trail pheromone can reduce the number of foraging ants in treated plots compared to controls. These studies show the potential for

using pheromones to control ants but more research is needed to access the effectiveness of these techniques with additional ant species.

Recently we identified 2,5-dimethyl-3-(2-methylbutyl)pyrazine (2-MeBu-diMePy) and 3-methyl-2-(2-methylbutyl)pyrazine (2-MeBu-MePy) as alarm pheromone components of *W. auropunctata* (Showalter *et al.* 2009). Both pyrazines induced attraction, arrestment and increased locomotion in lab bioassays. However, we were interested to observe whether *W. auropunctata* would respond to these pyrazines in natural environments.

We report here a series of field experiments that assessed the attraction and arrestment of *W. auropunctata* with the use of 2-MeBu-diMePy and 2-MeBu-MePy. We also compared the attraction capability of 2-MeBu-diMePy to that of peanut butter, which is widely used as a detection tool, and conducted a series of feeding experiments to assess whether 2-MeBu-diMePy could increase the consumption of food baits.

MATERIALS AND METHODS

Insects and Field Location

All field tests were conducted in a macadamia nut orchard on the island of Hawaii, Papaikou, HI (GPS coordinates: 19.787029, -155.124443), from 12 May 2009 to 26 May 2009. Tests were performed in macadamia nut trees where *W. auropunctata* trails were readily observed. The highest numbers of ants were found in heavily moss-covered trees, with higher activity periods coinciding with cooler temperatures. Average daily temperatures varied from 22-26 °C and relative humidity from 65-81 %.

Experiments 1-3

The following methodology was used for Experiments 1-3. Four map pins were used to define a 4 x 4 cm square counting area located 2 cm from an observed *W. auropunctata* trail. A treated rubber septum (13 mm snap-on stopper rubber septa, Wheaton, Millville, NJ) was pinned to the center of each counting area. Counting areas were limited to one per tree and remained fixed throughout an experiment. Treatments were rotated so that each tree/ant trail was exposed to every treatment. This helped control for variation in ant numbers and activity. Following attractive pheromone treatments, residual ant activity was sometimes observed in the counting area. In these

cases, more rest time was allowed or ants were blown off the counting area between replicates. Despite these measures, some residual ant activity due to previous treatments is suspected.

Experiment 1 assessed the concentration at which the pheromone is most active. 2-MeBu-diMePy lures were prepared on rubber septa at 100 ng, 10 μg, and 1 mg doses and compared to a CH_2Cl_2 control (120 replicates). Ants within the marked areas were counted at 5-min. intervals for 30 min.

Experiment 2 examined the relative attractancy of both *W. auropunctata* pheromone pyrazines. Four treatments were assayed: 1 mg 2-MeBu-diMePy, 1 mg 2-MeBu-MePy, and blends of 1 mg : 100 μg and 1 mg : 10 μg of 2-MeBu-diMePy to 2-MeBu-MePy. The ratios reflect concentrations found in ant extracts (Showalter *et al.* 2009). Initially, ant counts were made every 5 min. for 30 min., as in Experiments 1 and 3. However, on the day of this experiment most test areas were overrun by hundreds of ants within a few minutes of beginning a trial, making accurate ant counts extremely difficult. To address this problem, the time to attract a given number of ants (10, 20, and 30 ants) was recorded to assess initial attraction (16 replicates for each number of ants). If 30 ants were not attracted within 10 min., only the maximum number of ants present during the 10 min. was recorded. The time to attract ants in these cases was assigned as 10 min. for purposes of analysis because insufficient ants were attracted for an exact time recording.

Experiment 3 measured the attractancy of 2-MeBu-diMePy against peanut butter (Safeway Brand creamy), a widely used survey tool. Rubber septa, treated with 1 mg 2-MeBu-diMePy (60 replicates), filled with peanut butter (55 replicates), or treated with CH_2Cl_2 alone (60 replicates), were pinned to the center of the marked area. Ants within the marked area were counted at 5-min. intervals for 30 min.

Experiment 4

This experiment was designed to assess the ant population monitoring potential of 2-MeBu-diMePy in combination with a sticky catch material (Tanglefoot). A thin layer of Tanglefoot (The Tanglefoot Company, Grand Rapids, MI) was applied in ~5 x 5 cm squares on trees, 2-3 cm from ant trails. Rubber septa treated with 1 mg 2-MeBu-diMePy, peanut butter, or CH_2Cl_2 controls were placed in the center of the Tanglefoot square (8 replicates).

Ants were attracted by some lures but were not caught in the Tanglefoot. Therefore, the number of ants within 1 cm of the Tanglefoot square perimeter was observed and recorded. Counts were taken at intervals over 8 days.

Experiment 5

This experiment assessed the impact of 2-MeBu-diMePy on feeding behavior. Experiments 5.1-5.3 used feeding vials, as adapted from Greenberg and Klotz (2000). The feeding apparatus consisted of a 1.5 ml glass vial (12 x 32 mm clear glass crimp top vial, Supelco Inc. Bellafonte, PA) with a 3 x 3 cm square of conically perforated membrane (Weed Block, Easy Gardener Products, Inc., Waco, TX) placed between the previously filled vial and crimp lid (13 mm aluminum crimp seal without septum, Supelco Inc. Bellafonte, PA). Vials were filled with solutions of 2 g hydrolyzed protein/40 ml H_20 or 10 g sucrose/40 ml H_20.

We tried multiple methods of attaching the vials to the trees. The most reliable technique was Velcro in combination with epoxy adhesive. The conjoining sides of the Velcro were separated, and one half was epoxied to a chosen location in a tree, while the corresponding half was epoxied to the glass vial. This method allows for optimal vial placement (sheltered and vertical) and ease of setup and vial recovery.

To account for the different evaporation rates of sugar and protein solutions, evaporation controls were also set out in the field. These consisted of the same basic feeding vial apparatus, placed in a plastic container with a mesh lid. This excluded the ants, but also exposed the evaporation controls to the same temperature and humidity as the treatments. The data from the evaporation controls were subtracted from the differences in feeding vial weights to calculate the actual liquid consumed.

A preliminary trial, Experiment 5.1, was conducted to determine if these *W. auropunctata* populations displayed a preference for sugar or protein food sources. Feeding behavior was determined by measuring consumption of three liquid food sources: a sugar solution (10 g sugar/40 ml H_20), a protein solution (2 g protein/40 ml H_20), and a sugar/protein solution (an equal combination of the two). Experiments 5.2 and 5.3 assessed the impact of 2-MeBu-diMePy on the consumption of protein and sugar solutions, respectively. Initially, pheromone was applied directly to the membrane of the feeding

vial. However, in subsequent trials rubber septa treated with the pheromone were glued to the vials. Both treatments received 1 mg 2-MeBu-diMePy and were compared to a CH_2Cl_2 treated control. The vials were weighed before and after field exposure to *W. auropunctata*.

Experiment 5.4 assessed the impact of 2-MeBu-diMePy on ant consumption of peanut butter. Rubber septa treated with 1 mg 2-MeBu-diMePy or CH_2Cl_2 controls were hot-glued onto peanut butter-filled plastic caps (6 replicates). These were attached to the trees in the same epoxy/Velcro method as Experiment 5.1-5.3. Peanut butter-filled caps were weighed before and after field exposure.

Analysis

All results were analyzed using ANOVA followed by Tukey's HSD test (alpha = 0.05), with the exceptions of data from Experiments 5.2-5.4, in which *t*-Tests were performed. All analyses of significance were made at the $P < 0.05$ level. All statistical analyses were performed using SPSS version 15.0 (SPSS, Inc. Chicago, IL).

RESULTS

In Experiment 1, the most active pheromone concentration was determined (Fig. 1). The number of ants observed in counting areas was greatest with 1 mg lures, while only the 100 ng dose was not significantly greater than the control (ANOVA: $F = 111.044$, df = 3, $P < 0.001$). When an attractive lure was placed in a counting area, *W. auropunctata* workers generally began to respond by orienting toward the pheromone source within a few minutes of placement. 2-MeBu-diMePy lures appeared to increase ant locomotion compared to the normal pace of ants travelling along trails. Ant distribution within the counting area was non-random, with many more ants aggregating on or in close proximity to the pheromone-treated septa.

Experiment 2 measured the relative attractancy of the two *W. auropunctata* mandibular pyrazines (Table 1). There were no significant differences between pyrazine treatments for the time to 10 ants (ANOVA: $F = 0.115$, df = 2, $P = 0.951$), 20 ants (ANOVA: $F = 0.095$, df = 2, $P = 0.963$), or 30 ants (ANOVA: $F = 0.123$, df = 2, $P = 0.946$).

In Experiment 3, pheromone attractancy was measured against peanut butter (Fig. 2). More ants were observed with the 1 mg 2-MeBu-diMePy lure

Table 1. Experiment 2, relative attractiveness of *W. auropunctata* mandibular pyrazines.

	Time in seconds (mean ± SE)* for a given # of ants to be observed in recording area			
	2-MeBu-diMePy	2-MeBu-MePy	2-MeBu-diMePy/ 2-MeBu-MePy (10:1)	2-MeBu-diMePy/ 2-MeBu-MePy (100:1)
10 ants	219 ± 49 a	206 ± 53 a	209 ± 49 a	245 ± 56 a
20 ants	350 ± 59 a	390 ± 52 a	368 ± 54 a	375 ± 52 a
30 ants	427 ± 49 a	461 ± 46 a	423 ± 51 a	434 ± 50 a

*Means within a row followed by the same letter are not significantly different ($P > 0.05$) using Tukey's HSD test.

Table 2. Experiment 5, food material consumed by *W. auropunctata*.

	Amount consumed in grams (mean ± SE)			
Exp. 5.1*	Protein & Sugar	Protein	Sugar	Control
	1.2 ± 0.2 a	1.1 ± 0.1 a	0.14 ± 0.03 b	0.12 ± 0.02 b
Exp. 5.2†	Protein		Protein & 2-MeBu-diMePy	
	0.33 ± 0.06 a		0.14 ± 0.03 b	
Exp. 5.3†	Sugar		Sugar & 2-MeBu-diMePy	
	0.3 ± 0.1 a		0.09 ± 0.03 b	
Exp. 5.4†	Peanut Butter		Peanut Butter & 2-MeBu-diMePy	
	0.11 ± 0.05 a		0.019 ± 0.006 a	

*Means within a row followed by the same letter are not significantly different ($P > 0.05$) using Tukey's HSD test.
†Means within a row followed by the same letter are not significantly different ($P > 0.05$) using a *t*-test.

than either the peanut butter or control, which were not significantly different from each other (ANOVA: $F = 154.315$, df = 2, $P < 0.001$).

Experiment 4 attempted to assess the ant population monitoring potential of 2-MeBu-diMePy in combination with Tanglefoot, but was converted into a longevity study of pheromone attractancy in comparison with peanut butter attractancy (Fig. 3). The pheromone was found to have significantly higher attractancy at every time interval, but no significant difference was found between peanut butter and control. In contrast to the short-term observa-

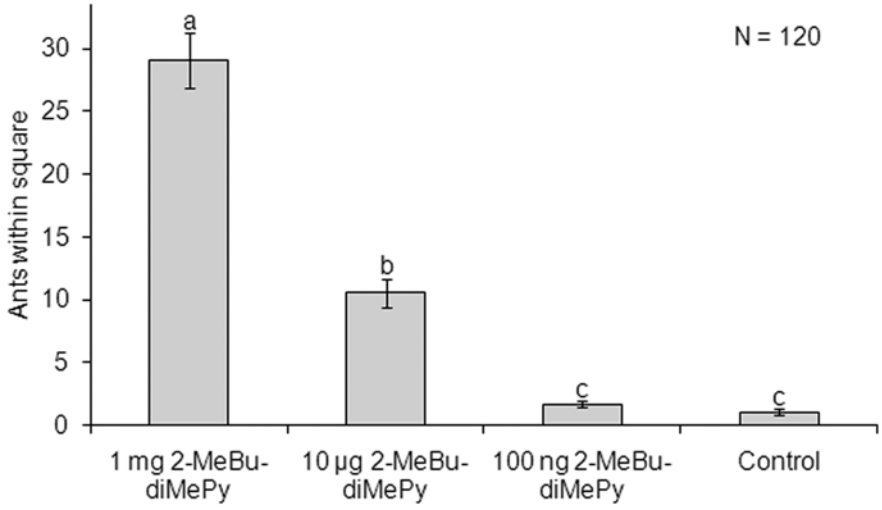

Fig. 1. Experiment 1, numbers (mean ± SE) of *W. auropunctata* counted in defined area for each treatment at 5-minute intervals. Letters represent significant differences ($P < 0.05$) between treatments (ANOVA, followed by Tukey's HSD).

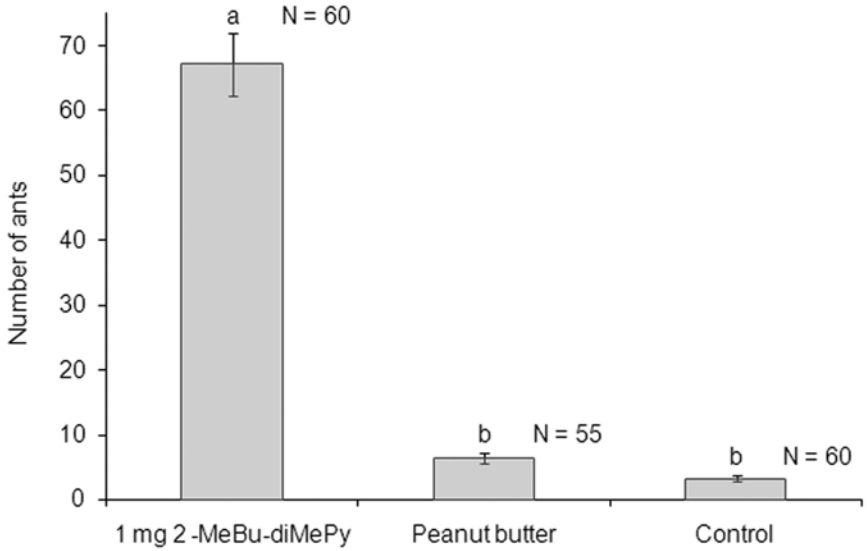

Fig. 2. Experiment 3, numbers (mean ± SE) of *W. auropunctata* counted in defined area for each treatment at 5-minute intervals. Letters represent significant differences ($P < 0.05$) between treatments (ANOVA, followed by Tukey's HSD).

tions of Experiments 1-3, in which ant locomotion seemed to increase, *W. auropunctata* surrounding pheromone-treated lures in Experiment 4 were largely quiescent.

Experiment 5 consisted of a series of trials designed to measure the impact of the pheromone on feeding activity and to determine whether a preference for sugar or protein food sources exists (Table 2). Experiment 5.1 showed that ants consumed a significantly greater amount of both protein and protein/sugar solution than either sugar solution or control (ANOVA: $F = 27.976$, df = 3, $P < 0.001$). In Experiment 5.2, ants consumed significantly less protein solution from vials treated with 2-MeBu-diMePy in comparison to protein solution alone (t-test: $P = 0.008$). Experiment 5.3 showed the same effect with a sugar solution, with ants consuming significantly less sugar solution from 2-MeBu-diMePy treated feeding vials vs. sugar solution alone (t-test: $P = 0.048$). Experiment 5.4 showed a non-significant decrease in the consumption of peanut butter in baits treated with 2-MeBu-diMePy compared with peanut butter alone (t-test: $P = 0.067$). It was noted that while pheromone-treated

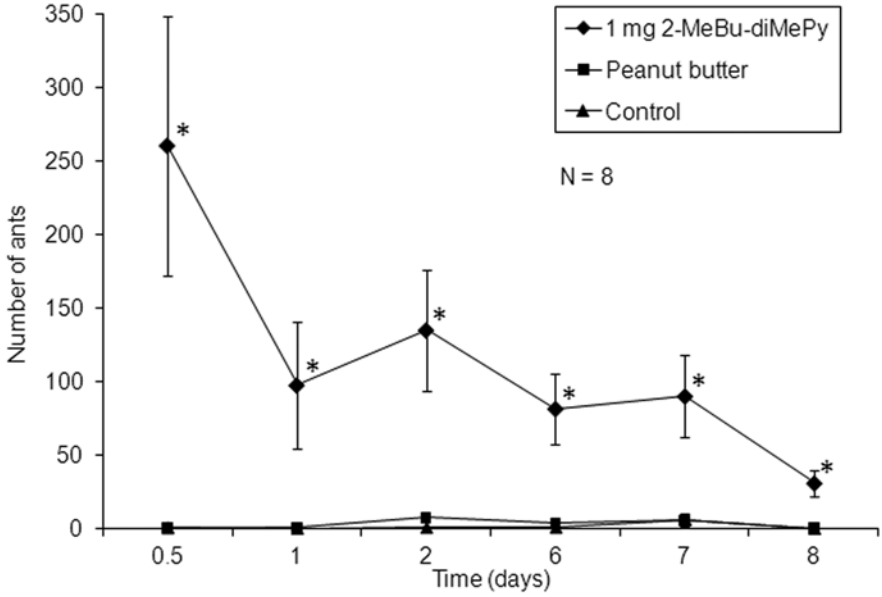

Fig. 3. Experiment 4, numbers (mean ± SE) of *W. auropunctata* within 1 cm of the Tanglefoot square perimeter at given time intervals for each treatment. Asterisks identify treatments significantly different ($P < 0.05$) from others at a given time interval (ANOVA, followed by Tukey's HSD).

baits were often the first to be discovered and quickly became surrounded by ants, these ants did not readily consume the bait or appear to recruit other *W. auropunctata* at the same rate as ants at untreated baits.

DISCUSSION

Field testing with 2-MeBu-diMePy and 2-MeBu-MePy supports previous laboratory bioassay results (Showalter *et al.* 2009), which showed that *W. auropunctata* are attracted and arrested by both ant pyrazines. 2-MeBu-diMePy and 2-MeBu-MePy attracted and arrested more ants than controls in every test performed. While the relative importance of attraction and arrestment behavioral modalities is difficult to quantify in Experiments 1-4, both are likely to have contributed to the number of ants counted in defined areas. Attraction appeared to be most important in Experiments 1-3, while many of the ants counted in Experiment 4 seemed to be arrested after an initial attraction.

2-MeBu-diMePy is the primary alarm pheromone component found in *W. auropunctata* and therefore was the initial focus of this field research. Increasing amounts of 2-MeBu-diMePy in Experiment 1 showed an attraction and arrestment dose response by *W. auropunctata* workers with a maximum response to the 1 mg lure (Fig. 1). This amount of pheromone is considerably higher than the ~200 ng of the alarm pyrazine that Howard *et al.* (1982) found in individual *W. auropunctata* workers. However, we did not quantify the absolute amount of 2-MeBu-diMePy released per ant when alarmed or the release rates of the lures in our study. Therefore, strict comparisons between the activity of 2-MeBu-diMePy and the alarm pheromone released by ants are not possible. A reasonable assumption may be that at least the 1 mg dose releases amounts of pheromone some orders of magnitude above that which is likely to be encountered by ants naturally. Irrespective of the relative activity of various concentration of 2-MeBu-diMePy, the attractiveness of this compound makes it a good candidate for use ing monitoring *W. auropunctata* populations.

Both alarm pheromone components previously identified in *W. auropunctata* (Showalter *et al.* 2009) were shown to be behaviorally active in laboratory bioassays. However, the bioassay results did not consistently indicate either a most attractive pyrazine or blend of pyrazines. Such pheromone attrac-

tion is often mediated by multiple chemical components, and the ability to produce a blend of chemical signals allows for greater specificity in intraspecies communication, complex chemical messages, and the possibility of enhanced signaling through synergistic effects (Hölldobler & Wilson 1990). Although signal complexity is common in nature, studying the interactions of component compounds can be difficult, particularly when concentration and component ratios may need to be adjusted to find an optimal attractant blend. An example of this complexity can be seen in the trail pheromone of the ant *Tetramorium meridionale* Emery, whose trail pheromone's four components are required in combination to reproduce activity similar to live ant secretions (Jackson *et al.* 1990).

To test the synergistic effects of 2-MeBu-diMePy and 2-MeBu-MePy (Experiment 2), we examined their relative attractancy by testing these pyrazines singly and in blends based on ratios found in *W. auropunctata* workers (Showalter *et al.* 2009). There was no significant difference in attraction between pheromone components at any of their tested ratios, pointing to a lack of synergistic effects between the components. This is unusual since examples of pheromone synergism predominate in the literature; however, non-synergistic interactions have also been reported. An example is the gregarious desert locust *Schistocerca gregaria* (Forskål), which produces an oviposition aggregation pheromone consisting of two major components that are equally active individually and in combination, and thus not synergistic (Rai *et al.* 1997).

Experiment 3 was designed to compare the attractiveness of 2-MeBu-diMePy to peanut butter, a bait commonly used to survey for *W. auropunctata*. Peanut butter releases a number of volatile alkylpyrazines, notably 2,5-dimethylpyrazine and 2,5-dimethyl-3-ethylpyrazine (Joo & Ho 1997), which are similar in structure to the pyrazines found in *W. auropunctata*. Surprisingly, given the release of alkylpyrazines and previous reports of attractancy (Williams & Whelan 1992), peanut butter was not found to attract significantly higher numbers of ants than the negative control. This was further supported by the results of Experiment 4, which again showed peanut butter did not attract more ants than the negative control. This discrepancy with previous reports (Williams & Whelan 1992) probably results from differences in bioassay methodology. Observations of peanut butter discovery by

ants, during Experiment 5.4, indicate that *W. auropunctata* workers quickly swarm the food resources once they are discovered, but that initial discovery is achieved through somewhat random searching and is not aided by volatile cues. This conclusion is most strongly supported by the results of Experiment 4, in which ants were prevented from contacting the peanut butter by the surrounding Tanglefoot, and in which case no attraction was observed. The strong preference shown for peanut butter (Williams & Whelan 1992) is likely due to recruitment mediated by the ants themselves (e.g. physical and pheromonal recruitment).

The original purpose of Experiment 4 was to assess whether 2-MeBu-diMePy could be used in combination with Tanglefoot to monitor *W. auropunctata* populations without constant observation. However, *W. auropunctata* were cautious when approaching lures surrounded by Tanglefoot and did not become entangled. This result presents serious difficulties in using 2-MeBu-diMePy as a detection tool. However, continuing observations of these lures showed that they attracted and arrested ants for up to 8 days (Fig. 3). This longevity compares favorably with lures currently used for detection, such as peanut butter, which often deteriorate when in the field for more than a few days.

Of primary interest in researching *W. auropunctata* pheromones is their potential use in monitoring and controling this invasive pest. One possible control application would be the inclusion of 2-MeBu-diMePy in insecticidal baits to increase feeding (Hughes *et al.* 2002). In Experiments 5.2 and 5.3, however, ants consumed significantly less sugar and protein from feeding vials treated with 2-MeBu-diMePy than from those with protein or sugar alone. These results were not consistent with previous studies of ant pheromones, which increased or did not affect consumption of baits treated with pheromone (Greenberg & Klotz 2000; Hughes & Goulson 2002; Hughes *et al.* 2002; Robinson & Cherrett 1978).

Alarm and trail pheromones as bait enhancer candidates have consistently been shown to induce attraction (Greenberg & Klotz 2000; Hughes *et al.* 2002). However, consumption of bait does not always increase proportionally with the number of ants attracted, as has been shown through ant responses to alarm pheromones. Bait removal is likely to increase merely because a larger number of ants in an area increases the likelihood that more bait will be removed, but the increase is not always proportional to the number of ants

attracted (Robinson & Cherrett 1978). In our results, however, the pyrazine actually decreases bait removal, suggesting that including the alarm pheromone in *W. auropunctata* baits is not necessarily an effective way to increase bait consumption and therefore aid in ant control.

The decrease in the amount of bait consumed by ants at pheromone-treated stations suggests that the alarm pheromones may inhibit normal responses to a food resource. This is particularly supported by the observation that *W. auropunctata* workers seemed to find treated baits more quickly, but do not recruit other ants to the same extent as workers at untreated baits. A hierarchy of behavior may exist by which some behaviors supersede others once elicited. In this case, alarm behavior may take precedence over feeding or retrieval of food items. An example of this behavioral hierarchy was noted by Moser *et al.* (1968) and Blum *et al.* (1968) who both observed that *Atta* spp. often dropped their loads or were less likely to transport bait when alarmed by synthetic pheromone. Hughes and Goulson (2001) present another possible reason that alarm pheromone-treated baits can be more attractive but less consumed. In a study with grass-cutting ants, they found that the main caste to respond to an alarm pheromone is the minor worker, which may be too small to transport bait (Hughes & Goulson 2001). Size is unlikely to be a problem for *W. auropunctata*, however, because it appears to have only one worker caste, which is capable of carrying the bait used in our studies.

Although our field tests showed the *W. auropunctata* alarm pheromone to be not particularly useful for increasing consumption of food baits or trapping ants with Tanglefoot, they revealed that 2-MeBu-diMePy is significantly more attractive than peanut butter. This quality may be instrumental in developing other control applications, such as direct disruption of trail following behavior (Suckling *et al.* 2008) or a different trapping device for detection.

ACKNOWLEDGMENTS

We would like to thank Janice Nagata and Esther Schneider for their assistance in conducting field experiments and collecting ants. We thank Peter Follet for his help in locating a suitable test site. This project was funded in part by a grant from the Hawaii Invasive Species Council Research and Technology Grant Program (Agreement # 58148).

REFERENCES

Abedrabbo, S. 1994. Control of the little fire ant, *Wasmannia auropunctata*, on Santa Fe Island in the Galapagos Islands. In: Williams, D.F., editor. Exotic ants: Biology, impact, and control of introduced species. Boulder, CO: Westview Studies in Insect Biology. p 219-227.

Blum, M.S., F. Padovani & E. Amante. 1968. Alkanones and terpenes in the mandibular glands of *Atta* species. Comparative Biochemistry and Physiology 26:291-299.

Causton, C.E., C.R. Sevilla & S.D. Porter. 2005. Eradication of the Little Fire Ant, *Wasmannia auropunctata* (Hymenoptera: Formicidae), from Marchena Island, Galapagos: On the edge of success? Florida Entomologist 88(2):159-168.

Conant, P. 2000. *Wasmannia auropunctata* (Hymenoptera: Formicidae): established on the Island of Hawai'i. Bishop Museum Occasional Papers 64:21.

de Souza, A.L.B., J.H.C. Delabie & H.G. Fowler. 1998. Wasmannia spp. (Hym., Formicidae) and insect damages to cocoa in Brazilian farms. Journal of Applied Entomology 122:339-341.

Greenberg, L. & J.H. Klotz. 2000. Argentine ant (Hymenoptera: Formicidae) trail pheromone enhances consumption of liquid sucrose solution. Journal of Economic Entomology 93(1):119-122.

Heraty, J.M. 1994. Biology and importance of two Eucharitid parasites of *Wasmannia* and *Solenopsis*. In: Williams, D.F., editor. Exotic ants: Biology, impact, and control of introduced species. Boulder, CO: Westview Press. p 104-120.

Hölldobler, B. & E.O. Wilson. 1990. The ants. Cambridge, Mass.: Harvard University Press

Howard, D.F., M.S. Blum, T.H. Jones & M.D. Tomalski. 1982. Behavioral responses to an alkylpyrazine from the mandibular gland of the ant *Wasmannia auropunctata*. Insectes Sociaux 29:369-374.

Hughes, W.O.H. & D. Goulson. 2001. Polyethism and the importance of context in the alarm reaction of the grass-cutting ant, *Atta capiguara*. Behavioral Ecology and Sociobiology 49:503-508.

Hughes, W.O.H. & D. Goulson. 2002. The use of alarm pheromones to enhance bait harvest by grass-cutting ants. Bulletin of Entomological Research 92:213-218.

Hughes, W.O.H., P.E. Howse, E.F. Vilela, J.J. Knapp & D. Goulson. 2002. Field evaluation of potential of alarm pheromone compounds to enhance baits for control of grass-cutting ants (Hymenoptera: Formicidae). Journal of Economic Entomology 95(3):537-543.

Jackson, B.D., S.J. Keegans, E.D. Morgan, M.C. Cammaerts & R. Cammaerts. 1990. Trail pheromone of the ant *Tetramorium meridionale*. Naturwissenschaften 77:294-296.

Johnson, D.W. 1988. Eucharitidae (Hymenoptera: Chalcidoidea): Biology and potential for biological control. Florida Entomologist 71:528-537.

Joo, K. & C.-T. Ho. 1997. Quantitative analysis of alkylpyrazines in regular- and low-fat commercial peanut butter preparations. Bioscience, Biotechnology, and Biochemistry 61(1):171-173.

Jourdan, H., L. Bonnet de Larbogne & J. Chazeau. 2002. The recent introduction of the neotropical ant *Wasmannia auropunctata* (Roger) into Vanuatu archipelago (Southwest Pacific). Sociobiology 40(3):483-509.

Kirschenbaum, R. & J.K. Grace. 2007. Dominant ant species in four habitats in Hawaii (Hymenoptera: Formicidae). Sociobiology 50(3):1069-1073.

Le Breton, J., J. Chazeau & H. Jourdan. 2003. Immediate impacts of invasion by *Wasmannia auropunctata* (Hymenoptera: Formicidae) on native litter ant fauna in a New Caledonian rainforest. Journal of Austrailian Ecology 28 (2):204-209.

Lowe, S., M. Browne & S. Boudjelas. 2000. 100 of the world's worst invasive alien species. Aliens 12:S1-S12.

Mann, W.M. 1918. Myrmecophilous insects from Cuba. Psyche 25:104-106.

Moser, J.C., R.C. Brownlee & R. Silverstein. 1968. Alarm pheromones of the ant *Atta texana*. Journal of Insect Physiology 14:529-535.

Rai, M.M., A. Hassanali, R.K. Saini, H. Odongo & H. Kahoro. 1997. Identification of components of the oviposition aggregation pheromone of the gregarious desert locust, *Schistocerca gregaria* (Forskal). Journal of Insect Physiology 43(1):83-87.

Robinson, S.W. & J.M. Cherrett. 1978. The possible use of methyl 4-methylpyrrole-2-carboxylate, an ant trail pheromone, as a component of an improved bait for leaf-cutting ant (Hymenoptera: Formicidae) control. Bulletin of Entomological Research 68:159-170.

Robinson, S.W., A.R. Jutsum, J.M. Cherrett & R.J. Quinlan. 1982. Field evaluation of methyl 4-methylpyrrole-2-carboxylate, an ant trail pheromone, as a component of baits for leaf-cutting ant (Hymenoptera: Formicidae) control. Bulletin of Entomological Research 72:345-356.

Showalter, D.N., E.J. Troyer, M. Aklu, E.B. Jang & M.S. Siderhurst. 2009. Alkylpyrazines: Alarm pheromone components of little fire ant, *Wasmannia auropunctata*. - Submitted to Insectes Sociaux.

Smith, M.R. 1965. House-infesting ants of the eastern United States. Their recognition, biology, and economic importance. 1-105 p.

Spencer, H. 1941. The small fire ant *Wasmannia* in citrus groves: A preliminary report. Florida Entomologist 24(1):6-14.

Suckling, D.M., R.W. Peck, L.M. Manning, L.D. Stringer, J. Cappadonna & A.M. El-Sayed. 2008. Pheromone disruption of Argentine ant trail integrity. Journal of Chemical Ecology 34:1602-1609.

Tatsuki, S., M. Teryama, Y. Tanaka & T. Fukumoto; 2005. Behavior-disrupting agent and behavior disrupting method of Argentine ant. USA.

Ulloa-Chacón, P., D. Cherix & R. Meier. 1991. Bibliografía de la hormiga colorada *Wasmannia auropunctata* (Roger) (Hymenoptera: Formicidae). Noticias de Galápagos 50:8-12.

Vilela, E.F. & P.E. Howse. 1988. Pheromone performance as an attractive component in baits for the control of the leafcutting ant *Atta sexdens rubropilosa* Forel, 1908 (Hymenoptera: Formicidae). Anais da Sociedade de Entomológia do Brasil 17:107-124.

Walker, K.L. 2006. Impact of the little fire ant, *Wasmannia auropunctata*, on native forest ants in Gabon. Biotropica 38(5):666-673.

Wetterer, J.K. 1997. Alien ants of the Pacific islands. Aliens 6:3-4.

Wetterer, J.K. & S.D. Porter. 2003. The little fire ant, *Wasmannia auropunctata*: Distribution, impact and control. Sociobiology 41(3):1-41.

Wetterer, J.K., P.D. Walsh & L.J.T. White. 1999. *Wasmannia auropunctata* (Roger) (Hymenoptera: Formicidae), a destructive tramp-ant, in wildlife refuges of Gabon. African Entomology 7(2):1-3.

Williams, D.F. & P.M. Whelan. 1992. Bait attraction of the introduced pest ant *Wasmannia auropunctata* (Hymenoptera: Formicidae) in the Galapagos Islands. Journal of Entomological Science 27(1):29-34.