Field Evaluation of Potential of Alarm Pheromone Compounds to Enhance Baits for Control of Grass-Cutting Ants (Hymenoptera: Formicidae)

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ABSTRACT Leaf-cutting ants are important economic pests of the Neotropics, and the most common method of control involves the use of insecticidal baits. Baits that are currently available exhibit low attractiveness to grass-cutting species, thus there is a need to develop improved baits. The potential for using alarm pheromone compounds to enhance the attractiveness and subsequent harvest of baits was examined for two economically important species of grass-cutting ant, *Atta bisphaerica* (Forel) and *Atta capiguara* (Goncalves). Compounds of the alarm pheromone were applied to rubber septa that were then sealed inside plastic sachets together with citrus pulp-based bait. The best candidate compound for bait enhancement was 4-methyl-3-heptanone. This compound significantly increased the attractiveness of bait sachets to both species. It also appeared to improve the discovery of nearby unenhanced sachets. However, 4-methyl-3-heptanone resulted in only a slight and nonsignificant improvement in bait harvest. Enhanced and unenhanced bait sachets were applied at a number of positions to obtain an improvement in harvest, but without success. The possible reasons for the lack of an enhancement of harvest and the potential for using alarm pheromone compounds as leaf-cutting ant bait enhancers are discussed.

KEY WORDS leaf-cutting ants, alarm pheromone, bait enhancement

LEAF-CUTTING ANTS (Hymenoptera: Formicidae: Attini: *Atta* and *Acromyrmex*) are the dominant herbivores of the Neotropics (Hölldobler and Wilson 1990). They can consume between 12 and 17% of total leaf production in some areas (Cherrett 1989). This amount, combined with their highly catholic diet, has resulted in them becoming one of the most destructive pests in these regions (Weber 1972, Cherrett 1986a). Approximately one-quarter of all leaf-cutting ant species have specialized in foraging on monocotyledonous plants and consequently are known as the grass-cutting ants (Fowler et al. 1986). These species are significant pests of monocotyledonous crops such as sugarcane and of pasture (Fowler et al. 1986, Precetti et al. 1988, Della Lucia 1997). It has been estimated that normal nest densities of one grass-cutting species, *Atta capiguara* (Goncalves), can reduce the number of head of cattle that an area can support by 10–30% (Fowler et al. 1986). Furthermore, the distributions and impact of these grass-cutting species is growing in line with the spread of ranching in South America (Weber 1982, Fowler 1983, Cherrett 1986b).

At present, the most popular method of controlling leaf-cutting ants is the use of toxic baits (Vilela 1986, Della Lucia 1997). These baits are based upon a matrix of citrus pulp that is attractive to species that forage on dicotyledonous plants (Cherrett and Seaforth 1970, Mudd et al. 1978). However, such baits are only mildly attractive to grass-cutting ants and so the baits are of much lower efficacy against these species (Fowler et al. 1986, Lapointe et al. 1993, Zanuncio et al. 1993, Della Lucia 1997; unpublished data). There is therefore a great need to develop improved baits for the control of grass-cutting species in particular, with the low attractiveness of present baits being the most pressing problem that needs to be addressed.

Communication in leaf-cutting ants, and in social insects in general, is based primarily on the use of pheromones (Wilson 1965). Many of these pheromones have attractive properties, giving them obvious potential in bait enhancement, which was first recognized for leaf-cutting ants by Moser (1967). Since then, a number of pheromones has been assessed as potential bait enhancers. Robinson and Cherrett (1974) applied a range of brood extracts to filter paper discs but failed to detect any improvement in transport back to the nest by *Atta cephalotes* (L.). Later, the main components of the trail pheromones of *Atta*

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sexdens (L.), *A. cephalotes,* and *Acromyrmex octospinosus* (Reich) were tested both in laboratory and field experiments (Robinson and Cherrett 1978, Robinson et al. 1982, Vilela and Howse 1988). Although trail pheromone compounds enhanced the discovery and transport of filter paper discs that were not inherently attractive, they did not enhance citrus pulp bait because it was already attractive. Trials with the leaf-marking pheromone of *A. cephalotes* produced similar results (Vilela and Howse 1988, Howse 1990).

The use of alarm pheromones in bait enhancement is somewhat counter-intuitive. Their potential comes from the main behavioral response of leaf-cutting ants to their alarm pheromone being attraction to the source (Moser et al. 1968, Hughes et al. 2001a). Trials with *A. octospinosus* (Knapp 1987) and on *A. sexdens rubropilosa* (Forel) (Knapp 1995) have demonstrated that enhancement with certain alarm pheromone components can improve the discovery and harvest of baits by these species. 4-Methyl-3-heptanone is the most behaviorally active component of the alarm pheromones of *Atta bisphaerica* (Forel), *A. capiguara, A. sexdens rubropilosa, A. texana* (Buckley), *A. cephalotes* and *A. laevigata* (Fr. Smith) (Blum et al. 1968, Moser et al. 1968, Riley et al. 1974, Knapp 1995, Hughes et al. 2001a). It is also the most abundant compound in a further two species of [*Atta* (*A. colombica* (Guerin) and *A. robusta* (Borgm)] (Blum et al. 1968) and seems likely to be the main component of the alarm pheromone throughout the *Atta* genus. In this study we aimed to establish whether alarm pheromone compounds could be used to enhance baits applied against two grass-cutting species, *Atta bisphaerica* (Forel) and *A. capiguara* (Goncalves). Both of these species are important pests of pasture and monocotyledonous crops in southern Brazil (Fowler et al. 1986, Precetti et al. 1988), and the compositions of their alarm pheromones have recently been determined (Hughes et al. 2001b). Enhancement may simply involve improved attractiveness of the bait but should ultimately result in improved harvest as well. Following the previous studies by Knapp (1987, 1995), we applied the bait and pheromone sources in sealed plastic sachets to provide them with some protection against the environment and nontarget organisms.

Materials and Methods

General Methodology. These experiments were carried out during March and October 1996 and March 1998 in the state of Minas Gerais, Brazil. The experiments with *A. bisphaerica* were conducted on pastures within 40 km of Viçosa in southeastern Minas Gerais, and the *A. capiguara* experiments were conducted on pastures located within 30 km of Capinópolis, in western Minas Gerais. All of the nests used were mature, with mound surface areas of at least 10 m^2, and all the foraging trails used were observed before the experiments to ensure that they had good activity (>25 ants per minute passing a point 1 m from the trail entrance).

Bait and pheromone sources were applied in sachets made from black, polythene sheets through which both pheromone and bait odors could diffuse (Cherrett 1986c, Knapp 1995). Two 7 by 7-cm sections of this material were heat-sealed along three edges to make a sachet. Ten grams of Mirex-S (0.3% sulfluramid [AI]) (Attakill, SP, Brazil) citrus pulp-based bait was then placed inside, the pheromone source added, and the sachet immediately heat-sealed along its fourth edge. The pheromone sources used were rubber septa (Sigma, Dorset, UK). Septa were loaded with 50 μl of the test compound, because this quantity was used successfully in earlier studies (Knapp 1995; W.O.H.H., unpublished data). The septa were left for 1 h before being placed in the sachet to allow the chemical to be absorbed into the rubber. Control sachets were prepared using 10 g of bait and blank rubber septa with no chemical added. All sachets were applied within 2 h of being sealed. Colony mortality was not monitored and nests were never used more than once in case the toxic bait had any affect on the ants foraging behavior.

Comparison of Candidate Compounds for Bait Enhancement. Four of the main alarm pheromone compounds of *A. bisphaerica* were tested with this species to establish which had the best potential as a bait enhancer. Five trails from each of nine colonies were used to give 45 replicate trails in total. At each trail, a 10-cm-diameter circle was cleared of vegetation to the side of the entrance hole. This clearance was done 24 h before the experiment so that it did not interfere with ant activity during the experiment. The bait sachets were then placed in the center of these cleared circles at the start of the main foraging period ($\approx$1800 hours). Each treatment was applied to one trail from each nest in a randomized manner, with an unenhanced sachet being applied at the fifth trail. Instantaneous counts were made of the number of ants within the 10 cm circles immediately before application of the sachets (time 0), and at 1 min, and 0.75, 1.5, 2.25, and 15 h after application. Any bait remaining after this time was removed and weighed to calculate the proportion of bait applied that was harvested. Due to practical limitations, the experiment was carried out in two parts with four replicate nests being tested 1 wk after the first five. In the intervening period ant activity had decreased from a nocturnal to a crepuscular pattern. Sachets in the last four replicates were therefore left in place for longer and removed at 22 h after application.

The ant counts were log $(x + 1)$ transformed and analyzed with a repeated measures analysis of variance (ANOVA), under the null hypothesis that treatment did not affect the numbers of ants counted over the experimental period. The proportions of bait harvested by the end of the experiment were arcsine transformed and analyzed with a one-way factorial ANOVA.

Response of *A. capiguara* to Bait Sachets Enhanced with 4-Methyl-3-heptanone. The most effective compound in the previous experiment, 4-methyl-3-heptanone, was tested with *A. capiguara* to examine whether it would have a similar effect on this species.

The experiment also examined whether enhancement would still occur when the bait was applied further away from the entrance hole. In the previous experiment the bait was applied only 5 cm from the hole; whereas in this experiment, bait was placed either 15 or 50 cm to the side of the foraging trail at a point 1 m away from the entrance hole. Ten nests were used and two trails were selected per nest, one for each of the distances to be tested. Fifty centimeter long "roads" were cleared of vegetation on either side of each trail, perpendicular to it at a point 1 m from the entrance hole. The sachets were placed on these roads as test-blank pairs, with the sachet enhanced with 4-methyl-3-heptanone (test) being on the road to one side of the trail and the unenhanced sachet (blank) on the other. The enhanced and unenhanced sachets within a pair were therefore not independent of one another. In 10 replicates the sachets were placed 15 cm from the trail, and in the other 10 they were 50 cm from the trail. The nests used had a crepuscular pattern of foraging activity with short periods of activity, rather than the nocturnal pattern exhibited by the colonies in the previous experiment. Sachets were therefore left in place for a longer period of time, 36 h, to expose them to a roughly equivalent number of ant-foraging hours. All sachets were placed at the start of the evening foraging period (≈1900 hours).

Instantaneous counts were made of the numbers of ants within cleared 10 cm circles centered on the sachets before (time 0) and at 0.75, 1.5, 2.25, 3, 12, 24, and 36 h after application. After 36 h, the sachets were ranked for harvesting activity. The scale used was from 0–5: 0, sachet untouched; 1, sachet slightly cut; 2, sachet cut and opened; 3, sachet opened and some bait transported; 4, sachet heavily cut and large quantities of bait transported; and 5, all bait transported.

A repeated measures ANOVA was used to analyze the log $(x + 1)$ transformed ant count data, to examine whether the numbers of ants differed between the distances over the course of the experiment. Because the test and blank sachets were not independent they were analyzed separately. A matched pairs t-test was used to compare the number of ants counted at the test and blank sachets at the time of peak response, 2.25 h after application. The harvesting activity ranks of the test and blank sachets at the end of the experiment were compared with a Wilcoxon's signed ranks test, for each of the distances separately. A Mann-Whitney U test compared the harvesting activity at the two distances and was done separately for the test and blank sachets. Spearman's rank correlations were used to explore the relationships between bait harvest and the number of ants responding for the test and blank sachets.

Effect of Position on Sachet Enhancement. The previous experiments tested bait applied at only one or two positions. Trials were now carried out to examine further the influence of position on bait harvest and enhancement, and to establish the optimum position for the placement of bait. In the first part of this experiment, bait sachets enhanced with 4-methyl-3-heptanone and unenhanced sachets were tested with the original test species, *A. bisphaerica*, at three different positions. These sachets were beside the entrance hole (near entrance), beside the trail 1 m from the entrance hole (trail), and 15 cm from the trail but connected to the trail by a road cleared of vegetation (road). Two trails were selected for each nest. The control sachet was randomly allocated to one trail and the test sachet to the other. Twenty-eight nests were tested at the near entrance, 12 at the road, and 15 at the trail. The positions were tested at different nests because the nests used generally had only two trails with good activity and they could not be reused because of the potential for bait toxins affecting the foraging behavior of the ants. Ten-centimeter-diameter circles were cleared of vegetation at each of the positions at least 24 h before the experiment. The nests had a crepuscular pattern of foraging activity during the experiment, and all sachets were placed at the start of the evening foraging period (≈1800 hours). Instantaneous counts of the number of ants within the circles were made immediately before the experiment (time 0) and at 1 min, and 0.5, and 13 h after application. The experiment was only continued for 13 h so as to limit the ants to a single foraging period during which the effect of enhancement was likely to be greatest. After 13 h, the sachets were removed and any bait remaining was weighed. The three positions were tested on different nights and were analyzed separately. The ant count data were log $(x + 1)$ transformed and analyzed with a repeated measures ANOVA. The proportions of bait harvested from the test and control sachets were arcsine transformed and compared with t-tests for independent samples because the test and control sachets were applied to different trails.

The same experiment was carried out with *A. capiguara.* The near entrance and road positions were replicated with five nests each, and four nests used for the trail position. All sachets were placed at the start of the main foraging period (≈1900 hours). Counts of ant activity were not made in these replicates. The sachets were left in place for 36 h to give the ants access to the sachets for several foraging periods, as in the earlier experiment with *A. capiguara.* The levels of harvesting activity that had occurred were ranked after this time. The ranked harvesting activity at the enhanced and unenhanced sachets were compared with a Mann-Whitney U test at each position.

Results

Comparison of Candidate Compounds for Bait Enhancement. There was no difference between the two sets of replicates in either ant activity ($F = 1.19$; df = 20, 176; $P = 0.27$) or bait harvest ($F = 0.92$; df = 4,35; $P = 0.47$). The results for the two sets of replicates were therefore pooled. Treatment significantly affected the way the number of ants near the sachets changed over the course of the experiment ($F = 2.36$; df = 20, 201; $P = 0.002$). Ant numbers near the control sachets increased only marginally after application, and to only a low level near the sachets enhanced with 2-heptanone, 3-octanone or 4-methyl-2-hexanone

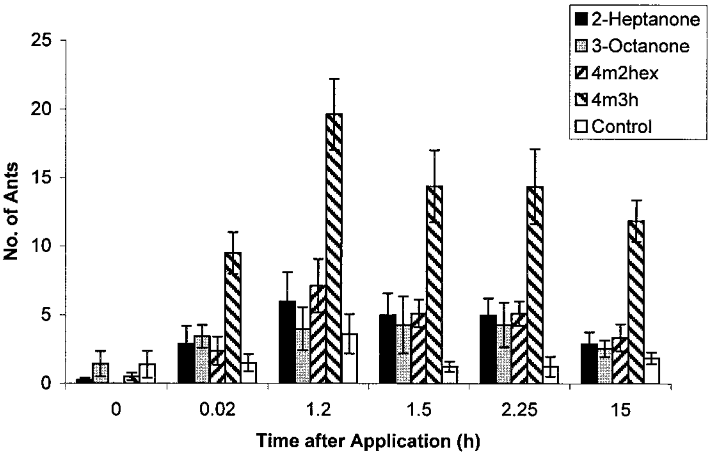

Fig. 1. Response of *A. bisphaerica* to bait sachets in the first experiment. Ant activity was recorded as the mean ± SE number of ants within a 10-cm circle centered on the sachet. Sachets were enhanced with 50 μl of 2-heptanone, 3-octanone, 4-methyl-2-hexanone (4m2hex), or 4-methyl-3-heptanone (4m3h), or were unenhanced (control).

(Fig. 1). However, the 4-methyl-3-heptanone treatment rapidly attracted a large number of ants. The ants bit at the sachets in all treatments and often cut holes in them. This cutting exposed the bait, which other individuals would subsequently transport to the nest. Frequently, the septa and pieces of the sachet were transported. Ants near the 4-methyl-3-heptanone sachets appeared more agitated than those in the other treatments and attacked the sachets in a more aggressive manner.

Approximately twice as much bait was harvested from the sachets enhanced with 4-methyl-3-heptanone as from the unenhanced sachets (69.3 ± 13.6 and 33 ± 14.9%, respectively), with the other treatments being intermediate (2-heptanone, 37.8 ± 16.1%; 3-octanone, 44.2 ± 17.3%; 4-methyl-2-hexanone, 52.6 ± 15.9%). However, this difference between treatments was not significant ($F = 0.86$; df = 4, 40; $P = 0.49$).

Response of *A. capiguara* to Bait Sachets Enhanced with 4-Methyl-3-heptanone. Distance from the trail did not significantly affect the way ant numbers changed near either the blanks ($F = 0.66$; df = 7, 126; $P = 0.71$) or the tests ($F = 0.87$; df = 7, 126; $P = 0.53$), possibly due to the extremely high variances. The number of ants near both the blank ($F = 3.45$; df = 7, 126; $P = 0.002$) and test ($F = 6.05$; df = 7, 126; $P < 0.001$) sachets increased significantly after application of the sachets at both distances from the trail (Fig. 2). Although numbers at 15 and 50 cm were not significantly different (blanks: $F = 2.84$; df = 1, 18; $P = 0.109$; tests: $F = 1.70$; df = 1, 18; $P = 0.208$), they increased faster and to a higher level when the sachets were only 15 cm from the trail. More ants were consistently

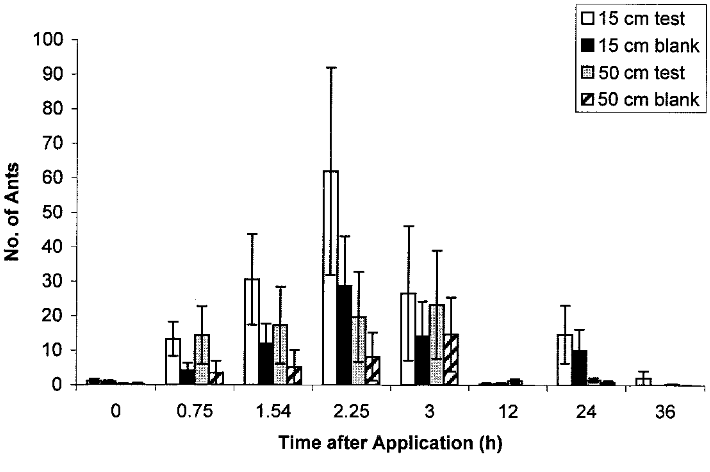

Fig. 2. Response of *A. capiguara* to bait sachets in the second experiment. Ant activity was recorded as the mean ± SE number of ants within a 10-cm circle centered on the sachet. Sachets were placed either 15 or 50 cm from the trail. They were either enhanced with 50 μl of 4-methyl-3-heptanone (test), or were unenhanced (blank) and were placed as test-blank pairs.

attracted to the test sachets than to the controls, and the difference at 2.25 h after application was significant at 50 cm from the trail ($t = 2.46$, df = 9, $P = 0.036$) though not at 15 cm ($t = 1.45$, df = 9, $P = 0.18$). Ants were still attracted to sachets at 15 cm from the trail at the start of the next evening foraging period, 24 h after application.

A slightly greater level of harvesting activity occurred at the test sachets than at the controls at both 15 cm (test rank = 3.7 ± 0.7, blank rank = 3.1 ± 0.8; $Z = 1.34, N = 10, P = 0.18$) and 50 cm (test rank = 1.5 ± 0.8, blank rank = 1.0 ± 0.7; $Z = 1.00, N = 10, P = 0.317$) from the trail, but these differences were not significant. Significantly more harvesting activity was directed at the sachets at 15 cm compared with those at 50 cm, both for the tests ($U = 26.5, N = 20, P = 0.045$) and the blanks ($U = 26.0$, $N = 20$, $P = 0.039$). Harvesting activity at the test and blank sachets was highly correlated at both 15 cm ($r_s = 0.76, N = 10, P = 0.01$) and 50 cm ($r_s = 0.76, N = 10, P = 0.01$) from the trail. The numbers of ants at the test and blank sachets was correlated at 2.25 h after application (15 cm: $r_s = 0.66$, $N = 10, P = 0.036$; 50 cm: $r_s = 0.77, N = 10, P = 0.009$). At both distances, harvesting activity was correlated with the number of ants at the sachets 2.25 h after application (15 cm: $r_s = 0.53, N = 20, P = 0.016$; 50 cm $r_s = 0.63, N = 20, P = 0.003$). Ants near the test sachets appeared more agitated and frequently exhibited alarm behaviors. They also cut the test sachets more rapidly than they did the blank sachets. The ants generally discovered the test sachet first and became alarmed. There was then an increase in activity and exploration in the general area, which resulted in the ants rapidly discovering the unenhanced sachet as well.

Effect of Position on Sachet Enhancement. In the *A. bisphaerica* replicates, the enhanced and control sachets differed significantly in the way ant numbers at the sachets changed over time at the near entrance ($F = 4.53$; df = 3, 162; $P = 0.004$) and the road ($F = 5.66$; df = 3, 66; $P = 0.002$), with more ants being attracted to the enhanced sachets. The response at the near entrance was extremely rapid, with most of the increase in ant numbers occurring during the first minute after application (Fig. 3A). At the road the response was slower with most of the increase occurring between 1 and 30 min after application (Fig. 3B). More ants were associated overall with the test sachets at the trail ($F = 7.91$; df = 1, 28; $P = 0.009$) (Fig. 3C), but the treatments did not significantly differ in the way ant numbers changed during the experiment ($F = 1.86$; df = 3, 84; $P = 0.14$). At the trail, the number of ants at both the test and control sachets increased significantly ($F = 60.7$; df = 3, 84; $P < 0.001$), largely between 1 and 30 min after application, although numbers near the enhanced sachets did increase slightly quicker. Thirteen hours after application the test sachets were still more attractive than the controls at the near entrance, and, to a lesser degree, at the other positions as well.

More bait was harvested from the enhanced sachets than the controls at the near entrance (70.9 ± 8.2 versus 57.3 ± 9.5%, $t = 0.97$, df = 54, $P = 0.34$) and trail (81.1 ± 9.3 versus 73.3 ± 11.8%, $t = 0.49$, df = 28, $P = 0.63$), but these differences were not significant. There was no difference in harvest between enhanced and unenhanced sachets at the road (41.7 ± 14.9% of both enhanced and unenhanced sachets). In the *A. capiguara* replicates, there were also no significant differences between the harvesting activity directed at the test and control sachets at any of the positions tested (near entrance: 3.8 ± 1.0 versus 4.3 ± 0.8, $U = 8.5$, $N = 9$, $P = 0.66$; road: 2.5 ± 1.4 versus 3.8 ± 1.3, $U = 6.0, N = 8, P = 0.5$; trail: 4.4 ± 0.6 versus 2.0 ± 1.8, $U = 3.5$, $N = 9$, $P = 0.08$; test and control ranks, respectively).

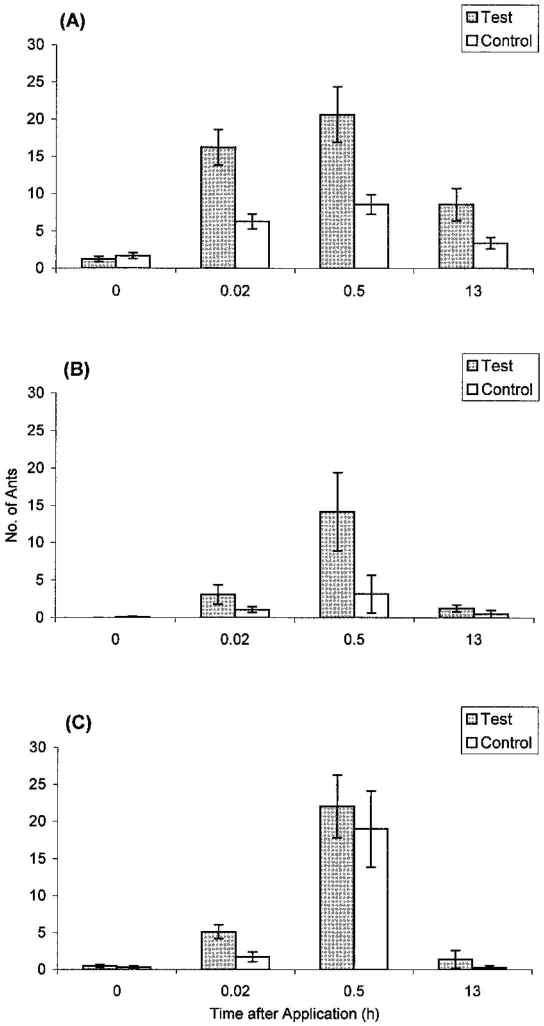

Fig. 3. Response of *A. bisphaerica* to bait sachets during the third experiment. Ant activity was recorded as the mean ± SE number of ants within a 10-cm circle centered on the sachet. Sachets were placed at three different positions. (A) Near entrance. (B) Road. (C) Trail. They were either enhanced with 50 μl of 4-methyl-3-heptanone (test) or were unenhanced (control).

Discussion

In all of the experiments with *A. bisphaerica* and *A. capiguara*, bait sachets enhanced with 4-methyl-3-heptanone were associated with significantly greater aggregations of ants than were unenhanced sachets. The level of the effect varied but was as much as five times. 4-Methyl-3-heptanone is the most behaviorally active of the main components of the alarm pheromones of *A. bisphaerica* and *A. capiguara*, being responsible for attraction as well as the full range of alarm behaviors (Hughes et al. 2001a). It appears that the chemical stimulates the same behaviors when incorporated in bait sachets. Ants are attracted to the enhanced sachets and show alarm behavior. Either because of their alarm or because they smell the bait inside, they then begin biting and cutting the sachet. Eventually, they exposed the bait, which they then begin harvesting. Because 4-methyl-3-heptanone is the main alarm pheromone component throughout the *Atta* genus, it seems likely that it will improve bait attractiveness to other species of *Atta* as well as *A. bisphaerica* and *A. capiguara.* Indeed, of the components tested as bait enhancers with *A. sexdens rubropilosa* by Knapp (1995), 4-methyl-3-heptanone was identified as the most effective compound for this species, too.

The results also suggest mechanisms by which the effectiveness of 4-methyl-3-heptanone as a bait enhancer may be increased further. In the second experiment, enhanced and unenhanced bait sachets were applied in proximity. It appeared that the enhanced sachet not only attracted ants but also stimulated a general increase in ant activity in the area, which resulted in them rapidly discovering the unenhanced sachet as well. The presence of pheromone enhanced bait may thus influence the discovery and harvest of unenhanced bait placed nearby. Robinson et al. (1982) noted that pick up of control bait appeared to be affected by treated bait placed nearby. The effect of enhanced bait on nearby unenhanced bait has potential applications. It may be possible to improve the harvest from normal bait by placing it close to enhanced bait. Because enhanced bait will be more expensive than standard bait, this application method could reduce the overall cost of treatment.

The attractant effect of 4-methyl-3-heptanone-enhanced sachets appeared to take place again at the start of the next foraging period. Although the ketone would normally have volatized from the rubber septa within this time (W.O.H.H., unpublished data), the plastic lining of the sachet may act as a second control-release mechanism (Cherrett 1986c; Knapp 1987, 1995). This mechanism could mean that pheromone was still released at the start of the next foraging period, ≈24 h after application. The attraction at this time could have been due to the persistence of trail pheromone paths laid by ants responding during the earlier period of activity

In addition, the position of the bait was important. The ant response in the second experiment was greater when the bait was placed closer to the trail. However, a substantial response was stimulated even 50 cm from the trail. The response was more rapid when the sachets were applied close to the trail entrance as opposed to 1 m away. Many ants are more aggressive closer to their nest (Shorey 1973, Jutsum 1979) and the trail traffic is also higher closer to the entrance hole (Hughes and Goulson 2001). This position, therefore, appears to be the optimum location for the application of pheromone enhanced bait, although good effects can still be achieved further from the entrance hole.

Enhancement of bait harvest was less conclusive. There was a slightly higher level of bait harvest from sachets enhanced with 4-methyl-3-heptanone, but the effect was small and not statistically significant in any one experiment. However, the effect was consistent, and with larger sample sizes might have reached statistical significance. Enhanced sachets attracted substantially more ants, and in the second experiment bait harvest was correlated with the number of ants attracted, so why was there not a greater improvement in harvest? There are a number of possible reasons. The main caste to respond during an alarm reaction on trails are minor workers (Hughes and Goulson 2001) and it may be that these ants are too small to cut the sachet or to transport bait. Blum et al. (1968) and Knapp (1995) observed that transporting ants often drop their loads when alarmed. It is, therefore, possible that alarmed ants may inherently be less likely to transport bait. One of the most likely reasons though is that grass-cutting ants do not find citrus pulp bait very attractive as a food source (Fowler et al. 1986, Lapointe et al. 1993, Della Lucia 1997). Thus, although enhancement with 4-methyl-3-heptanone may increase the discovery of bait, the ants may still choose not to transport it. Future work should clearly address these issues. In addition, bait and pheromone in these experiments was applied within sachets and the plastic barrier is likely to have disrupted the relationship between ant attraction and bait harvest. Better enhancement may be achieved by applying the bait in a manner that allows the ants direct access to it.

Nevertheless, alarm pheromone compounds do show significant potential as bait enhancers. The results clearly demonstrate that 4-methyl-3-heptanone can achieve the first objective of a bait enhancer, that of increasing the attractiveness of the bait to the ants. It has the capability to do this not only for *A. bisphaerica* and *A. capiguara,* but probably for other species of *Atta* as well. If the reasons why this increased attractiveness did not result in a similar increase in bait harvest can be elucidated and resolved, enhancement with alarm pheromone compounds may result in substantially improved baits for the control of leaf-cutting ants.

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