The use of alarm pheromones to enhance bait harvest by grass-cutting ants

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Abstract

The enhancement of bait for the control of grass-cutting ants was investigated using two species of grass-cutting ant, *Atta bisphaerica* (Forel) and *Atta capiguara* (Gonçalves) (Hymenoptera: Formicidae). Bait was applied in loose piles to obtain a direct relationship between ant attraction and bait harvest. Enhancement with alarm pheromone compounds significantly increased the attractiveness and harvest of bait under certain conditions. A large proportion of the ants attracted to the enhanced bait were minor workers. These ants rarely transport bait because of their small size, and so it may be possible to increase the effect of bait enhancement by using smaller bait granules. Foragers of *A. capiguara* were less inclined to transport citrus-pulp bait than were those of *Atta laevigata* (Fr. Smith), a species that also harvests dicotyledonous plants. This emphasizes the importance of developing a bait matrix that is more acceptable to grass-cutting species. Nevertheless, the results suggest that alarm pheromone compounds have significant potential to improve the efficacy of baits for the control of grass-cutting ants.

Introduction

Leaf-cutting ants (Hymenoptera: Formicidae: Attini: *Atta* and *Acromyrmex*) are important economic pests of the neotropics. Between 12 and 17% of the total herbivory in some areas can be attributed to leaf-cutting ants (Cherrett, 1989) and this can rise to 50% in agroecosystems (Blanton & Ewel, 1985). Ten of the 28 species of *Atta* and *Acromyrmex* have specialized in foraging on monocotyledonous plants and are termed grass-cutting ants (Fowler et al., 1986). These species are significant pests of pasture in South America, as well as of monocotyledonous crops such as sugarcane (Fowler et al., 1986; Precetti et al., 1988; Della Lucia, 1997). It has been estimated that one grass-cutting species, *Atta capiguara* (Gonçalves), at normal nest densities can reduce the number of head of cattle that an area can support by 10–30% (Fowler et al., 1986).

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The most common method of leaf-cutting ant control at present is the use of toxic baits (Vilela, 1986; Della Lucia, 1997). These can be distributed near the ant nest or in areas where damage is occurring, and have a slow acting insecticide formulated in a matrix of citrus pulp. The ants transport the baits back to the nest where the toxin circulates throughout the colony (Echols, 1966). Such baits can be extremely effective against species that forage on dicotyledonous plants (Zanuncio et al., 1992; 1996; Della Lucia, 1997). However, grass-cutting ants are only mildly attracted to citrus pulp-based baits and such baits are of low efficacy against these species (Fowler et al., 1986; Lapointe et al., 1993; Zanuncio et al., 1993; Della Lucia, 1997; personal observation). There is therefore a significant need for the development of baits that are more attractive to grass-cutting ants.

One possible method of increasing the attractiveness of baits is pheromone enhancement. Communication in leaf-cutting ants relies primarily upon the use of pheromones and a number of these have attractive properties. The incorporation of the attractive components from one or more of these pheromones could therefore enhance the attractiveness and harvest of bait. Several candidate pheromones have been examined with this view in mind, but without success. Robinson & Cherrett (1974) applied a range
of brood extracts to filter paper discs but failed to find any improvement in transport back to the nest by *Atta cephalotes* (Linnaeus). Possibly the pheromone with the most obvious potential as a bait enhancer is the trail pheromone, and the main components of this have been tested in both laboratory and the field experiments with *Atta sexdens* (Linnaeus), *A. cephalotes* and *Acromyrmex octospinosus* (Reich) (Robinson & Cherrett, 1978; Robinson *et al.*, 1982; Vilela & Hownse, 1988). Although trail pheromone compounds did improve the discovery and transport of filter paper discs that were not inherently attractive, they did not enhance citrus pulp bait, presumably because it was already attractive to these species. The leaf-marking pheromone of *Atta cephalotes* has also been examined, but no improvement in bait transport was found for probably the same reason (Vilela & Hownse, 1988; Hownse, 1990). However, components from the alarm pheromone have been found to show some potential. Leaf-cutting ants exhibit an aggressive alarm reaction during which they release an alarm pheromone from their mandibular glands (Blum *et al.*, 1968; Moser *et al.*, 1968; Knapp, 1995). One of the main behavioural responses of the ants to their alarm pheromone is attraction to the source (Moser *et al.*, 1968; Hughes *et al.*, 2001a). Experiments with *Acromyrmex octospinosus* (Knapp, 1987) and *Atta sexdens rubropilosa* (Forell) (Knapp, 1995) have found that enhancement with certain alarm pheromone components can improve the discovery and harvest of baits by these species.

The compositions of the alarm pheromones of two grass-cutting species, *Atta bisphaerica* Forel and *A. capigurua*, have recently been established (Hughes *et al.*, 2001b) and the main behaviourally-active component found to be 4-methyl-3-heptanone (Hughes *et al.*, 2001a). When this compound was added to bait sealed inside plastic sachets that the ants could readily cut open, it was found to substantially increase the attractiveness of the bait (Hughes *et al.*, in press). However, there was not found to be any improvement in bait harvest. In the present study, bait was applied in loose piles to obtain a more rapid response that was predicted to result in a closer relationship between ant attraction and bait harvest than that exhibited when bait is applied in sealed sachets. A series of experiments with *A. bisphaerica* and *A. capigurua* were carried out in an attempt to obtain an enhancement of bait harvest and to examine the reasons why such an improvement may not occur. Although 4-methyl-3-heptanone is the most behaviourally-active component, it is not as active as the natural alarm pheromone (Hughes *et al.*, 2001a), and while an alarm response can occur at some distance along the trail the response is greatest close to the entrance hole (Hughes & Goulson, 2001). Various source/position combinations of 4-methyl-3-heptanone and natural alarm pheromone were tested at positions near the entrance and further along the trail. The response of *A. capigurua* was also compared with that of a generalist species, *Atta laevigata* (F. Smith), which is known to harvest citrus pulp baits readily.

Materials and methods

All of the following experiments were carried out between March and May 1998. Replicates with *A. bisphaerica* were carried out near Viçosa, Minas Gerais, and Botucatu, São Paulo, in Brazil. The experiments with *A. capigurua* and *A. laevigata* used nests located near Botucatu and near Capinópolis, Minas Gerais. Only trails with good activity (at least 25 ants per minute) from mature nests were used. Bait was placed at two positions during these experiments: 10 cm to one side of the trail entrance hole (‘near entrance’ position) and 10 cm to one side of the trail at a point 1 m from the entrance hole (‘trail’ position). The general experimental method involved 10 cm diameter circles being cleared of vegetation at the position to be tested, 24 h prior to usage. One hundred granules of Mirex 5 bait (3 g kg\(^{-1}\) ai sulfufradimet (Attakill, São Paulo, Brazil) were placed in a pile at the centre of the 10 cm diameter circle. Only full size granules were used (approximately 7 mm length, 2 mm diameter and 35 mg weight). An alarm source was then immediately placed on top of the pile at its centre. Alarm sources were either 10 µl of 4-methyl-3-heptanone (racemic, 99% purity, ChemSampCo, South Carolina, USA) loaded on to a cigarette filter, or a crushed ant head. Although it is the S enantiomer that is thought to be found in *Atta* species, the behavioural response is not inhibited by the presence of the R enantiomer (Riley *et al.*, 1974; Nascimento *et al.*, 1997). Where crushed heads were used, a forager with an estimated head width of between 2.5 and 3.0 mm was removed from the trail using forceps. The head was separated from the body, placed at the centre of the bait pile, and crushed using the forceps. The forceps were cleaned in dichloromethane and the solvent allowed to evaporate prior to usage. Bait piles with a blank cigarette filter were used as controls. Enhanced and control bait piles were tested on separate trails from the same nest.

**Pheromone enhancement of bait piles**

Three source/position combinations were tested with *A. bisphaerica* to establish if any resulted in an enhancement of attraction or harvest; crushed ant head at the trail position (replicated 11 times), 4-methyl-3-heptanone at the trail position (replicated 11 times), and 4-methyl-3-heptanone at the near entrance position (replicated 19 times). Following the results of the *A. bisphaerica* replicates, two of the source/position combinations were tested with *A. capigurua*: crushed ant head at the trail position (replicated 24 times) and 4-methyl-3-heptanone at the near entrance position (replicated 10 times).

Instantaneous counts were made of the number of ants within the circles 5 min after application of the bait and source. The ant counts were log (x + 1) transformed and the numbers at the test and control piles compared with t-tests. Ants counted were classified as either minor workers (estimated head width < 1.4 mm) or foragers (estimated head width > 1.4 mm). The proportions of the ants counted that were minor workers were compared using G tests, with G adjusted with William’s correction factor because there was only one degree of freedom (Fowler & Cohen, 1990). The numbers of bait granules remaining within the circles were counted at 45 min after application and compared using t-tests.

**Detailed comparison of the response of A. capigurua and A. laevigata**

In this experiment crushed ant heads were used as the sources and placed at the trail position as before. Both tests and controls were replicated five times with each species. Instantaneous counts of the number of ants within the circles were made prior to application. In the *A. capigurua* replicates,
counts were then made at 2, 5, 15, 30 and 45 min after application. When *A. laevigata* was tested they were only made at 2, 5 and 10 min after application because all the bait had been harvested by this time. In addition, the total numbers of ants that contacted the bait and the numbers that transported the bait were recorded during the 2 min preceding each count. Ants that touched the pile of bait granules in any way were recorded as having 'contacted' it. When an ant then grasped a bait granule between its mandibles, and, either by lifting or dragging, moved it out of the 10 cm circle, it was recorded as having 'transported' the bait. Separate counts were made for foragers and minor workers.

The numbers of ants responding to the control and test bait piles were log (x+1) transformed and analysed with a repeated measures ANOVA. The proportions of foragers and minors in the ants responding were compared with G-tests, as were the proportions of ant contacts that transported the bait.

**Results**

**Pheromone enhancement of bait piles**

All of the source/position combinations resulted in significantly more ants being attracted to the enhanced bait piles than to the control piles (fig. 1). Of the combinations tested with *A. bisphaerica*, 4-methyl-3-heptanone at the trail position provided the smallest improvement and so this combination was not tested with *A. capiguara*. In every case, a greater proportion of the ants responding at the enhanced piles were minor workers compared with those at the control piles (fig. 2). In all of the source/position combinations more bait was harvested from the test piles than from the controls (fig. 3). This difference was only statistically significant when the bait was enhanced with 4-methyl-3-heptanone and placed at the near entrance position, although it was just marginally non-significant when enhanced with crushed heads and placed by *A. bisphaerica* trails (*P = 0.052*). Power analysis of the latter source/position combination reveals that there was only a 50% probability of not making a type II error and the sample size would have had to be doubled to increase the power to 80%. Overall, 40–57% of the control bait piles were harvested, and 57–89% of the test piles.

**Detailed comparison of the response of *A. capiguara* and *A. laevigata***

The reaction of *A. capiguara* to the bait piles was as previously described, with significantly more ants being attracted to the enhanced bait (*F* = 13.2, *P < 0.001) (fig. 4a). Treatment did not significantly affect the change in the number of *A. laevigata* workers near the bait during the experiment (*F* = 1.07, *P = 0.38) (fig. 4b). Numbers at the test and control piles were not significantly different (*F* = 0.54, *P = 0.48), and increased significantly at both (*F* = 50.2, *P < 0.001). In both species, a significantly greater proportion of the ants attracted to the test piles were minors compared to the controls (*A. capiguara*: 79.5 ± 5.9%, *P = 58.9 ± 14.9%, *G* = 11.0, *df* = 1, *P < 0.001; *A. laevigata*: 43.5 ± 5.6% vs. 24.3 ± 4.6%, *G* = 19.3, *df* = 1, *P < 0.001). Overall, foragers were more likely than minor workers to transport bait (*A. capiguara*: *G* = 136.6, *df* = 1, *P < 0.001; *A. laevigata*: *G* = 294.8, *df* = 1, *P < 0.001) (fig. 5). Minors of *A. capiguara* only transported bait in

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Fig. 1. Response to bait piles during the first experiment. Three source/position combinations were tested: crushed head/trail, 4-methyl-3-heptanone/trail, and 4-methyl-3-heptanone/near entrance. Bait piles were either enhanced with the alarm source (a), or were unenhanced (b). (*P < 0.05; **P < 0.01; ***P < 0.001; ns, *P > 0.05; t-tests.*)

Fig. 2. Proportions of ants responding to bait piles during the first experiment that were minor workers. Three source/position combinations were tested: crushed head/trail, 4-methyl-3-heptanone/trail, and 4-methyl-3-heptanone/near entrance. Bait piles were either enhanced with the alarm source (a), or were unenhanced (b). (*P < 0.05; **P < 0.01; ***P < 0.001; ns, *P > 0.05; G-tests.*)

Fig. 3. Bait harvested after 45 min. Three source/position combinations were tested: crushed head/trail, 4-methyl-3-heptanone/trail, 4-methyl-3-heptanone/near entrance. They were either enhanced with the alarm source (a), or were unenhanced (b). (*P < 0.05; **P < 0.01; ***P < 0.001; ns, *P > 0.05; t-tests.*)
Fig. 4. Numbers of (a) *Atta cephalotes*, and (b) *A. laevigata*, ants responding to bait piles during the second experiment. Bait piles were placed at the trail position and were either enhanced with a crushed head (---) or left unenhanced (-- --).

less than 5% of contacts. Ants contacting the bait were more likely to transport it if it was not associated with alarm pheromone (*A. cephalotes*: $G_{adj} = 35.2$, df = 1, $P < 0.001$; *A. laevigata*: $G_{adj} = 15.3$, df = 1, $P < 0.001$). The most obvious difference between the species was the harvest of bait. All of the bait was harvested by *A. laevigata* within 10 min of application. Even 45 min after application an average of about 50% of the *A. cephalotes* bait remained, with harvesting still in progress. *Atta laevigata* foragers were significantly more likely than *A. cephalotes* foragers to transport bait they contacted ($G_{adj} = 11.5$, df = 1, $P < 0.001$) (fig. 5).

**Discussion**

Enhancement of bait piles either with 4-methyl-3-heptanone or with crushed ant heads resulted in significantly greater numbers of ants being attracted to them than to unenhanced bait piles. The alarm response stimulated was similar to that described previously for various species of *Atta* (Blum et al., 1968; Moser et al., 1968; Knapp, 1995; Hughes et al., 2001a) and in agreement with results obtained previously using bait in sachets (Hughes et al., in press). In both *A. bisphaerica* and *A. cephalotes*, crushed heads resulted in a greater response than that stimulated by 4-methyl-3-heptanone. The alarm pheromone of leaf-cutting ants is made up of a complex mixture of compounds (Nascimento et al., 1993; Hernández et al., 1999; Hughes et al., 2001b). Although 4-methyl-3-heptanone is the most behaviourally-active component in every species of *Atta* examined, the complete alarm pheromone produced by crushed ant heads stimulates a greater response than does 4-methyl-3-heptanone alone (Hughes et al., 2001a). It therefore appear that by incorporating other components of the alarm pheromone together with 4-methyl-3-heptanone, it may be possible to obtain a greater improvement in bait attraction than that stimulated by the ketone alone.

Unlike most previous attempts to enhance leaf-cutting ant baits with pheromones (Robinson & Cherrett, 1974, 1978; Robinson et al., 1982; Vilela & Howse, 1988; Hughes et al., in press), these experiments with bait piles found an improvement in bait harvest. The application of natural alarm pheromone with bait piles at 1 m along the trail resulted in a substantial but non-significant increase in harvest, although the power of the test with *A. bisphaerica* was low. However, the addition of 4-methyl-3-heptanone to bait piles applied near the entrance holes caused a significant increase in harvest by both *A. bisphaerica* and *A. cephalotes*. In this treatment, 80.0% of the enhanced bait was harvested by *A. bisphaerica* (compared with 57.4% of the control bait), and 89.1% by *A. cephalotes* (compared with 50.5% of the control bait). As proportions of the quantity harvested from the unenhanced piles, enhancement with 4-methyl-3-heptanone resulted in 39.4% more bait being harvested by *A. bisphaerica* and 76.6% more by *A. cephalotes*.

In spite of the significance of these results, they do raise the question of why the effect of enhancement on bait
harvest was not even greater. Enhancement resulted in as much as ten times as many ants being attracted to the enhanced bait as to the controls, yet the level of harvest was only 39–76% greater at most. Minor workers are the main group of ants to respond to alarm pheromone and are not normally involved in the harvest of food (Stradling, 1978; Wilson, 1980; Hughes & Goulson, 2001). These ants were found to be less inclined to transport bait particles than their larger nestmates. However, this may be at least partly due to the size of the granules which were very large relative to the size of the minor workers, and therefore difficult for these ants to manipulate. Minor workers will transport pieces of vegetation that are small enough for them to manipulate, although this is rare (Hughes & Goulson, 2001; personal observation). The use of granules small enough for minor workers to transport may therefore increase the effect of enhancement on bait harvest.

In addition, the ants attracted to bait by an alarm pheromone compound will inherently be alarmed. The results of the second experiment showed that alarmed ants are less likely to transport bait than unalarmed individuals. This negative effect though, is not necessarily as problematic as it appears. The level of alarm can decrease quite rapidly (Hughes et al., 2001a), so the pick-up rate would be expected to increase in a corresponding manner. Such an increase in the pick-up rate over time by A. capigurara foragers was seen at the enhanced bait piles, with the proportion of contacts by foragers that resulted in bait being transported being the same at the test and control piles after 30 min. In the A. laevigata replicates all the bait was harvested within 10 min. The large difference between the test and control piles in the proportion of A. laevigata foragers contacting the bait that then transported it suggests that this was too short a time span for a sufficient decline in alarm behaviour to have occurred. The effect of alarmed ants being less likely to transport bait appears to be outweighed by the benefit gained from attracting a larger number of ants to the bait more quickly.

When the response of A. capigurara to loose bait piles was compared with that of A. laevigata, an important difference emerged. Atta laevigata harvests both monocotyledonous and dicotyledonous plants (Cherrett, 1986; Fowler et al., 1986), and will readily harvest baits based upon a citrus pulp matrix. Ants of this species were attracted in large numbers even to the unenhanced bait and were far more likely to transport bait than A. capigurara. As a result the bait was harvested considerably quicker by A. laevigata. This clearly shows the importance of using bait based on a matrix that is acceptable to the species concerned. In fact, harvest of the control piles by A. laevigata was so rapid that no improvement due to the increased attractiveness of enhanced bait could be detected.

In conclusion, the results presented here demonstrate that the addition of a source of either natural alarm pheromone or 4-methyl-3-heptanone alone can increase the attractiveness of bait piles to A. bisphearaica and A. capigurara. They also establish that such an improvement in attractiveness can produce a significant increase in bait harvest. Although this improvement was only statistically significant when bait was applied close to the trail entrance, this was partly due to the small number of replicates and the relative crudity of the experiments. It is highly likely that the effect can be considerably improved by measures such as the use of smaller bait granules, the incorporation of additional compounds from the alarm pheromone together with 4-methyl-3-heptanone, or by the development of a bait matrix that is more acceptable to grass-cutting ant species. Enhancement of bait with alarm pheromone compounds therefore has significant potential to substantially improve the harvest of bait by A. bisphearaica and A. capigurara, and probably other species of Atta as well.

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