STUDIES ON THE USE OF LEAF-CUTTING ANT SCENT TRAIL PHEROMONES AS ATTRACTANTS IN BAITS

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INTRODUCTION Since the first reported successful use of pheromones in insect control by Gaston et. al. in 1967, interest has been growing steadily. Baiting traps with lepidopteran sex pheromones to lure males to their death, or saturating areas with sex pheromones to confuse the process of mate location are the best known techniques. Both of these have been shown to work, either in field trials or on a laboratory scale and the topic has been well reviewed by Jacobson (1972).

It has recently been suggested that pheromones other than sex pheromones might play some part in the control of insect pests as components of attractive baits. Glancey et. al. (1970) suggested that in the fire ant *Solenopsis saevissima* (Fr. Smith) pheromones may control brood recognition, and he demonstrated that hexane extracts of brood added to clay granules or corn cob grits caused these materials to be returned to the nest and treated as brood for a period of hours. Robinson and Cherrett (unpublished) attempted to extract brood pheromones from the leaf-cutting ant *Atta cephalotes* (L.) with a view to incorporating them in an attractive bait. Unfortunately the success obtained by Glancey et al. with the fire ant could not be repeated with *Atta cephalotes*.

The possibility of using trail pheromones as a component of an attractive bait has been suggested by Moser (1967) and Lewis (1972). Previous work (Blum et. al., 1964) has shown that, in the laboratory, Attines will readily follow trails made from poison sac extracts of related species, and it would appear that any useful trail pheromones isolated from Attine ants might be active against a wide range of Attine pest species.

It became possible to test this hypothesis with the isolation and identification of a volatile component from the *Atta texana* trail secretion and its subsequent manufacture by Tumlinson et. al. (1971). The possible use of this component (Methyl - 4 - methylpyrrole 2-carboxylate (M4MP2C)) has been tested by Robinson and Cherrett (unpublished) on three species of leaf-cutting ants *Atta cephalotes*, *Acromyrmex octospinosus* (Reich) and *Atta Sexdens* (L.).

The pick-up of discs treated with trail pheromone

Using a technique described by Cherrett and Seaforth (1970) 0.5 mm diameter filter paper discs were used as an artificial bait. Ten or twenty discs containing specific amounts of M4MP2C and an equal number of control discs were randomly spread on a clean glass sheet. This was then placed into an ant foraging area from which all leaf material had previously been removed. When either approximately half the total numbers of discs offered had been removed, or 90 minutes had elapsed, the numbers of discs of each type remaining were counted. It was found that in all three species tested there was no significant preference for pickup of discs containing M4MP2C ($P > 0.75$ for *Atta cephalotes*; $P > 0.25$ for *Atta sexdens* and $P = 0.5$ for *Acromyrmex octospinosus*). The experiment was repeated using sugar impregnated discs which were readily carried back to the nest (Mustapha, 1971). Discs containing pheromone in addition to the sugar were preferentially picked up and carried back to the nest by all three species. The discs containing the trail pheromone became more attractive as greater amounts of pheromone were added, until a maximum was reached, after which the ants were repelled.

<u>The response of workers to trail pheromone on sugar impregnated discs</u>

Preliminary investigations into the reasons for this increased pickup of discs were made by counting the number of investigations that the ants made of each type of disc before picking them up. Attractive discs with and without the pyrrole were compared directly. The number of disc investigations per disc pickup was not significantly different in each type of disc, although the total number of investigations was increased for discs containing the pyrrole. From these data Robinson and Cherrett (unpublished) have suggested that the pyrrole was acting purely as an attractant and thereby increasing the effective size of the bait particle. Using an equation modified from Hunter and Symonds (1970), for use in calculating an optimum distribution pattern for slug bait, it is possible to estimate the effective increase in the radius of the bait particle. The ants are assumed to be foraging over the test plate at random, and although it is well known that leaf-cutting ants follow chemical trails whilst foraging (Blum et. al. 1964), this assumption of random searching is probably reasonable in the present experimental situation, within the confines of the glass plate. It seems unlikely that in such a small area the ants could use chemical trails effectively, since the search area would quickly become saturated with spots of trail compound.

Consequently if P = the probability that an ant will, by chance, find a piece of bait within a given time, X = the effective size (radius) of the bait particle, Y = the distance moved by an ant in that unit of time and A = the area in which each piece of bait is placed.

Then
$$P = 1 - e^{\frac{-2xy}{A}} \qquad (1)$$

From equation (1)
$$\log_e (1 - P) = \frac{-2xy}{A}$$

Both types of discs were being compared at the same time so if P_1, X_1, Y_1 and A_1 refer to the discs containing the pyrrole and P_2, X_2, Y_2 and A_2 refer to the control discs, then:-

$$\frac{\log_e (1-P_1)}{\log_e (1-P_2)} = \frac{-2X_1 Y_1 A_2}{-2X_2 Y_2 A_1} \qquad (2)$$

Since both types of disc were placed in the same area at the same time, A_1 and A_2 can be assumed to be equal, and Y_1 and Y_2 can be assumed to be equal, thus equation (2) becomes

$$\frac{\log_e (1-P_1)}{\log_e (1-P_2)} = \frac{X_1}{X_2} \qquad (3)$$

Using equation (3) the effective sizes of the bait particles were calculated using the preference data obtained by Robinson and Cherrett (unpublished). P was calculated by making P_1 and P_2 equivalent to the proportions of each type of disc taken when $P_1 + P_2 = 1$. The effective increases in radius of the bait pieces as a result of adding pheromone are shown for the three species tested in Figure 1.

As discs once removed by the ants were not replaced and consequently ants arriving late on the foraging area would encounter a smaller proportion of the pheromone-trated discs, this method of recording preferences is considered to be fairly rigorous, and would tend to underestimate the real increase in effective bait size (Cherrett and Seaforth, 1970). Thus these preliminary experiments indicate that M4MP2C would be useful in the manufacture of an improved artificial bait for leaf-cutting ant control.

The response of workers to the trail pheromone on citrus pulp

Preliminary field trials with a bait using citrus pulp as the primary arrestive material have been successful against some of the pest species found in the West Indies (Lewis, 1972; Lewis, in press) and the next step was to see if the results using the trail pheromone on paper discs could be repeated with the citrus pulp matrix.

Two pieces of dry citrus pulp were offered to the ants, one on either end of a 2.5 x 7.5 cm glass microscope slide. One of the pieces contained a specific amount of the pyrrole dissolved in hexane, the other piece was used as a hexane control. Glass slides were placed individually into an ant foraging area and with the one species tested (*Atta sexdens*), an increase in effective bait size was obtained for a comparable range of pheromone concentrations, to those used on filter paper discs. The results (Figure 1) show that the increase in effective bait size obtained was slightly less, with glass slides the maximum increase being 2.5 time compared with 3.5 for paper discs.

The persistence of the trail pheromone on citrus pulp

To investigate how long active concentrations of the pyrrole would stay on the citrus pulp bait, six individual pieces of citrus pulp (weight range 40 to 90 mg) were placed in small pits (2.5 cm diam; 1 cm depth) drilled into a perspex block (30 x 12 cm). Three of the pieces of pulp each had 0.4 µg of the pyrrole dissolved in 10µl of hexane applied to them. The remaining three pieces were used as a control and had 10µ of hexane added. The pits containing the pulp were then covered with a fine gauze of phosphor-bronze wire through which the ants were unable to pass. Pits were prepared and stored at 27°C and 80-90% relative humidity and after varying periods of time the perspex blocks were placed into the ant foraging areas. The ants were allowed approximately 30 minutes to settle down and counts were subsequently taken during a period of approximately 90 minutes.

The numbers of ants with either their whole bodies over the gauze or with their antennae investigating the gauze were counted for each pit. This was continued until a total of thirty counts was obtained for each pit, at each time interval. The results were pooled to give a single count for pits containing pulp and pyrrole, and control pits containing plain pulp.

Figure 2 shows the pooled data from several experiments showing the numbers of ants on pits containing pulp and pyrrole as a percentage of the numbers of ants on all pits, plotted against time in hours after the pyrrole was added to the pulp.

From figure 2 it can be seen that although M4MP2C is designated as a volatile component of the Atta texana trail-following secretion, the ants were able to detect it on the pulp for a considerable period of time after it had been added.

DISCUSSION Laboratory work has shown that M4MP2C can effectively increase the effective size of pieces of bait for the leaf-cutting ants Atta cephalotes, Atta sexdens and Acromyrmex octospinosus and enhance their chance of being found. It is also possible that the addition of M4MP2C to the bait may increase the foraging activity of the nest (unpublished personal observations). These two factors should combine to increase the effectiveness of the bait as they both increase its chance of being found. The cost of adding M4MP2C to the bait depends on (a) the cost of formulation and this should be negligible since Robinson and Cherrett (unpublished) have shown that the pyrrole can be added to paper disc baits dissolved in soya bean oil, and soya bean oil is at present a component of the citrus pulp bait successfully tested by Lewis (1972, In press) and (b) the cost of buying M4MP2C on a commercial scale. Although M4MP2C is manufactured commercially* it has not so far been possible to obtain price quotations for large quantities. From the result shown in figure 1 the optimum quantity of M4MP2C to be added to the bait would be approximately 1ppm by weight or 1 mg of M4MP2C in 1kg of pulp. From the results already obtained in the laboratory it does seem possible that the leaf-cutting ant trail pheromone M4MP2C might play some part in the formulation of an attractive bait to control these pest animals, although the practicality of this method of control is not yet clear.

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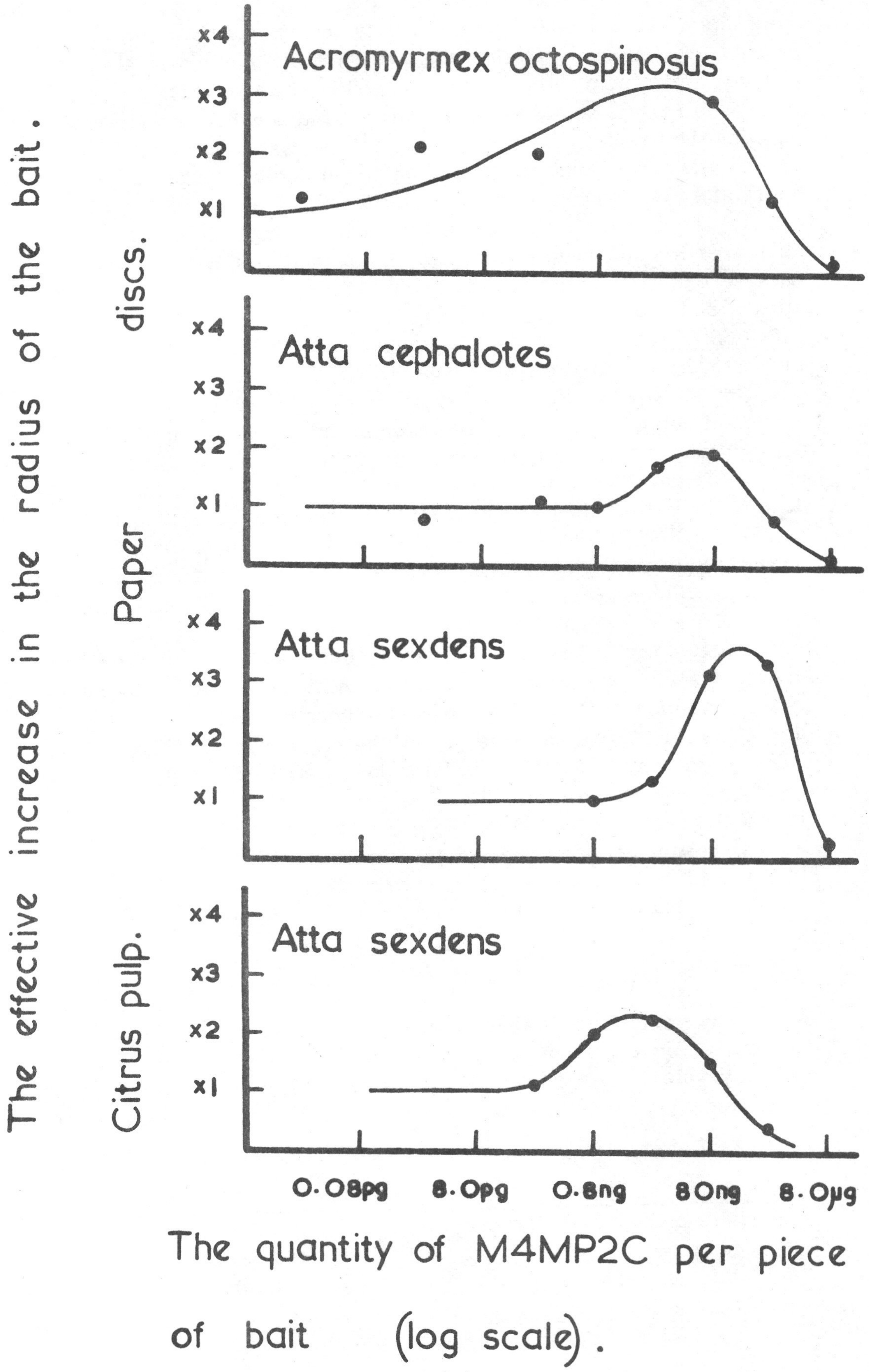
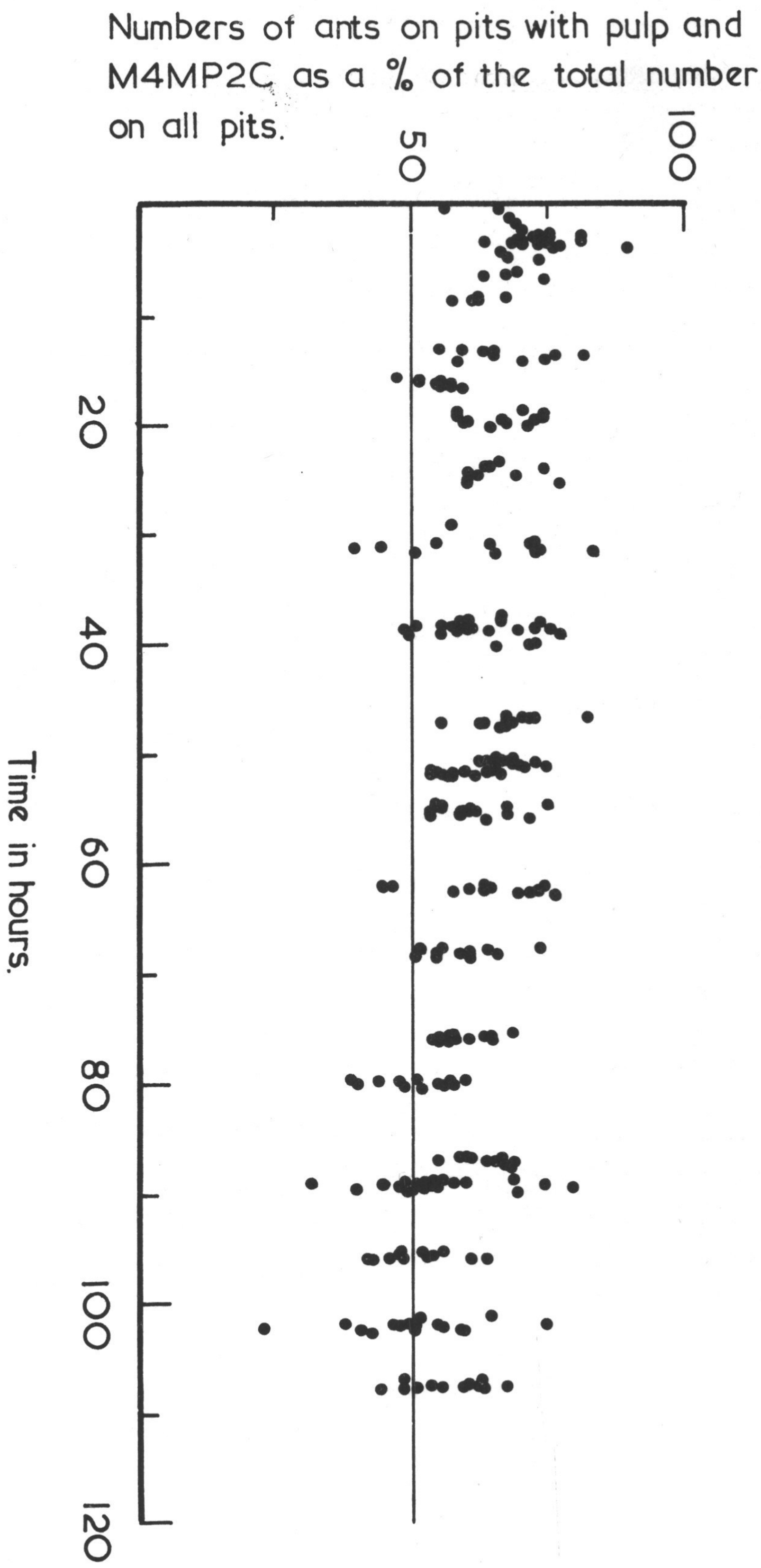

The effective increase in the radius of the bait.
Paper discs.
Citrus pulp.
Acromyrmex octospinosus
Atta cephalotes
Atta sexdens
Atta sexdens
x4
x3
x2
x1
0.08pg
8.0pg
0.8ng
80ng
8.0µg
The quantity of M4MP2C per piece of bait (log scale).


Numbers of ants on pits with pulp and M4MP2C as a % of the total number on all pits.
50
100
20
40
60
80
100
120
Time in hours.

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