METHOD AND PROCEDURE FOR EVALUATING BIOLOGICAL PERFORMANCE OF PHARAOH ANT, *MONOMORIUM PHARAONIS* (HYMENOPTERA: FORMICIDAE), BAITS

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Abstract - Pharaoh ants, *Monomorium pharaonis*, are significant pests associated with man and his environment. Chemical sprays are not ideal control methods if they cause the ants to disperse. There is a preference for bait treatments that provide a means to use ant biology to transfer slow-acting compounds into the nest and kill it at the source. Many factors can influence bait performance, including the feeding preference of the target species and the composition of the bait matrix. Since the majority of the bait is composed of various foodstuffs, the quality of those ingredients, and their attractiveness over time must be maintained. Correct bait placement, treatment density, monitoring are essential operational parameters, while any contamination of the bait stations, themselves may effectively repel ants, independent of the palatability of the bait matrix. A bioassay has been developed (based on the methods of Rupes) which allows rapid assessment of bait performance. New ant colonies are established in the main breeding culture and allowed to acclimatise for a week, or more, before introducing baits. These baits may be from production batches prior to distribution, or from the field, or may be new bait candidates (eg new formulations, or new active ingredients). By introducing the baits into a system where alternative food is abundant, and the ant colony is established, the conditions may be considered difficult, even in a confined space under laboratory conditions. Results to date show that where concerns have been raised about bait performance in the field, these are rarely attributable to the product itself (or alone). The test system has wider use as a model for establishing the bait potential of novel active ingredients since actively foraging colonies of a social insect can be established quickly and easily to determine such critical factors as repellency of the ai, delayed action, formulation palatability, behavioural effects on foragers and indirect effects via transfer of lethal doses to non-foraging stages.
Key words - Social behaviour, colony, trophallaxis, foraging, repellent, nest

INTRODUCTION

The Pharaoh's ant, *Monomorium pharaonis* L., is believed to have originated in North Africa – Middle East and is now found throughout the world (Edwards, 1986). Its preference for warm, humid environments tends to restrict its distribution to heated buildings in temperate climates. Within those buildings, unlike outdoor species, Pharaoh's ant activity (and, hence, the longevity and visibility of the infestation) is relatively unaffected by the seasons, although activity may be encouraged when central heating systems are turned on in the autumn.

Pharaoh's ant colonies range in size from a few dozen to hundreds of thousands with multiple queens. The foraging population accounts for approximately 10% of the total nest members. Generally, proteinaceous food sources are preferred although these ants will consume a wide variety of foods including chocolate, blood and dead insects. New nests are formed by budding, which may be in response to limited food availability some form of mechanical or chemical disturbance. Workers carry larval stages to a new site and rear new queens and males (Edwards, 1986).

Infestations may spread by way of service ducts, within wall and ceiling voids, etc. as a result of the ease with which the colony buds, and the wide foraging area that may be associated with each nest. Consequently, Pharaoh's ants pose a health risk due to the possibility of mechanically carrying pathogens such as *Streptococcus* and *Staphylococcus* sp. from unhygienic areas such as drains, decaying vegetable matter etc., to other foraging sites in kitchens, or to contaminate sterile supplies in hospitals.

Control measures must seek to eliminate the nests since spray treatments that might encourage dispersal and colony budding may exacerbate the pest problem. Consequently, some form of baiting is the preferred response. The same properties that characterise good baits for other public health pests, including cockroaches and termites, are required for *M. pharaonis*, which are a slow-acting, non-repel-

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lent active ingredient formulated in a palatabe bait base that remains attractive when alternative food sources are present, and is transferred to other members of the nest. Hydramethylnon, formulated at 0.95% active ingredient in Maxforce® Pharaoh Ant Killer (MPAK) has proven to be a suitable insecticide (Lucas and Invest, 1993; Short *et al.*, 1993).

The initial objective of the present research was to develop a test method to evaluate and compare candidate active ingredients and bait products against Pharaoh's ant nests. However, with the success of MPAK in the market place came occasional reports difficulties achieving control in the field. From past experience we were aware that "control failure" is often attributed to insecticide products when, in fact, there may be a number of factors involved. With more conventional pest control products, such as liquid surface sprays, chemical analysis can determine whether a sample is within specification for active ingredient, particle size, dispersion etc. With baits this is more problematic when a variety of foodstuffs constitute 99% of the product. Therefore, we required a facility to examine the biological performance of MPAK bait stations before and after distribution as part of quality assurance procedures.

MATERIALS AND METHODS

Insect culture

Pharaoh's ants, *Monomorium pharaonis* L. were obtained from the Schering CAMCO laboratories, in Chesterford Park, UK, in 1994 and have been maintained on a diet of liquid glucose, beef liver extract mixed with sucrose, and dead German cockroaches (*Blattella germanica*).

Nest preparation

Wooden (untreated beech sapwood) nesting boxes were placed in the colony boxes for 7 – 14 days and only those that had become occupied by 200 - 500 workers and queens, and where eggs had already been laid, were selected for test. These fresh nests were transferred to a plastic box, placed inside a plastic bowl which had its sides coated with Fluon (50% PTFE suspension) and strips of double sided sticky tape around the rim. Plastic mesh was placed over the tape as a further barrier to prevent any ants from escaping and the entire apparatus was placed in a large tray containing paraffin oil (Figure 1). Within the inner box, the nests were provided with a water pot, containing cotton wool saturated with water, and a smaller pot containing the dry sucrose and liver diet. Outside the inner container was a pot of dead *Blattella* to encourage ants to forage away from the nest. The nests were allowed 7 days to acclimatise to these conditions in a laboratory at 27 ± 2 ºC, 50+/- 10% relative humidity, with a 12 hour photoperiod.

Baits

Maxforce® Pharaoh's Ant Killer (MPAK) bait stations (0.95% w/w hydramethylnon) from: stock predistribution; customer stock, unopened; and removed from sites where complete control had been problematical. Experimental bait matrices comprised active ingredient mixed with the dry sucrose + liver extract food source (as provided in normal culturing) and a honey + peanut butter + dead cockroach mix (1:1:1) (from discussions with Prof. Peter Miller, University of Technology, Sydney)

Assessment

Once the experimental nest had acclimatised, either a bait station, or an experimental bait was introduced into the outer box, near the pot of cockroaches (Figure 1). Bait performance was assessed at weekly intervals. Initial attempts to estimate how many workers, queens etc., were present at each assesment point were abandoned as impractical and, instead, a scoring system was used to monitor nest activity during the test period: Grade 0 - no apparent effect upon the nest; Grade 1 - 10-30% ants killed; Grade 2 - 30-50% ants killed; Grade 3 - 50-80% ants killed; Grade 4 - 80-99% ants killed; Grade 5 - no survivors.

Each assessment score was accompanied by comments on presence of eggs, queens, workers tending brood etc. Tests were usually monitored until 4 weeks after the baits were introduced although, in

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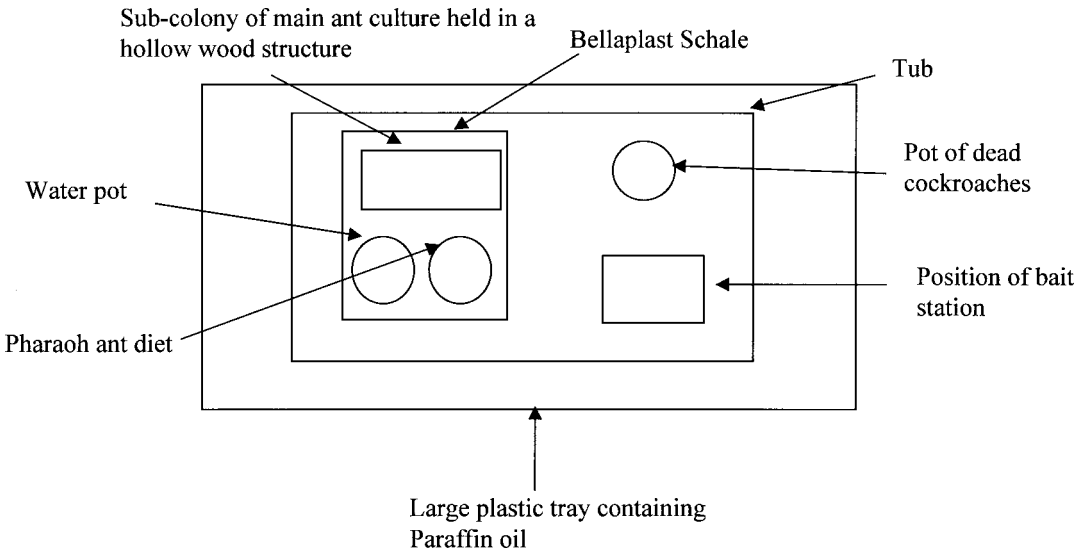

Figure 1. Diagram of bioassay container.

some cases, monitoring was continued to confirm whether a few surviving workers could rear[1] brood and enable the nest to recover.

RESULTS

Reference standard sample

The results of tests conducted on the standard MPAK sample, during 1998, are given in Figure 2. In total, 20 samples were tested on 7 different occasions. Four nests were killed within 2 weeks, and 11 by the end of week 3. Only 2 contained any survivors at the 4 week assessment and, in both cases, the few remining workers were dead the following week. The data for time to reach grade 4 (from which nests very rarely recovered) showed that 17 of the 20 nests were severely affected within one week of exposure.

Investigations of control problems

As part of the investigations into control problems which could have been due to the quality of the product, MPAK bait stations were returned to the AgrEvo laboratories in Frankfurt from 5 sources during 1998: Czech Republic, after poor performance in Dr Rupes' laboratory tests (National Institute of Public Health, Prague); two sites in Germany (used baits were compared with unused baits from unopened boxes in the customer's store); and sites in the UK and Holland (only baits from unopened boxes in the customer store were evaluated). Additional information was obtained from site visits, where appropriate.

Our bioassay is based upon that developed by Dr Rupes. Figure 3 shows that 2 of the 3 bait stations from the Czech Republic that we tested performed satisfactorily by the end of the normal 4 week period. The third was performing very poorly until the 4th week but the colony was eliminated the following week. Although, in our assays, these baits succeeded in killing the nests in 4-5 weeks, the poor performance over the first 3 weeks was atypical and agreed with the concerns raised in Dr Rupes' laboratory.

All baits from the UK (6 samples) and Holland (3 samples) eliminated nests within the 4 week exposure period. Five of the 9 nests were dead by the end of the third week - similar to the performance of the standard (see Figure 2).

When baits collected from the sites, in Germany, were compared with unused samples, two significant features emerged in both cases. The pattern in Figure 4 shows the worst of the two cases – where

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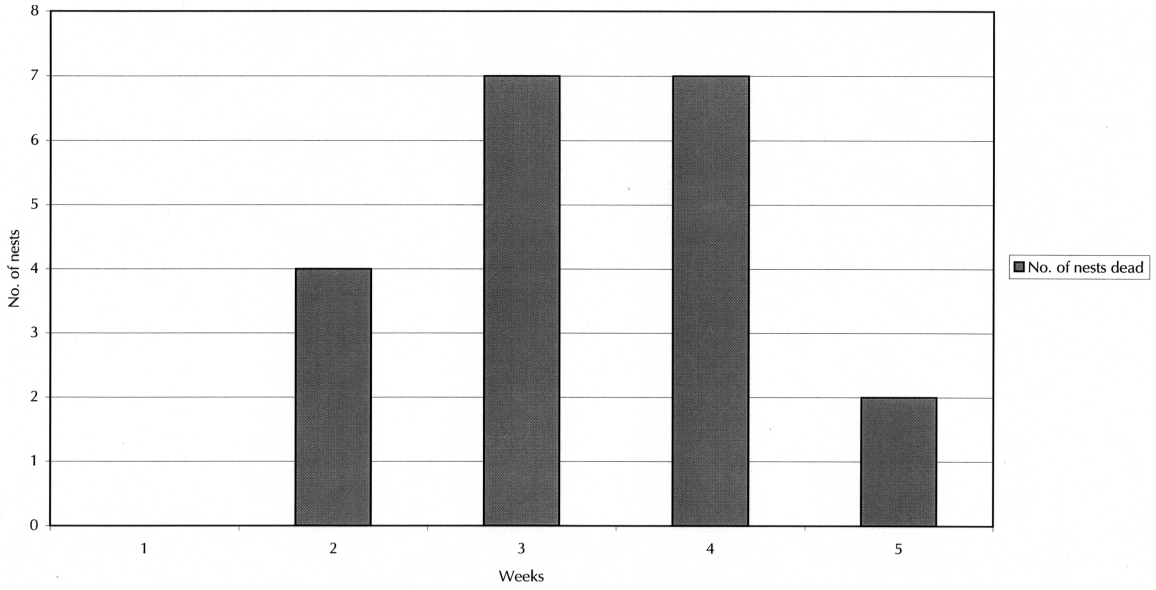

Figure 2. Time (weeks) to eliminate Monomorium pharaonis nests using reference standard MPAK in laboratory tests during 1998 (total of 20 bait stations).

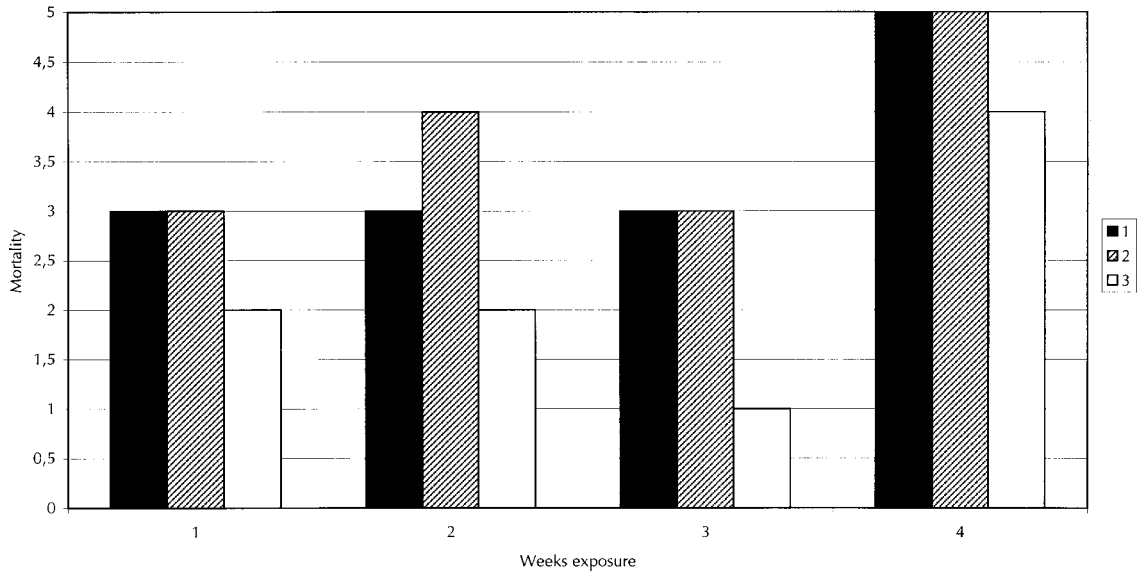

Figure 3. Mortality of Monomorium pharaonis exposed to three bait stations (MPAK) from the Czech Republic.

one of the baits collected from the site, clearly failed to eliminate the nest. One of the 6 baits collected from the other site also performed poorly, but was grade 4 at the end of week 4. All the baits from the unopened boxes (4 from one customer, and 6 from the other) reduced the nests to grade 4 within the first week, and all were dead by the end of week 4 of the bioassay.

Experimental baits

The influence of bait composition was clear in the comparison given in Figure 5. Using the same concentration of an experimental compound, performance was either excellent, when mixed with the honey + peanut butter + cockroach, or very poor, when mixed with one of the food sources normally provided to the colony as part of the standard rearing procedure.

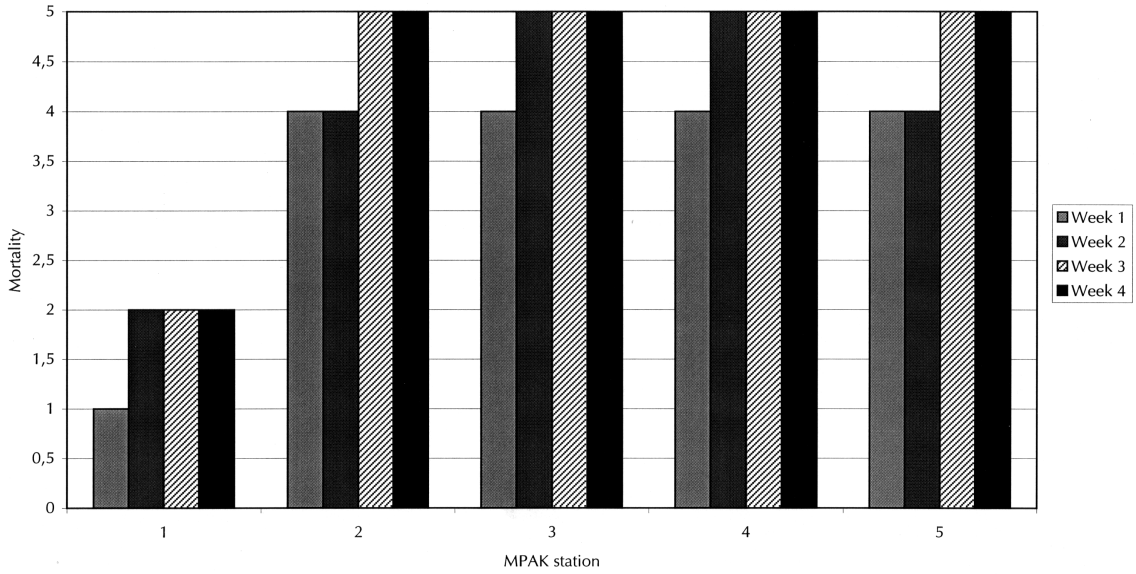

Figure 4. Mortality of Monomorium pharaonis exposed to five used bait stations collected from a site in Germany.

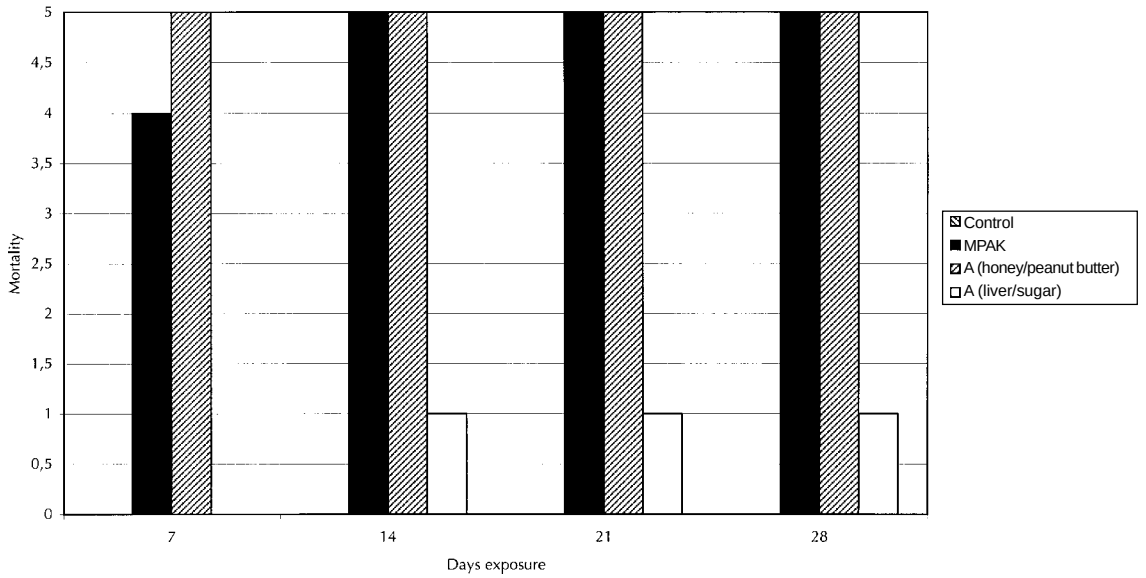

Figure 5. Mortality of Monomorium pharaonis colonies exposed to MPAK and an experimental compound (A) in two bait matrices.

DISCUSSION

During the course of the studies conducted over the past year, the reference baits have often eliminated the test colonies within 3 weeks, and very rarely take longer than 4 weeks to eliminate nests under test conditions (Figure 2). Therefore, while it is possible that ant and nest behaviour vary between nests and test dates, this variation does not appear to be substantial, and cannot be the sole, or even prime, explanation for poor results recorded in tests using MPAK from other batches, or returned from customers. Indeed, with the exception of the unusually slow speed of action of the baits from the Czech Republic (Figure 3), MPAK samples performed well when taken from their packaging. Those returned from field sites usually performed well, but with noticeable exceptions (Figure 4). This was not surpris-

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ing, given that they had been exposed to field conditions, some of which could have been detrimental to the bait.

Pharaoh's ants are known to feed on a variety of foodstuffs (Edwards, 1986) and the palatability of any bait is a key component of treatment success , independent of the inherent activity of the active ingredient. A clear example was demonstrated in Figure 5 where the same insecticide, presented in two bait formulations, at the same concentration, caused very different effects. Interestingly, the food mix that was a standard component of the diet proved to be a poor bait. This may reflect the foraging behaviour of workers if some essential nutrients are obtained from that food source, but most foragers visit the dead cockroaches or, alternatively, in this crude bait, the formulation or physico-chemical properties of the insecticide were inappropriate.

Since palatability is critical to bait performance, anything that reduces it may put treatment success at risk. In the field, contamination of a bait station with cleaning fluids, insecticides or other chemicals, could render it unattractive or repellent. Excess moisture or water entering the station could adversely affect some of the foodstuffs in the bait matrix. The same argument could apply to baits that have not been stored as directed, or have exceeded their shelf life. Hence, the rationale behind testing used and unused baits from the German sites (Figure 4). In those instances, freshly opened baits always performed satisfactorily.

Several significant operational factors were identified where control problems were investigated. In one instance, the manageress at an infested site had noticed ants congegating around bait stations, and then sprayed them with an insecticidal aerosol, thereby rendering them repellent to any future visitors. In others, the use of sticky pads placed in the centre of the base (for placement on vertical surfaces, for example) contrary to the recommendation to place pads at the edges. This may have impeded access to the bait by lifting the station above the substrate. Thinner pads, or double-sided tape are possible alternatives. A third feature, highlighted in some of the investigations, was the bait station density and the extent of the total area treated. In one instance, bait station density, upon investigation and inspection of the site, was only 1 per $30m^2$: well below the recommended 1 per 10 m^2 (or more for heavy or persistent infestations). When this was combined with reports that ants were noticed foraging outside buildings there was the possibility that pockets or sources of the infestation were not being treated thereby compromising the chances of treatment success.

Pre- and post-treatment monitoring are important components of any successful baiting program. Monitoring tools include peanut butter-baited white index cards (Oi *et al.*, 1994; Williams and Vail, 1994) and tubes containing liver (Lucas and Invest, 1993; Short *et al.*, 1993). These baits are used to identify the extent of the infestation, and can be used to demonstrate effects on the ants by quantifying reductions in foraging activity. Thus, Williams and Vail (1994) were able to detect the return of foragers to monitoring bait sites 4-6 weeks after the disappearance of foragers from those locations. Where ants are observed outside buildings, placing bait stations around the exterior of the premises may contribute, significantly, to control, even to the extent that indoor treatments are unnecessary (Oi *et al.*, 1994). Most importantly, the baiting program must encompass the infestation whether or not it extends beyond a structure so that the infestation does not become fragmented – small, scattered infestations being particularly difficult ot control.

The success of baits for cockroach and Pharaoh's ant control has, perhaps, lulled us into a false sense of security. These products are easy to apply – no mixing required, minimal protective clothing, ready-to-use etc. At the time Maxforce® was introduced in the UK, there was considerable scepticism about the level of control that could be achieved, but that was soon replaced with a high level of confidence based on the evidence of numerous cases where control was achieved. Indeed pest elimination (cockroach and ant), was demonstrated where there had been an history of persistent infestations (Lucas and Invest, 1993). The availability of bait stations as consumer products has, perhaps, implied that control is easy to achieve when, in fact, just as much professionalism is required in the correct placement and use of these as for any other pest control products. This is especially true of the control

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of Pharaoh's ants. A reproducible test to assay performance of Pharaoh's ant baits is now available, and in use, to assist the development and support of these products.

REFERENCES CITED

Edwards, J. P. 1986. The Biology, Economic Importance and Control of the Pharaoh's Ant, *Monomorium pharaonis* (L.). In Bradleigh Vinson, ed. Economic impact and control of social insects, New York: Praeger, pp 257-271

Lucas, J. R. and J.F. Invest. 1993. Factors involved in the successful use of hydramethylnon baits in household and industrial pest control. In: Wildey, K. B. and Robinson, W. H. eds., Proc. 1st Int. Conf. Insect Pests in the Urban Environment. pp 99-106

Oi, D. H., K. M. Vail, D. F. Williams, and D. Bieman. 1994. Indoor and outdoor foraging locations of Pharaoh ants (Hymenoptera: Formicidae) and control strategies using bait stations. Florida Entomol. 77:85-91.

Short, J. E., I. A. Grieg, and J. P. Edwards. 1993. A laboratory comparison of two baits as control agents for the Pharaoh's ant (*Monomorium pharaonis*) and evaluation of Hydramethylnon baits against a field infestation. In: K.B. Wildey and W.H. Robinson, eds., Proc. 1st Int. Conf. Insect Pests in the Urban Environment. p 477.

Williams, D. F and K. M. Vail. 1994. Control of a natural infestation of the Pharaoh ant (Hymenoptera: Formicidae) with a corn grit bait of fenoxycarb. J. Econ. Entomol. 87:08-115.