PHEROMONE ENHANCED BAITS FOR PEST ANT CONTROL: CURRENT STATUS AND FUTURE PROSPECTS

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Abstract—The utility of pheromone enhanced ant baits was investigated using fire ants as the model organism. The enhanced bait consisted of hydramethylnon (active ingredient), soybean oil (phagostimulant and AI solvent), defatted corn grit (carrier), and "invictolide", a component of the fire ant queen recognition pheromone. The major hurdles that had to be overcome in this work were the many variables in the field that contributed to the high variance in bait efficacy, which makes detection of differences between standard and treatment difficult. Consideration of these variables was essential in this work. The enhanced bait was discovered almost twice as fast as standard bait particles. In addition, controlled field studies using single mound treatments demonstrated that besides being discovered faster, more active ingredient was getting to each target colony, because more bait particles were discovered. Thus, this work has demonstrated that pheromone enhanced ant baits offer several advantages over standard baits, especially the potential reduction of insecticide usage. Opposing these advantages are considerations for commercialization, among these are shelf life and the economics of the new pheromone enhanced formulation.

INTRODUCTION

Fire Ants as a Pest and Model System.

The fire ant, *Solenopsis invicta* Buren, was accidently introduced into the United States in the late 1930's and currently inhabit over 150 million hectares in Puerto Rico and twelve southern states from Texas to Virginia (Lofgren, 1986a). It has also been reported in California, Arizona, New Mexico, and Maryland. Mature colonies may contain 250,000 workers and reach infestation rates of over 130 mounds per hectare. With proliferation of the polygyne form (multiple queen colonies) of *S. invicta* in the Southern states in the last decade, it is common to find many millions of fire ants per hectare existing in one super colony with hundreds of small mounds (Porter, 1992). Fire ants are highly aggressive when their nests are disturbed, which often results in painful stings to humans. Approximately 30% of the people in the infested areas are stung each year and about 1% of the population may develop hypersensitivity to the ant's venom and require some type of medical care (Adams, 1986).

The fire ant adversely affects the yields of several important agricultural crops (Adams, 1986). Soybean yield reductions are associated with fire ants feeding on germinating seeds, thus lowering plant density and causing crop loss of over $100 million. Other affected crops include corn, potatoes, eggplant, and okra. Fire ants seriously damage young citrus trees by girdling the trunk and feeding on newly set fruit, and other plant tissue.

The fire ant is an opportunistic omnivore and because of its large numbers (especially the polygyne form) it has a dramatic impact on wildlife (Porter *et al.*, 1988). The faunal diversity in infested areas is greatly diminished. Remarkably, fire ants appear to be attracted to electrical switches, chewing off insulation, and shorting out contacts in a wide variety of electrical equipment, from telephone switch boxes to air conditioning units (Mackay *et al.*, 1992).

This impact on agriculture and the public provides an exceptional driving force for the study of this pest ant and the development of control methods that have minimal impact on the environment. In addition, the fire ant exists in the United States in monogyne and polygyne population forms (many pest ants are polygyne), thus it is an excellent model for the control of other pest ant species. This is exemplified by the novel bait toxicants originally discovered for fire ant control and now used for the control of household pest ants and cockroaches, e.g. hydramethylnon, fenoxycarb, and sulfluramide (Lofgren, 1986b).

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Toxic Baits

The development of the bait toxicant concept was an important step in minimizing the use of the residual insecticides of the past and decreasing the amount of chemical applied to the environment today. Currently, baits offer improved control and use less insecticide than alternative mound drenches (see Williams, 1994). The use of toxic baits places special constraints on potential toxicants. It is essential that the insecticide exhibit delayed toxicity (less than 15% mortality after 24 hours), because only a small percentage of workers are engaged in foraging (<10%). There must be time for distribution of the toxicant/phagostimulant to all colony members. In addition, a toxicant must exhibit activity over a 10 to 100 fold range of concentrations, because during worker / worker transfer via trophallaxis the toxicant becomes diluted. The other bait components are a toxicant solvent/phagostimulant (usually soybean oil), and an inert carrier that absorbs the oil (a corn grit product). The carrier makes it possible to use commercial spreaders to distribute the bait (Williams, 1994).

Despite the positive aspects of baits, they do contain a general phagostimulant, soybean oil, and thus affect non-target ant species, as well as fire ants. Non-target ant species are important as competitors in the foraging arena and as predators of newly mated fire ant queens. In addition, the foraging arena is very complex and although the fire ant is an exceptional foraging ant, it discovers only a percentage of the bait particles. Data is presented hereon the more precise targeting of fire ant baits using attractant pheromones and some of the consequences of achieving this goal.

METHODS AND MATERIALS

Bait Discovery Bioassay

Experimental sites were fire ant infested grassy areas (>1/2 ha.), maintained through mowing. The monogyne/polygyne status of the fire ant population was determined by observation of mound characteristics, worker size distribution, search for queens, and by an aggression bioassay (Morel *et al.*, 1990). All sites were in the vicinity of Gainesville, Florida.

Blank pregel defatted corn grits were sieved through American standard sieves #10 (2.0 mm) and #12 (1.7 mm). Particles remaining in the #12 sieve were used for subsequent bioassays. The neat pheromone (racemic invictolide; Figure 1; Nitto Denko Co., Japan) was weighed and an appropriate amount of soybean oil/hydramethylnon (as used in standard bait formulation; American Cyanamid Company, Princetown, NJ) was added to give a solution containing 100 queen equivalents (QE) of invictolide in 0.61 μl. The amount of oil per grit was determined to be 0.61 μl and 1 QE of racemic invictolide is 8 ng. This solution was diluted with the soybean oil/hydramethylnon solution to give other concentrations. Then, 0.61 μl of the pheromone/oil formulation was applied to each grit via syringe, as needed. Treatments and hexane controls were prepared in groups of five and placed in separate vials. The contents of the separate vials containing the treatment and control were unknown to the observers.

Each bioassay arena consisted of two drop sites chosen directly in front of the observer, roughly 30 cm apart. One control and one pheromone treated bait particle were simultaneously placed in the selected sites without disturbance to the surrounding area. The positions of the two bait particles (treatment and control) were determined randomly. The time to first ant discovery was recorded for each pair of bait particles. The test was terminated after both particles had been discovered. If both particles were not discovered within 15 minutes the test was terminated. The process was repeated at other locations if needed to obtain the desired number of replicates.

Paired Single Mound Field Tests.

All test sites were pasture land with grazing cattle. The type of fire ant population present (monogyne versus polygyne) was determined as described above. Only monogyne population field sites were used in the work presented here.

Once a test site was found to be satisfactory, 30 to 40 mounds were surveyed by observing the mound size (height and diameter), then the population index (PI) of each colony was estimated by

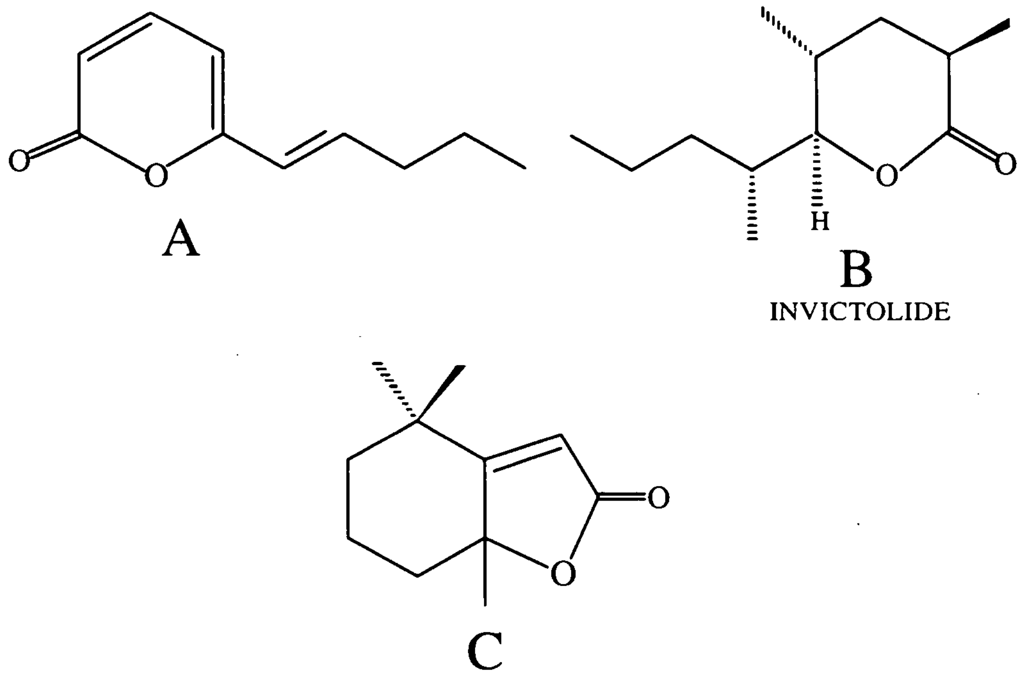

Figure 1. Fire ant queen pheromone components: A=(E)-6-(1-pentyl)-2H-pyran-2-one; B=invictolide; C=dihydroactinidiolide (Rocca *et al.*, 1983a,b).

digging up a portion of the mound (after Banks and Lofgren, 1991). The mound depth, mound size, and ant density were recorded, as were the presence or absence of worker brood, the presence or absence of sexual brood or alates, and general shape of the mound. This information and mound location was used to match mounds.

Baits were prepared using blank corn grits, hydramethylnon / soybean oil, and blank soybean oil (American Cyanamid Company, Princeton, NJ). The standard hydramethylnon-soybean oil solution was diluted to 33.3% active ingredient using the blank soybean oil. Half the 33.3% strength oil-concentrate was dosed with racemic invictolide (neat) at approximately 25 ng (3.33 QE) per bait particle. The oils were then added to the blank corn grits at a 20% (W/W) loading, blended thoroughly, and left to equilibrate at room temperature overnight or longer.

One of the mounds of a pair was assigned a treatment, standard or standard plus pheromone, by the flip of a coin. The second mound of the pair received the opposite treatment. Applications were made with a broadcast spreader. The target mounds became the center of a 15 m treatment square. Bait was applied to this area at a rate in agreement with the dose specified on the label (between 2 and 3 lbs per hectare). The pairs were treated at as close to the same time as possible, usually within 15 minutes of each other.

Mounds were checked at regular intervals after application to look for changes in the PI. Generally, evaluations were made after 3, 7, 10, 14, 17, and 21 days. Each mound was considered separately during the evaluation process (the PI of one mound in a pair was not linked with the other). Observations and PI determinations were made without knowledge of the treatment used. Eleven replicate pairs were set up in this experiment.

RESULTS AND DISCUSSION

The development of a bait formulation targeted specifically at *S. invicta* has many associated advantages. The basic premise is that addition of species-specific attractant pheromones to baits will enable *S. invicta* workers to find and retrieve significantly more bait particles (active ingredient), than with the non-enhanced bait. If this is true, the bait will be more effective because the fire ant will find more bait particles, and there will be fewer particles available for non-target ants and other organisms. Non-target ants are good predators of fire ant newly mated queens and less toxicant effects on them could result in slower reinfestation rates. What are the pheromone possibilities?

Fire Ant Pheromones

The following social insect behavioral responses are pheromone mediated: alarm, attraction, recruitment to a new food source or nest site, trail following, brood care, aid at molting, recognition of nestmates, caste regulation, control of competing reproductives, worker and sexual excitant during mating flights, and the rendezvous of male and female sexuals during mating flights (Vander Meer, 1983). We know little about some of these areas, for example, mating flight pheromones (sex pheromones, excitants, etc.) and caste regulation in fire ants. These areas have potential in control strategies, but they require much basic research. Assistance at molting and brood care may be pheromone controlled. However, the existence of brood pheromones has not been unambiguously demonstrated (Morel and Vander Meer, 1988).

Information on fire ant recruitment pheromone chemistry and associated behaviors is extensive. The Dufour's gland, attached to the base of the sting apparatus, produces the pheromone (Wilson, 1959). A worker lays a trail by releasing the Dufour's gland contents through the sting, which is periodically touched to the surface on which the ant is walking. We have reduced the recruitment process to three sub-categories: attraction, orientation induction, and trail following (Vander Meer *et al.,* 1988; Vander Meer *et al.,* 1981; Vander Meer *et al.,* 1990; Vander Meer, 1986). Behaviors elicited by "recruitment" pheromones are context dependent. For example, alarm, migration to a new nest site, and attraction to a distressed worker have been observed (Wilson, 1962). Most pertinent to the present work is the incorporation of the element(s) responsible for worker attraction to baits. The attractant associated with recruitment has two components, but preliminary studies show that at greater than physiological levels a single bicyclic homosesquiterpene component significantly attracts workers (Vander Meer, unpublished). The utility of this pheromone in integrated management of the fire ant awaits the availability of the compound through synthesis.

The poison gland of fire ant queens produces a worker attractant (Vander Meer *et al.,* 1980). The product is stored in the poison sac. The queen has control over the release of this pheromone through the sting apparatus (Vander Meer and Morel, 1995). Three components isolated from extracted queens elicit a wide variety of worker behaviors, including attraction (Glancey *et al.,* 1983). These compounds (Figure 1) have been identified and synthesized (Rocca *et al.,* 1983a,b; Mori and Nakazono, 1986a,b).

We have determined, using an olfactometer (Vander Meer *et al.,* 1988), that invictolide itself elicits significant worker attraction, as does the racemic mixture (available from Nitto Denko, Co., Japan; Vander Meer, unpublished). Reducing the number of components from three to one and determining that optical purity is not necessary, represent a major advance toward potential commercialization. Racemic invictolide was, therefore, chosen as the model pheromone to test the utility of pheromone enhanced fire ant baits.

Bait Discovery

The mean discovery time for the pheromone treated bait particles was 30 percent less than the discovery time for the standard bait particle. The data were not normally distributed and were log transformed. Comparison by paired t-test showed the differences to be significant (D.F. = 104; $t_{2.64}$; $P<0.005$). This is graphically presented in Figure 2. Pheromone enhanced bait particles were discovered first over 66 percent of the time (Figure 3). Pheromone concentrations of ten and one QE also gave a significant reduction of discovery time. These results demonstrate that addition of the pheromone to a bait particle significantly decreases the time it takes fire ant workers to discover it, even at one QE/particle.

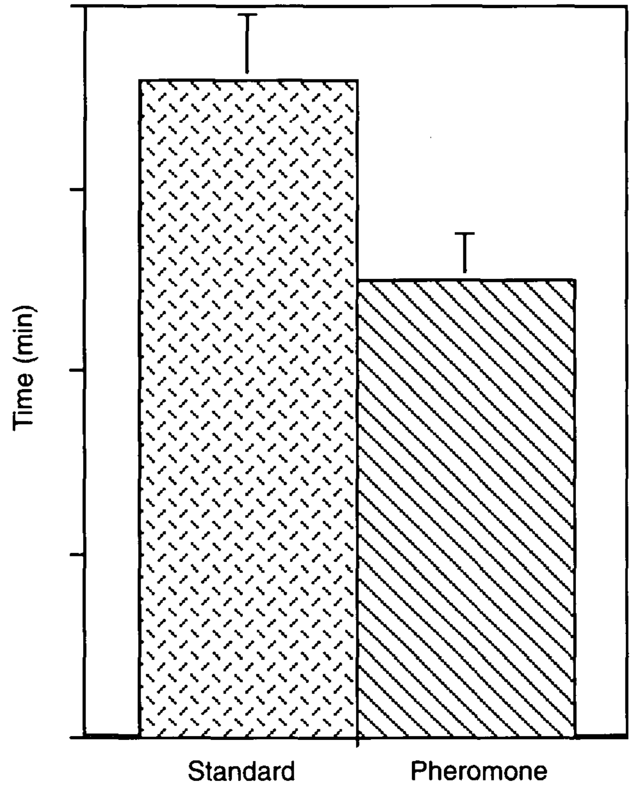

Figure 2. Mean discovery time and SE for hydramethylnon bait particles with and without the addition of the queen pheromone, invictolide.

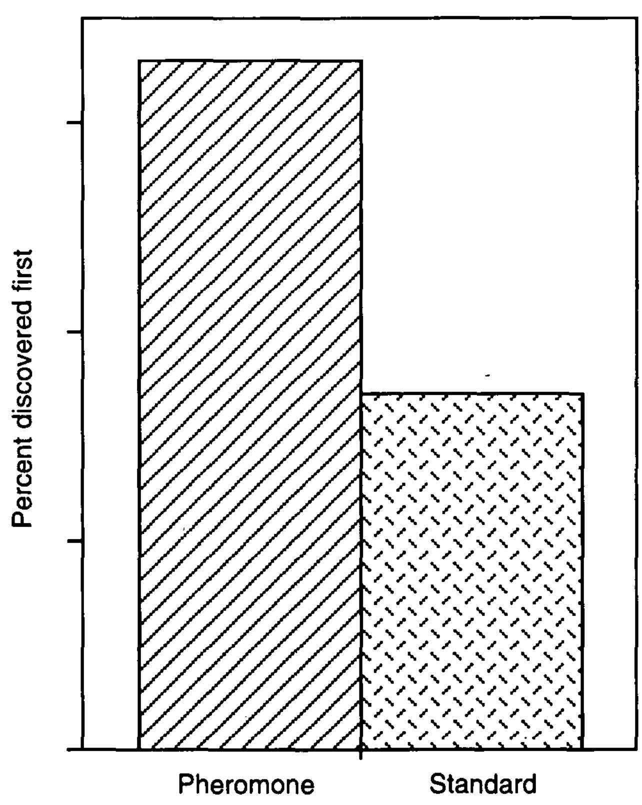

Figure 3. The percent of the time that fire ant workers found the pheromone treated or untreated hydramethylnon bait particle first.

Hydramethylnon Concentration/Activity.

In a previous study (Vander Meer, unpublished), laboratory fire ant colonies were fed precise amounts of hydramethylnon in soybean oil, based on their colony size. The amount of AI available to each worker was calculated using the following information: A. maximum field populations of 60 mature colonies per 0.5 hectare; B. worker populations of 200,000/colony; C. commercial bait (AMDRO) oil loading of 20%; D. The oil contained 4% hydramethylnon; E. 454 g of bait recommended per 0.5 hectare; and F. There are ca. 460,000 grits per 454g of bait. Replicate sets fire ant colonies received 100, 33.3, 10, or 3.3%, of the maximum estimated hydramethylnon dose and mortality was monitored over time. The results provide an envelop of mortality/time lines that depicts the results if colonies get 100% of the AI presented to them. For colonies of the same size, the difference in the mortality curves is strictly dependent on the amount of AI received. This concept and logic can be applied to the paired single mound field tests.

Paired Single Mound Field Tests.

One of the major hurdles in this work was the development of a rapid application method that could easily be switched from one treatment to another. The objective was to treat two mounds paired by Population Index with treatment and standard within as short a time as possible. This was accomplished through the adaptation of a small hand pushed spreader to effectively put out the required rate of 1 lb/acre (26.1 g/225 m^2). The two plots making up a pair were treated within 15 minutes of each other except on rare occasions when the paired mounds were treated within 30 minutes. The technique of matching treatment and standard pairs plus close to simultaneous applications, allow a pairwise statistical analysis. This means that individual replicate pairs are independent of each other, effectively eliminating the day and time of day variables.

The choice of 33.3% of the standard amount of active ingredient was based on our previous laboratory generated dose / activity studies. The intent was to make differences in the AI received more evident, e.g. Figure 5, the difference between 10 and 33% is greater than 33 and 100%. All field tests were carried out with twice weekly evaluations. A dramatic decrease in the target mound PI was observed by 21 days.

The results are shown in Figure 4 and are from eleven paired replicates. Pheromone enhanced bait was significantly better than standard at three of the six evaluation times. In all cases, pheromone enhanced bait had a lower mean percent remaining population. The percent difference between the pheromone enhanced bait and standard ranged from 8.6 to 23.3 percent.

If the pheromone enhanced bait was not effective, then it would be expected that for the individual replicates over the six evaluation periods there would be ties and an equal number of cases where enhanced and non-enhanced bait gave better results. Out of the 66 replicate evaluations 48 were in favor of the pheromone enhanced bait and only eight were in favor of the non-enhanced bait. Chi-squared analysis showed this result to be highly significant (X^2=17.857; DF=1; =0.0001).

For the field results it is possible to estimate the amount of AI received by the target colonies by comparison with results obtained in the controlled laboratory studies mentioned above, Figure 5. In the paired single mound studies presented above, we do not know how much AI the target colonies received. However, the application rate would give the target colony the full 33.3% AI expected if they received 100% of the oil and AI absorbed into the bait particles. We know from other laboratory studies that workers are only able to extract 85–90% of the oil from grit particles. Thus, the maximum obtainable AI is 28–30% assuming 100% bait recovery. A direct comparison of the laboratory (result if 100% of AI is consumed; theoretical) and field experiments (actual) is informative.

Figure 5 shows an overlay of the laboratory results for 100, 33.3, 10, and 3.3 percent AI, along with field results using 33.3% AI and pheromone enhanced 33.3% AI bait used against single monogyne colonies. The general shape of the population decline matches that found in the laboratory study. The difference between pheromone enhanced and non-enhanced bait indicates that the fire ant workers are finding more pheromone enhanced bait particles than non-enhanced bait particles and therefore get a higher dose of hydramethylnon than the colonies treated with non-enhanced bait.

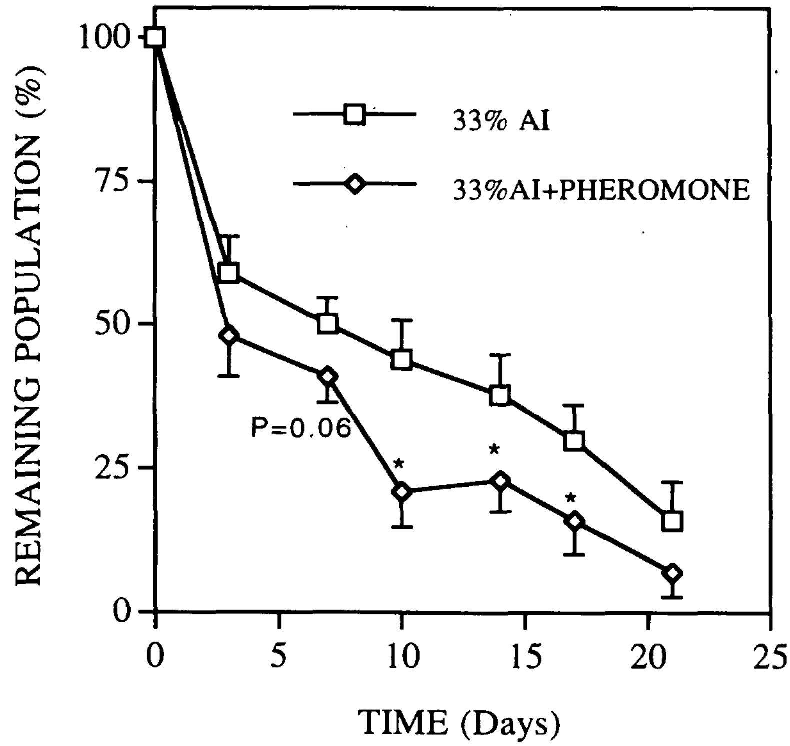

Figure 4. Percent surviving population versus time after treatment for 33.3% hydramethylnon bait with and without racemic invictolide (3.3 QE/particle). A * indicates significantly different (P < 0.05) paired results for the indicated time periods.

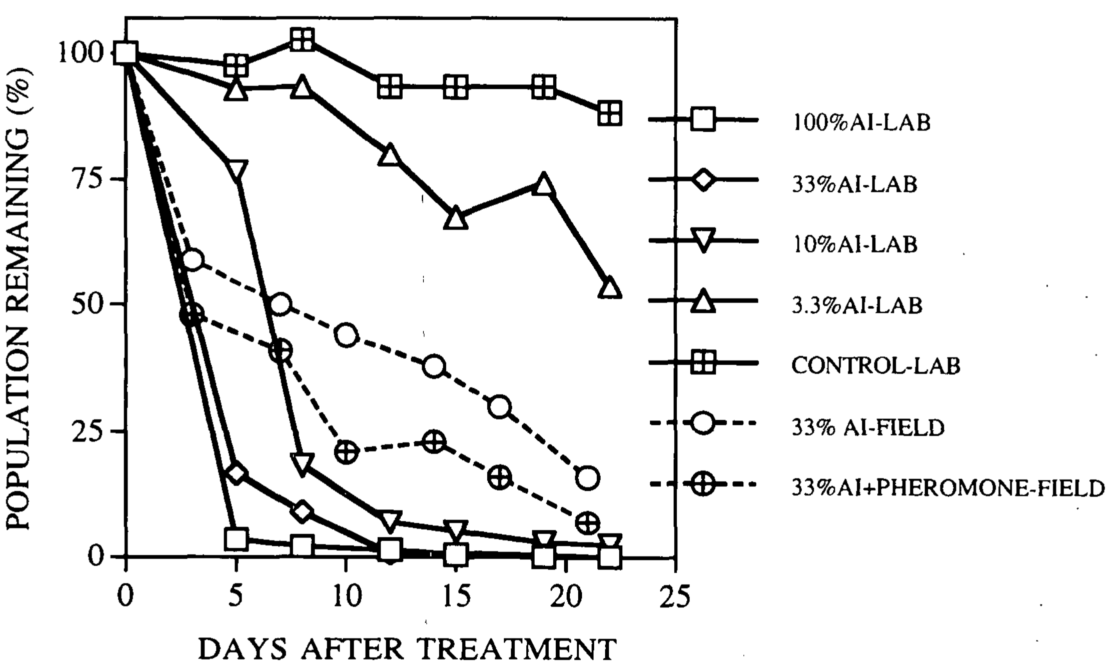

Figure 5. Comparison of laboratory dose/activity results (100, 33.3, 10. and 3.3 % AI) and paired single mound field data using the equivalent of 33.3% AI bait and 33.3% bait+3.3 QE Invictolide/particle.

We demonstrated that addition of racemic invictolide to fire ant bait particles significantly increased the speed of fire ant bait discovery. It was hypothesized that increased speed of discovery means that through bait attraction, more of the bait particles will be found by the fire ant, leading to greater efficacy and fewer effects on non-target ants. The data presented above have demonstrated, for the first time, increased efficacy of pheromone enhanced fire ant bait. In addition, the data provide evidence that the fire ant is not as effective at discovering bait particles as previously thought and that addition of pheromone increases the bait discovery process to the point where significant differences in efficacy can be observed. With the increased efficiency of bait discovery and retrieval by the fire ant must come the added benefit of increased species-specificity, since fewer bait particles will be available for non-target ant species. The comparative effects of pheromone enhanced versus non-enhanced bait on nontarget ant species must be addressed in separate carefully designed experiments.

Although there are measurable advantages to pheromone enhanced baits the actual increase in efficacy (taking a good bait, >90% and making it ≤10% better) is not great. Thus, marketing advantages, economics, and shelf life are some of the commercialization decision making considerations. Currently, invictolide is a costly bait additive; however, we are continuing laboratory and field studies with other fire ant pheromones and formulations that may make the balance sheet more favorable toward commercialization.

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