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CONTROL OF FIRE ANTS IN CALIFORNIA ALMOND ORCHARDS

A Final Report Submitted to The Almond Board of California

by

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ABSTRACT. The objective of the study was to formulate a microbial pesticide product, develop a delivery system and to conduct a field test in a California almond production system. The microbial agent evaluated as a biological pesticide for the southern fire ant, *Solenopsis xyloni*, was the fungus *Beauveria bassiana* (strain 447). Results from our laboratory tests conducted in California showed that the *B. bassiana* 447 is extremely virulent for the southern fire ant. By 7 days following treatment all ants treated with fungus were dead and more than 95% of these ants produced viable spores within 4 days.

Seventy combinations of attractants/inert materials were offered to *S. xyloni* in lab tests. Results showed combinations including oils, almond, rice, corn grit and silicates were adequate for delivery of fungal spores to ants. Small *S. xyloni* colonies were exposed to the best combinations mixed with *B. bassiana* conidia and showed 89.6% mortality within 14 days.

The formulation which gave the best result in laboratory experiments was used in further experiments in the field in California. In the first field experiment on Paramount Farms in Shafter, California in March 1994, four fungal formulations and four different methods of application were tested in small plots of almond trees. In plots treated with fungus, levels of ant infection rose to a maximum of 20%, 3 to 5 days after application. An almond based bait gave best results in terms of reduction of ant activity at tuna baits and in terms of lower damage to almonds placed on the ground. Application of bait in rings around ant nest openings was chosen as the method for the second field experiment.

The second test, conducted in May and June 1994 at Paramount Farms, compared control of southern fire ants using 4 different products: Lorsban, Amdro, Logic and a formulation of a microbial pesticide containing fungal spores. The bait with fungus was foraged readily by fire ants and fungal infection of ants within nests was observed within less than a week after treatment. With a single fungus treatment, more than 50% of the fire ants in the nests were killed within 4 weeks after treatment. However, this level of population reduction was not sufficient to preclude damage to almonds placed on the ground. None of the treatments gave good enough control of fire ant populations to eliminate the risk of severe damage at harvest time. Lorsban appeared to have more of a foraging repellent effect than to reduce fire ant populations. The chemical baits were foraged and had some effect in reducing fire ant populations. Fire ant populations must be reduced to lower levels than were observed in this study. Perhaps multiple applications of the fungus could achieve this. The results suggest that an effective microbial pesticide using fungus as the active biological control agent can be developed for southern fire ant in almonds.

INTRODUCTION

The intent of this study was to provide research results as an initial step in the development of an effective non-chemical pesticide to be used as a biological control for fire ants in California almond production systems. Some chemical baits already commercially available but not registered for use in almond production systems were tested but the primary objective of the research was to collect information necessary to evaluate the potential of a microbial product for biological control of fire ants in California almond production systems. The microbial agent evaluated as a biological pesticide was a strain of the entomopathogenic fungus Beauveria bassiana (strain 447). The fungus is not known to be toxic to mammals and offers a potentially safe and environmentally desirable biological control alternative to currently used chemical pesticides.

The species of fire ant that occurs in California almond orchards is a native species (Solenopsis xyloni) different from the introduced fire ant species that are pests in the southeastern USA and those native to South America. However, the strain of fungus isolated from South American fire ants does kill the species found in California almond orchards. Results from our laboratory tests conducted in California showed that B. bassiana 447 is extremely virulent for the southern fire ant, S. xyloni. This result suggested that an effective microbial pesticide using fungus as the active biological control agent can be developed for southern fire ant in almonds if the fungus could be infective under environmental conditions in areas of California where almonds are produced. These conditions are quite different than the field conditions under which the fire ant fungus had been tested in Florida and Brazil. A formulation of the biological pesticide and an appropriate delivery system must be developed specifically for fire ant control in California almonds. Therefore, the primary objective of this study was to formulate a microbial pesticide product, develop a delivery system and to conduct a field test in a California almond production system.

SUMMARY OF TESTS OF FUNGUS AGAINST FIRE ANTS IN CALIFORNIA

TEST 1: Initial Laboratory Bioassay

In November 1993, an experiment was done to test whether a formulation of the patented strain of fungus (B. bassiana 447) developed in Florida for biological control of the red imported fire ant, Solenopsis invicta, could infect, kill and reproduce on the Solenopsis species that is a pest in almond orchards in California. The test was designed by the principal investigator and carried out in cooperation with John Evans of CIPM and Doug Blair of Paramount Farming Company.

Approximately 100 southern fire ant workers were collected from each of 5 nests at 3 different locations in a heavily ant-infested almond orchard on the property of Paramount Farms. The almond trees were planted in the orchard 3 years prior and were producing some nuts but not at economically profitable levels. This orchard had not been treated with any pesticides for control of fire ants. Ants from 3 nests at each collection site were treated in the laboratory with a powder containing fungal spores and ants from 2 nests/site were used as a control (check) and were treated with only inert ingredients (corn starch, no fungus). Groups of about 100 ants were treated in small plastic cups and then transferred to artificial nest cells held within plastic containers. Dead ants were removed daily and placed into a separate plastic cup for each treatment and stored in the freezer.

By 7 days following treatment all ants treated with fungus were dead. After 9 days, all ants, including those in the controls were frozen and shipped to Florida, where they were surface sterilized and placed individually into micro wells and incubated. Ant cadavers were observed daily and all ants showing signs of B. bassiana fungal growth and sporulation were recorded as confirmed infected ants.

For the ants that were treated with fungus and died, more than 95% of the ants produced spores within 4 days of death. In the control (check) only a few ants (less than 1%) showed signs of fungal infection. These results clearly demonstrated that the patented strain of fungus for fire ants in Florida is extremely virulent for fire ants infesting almond orchards in California and that field tests of fungal formulations of the biological pesticide should be carried out.

TEST 2: Development and Evaluation of Bait Formulation in the Laboratory

To formulate a microbial pesticide for southern fire ant using fungus as the active ingredient, we needed to determine food components that would be foraged by S. xyloni. Southern fire ant colonies from Paramount Farms were collected in December 1993. The colonies were prepared by digging them up with soil, then extracting the ants from soil with a water drip technique. Extracted ants were placed in plastic cups containing cut pieces of moist foam to maintain humidity and serve as anchorage material to prevent excessive shaking of the ants during transportation. In the lab ants were transferred from the plastic cups into plastic trays normally used for rearing of ants in the laboratory. Several food materials including pureed vegetables and fruits, honey-agar, almond, almond paste and insects were offered to the different ant colonies. Colonies nested in artificial nest cells and began feeding within a short time.

Several attractant/inert combinations already selected for use with the red imported fire ant were offered to S. xyloni in choice tests. New combinations including some almond products likely to be attractive to the southern fire ants were also tested. Small colonies (0.5 g of workers) were established in trays with a nest cell and water dish. One to two days was allowed for the ants to get settled in the new environment before the experiment was initiated. Ants were offered 0.1-0.5g of different formulations on paper squares and the number of ants on each of the squares was counted at regular intervals for 24h.

Preliminary tests were conducted with several materials and those that triggered a positive foraging response by the ants were further tested in combination with other materials. A total of more than 70 combinations of attractants and inert materials were tried. Results demonstrated that the combinations including oils, almond, rice, corn grit and silicates were adequate for delivery of fungal spores to ant colonies.

The red imported fire ant has been attracted to baits with up to 20% of B. bassiana conidia, but higher concentrations of the fungus can lead to repellency of the ants and consequently lower mortality. A formulation containing 10% of B. bassiana conidia was used in our tests against the red imported fire ant and gave good results so we used a 10% concentration of conidia in the bait formulations for tests with the southern fire ant. Laboratory tests showed that this concentration was not repellent to S. xyloni and that foraging appeared to be normal.

Small S. xyloni colonies (30 workers) were exposed to three best attractant/inert combinations mixed with 10% B. bassiana conidia. Each colony received 0.05 g of one formulation, and 2 colonies were used per treatment. Three controls, one for each type of formulation, were used. Cadavers were removed and counted 5, 7, 14, 21, and 28 days after application of the treatments. At the end of the experiment, surviving ants were counted and cumulative mortality was calculated. The cadavers were surface sterilized, plated in micro-titer plates, and incubated under high humidity conditions to allow growth of the infecting fungus. After 7-10 days, ants showing signs of B. bassiana infection were counted and fungal infection was calculated for the different treatments. The best formulation gave 60.9% mortality after 7 days of exposure of the bait to the ants, with 62.2% of cadavers showing signs of Beauveria infection. On day 14, cumulative mortality was 89.6% .

The formulation which gave the best result in this laboratory experiment was chosen to be used in further experiments in the field in Florida and in California. This formulation is a starchy material to which oil attractants were added. To this basic formulation 10% of _B_. _bassiana_ conidia were added. The final formulation was a dry bait powder which is attractive to fire ants and can be easily carried into the nests by workers. This formulation will remain dry for a long period of time in the field even when exposed to moist soil and dew. Variations of this basic formulation, with the substitution of the attractive ingredient were used in later field studies in almond orchards.

TEST 3: Preliminary Field Evaluation of Powders, Baits and Delivery Systems

To test several application methods and delivery systems with fungal formulations developed for southern fire ant, we conducted preliminary field tests in Florida and then in California.

A) Field tests in Florida

Field tests in Florida with the southern fire ant could not be carried out because the species is not present in the state. Therefore, our Florida field tests of initial formulations and delivery systems were conducted using naturally occurring field colonies of the red imported fire ant, _S. invicta_.

Prior to beginning of the experiment, the field to be treated was scouted and the fire ant nests were flagged. Twenty-four nests were selected in a large field. Pre-treatment samples were taken to obtain baseline data on ant infection within fire ant nests and levels of ant activity.

The bait used in the field test consisted of 10% _B_. _bassiana_ conidia mixed with rice, oils and a synthetic silicate base. The bait was a powder and was applied manually in the field. Doses of the baits corresponded to a maximum of 10g of active ingredient per nest.

Three application techniques were tested: a) small quantities of the bait applied in a grid pattern around nests (Stations); b) same amount of bait but distributed in lines between nests (Lines); c) baits applied to form a ring surrounding the nest mound (Ring).

Acceptance of the bait was not evaluated due to weather conditions during the application of the bait and subsequent days during which a cold front maintained the temperature in the Gainesville area below 50°F during the day for 3-5 days following the application of the bait. The cold temperatures deterred fire ants from foraging the bait. The ants stayed in their nests to avoid contact with cold air during most of the 5-day experiment. Ant activity was minimal following the application of the bait to the field, and rain storms washed away some of the bait formulation. Because of low ant activity and cold weather, ant samples did not show any difference in infection level among the treatments. However, we were able to make some important observations of how the bait formulation persisted in the field and we found that the viability of the spores was good despite exposure to cold, sunlight and rain.

Before application in the field, more than 90% of the conidia were viable in the formulation. After 1 day of exposure to the climatic factors in the field, just a very slight reduction of viability of the material was observed in the formulation on top of the bait piles and to a lesser extent on the material at the center of the piles. After 6 days of exposure in the field, the viability of the material exposed to the sun and dew/rain on the top of the formulation piles was about 55%. Material protected at the bottom of the bait pile had viability almost as high as when applied in the field. Material at the center of the pile had intermediate viability. Survival of conidia in these piles in the field was good considering the incidence of sunlight directly on the exposed top of the formulation piles left in the field. Survival of conidia was even better at the bottom of the piles which were not affected by sunlight or extreme moisture.

These results demonstrate that even after exposure to rain, dew and sunlight, the B. bassiana conidia in the bait formulation were able to survive for a prolonged period of time in the field. Temperature and humidity conditions were not monitored quantitatively but the bait was in direct contact with moist soil and/or vegetation, and exposed directly to the sun. Due to the semi-hydrophobic nature of the bait formulation, rain could only wet the material that was directly exposed. Material at the center and bottom of the pile remained dry during the observation period. Conidial germination was not directly observed in the bait formulation in the field, but it is unlikely that it would have occurred in the dry formulation.

B) First field test in California

The first field test of fungal formulations and delivery systems in California with fire ants in almond orchards was expected to be initiated in February 1994, but due to winter weather conditions and other delays, experiments were not initiated until late in March 94. Four fungal formulations and four different methods of application were tested in small plots of almond trees (50-150 m^2 per plot) to determine how southern fire ant reacts to the fungus in the field. Tests with commercially available chemical baits (Logic & Amdro) were not tested in the first experiment and were used only in the second experiment described later in this report.

Experiments were conducted during the months of March and April at Paramount Farms in Shafter , CA with the objective of comparing the control of southern fire ants in almond orchards using 4 different formulations of B. bassiana conidia: a) Bait 1 with oils as attractants; b) Bait 2 with almonds as the attractant; c) Bait 3 with tuna fish as attractant; and d) a dry powder formulation without any attractant.

Two fields (Paramount Farms Ranch numbers 304 and 388) were used in this experiment. Field 304 is a young orchard (3rd year), not yet in commercial production. In this field there was a dense fire ant population distributed throughout the soil but nests were not well defined . Field 304 has a sprinkler irrigation system which seemed to be the most favorable for ant infestations. Field 388 is a mature orchard with a flood irrigation system. The fire ant population was less dense than in 304. In field 304 all types of formulation were used, while only bait 1 and the powder formulation were used in field 388.

In field 388, formulations were applied as rings around ant nest openings identified on the surface of the soil, on the berms and close to tree trunks. The control formulation consisted of the inert powder used in the dry powder fungal formulation. One hundred grams of the formulations were applied per nest/plot, in circles surrounding all the nest openings found in an area of ± 3.5 m radius from the main nest excavation area, which was marked with numbered flag.

In field 304, plots of 150 m^2 contained 4 trees in 3 rows. The plots were 10 m along the tree row direction (north/south) and 15 m across the rows (east/west). The central row of trees in the plot hat 2 trees within the treatment area, whereas the exterior rows had only 1 tree each. Plots were separated from each other by at least 60 m in any direction. Two application methods were used with the bait formulations and two with the powder formulation. The bait formulations were applied as: a) Stations, piles of formulation applied manually with spoons along lines parallel to the tree rows in the plots; or b) Lines, continuous lines of bait formulation applied with a line chalker along lines parallel to the tree lines in the plots. The powder formulations were applied as: a) Broadcast, uniform coverage of the treatment plots with powder formulation applied with fertilizer spreader, b) Grid, a grid of lines (applied with lime chalker) of the powder mainly along the tree rows but with lines crossing perpendicularly to the tree rows. The control plots in this field were treated with formulation similar to Bait 1 but without any fungal material. In these plots the material was also applied as stations along the tree lines.

At different time intervals before and after the application of treatments, live ants were collected from the populations found in the experimental plots. These ants were freeze-killed, surface-sterilized and samples of ± 100 ants were plated into micro-titer well plates. These plates were incubated at 25°C for 7-15 days. After this incubation period, and using microscopic observation, ants were scored as having or not having signs of Beauveria sporulation. Ants presenting these signs were scored as having been killed by the fungus and a percent infection was calculated in relation to the number of ants plated.

Before and after the application of the treatments, tubes containing a mixture of 50% mashed canned tuna and 50% 9- Lives Chicken Dinner cat food were placed in each of the treatment plots. Four tubes were used in plots in field 304, and 2 tubes in field 388 . These tubes were collected after 4 hours and the number of southern fire ant workers in each of the tubes was counted.

After the application of the treatments, samples of 10 undamaged almonds were placed under wire cages in each of the treatment plots for a period of 24 hours, following the removal of tuna bait tubes. Four samples were used in plots in field 304, and 2 samples in field 388. After this period, the almonds were collected and the damage caused by ant feeding was rated. Almonds were classified as undamaged, with moderate damage (less than 30% of the meat taken) and severely damaged (more than 30% of the meat taken).

1) Infection of live southern fire ants in plots treated with fungal bait formulation.

B. bassiana infection in ants collected in the control plots was high and followed trends observed in the treatment plots. This was probably due to the contamination of sample collection equipment and some degree of foraging of the control ants in the treated plots. Also, in field 388, one day after application of the fungal formulations, the field was treated with liquid fertilizer applied with atomizer. This equipment produced a great deal of wind in the almond orchard and caused the treatment materials to be blown across plots.

In the fungus-treated plots, levels of infection rose to a maximum slightly higher than 20%, 3 to 5 days after application of the fungal formulations and declined afterward (**Figures 1 and 2**). The powder formulations caused a faster increase in the infection rates whereas the infection in plots treated with bait formulations reached a peak 1-2 days later than that reached in the plots treated with powder formulations. In bait plots in field 388, infection rates were still high (above 10%) 14 days after application of the treatment, while in other treatments and in field 304 infection of live ants was almost not detectable.

2) Foraging southern fire ants in tuna traps in treatment plots prior to and up to 2 weeks after treatment.

In field 388, low levels of ant activity both in the tuna baits and the almond samples prevented detection of any differences among the treatments. In field 304, levels of ants at tuna traps after the application of the treatments were lower than that before the treatment for all treatments except Bait 1 in lines (**Figure 3**). In the control plots, ant activity as measured by number of ants at tuna baits almost doubled after treatment in relation to levels observed prior to treatment. Greatest reduction of ant activity was caused by powder treatments, which besides the mortality caused in the ant population also caused a lower activity due to a somewhat repellent effect of the presence of the powder on the surface of the soil. Reduction in ant activity observed in the bait plots was a result of reduction in the ant population because the fungal formulations in these cases are attractive and not repellent to the ants.

3) Proportion of almonds not damaged by southern fire ants in treatment plots 1 and 2 weeks after treatment.

The proportion of undamaged almonds in the samples exposed to foraging ants varied greatly among the samples, due of the low level of ant activity observed. In the control plots, almost 80% of the almonds were undamaged, whereas in the best treatments (Bait 2 stations and powder grid and broadcast) close to 90% of the almonds were undamaged (**Figure 4**). Bait 1 had the worst performance in terms of almond damage, with 40-50% of the nuts damaged in plots treated with this formulation.

4) Conclusions from first California field test.

The first field test served as a initial experience of the field situation in almond orchards in California. Many observations on the biology and behavior of the ants were made and these served as basis for future experimentation and development of baits and application. Although ant activity was not at levels that would allow better separation of treatment effects the results demonstrated an apparent superiority of Bait 2 (almonds) both in terms of reduction of ant activity at tuna baits and in terms of almond damage. Based on these results and the observations from this field experiment, Bait 2 and the application method of rings around the nest openings were chosen for future experiments as described below.

TEST 4: Field Evaluation of Fungal Formulation and Various Chemical Treatments

To compare levels of fire ant control achieved with various baits (chemical and biological) in California almond orchards, we conducted a second field test at Paramount Farms near Shafter, California. This comprehensive test conducted in California almond orchards was a field test of the "best" fungal formulation (bait) in comparison with commercially available chemical baits (not registered for use in almonds) and Lorsban liquid (which is currently registered). Field plots of almond trees were larger than in the first field test and the timing of the initiation of the test was selected for a period when the fire ants were more actively foraging.

Experiments were conducted during the months of May and June 1994 with the objective of comparing the control of southern fire ants in almond orchards using 4 different products: Lorsban (applied at 6 pints/acre), Amdro (1.5 lb/acre), Logic (1.5 lb/acre), and 26.4 lbs of a formulation of a microbial pesticide containing 2.64 lbs of B. bassiana conidia. The liquid insecticide (Lorsban) was applied with a spray rig mounted on a tractor and the application methodology that is normally used for application of this insecticide in almond orchards. The granular baits (Amdro and Logic) were applied over the entire area of the treatment plots with a hand-held spreader. The fungal formulation was applied manually to form rings around fire ant nest openings which could be located on the surface of the soil in the treatment plots.

At different time intervals before and after the application of treatments, live ants were collected from the nests found in the experimental plots. These ants were freeze-killed, surface-sterilized and samples of ± 100 ants were plated into micro-titer well plates. These plates were incubated at 25°C for 7-15 days. After this incubation period, ants were scored for signs of Beauveria sporulation. Ants presenting these signs were scored as having been killed by the fungus and a percent infection was calculated in relation to the number of ants plated.

Before and after the application of the treatments, 4 tubes containing a mixture of ±50% mashed tuna and 50% 9-Lives Chicken Dinner cat food were placed in each of the treatment plots. These tubes were collected after 4 hours and the number of southern fire ant workers in each of the tubes was counted.

After the tuna bait tubes were removed, 10 samples of 10 undamaged almonds were placed under wire cages in each of the treatment plots for a period of 48 hours. After this period, the almonds were collected and the damage caused by ant feeding was rated. Almonds were classified as undamaged, moderately damaged (less than 30% of the meat taken) and severely damaged (more than

30% of the meat taken).

A) Infection of live southern fire ants in plots treated with fungal bait formulation.

B. bassiana infection in ants collected before treatment and ants in the control plots was extremely low (less than 1 %) at all times. In contrast, in the fungus-treated plots levels of infection rose to a maximum of 30%, 3 days after application of the fungal bait and declined thereafter (**Figure 5**). Infection levels in the southern ant population were still above the background (control) level 28 days after the application of the fungal bait. Although the infection level one month after application was only 2%, this demonstrated that the fungus was still producing effects in the ant population, and under favorable conditions, infection levels could possibly rise again.

Although, live ant infection levels serve as an indication of how the fungus is affecting the ant population, it is probably an underestimation of the true effect of the fungus. Only ants that respond to disturbance are collected in samples used for infection estimation. Ants which are too sick to rush to the nest entrances when nests are disturbed are therefore not included in the sample. This would explain the sudden drop in the detected infection levels after the peak infection on day 3. As the fungal infection progresses in the ants, more ants become unable to respond to the disturbance and there is an apparent rapid drop in the infection rate. This sudden decrease in infection rate observed in the field populations coincides with the 4th-5th day peak in mortality observed in the laboratory experiments. It is reasonable to assume that infection rates in the field populations treated with B. bassiana are higher than indicated by the infection rate estimates. However, an unbiased sample of the ant population which would give a better estimate of the infection rates would probably require opening the nest and causing a disturbance in the population which would interfere with the results of the experiments.

B) Mortality of southern fire ants in nests treated with fungal bait.

In order to estimate cumulative ant mortality based on the infection data collected in the fungus-treated areas, the following assumptions were made:

1. Only infection rates on days 3, 5, 7, 14, 21 and 28 after treatment are used.

2. Ants found to be infected on any day will die within 1 day, therefore ants collected two days later represent ants which have acquired the fungal infection within the last 2

days.

3. Mortalities (based on infection rates) are applied on the surviving population after mortalities for previous days have been applied to the ant population.

Based on these assumptions, the estimated total ant mortality in the fungus-treated area reached more than 50% during the 4-week period that the populations were monitored (**Figure 6**). Mortality increased very rapidly during the first week after application but rate of mortality decreased afterward.

C) Foraging southern fire ants in tuna traps in treatment plots prior to and 4 weeks after treatment.

Levels of ants at tuna traps before the application of the treatments were low due to unusual weather (rain and cooler temperatures) at the time the experiment was initiated (**Figure 7**). One week after treatment number of ants trapped increased in the control, fungus and Logic plots, whereas numbers decreased in the Amdro and Lorsban plots. In these two treatments, ants trapped in tuna bait tubes remained low for two weeks and started to increase again 4 weeks after treatment. Number of ants in tuna traps were higher for the other treatments but started to decrease 4 weeks after treatment.

In the Lorsban and Amdro plots, although ants were not coming to the tuna traps in large numbers, when the nests were disturbed, large numbers of ants would come to the soil surface. Extra tuna traps were put in the plots after disturbance of the ant nests. Results from these samples showed higher numbers of ants, especially in the Lorsban plots where almost no ants had been collected in the regular tuna traps. This served as an indication that the ants were still present in the Amdro and Lorsban plots but were perhaps repelled by the presence of the chemical pesticide, and did not leave the nests unless disturbed.

D) Proportion of almonds not damaged by southern fire ants in treatment plots prior to and 4 weeks after treatment.

The proportion of undamaged shelled almonds placed on the ground to expose them to ant foraging in the experimental plots served as an indication of the level of damage the ant populations could cause (**Figure 8**). Again due to cold weather during the pre-treatment sampling, levels of undamaged almonds were high for the initial sample after treatment. Although conditions for ant foraging improved immediately following the application treatments, the levels of undamaged almonds increased slightly for fungus, Amdro and Lorsban treatments, indicating that the level of actual ant foraging decreased after application of these treatments because ant population levels declined. For Control and Logic treatments, proportion of undamaged almonds decreased steadily for the initial 3 weeks of the treatment leveling off at the fourth week of the experiment. After the initial decline in foraging observed in the fungus, Amdro and Lorsban plots, foraging also increased and consequently levels of undamaged almonds decreased in these treatments. Throughout the 4 weeks of the experiments, the level of damage was lower for the Lorsban treatment but the percent of damaged almonds was more than 60% at 3 weeks. This suggests that none of the treatments including Lorsban gave a high level of ant control.

E) Levels of damage to almonds in treatment plots prior to and 4 weeks after treatment.

During the pretreatment period, shelled almonds placed on the ground to expose them to the foraging activity of ants in the experimental plot were mostly undamaged by the ants (**Figure 9**). In the Control plots, proportion of moderately and severely damaged almonds increased in the first 3 weeks of the experiment and levelled off in the fourth week. In Logic plots, the proportion of almonds in the different damage classes followed a similar trend, with a slight increase in undamaged almonds and consequent decrease in severely and moderately damaged almonds during the last week of the experiment. In the fungus plots, proportion of undamaged almonds remained high and severely damaged almonds remained low in the first week of the experiment but levels of damage increased toward the end of the experimental period. In the Lorsban plots, levels of damaged almonds also increased after the first week, but most of the damage was classified as moderate, with a low proportion of severely damaged almonds in the samples. In the Amdro plots, after an initial decrease in the proportion of severely and moderately damaged almonds, the levels of damage increased and by the end of the experiment so that 80% of the almonds were damaged.

F) Fire ant populations and levels of damage to almonds in treatment plots at time of harvest.

Although not proposed in the project funded by the Almond Board proposal, some ant and almond samples were collected by personnel of CIPM and Paramount Farms at the time of harvest. In late August just prior to shaking trees and in early September when almonds were on the ground at time of harvest, data were collected on fire ant abundance in tuna-baited traps and damage to almonds in shells.

The baited traps indicated that although many fire ants were present in all plots, they did not forage consistently during the time almonds were on the ground. In the days just before trees were shaken, ant traps collected large and intermediate numbers of ant foragers in all treatment plots **(Figure 10)**. Comparison of numbers of ants in treatments for August 26th (heavy foraging) and 27th (light to intermediate foraging) show that means are nearly 10 times larger on the 26th. This figure shows that when foraging is heavy, it is difficult to see a treatment effect because all treatments have large numbers of ants but the numbers collected in the untreated control plot were about twice those collected in the fungus and chemical treated plots, suggesting a treatment effect in all treated plots.

However, because damage levels were very low (generally less than 1.5%) in all plots, statistically significant treatment effects in terms of damage could not be detected. These results suggest that ant population levels were reduced as a result of the treatments but that the residual populations were still at levels where almonds were at risk of being damaged severely. Observed damage levels were probably low because conditions were not favorable for intense ant foraging at the time the almonds were on the ground, not because any of the treatments eliminated ant populations or reduced their levels enough to eliminate risk of damage.

DISCUSSION

The experimental results from the second field experiment in California clearly show that all ant control products tested had some effect on the southern fire ant but none of the treatments gave good enough control of fire ant populations to eliminate the risk of severe damage at harvest time.

Points of Importance:

1. The bait with fungus was foraged readily by southern fire ant and fungal infection of ants within nests was observed within less than a week after treatment. With a single fungus treatment, we estimated that more than 50% of the fire ants in the nests were killed within 4 weeks after treatment. However, this level of population reduction was not sufficient to preclude damage to almonds placed on the ground after treatments.

2. The Amdro and Logic chemical baits were foraged and had some effect in reducing fire ant populations but not enough ants were killed in nests to seriously reduce the risk of high damage levels at harvest.

3. Throughout the 4 weeks of the experiments, the level of damage was lower for the Lorsban treatment but the percent of shelled damaged almonds that were placed on the ground to expose

them to ants was more than 60% at 3 weeks. Lorsban appears to have more of a foraging repellent effect than to reduce fire ant populations within nests. This control tactic seems only to treat the symptoms of the fire ant problem and does little for long term reduction of ant populations.

Interpretation of Results

Data gathered in late August at the time of harvest indicated that although many fire ants were present in all plots, they did not forage much during the time almonds were on the ground. In the days just before trees were shaken, ant traps collected large and intermediate numbers of ant foragers in all treatment plots. The numbers collected in the untreated control plot were about twice those collected in the fungus and chemical treated plots, suggesting some treatment effect in all treated plots. However, because damage levels were very low at harvest time (generally less than 1.5%) in all plots, statistically significant treatment effects in terms of damage could not be detected.

These results suggest that ant population levels were reduced as a result of the treatments but that the residual populations were still at levels where almonds were at risk of being damaged severely. Observed damage levels were probably low because conditions were not favorable for intense ant foraging at the time the almonds were on the ground, not because any of the treatments eliminated ant populations or reduced their levels to eliminate risk of damage. A bait formulation is needed to reduce fire ant populations to much lower levels than were observed in this study. Perhaps multiple applications of the fungus could achieve this.

The primary objective of this study was to collect information necessary to evaluate the potential of a microbial product for biological control of fire ants in California almond production systems. Although research results from this study are only preliminary, they are encouraging and lead us to believe that there is good potential for developing a bait with fungus for control of southern fire ant and that possibly it would be more cost effective and environmentally desirable method for controlling this serious insect pest in almond production systems.

A second important objective was to evaluate various fire ant control tactics that are being used to control other species of fire ants in other crop systems and in the urban setting as potential tactics in a short term control strategy for fire ants in California almond orchards. We attempted to evaluate levels of control given by each control tactic and the feasibility and compatibility of the delivery systems with current almond production practices. From the results of this study, we cannot say that Lorsban is an effective control tactic. Although Lorsban is being used widely at a cost of more than $30.00/ acre, there is no evidence that this control tactic is reducing fire ant population levels enough to avoid risk of severe damage at harvest.

The other chemical baits tested, Amdro from American Cyanamid and Logic from Ciba Geigy did not show very positive results in these trials. Amdro, according American Cyanamid will not be registered in California. Logic is in the process of registration in California but the results of our study do not indicate that this product would be more effective than Lorsban or a biological product with fungus. **The bottom line is that there is no adequate treatment tactic for southern fire ants in almonds at the present time.** Data from our study show that although some ants were killed in all treatments, ant population levels were probably still high enough to cause unacceptable levels of damage if ants foraged heavily when almonds were on the ground during harvest.

Taking into consideration timing of cultural practices, pollination, herbicide applications, costs and efficacy of formicides (ant pesticides) and environmental factors, development of a biological control product with fungus as the active ingredient would be highly desirable. The data from ant traps just prior to almond harvest suggests that the effects of the fungus treatment was equal to or somewhat better than the Lorsban treatment. However, it will take at least 1-2 years before such a biological product could be commercially available. Until alternatives to Lorsban are available, the only fire ant control strategy for California production systems is no control or continue to use Lorsban because it is the only product registered for use in almond orchards. Because Lorsban must be applied at very high dosages (6 pints/acre, which is very expensive $30/acre) and still does not appear to be effective in killing most ants in the nests, it is important to improve scouting efforts to time applications of the chemical when ants are in the period of peak foraging.

FINAL CONCLUSIONS

Our final conclusion is that a better fire ant control strategy for the short term and long term needs to be developed. Lorsban appears to have more of a foraging repellent effect than to reduce fire ant populations within nests. This control tactic seems only to treat the symptoms of the fire ant problem and does little for long term reduction of ant populations. A bait formulation is needed to reduce fire ant populations to much lower levels than were observed in this study. Perhaps multiple applications of the fungus could achieve this. The field tests showed that a fungal bait can reduce fire ant populations and that with improvements, through development and further research, could be efficacious at equal or lesser cost than the chemical pesticide currently being used. A plan should be prepared for development and registration of a biological control product that could be commercialized. Registration and production of a commercial product would take 1-2 years so this would be a longer term strategy. However, considering the cost and environmental effects of Lorsban, pursuing the development of a product using fungus as the active ingredient is justified. It seems reasonable to conclude that a fungal formulation could be applied at equal or lesser cost than Lorsban and give equal or better levels of fire ant control.

We have stated that a single application of the fungus bait killed more than 50% of fire ants in nests. This reduces the problem at the source. Perhaps multiple applications would reduce the source populations even more. The cost of producing the fungal bait is probably low enough to allow at least 2 applications for $30.00/ acre.

Based on the results from this field trial, CIPM has entered into an agreement with the University of Florida to develop this fungus into a biorational product which we feel will be of benefit to the almond industry. This program over the next few years will require the expenditure of millions of dollars for research, development and commercialization. The support of the Almond Board and others has allowed this to happen. CIPM would like to thank the Almond Board of California, Paramount Farming Company and Blue Diamond Growers for their support in the funding and execution of this research. Our particular thanks go to Dr. Joe MacIlvaine.

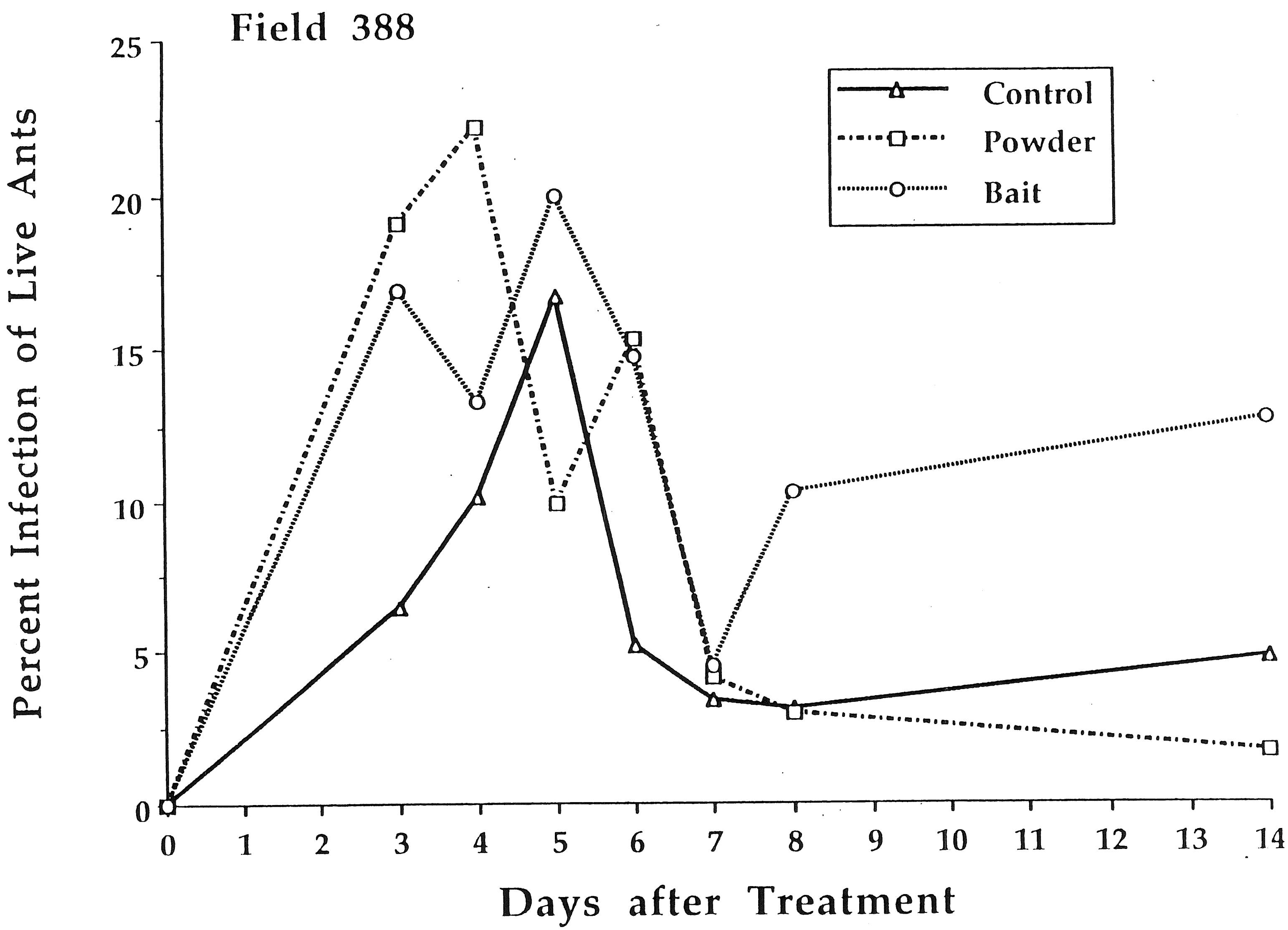

Figure 1. Mean percent *Beauveria bassiana* infection of live southern fire ants (*Solenopsis xyloni*) collected from nests in field 388 at Paramount Farms (Bakersfiled, CA) treated with control bait or formulations (powder or bait) containing 10% of conidia of *Beauveria bassiana* strain 477.

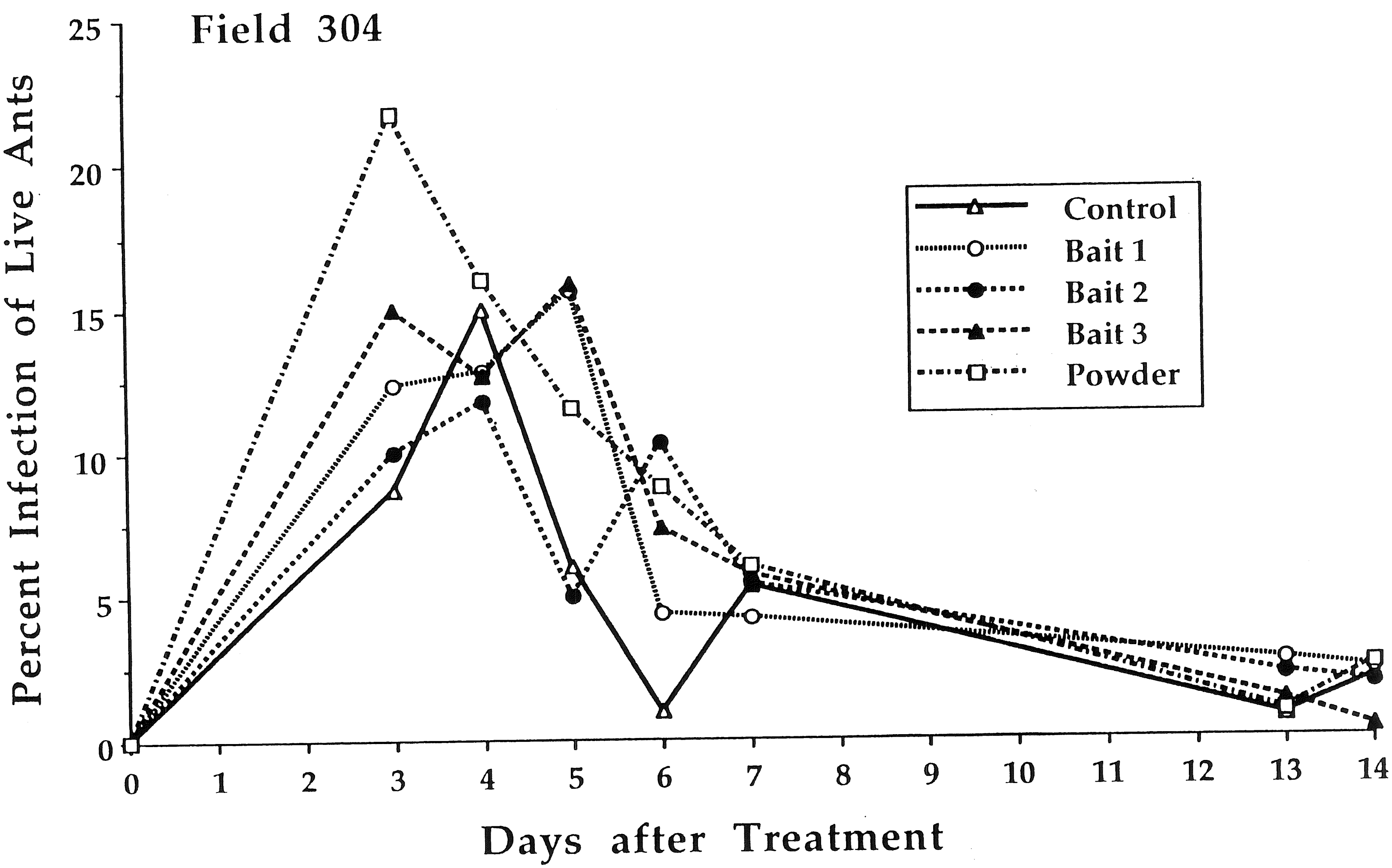

Figure 2. Mean percent *Beauveria bassiana* infection of live southern fire ants (*Solenopsis xyloni*) collected from nests in field 304 at Paramount Farms (Bakersfiled, CA) treated with control bait or formulations (powder or baits) containing 10% of conidia of *Beauveria bassiana* strain 477.

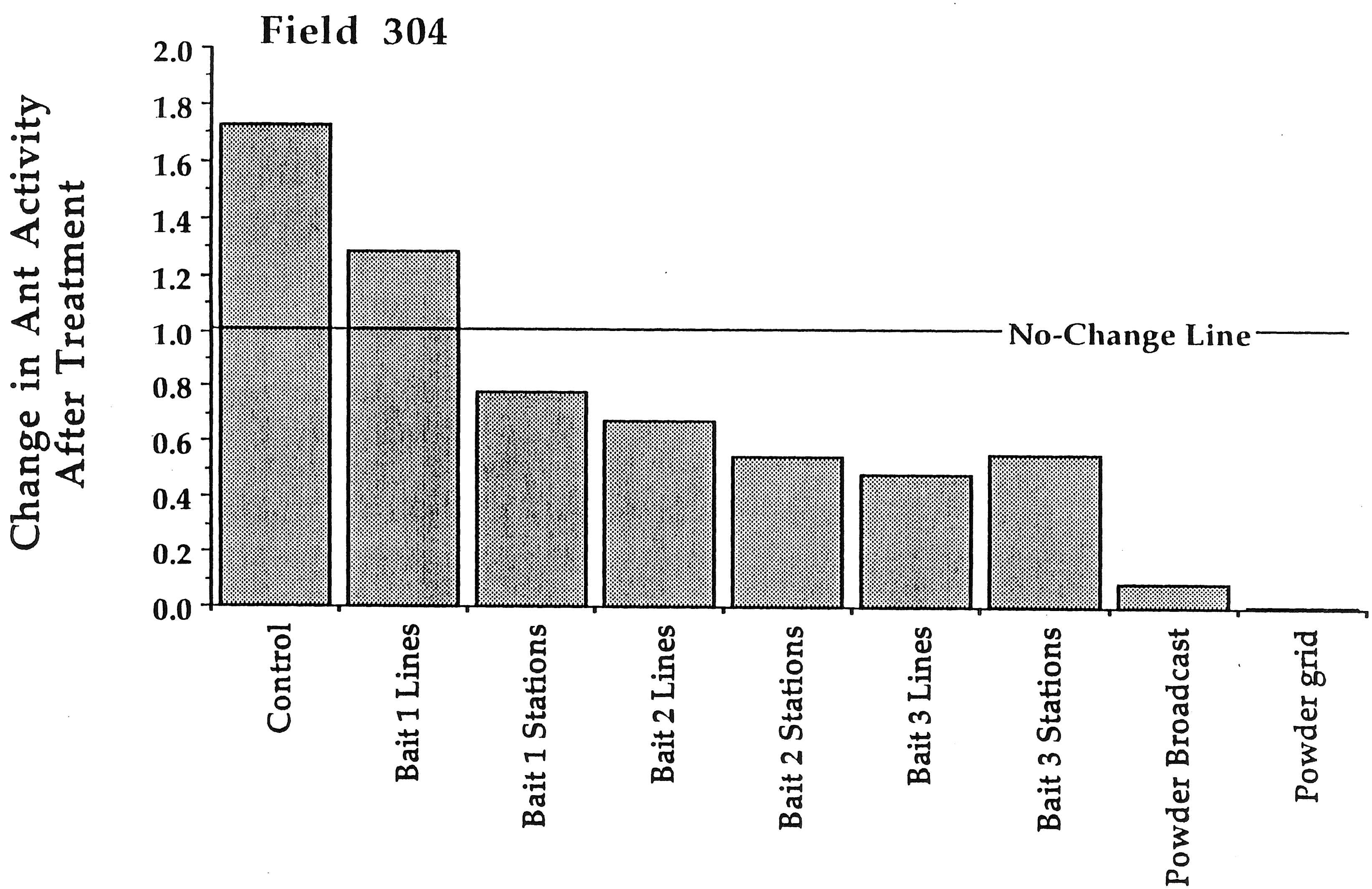

Figure 3. Change in activity (as determined by number of ants captured in tuna-baited traps) of southern fire ants (*Solenopsis xyloni*) in field 304 at Paramount Farms (Bakersfiled, CA) treated with control bait or formulations (powder or bait) containing 10% of conidia of *Beauveria bassiana* strain 477.

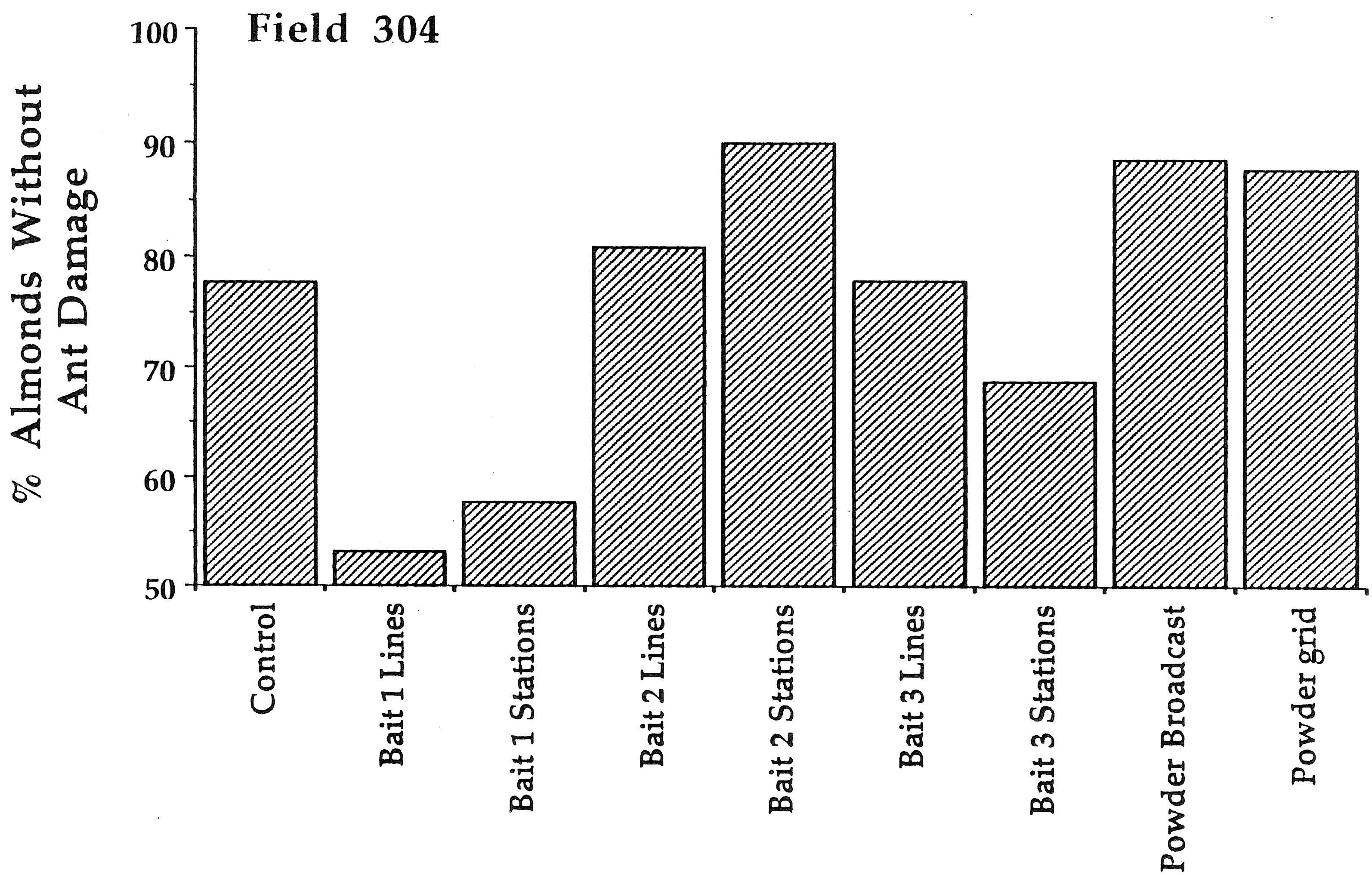

Figure 4. Mean proportion of undamaged almonds in samples exposed to ant foraging for a period of 24 h in plots in almond orchards at Paramount Farms (Bakersfiled, CA) (Field 304) treated with control bait or formulations (powder or bait) containing 10% of conidia of *Beauveria bassiana* strain 477.

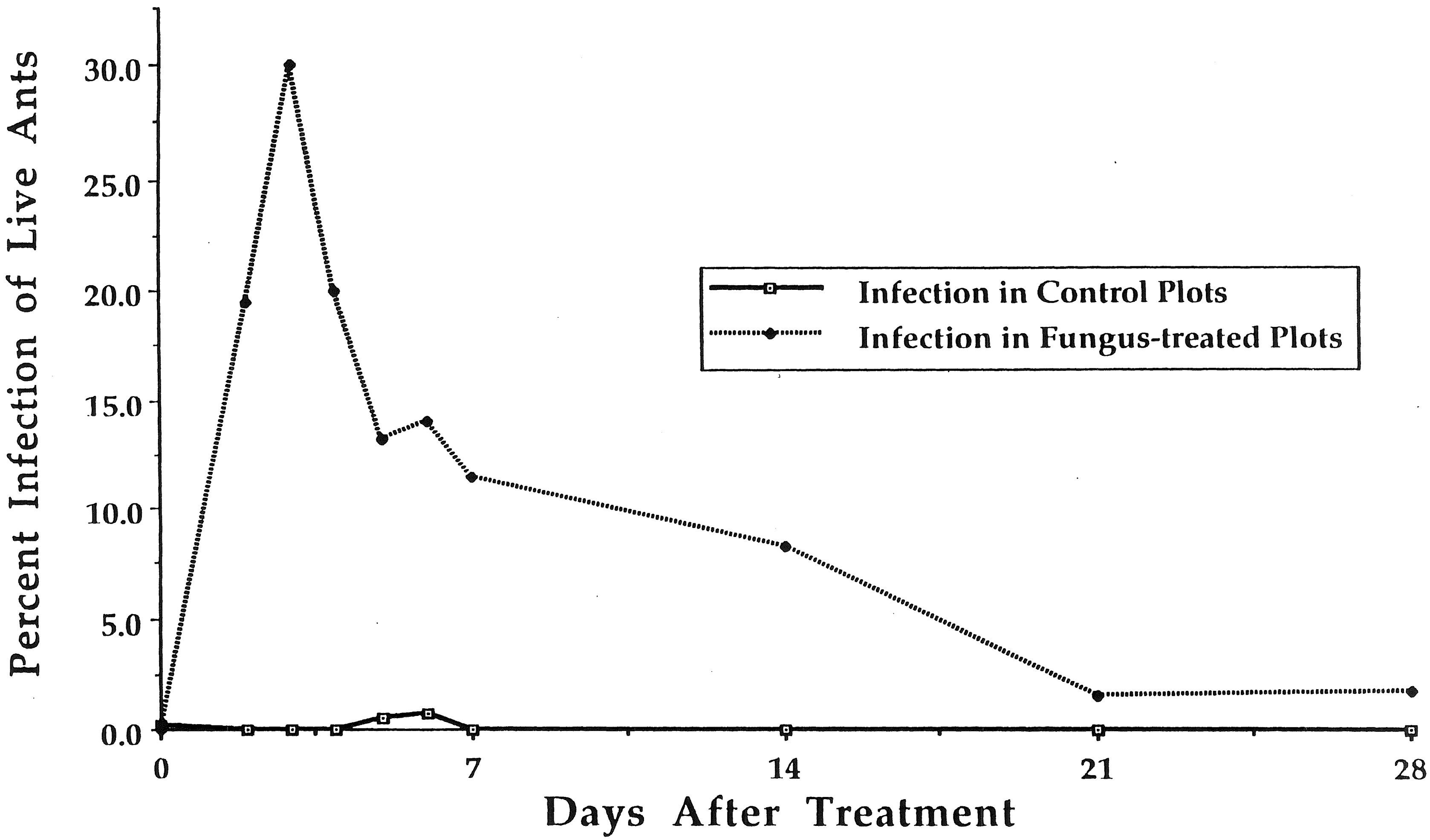

Figure 5. Mean percent *Beauveria bassiana* infection of live southern fire ants (*Solenopsis xyloni*) collected from field colonies in almond orchards at Paramount Farms (Bakersfiled, CA) (Field 370) treated with control or bait formulation containing 10% of conidia of *Beauveria bassiana* strain 477.

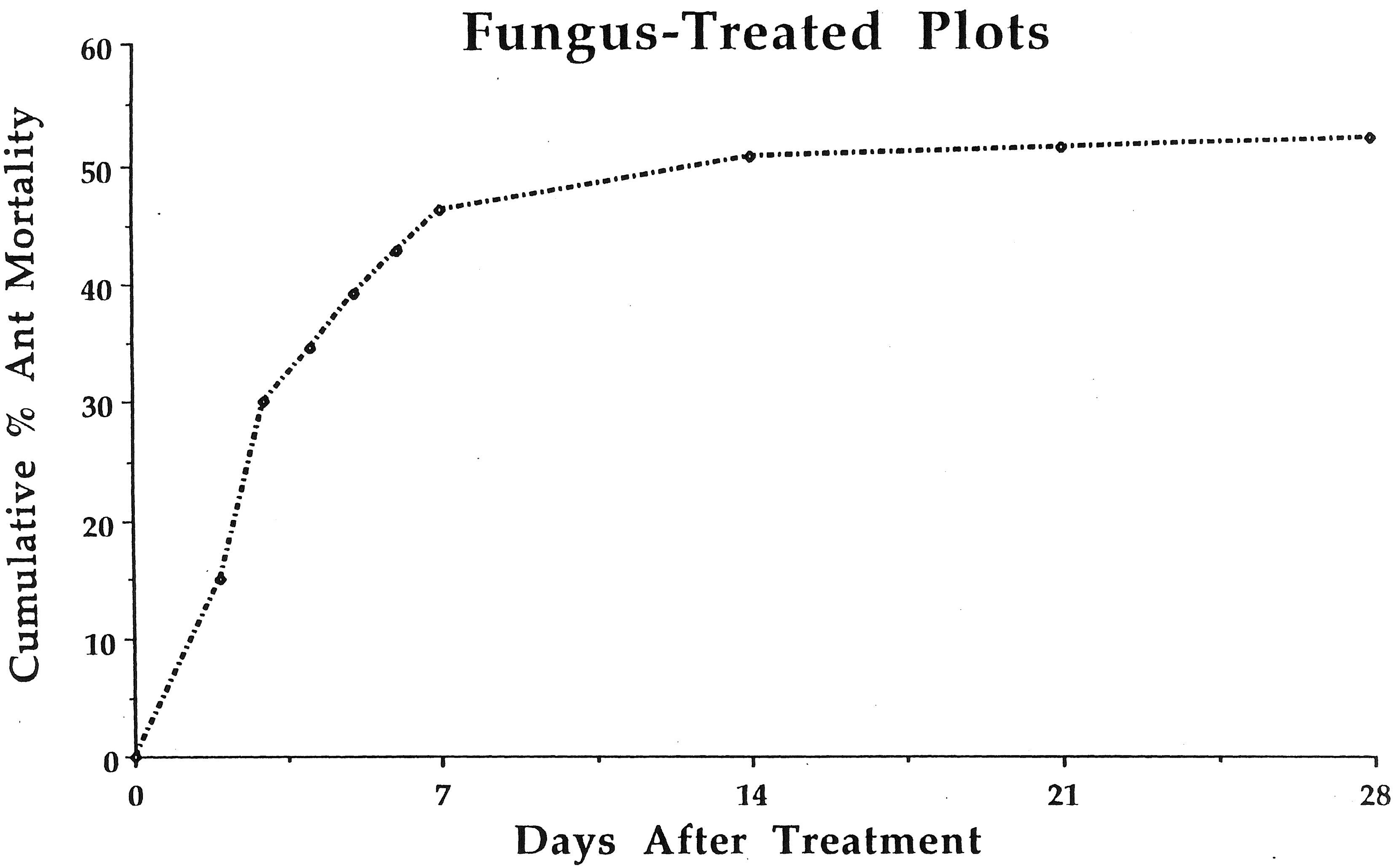

Figure 6. Estimated cumulative *Beauveria bassiana-induced* mortality of southern fire ants (*Solenopsis xyloni*) from field colonies in almond orchards at Paramount Farms (Bakersfiled, CA) (Field 370) treated with bait formulation containing 10% of conidia of *Beauveria bassiana* strain 477.

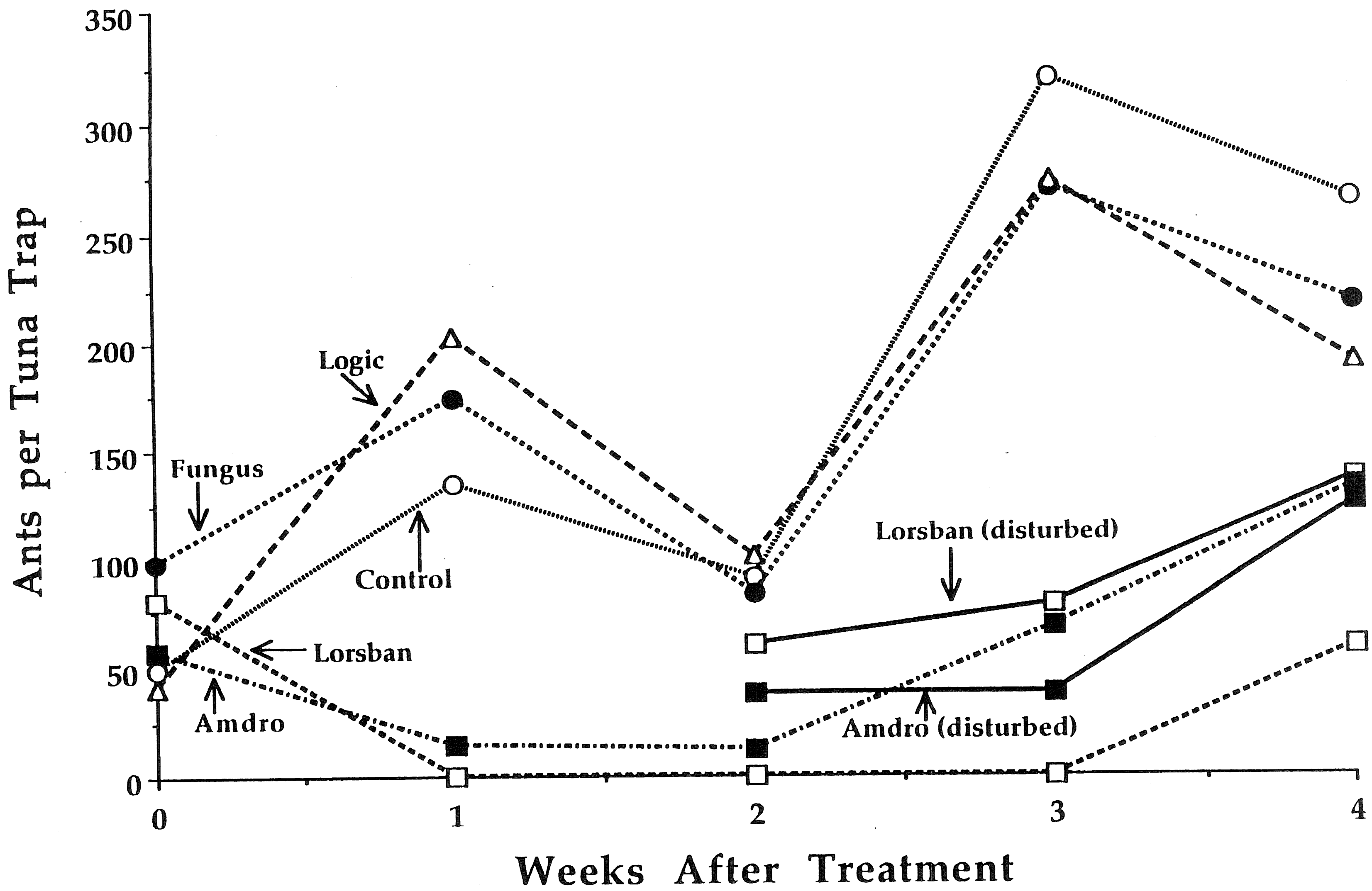

Figure 7. Mean number of southern fire ants (*Solenopsis xyloni*) trapped in tubes containing canned tuna/cat food mixture esposed to ant foraging for a period of 4 h in plots in almond orchards at Paramount Farms (Bakersfiled, CA) (Field 370) treated with different insecticides.

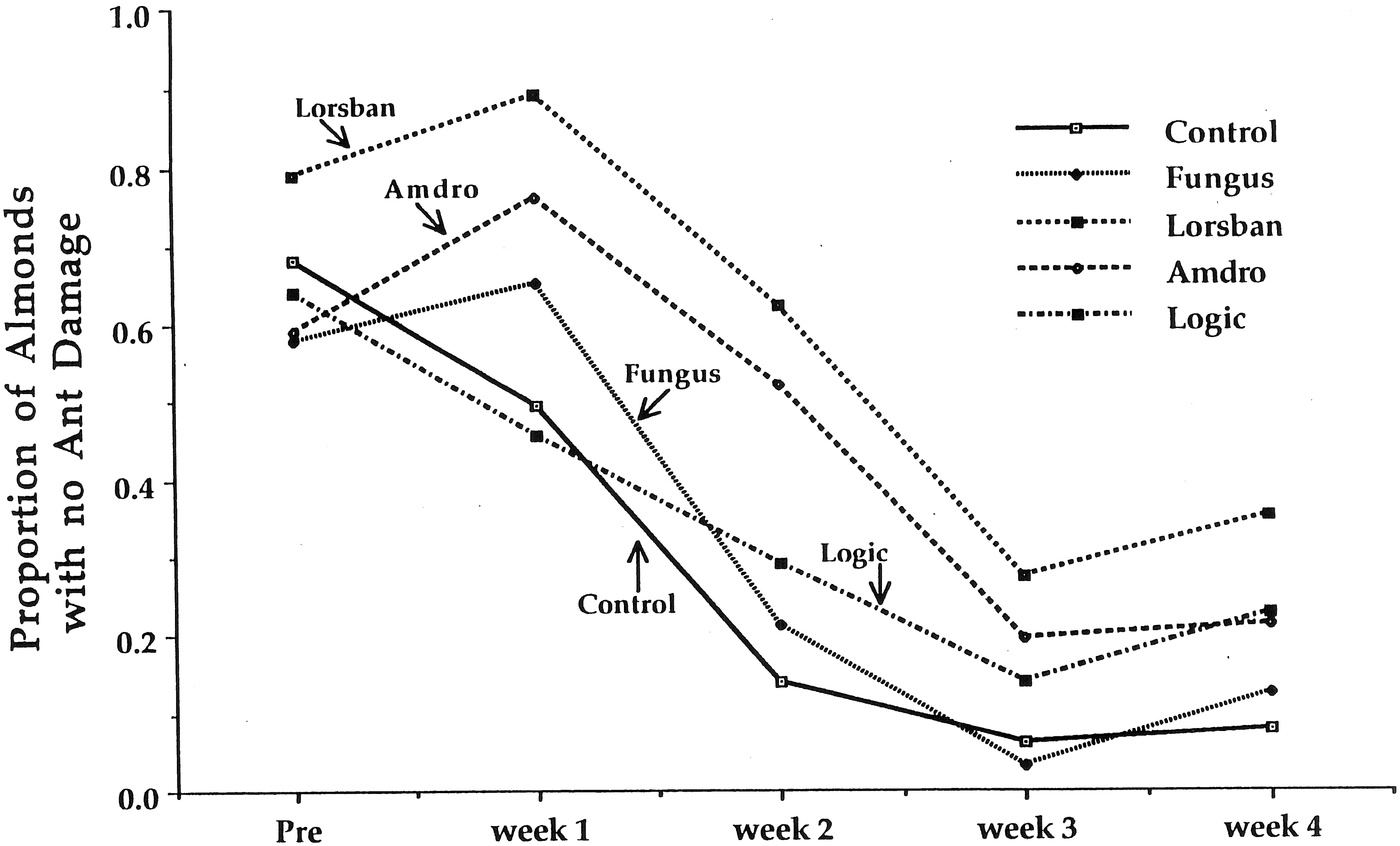

Figure 8. Mean proportion of undamaged almonds in samples exposed to ant foraging for a period of 48 h in plots in almond orchards at Paramount Farms (Bakersfiled, CA) (Field 370) treated with different insecticides.

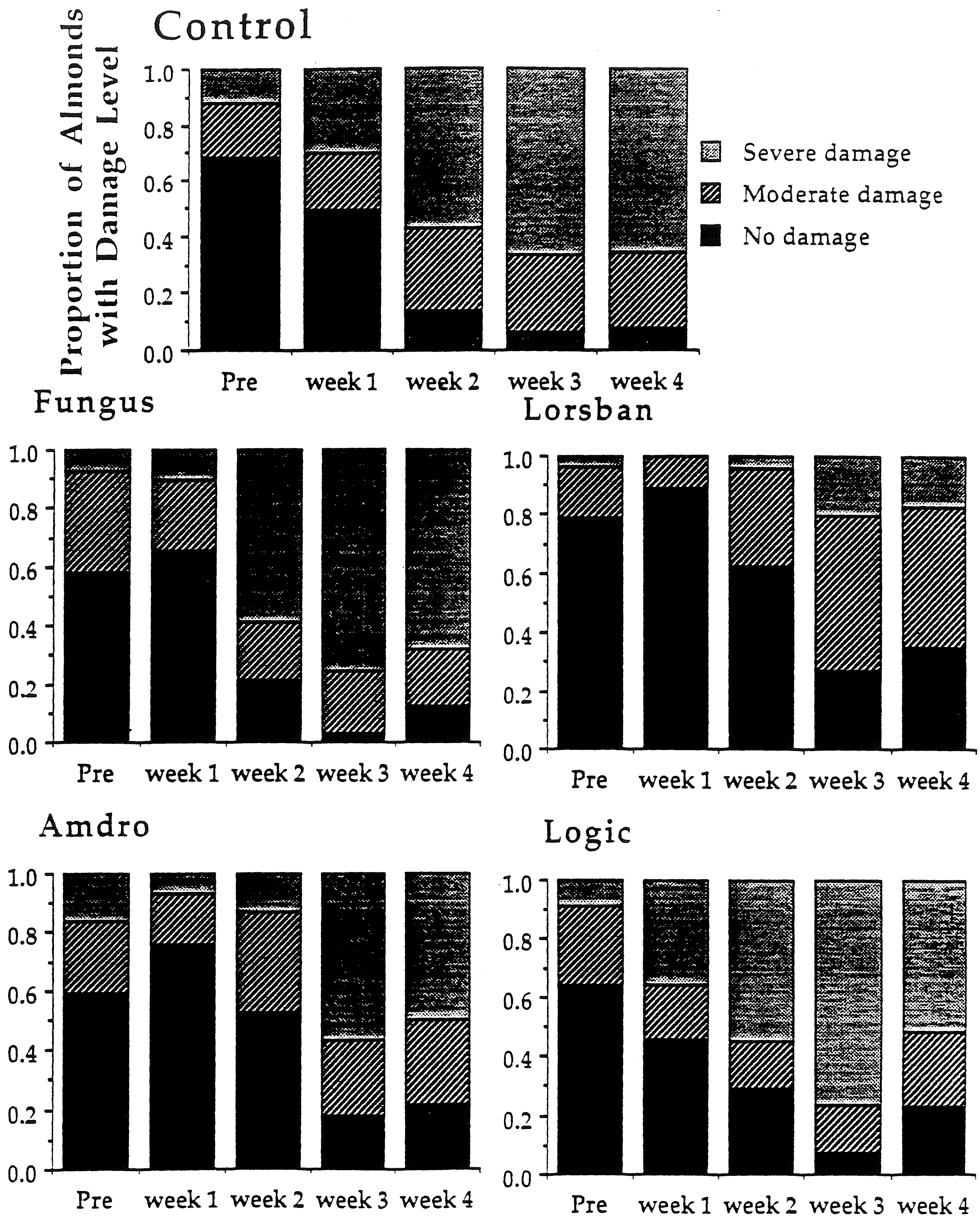

Figure 9. Mean proportion of almonds with different levels of damage in samples exposed to ant foraging for a period of 48 h in plots in almond orchards at Paramount Farms (Bakersfiled, CA) (Field 370) treated with different insecticides.

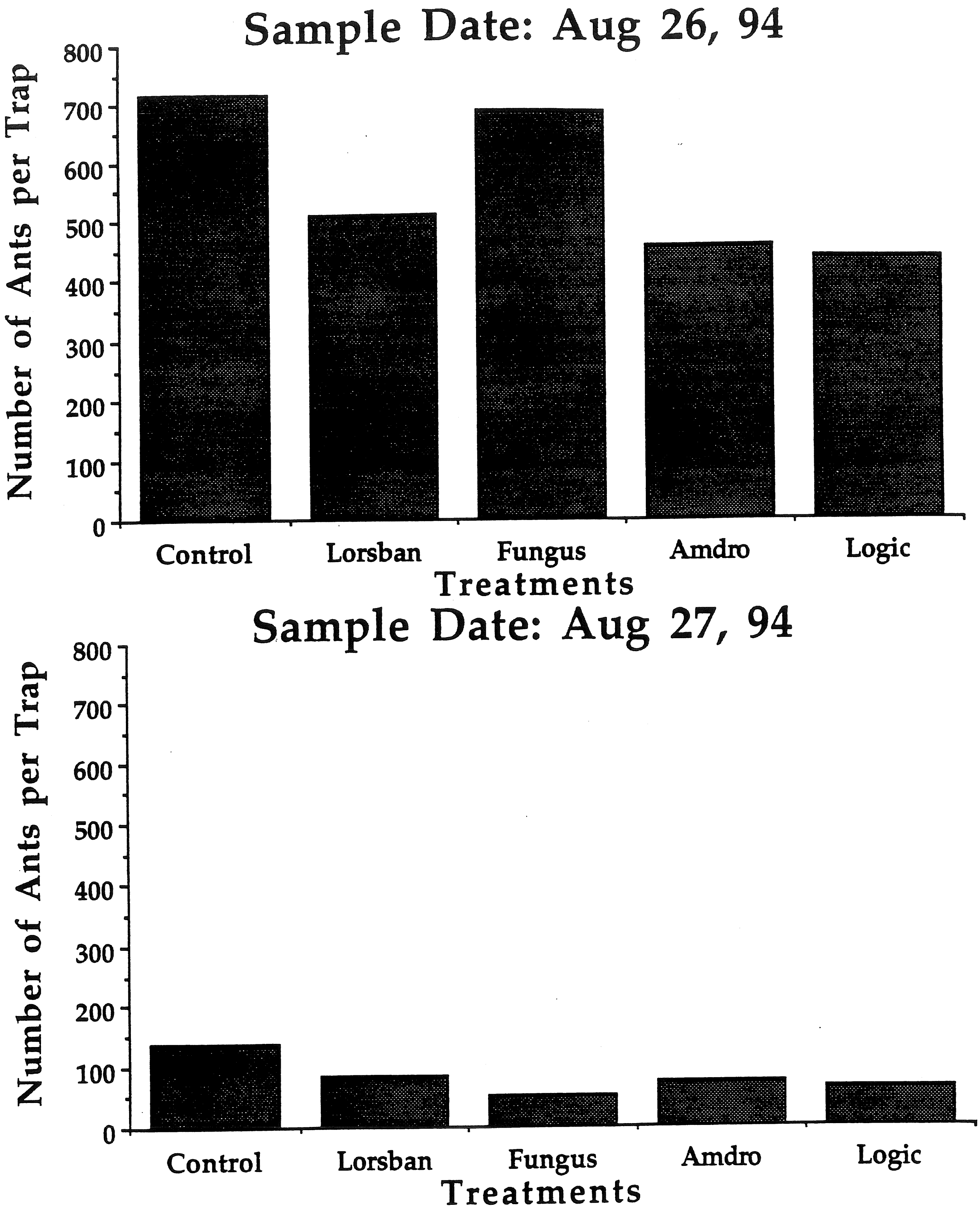

Figure 10. Comparison of number of ants in tuna traps on August 26 and 27, 1994, just prior to harvest, in plots in almond orchards at Paramount Farms (Bakersfiled, CA) (Field 370) treated with different insecticides.