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CONTROL OF FIRE ANTS IN CALIFORNIA ALMOND ORCHARDS

A Preliminary Final Report
Submitted to the Almond Board of California

by

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TABLE OF CONTENTS

ABSTRACT. The objective of this study was to develop a delivery system and test the efficacy of a microbial pesticide for control of fire ants in a California almond production system. The microbial agent evaluated as a biological pesticide for the southern fire ant, Solenopsis xyloni, was a bait formulation of the fungus Beauveria bassiana (strain 447). The fungal bait was applied on the ground surface at different times during the late spring and summer months when fire ants were thought to be actively foraging. Before application of fungal bait in the experimental area, infection of fire ants varied from 4 to 33%. This pre-treatment fungal infection lowered the ant population level compared to that from the previous year. The lower ant population made it more difficult to determine treatment effects but some patterns were observed. After the first application of fungal bait, the number of tubes with ants were fewer in all fungal bait plots. Number of ants declined after application of Lorsban and remained low until harvest. After treatment, high levels of fungal infection were mainly observed in the plots which received a second application of the fungus 3 weeks after the initial one. Despite low levels of the fire ant population, fungal bait applications did produce desirable effects, especially in plots which received the second application. The effects of the third application and levels of damage to almonds have not yet been analyzed but they will be after harvest is completed.

INTRODUCTION

The intent of this study was to provide research results as part of the development of an effective non-chemical pesticide to be used as a biological control for fire ants in California almond production systems. The primary objective of the research was to continue research started in the previous year and evaluate a microbial product for biological control of fire ants. The microbial agent evaluated as a biological pesticide was a strain of the entomopathogenic fungus Beauveria bassiana (strain 447) in a bait formulation. This bait produced encouraging results in 1994 field tests when applied during a period of aggressive ant foraging. The intent of the 1995 field test was to apply the fungal bait at different times when fire ants forage during spring and summer months. Also, larger treatment plots were used to simulate something closer to a production scenario.

SUMMARY OF FIELD TESTS IN 1994 AND 1995 TO EVALUATE A FUNGAL BAIT FORMULATION TO CONTROL FIRE ANTS IN A CALIFORNIA ALMOND ORCHARD

First Field Test (1994)

Four fungal formulations and four different methods of application were tested in small plots of almond trees during the months of March and April in two fields at Paramount Farms in

Shafter, CA. Formulations were applied as: a) rings around ant nest openings b) piles along lines; c) continuous lines, d) broadcast.

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. Greatest reduction of ant activity was caused by powder treatments, probably due to repellent effect of the presence of the powder on the surface of the soil. Reduction in ant activity observed in the bait plots resulted from reduction in the ant population by the fungal application. Results demonstrated an apparent superiority of a bait formulation.

Second Field Test (1994)

Various baits (chemical and biological) were used in a second field test at Paramount Farms near Shafter, CA. Experiments were conducted during the months of May and June 1994 using 4 different products: Lorsban, Amdro, Logic , and a bait containing <u>B. bassiana</u> conidia applied as rings around fire ant nest openings.

In the fungus-treated plots, levels of infection rose to a maximum of 30%, 3 days after application of the fungal bait but was only 2% one month after application. We estimated that total ant mortality in the fungus-treated area reached more than 50% during the 4-week period after the application. At the time of harvest, many fire ants were present in all plots but they did not forage consistently during the time almonds were on the ground. Numbers of ants 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, ant damage levels in the harvested product were very low (generally less than 1.5%) in all plots.

1995 Field Evaluation of Fungal Formulation

The 1995 field test was designed to test the efficacy of the best fungal bait formulation from 1994 in comparison to treatment with Lorsban. The scale of the experiment was much larger than in 1994. In total 10 acres were used in the experiment with treatment plots of 1/2 acre each. Fungal bait was applied in three different timing patterns. Some plots received a single treatment with fungal bait and others received two applications.

MATERIALS & METHODS

In the 1995 field test, half-acre plots containing approximately 60 trees were marked with colored surveying tape in ranch 370 of Paramount Farming Co. along Highway 43, south of Shafter (Township 28 South, Range 26 East). A total of 20 plots were marked in irrigation set 3 of section 7. Plots were set up for 5 treatments with 4 replications per treatment, one in each of 4 blocks **(Figure 1)**. Within each plot we marked the corners of the subplot designated as the sampling area and within the sampling area, samples of ants were obtained from 10 locations within a square in the center of the plot **(Figure 2)**.

The 5 treatments were: a) Control, with no application of any material for ant control; b) Lorsban, standard single application of 6 pints per acre; c) Fungal bait applied only once (F1); d) Fungal bait applied twice, the second application 3 weeks after the initial one (F2); and e) Fungal bait applied twice, the second application 1 week before shaking of the trees for nut harvest (F2-PreHarv).

Lorsban was applied with a conventional spray rig used for application of this material in almond orchards. The fungal bait was applied manually as piles along the berms at the rate of 10 kg of bait per acre. The bait contained 10% B. bassiana conidia so 1kg of fungal spores were applied per acre. Plots that received 2 applications received double this dose. Initial application of Lorsban and fungal bait was done on May 25, 1995. Second applications of the fungal bait were on June 17 and August 11, respectively.

Fire ants were sampled one month prior to application of the pesticide treatments and sampling continued until after the harvest of the nuts. Plots were sampled for ants by use of baited tubes. Baited tubes were prepared by cutting thin slices (± 0.5 cm thick) of hot dog and dropping one into each plastic tube. These tubes served as traps and were laid on the soil surface to allow ants to forage for 2 hours. After this time, the tubes were collected and capped quickly to trap the ants that were foraging on the hot dog bait. Within each plot, samples of ants were obtained from the 10 locations at the centers of the berms between trees. As the tubes were recovered, all 10 tubes from each plot were placed in a labeled plastic bag and then put into the freezer overnight to kill the ants. Samples of frozen ants were packed and shipped to our laboratory at the University of Florida.

In the laboratory, ants in each tube were counted, and 100 ants were surface sterilized and placed in wells of a microtiter well plate. These plates were incubated under moist conditions to allow development of fungus internally and eventually externally on the ants. After an incubation period of 7 to 10 days, ants with signs of B. bassiana growth were marked as being infected by this fungus. Percentage of infected ants was then calculated for each sample.

Block 1	Block 2	Block 3	Block 4
Plot 5 Lorsban	Plot 10 Lorsban	Plot 15 F2	Plot 20 F1
Plot 4 F2	Plot 9 F2	Plot 14 F2 -PreHarv	Plot 19 Control
Plot 3 F1	Plot 8 F2 -PreHarv	Plot 13 F1	Plot 18 Lorsban
Plot 2 Control	Plot 7 F1	Plot 12 Lorsban	Plot 17 F2 -PreHarv
Plot 1 F2 -PreHarv	Plot 6 Control	Plot 11 Control	Plot 16 F2

Figure 1. Experimental treatment plots in ranch 370 of Paramount Farms used for 1995 field experiment.

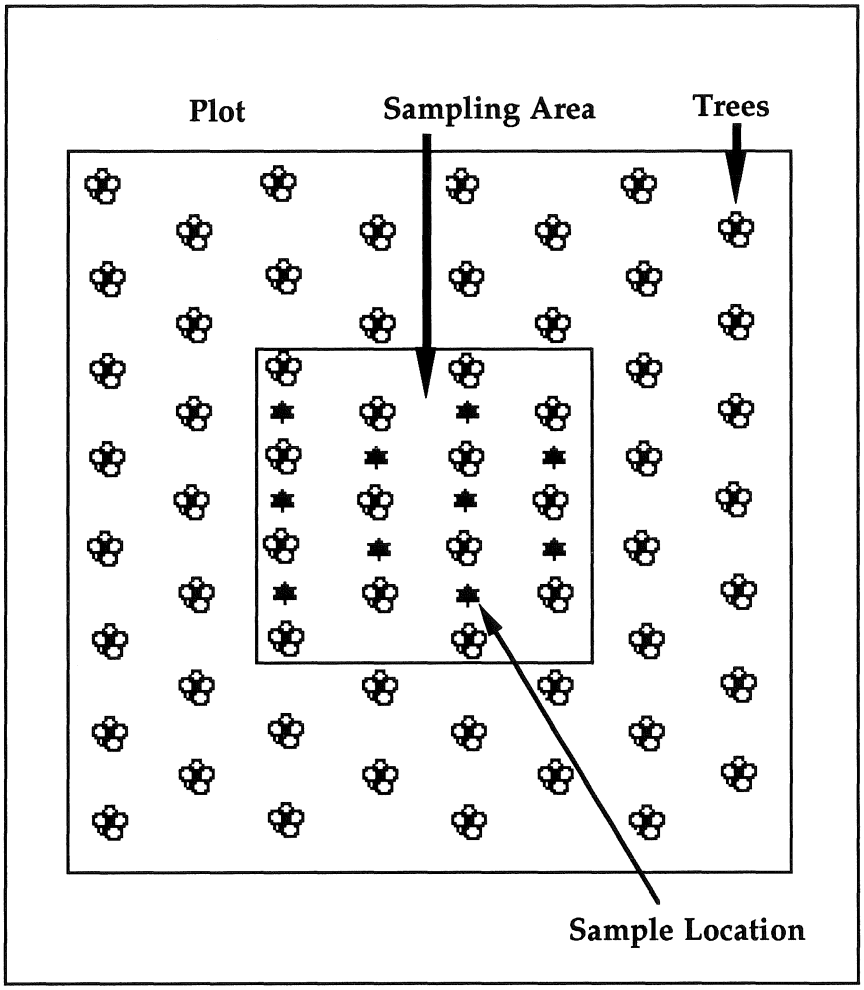

Figure 2. Sampling area and sample locations within treatment plots used for 1995 field experiment.

Also, samples of live ants were collected directly from nests found in the sampling area within the plots. These samples usually were taken a few days before and after the fungal applications, and occasionally at other times during the experiment. These ants were collected with a spatula and placed into a box lined with Fluon (slick material that prevents escape of the ants). These ants also were frozen and then processed as described earlier and plated into microtiter plates. Percent infection also was calculated for these ants.

RESULTS

A) Southern fire ant population levels

Ant populations in the experimental area were low this year in comparison to populations observed in the previous year. Number of hot dog tubes that were occupied by ants were usually below 5 per plot out of 10 tubes exposed to ant foraging **(Figure 3)**. Number of traps with ants dropped significantly on the Lorsban plots following application of the insecticide and remained low until harvest time. A decrease in the number of tubes with ants was observed for the fungal bait plots after the first application. Also, a decline in the number of traps with ants was observed in all plots during the July 3rd and 11th samples, probably due to weather conditions that were not adequate for ant foraging.

The low numbers of ants per hot dog trap also are indications of the lower ant population for this season compared to 1994. The maximum number of ants per tube was never higher than 90, with numbers usually below 50 ants per tube for all treatments **(Figure 4)**. During the 1994 experiments, numbers of ants per baited tube were usually in the 100 to 1000 ants per tube range. Number of ants in the baited traps declined after application of Lorsban and remained low for the duration of the experiment. Declines were also observed in the numbers of ants collected in traps from the fungal bait plots after the first and second applications. Of the fungal treatments, higher numbers of ants in the baited traps were observed in the plots that received only the first application of the bait. Among the fungal treatments, ant populations were lowest in the plots that received 2 applications, 3 weeks apart. Numbers of ants per trap in this treatment (after second application on June 17) were significantly lower than in the control plots, and not much higher than in the Lorsban plots.

B) Fungal infection of fire ants

Beauveria bassiana infection of ants collected in the hot dog traps was high in the beginning of the experiment, before any application of the fungus in the experimental area **(Figure 5)**. Infection rates during the period between April 24th and May 16th

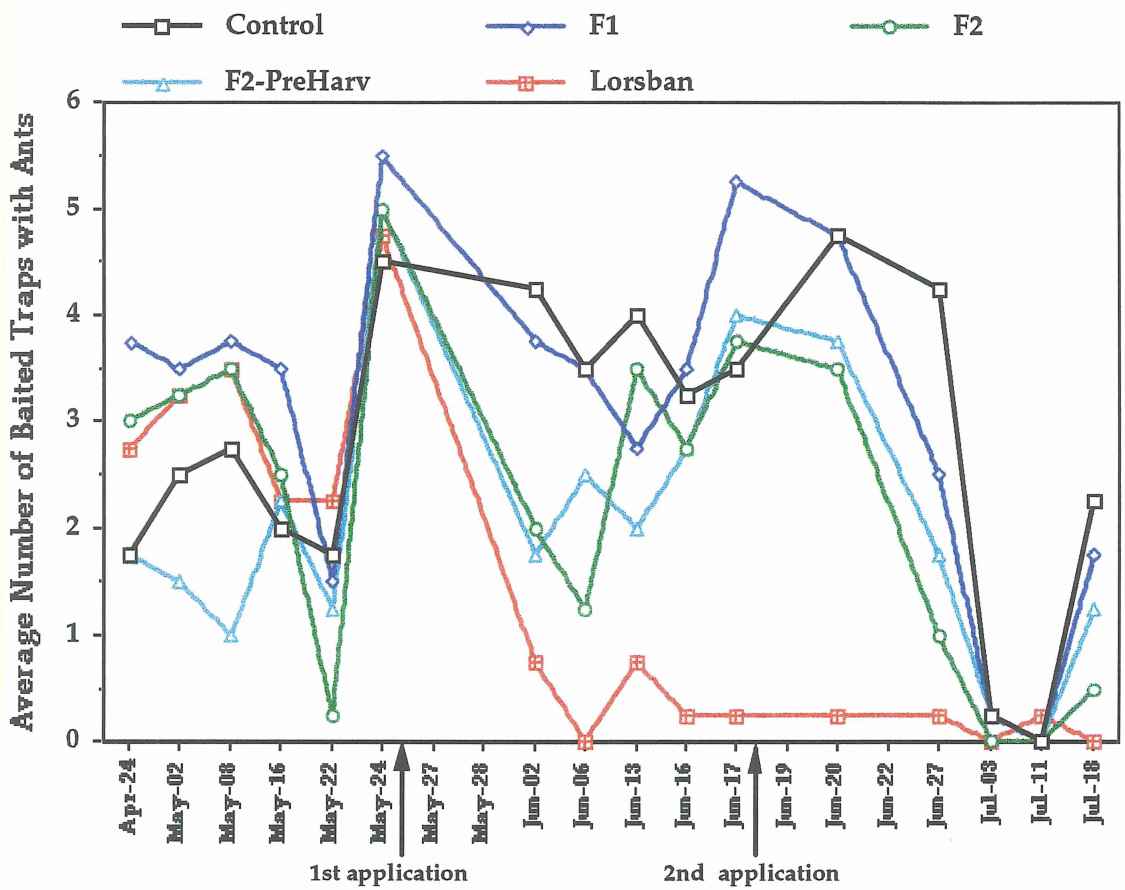

Figure 3. Average number of baited trap with ants for all treatments in 1995 field experiment at Paramount Farms. Control = no application for ant control; F1 = only first application of fungal bait; F2 = First and second applications of fungal bait; F2-PreHarv = first and third application (on Aug 11th) of fungal bait; Lorsban = one application of Lorsban on May 25th.

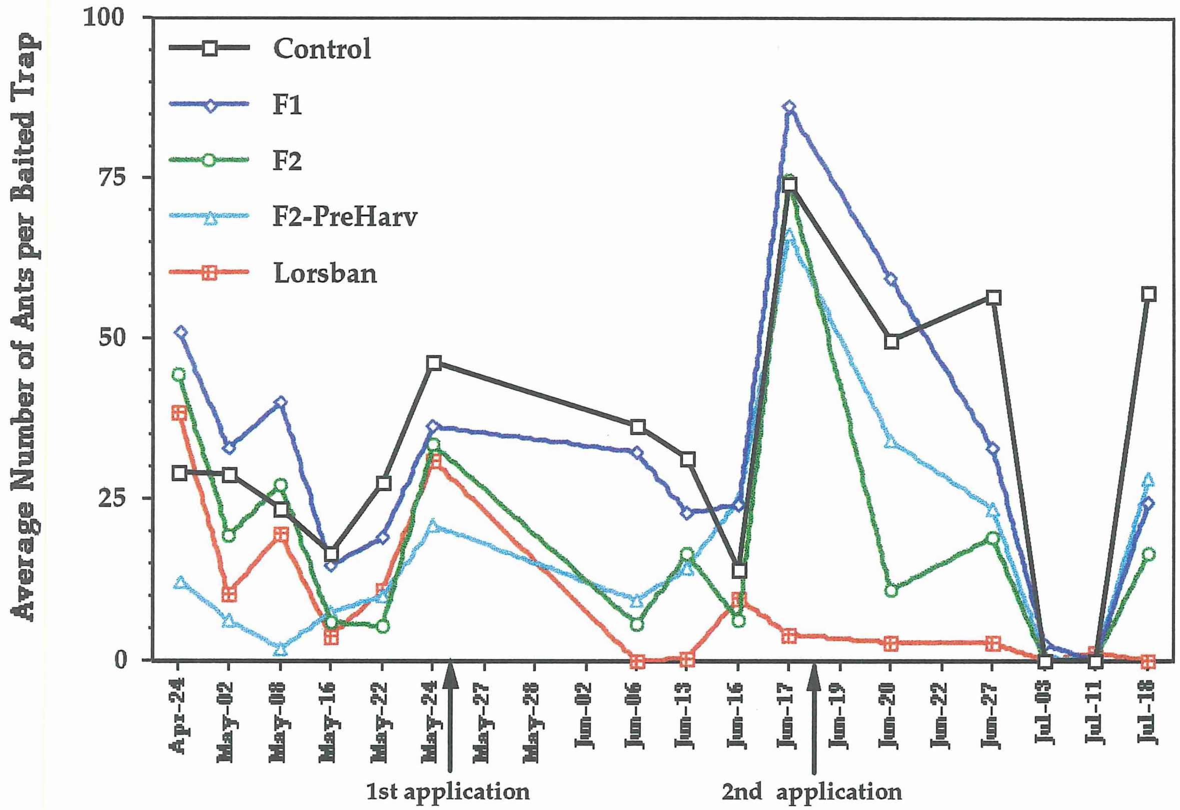

Figure 4. Average number of ants per baited trap for all treatments in 1995 field experiment at Paramount Farms. Control = no application for ant control; F1 = only first application of fungal bait; F2 = First and second applications of fungal bait; F2-PreHarv = first and third application (on Aug 11th) of fungal bait; Lorsban = one application of Lorsban on May 25th.

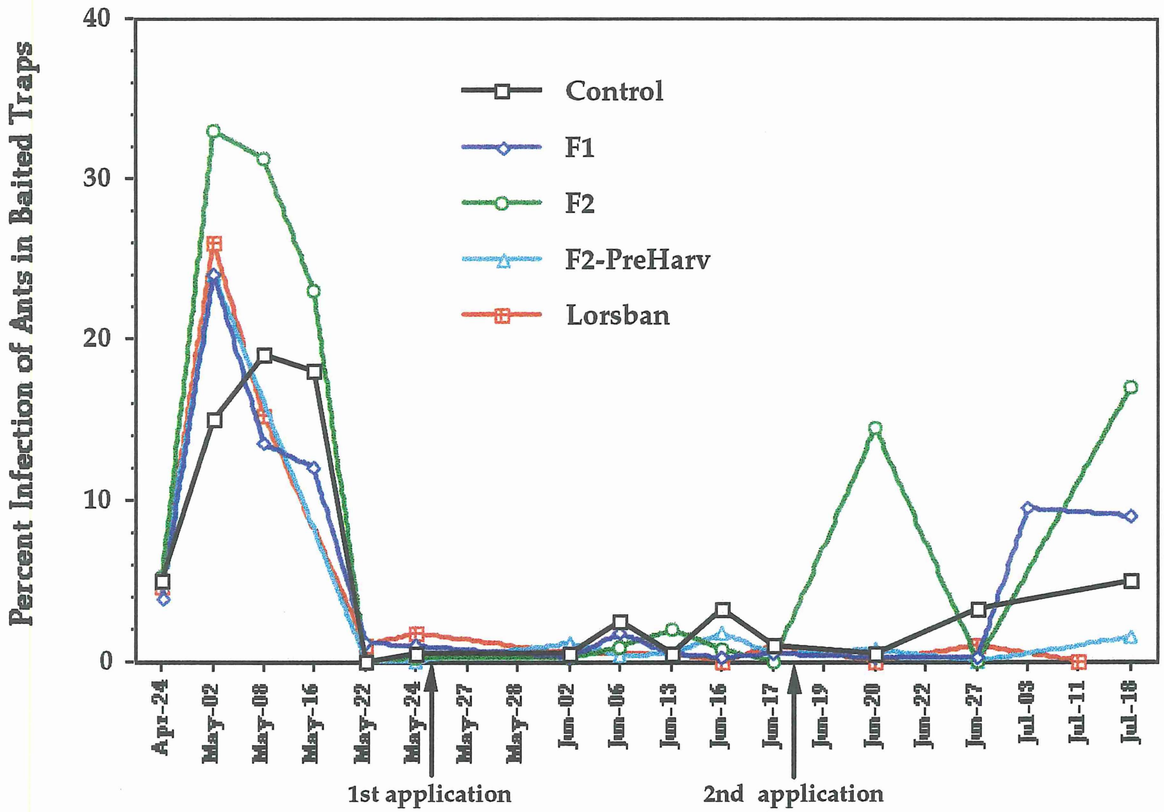

Figure 5. Percent infectionof ants in baited traps for all treatments in 1995 field experiment at Paramount Farms. Control = no application for ant control; F1 = only first application of fungal bait; F2 = First and second applications of fungal bait; F2-PreHarv = first and third application (on Aug 11th) of fungal bait; Lorsban = one application of Lorsban on May 25th.

varied from 4 to 33% for the different treatment plots, prior to application of treatment pesticides on the plots. On May 22 and 24, three and one days before the initial treatment of the field, the infection levels had fallen to normal background levels of less than 2 %. The levels of B. bassiana infection as detected from ants collected in the hot dog traps continued to be low in all plots until the second application of the fungal bait. No hot dog traps were placed in the plots until 7 days after the first application of the fungal bait. The second application of the fungal bait caused a significant increase in the infection of ants from the plots which received this application. High levels of infection were observed again for this treatment in the July 18th sample but not in the June 27th sample.

Figure 6 shows fungal infection of ants collected directly from nests in the experimental plots. In the control and plots treated with fungal bait, infection rates of 10 to 17 % were observed following the first application of the fungus and this infection level dropped to normal background levels by June 2nd. A second rise in infection was observed in ants collected after the second application of the fungal bait. These higher levels of infection were mainly observed in the plots which received the second application of the fungus 3 weeks after the initial one. However, high levels of infection also were observed in some of the other plots.

C) Levels of damage to almonds in treatment plots at time of harvest

These data will be presented after the harvest is completed and the data on damage have been summarized.

DISCUSSION

The number of ants in the experimental area was much lower in 1995 than in the 1994 field experiments. From the infection data, it appears that high infection of ants in April and early May contributed to lowering of the fire ant population in the experimental plots before the experiment was initiated. We cannot determine if this fungal infection was from an endemic strain of fungus or was related to our fungal treatment in 1994. This epizootic of B. bassiana that seems to have affected the ant population in the experimental area, may have been aided by the low temperatures and excessive moisture (rainfall) during the months of April and May 1995.

Despite the low level of the fire ant population, fungal bait applications seem to have produced desirable effects, especially in plots which received a second application 3 weeks after the initial one. When this preliminary report was written, no data were available for the preharvest fungal bait application or the level of ant damage in the nuts harvested from the plots.

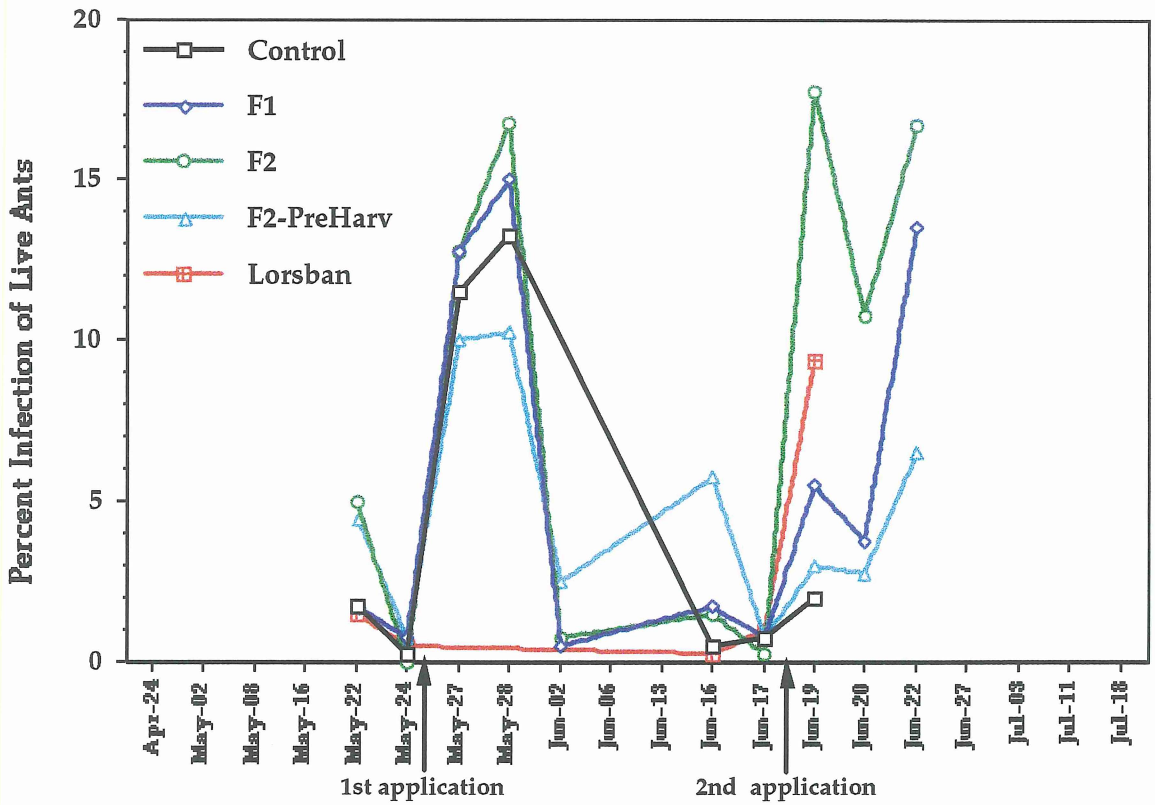

Figure 6. Percent infectionof live ants for all treatments in 1995 field experiment at Paramount Farms. Control = no application for ant control; F1 = only first application of fungal bait; F2 = First and second applications of fungal bait; F2-PreHarv = first and third application (on Aug 11th) of fungal bait; Lorsban = one application of Lorsban on May 25th.

This data will be included in the final version of our report, which will be presented at the Annual Meeting of the Almond Board.

ACKNOWLEDGEMENTS

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