Project no.: 2001-DO-00
Project title: Biological and Chemical Control of Ants
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Cooperating Personnel: Walt Bentley (UCCE/KAC)

FINAL REPORT

Objectives:

1) Determine the time required to reduce or eliminate: a) adult worker caste ants; b) immature ant stages (brood); and c) the queen(s) of individual colonies of the pavement ant that are provided access to various imported fire ant baits. Complete bait replications with southern fire ant colonies. (Continuation of Project Number: 2000-DO-00)

2) Continue determination of infection and impact of the imported fire ant pathogen, *Thelohania solenopsae*, on pavement and southern fire ant colonies (Project Number: 99-DO-00, Biological Control of Ants).

Justification of objectives:

1) The southern fire ant and the pavement ant can cause significant damage to almonds by feeding on kernels, which result in losses through downgrading and yield reduction. Current control options include applications of contact insecticides (e.g. Lorsban, Pounce) and use of ant baits (Clinch, Distance/Esteem). Adult ants cause kernel damage, while the amount of eggs, larvae, and pupae (brood) and the number of queens influence the re-infestation rate by ant colonies. The active ingredient in the ant bait Clinch™ (0.011% abamectin) sterilizes the queen in the red imported fire ant and studies funded by the Almond Board of California in 2000 indicated a similar mode of action occurs in the southern fire ant however data for the pavement ant is lacking. Knowledge of the time it takes to reduce or eliminate individual components of the ant colony structure for these pest ant species will provide a biological baseline to optimize the timing of ant bait applications to reduce kernel damage and minimize the need for additional treatments.

2) In the 1970's a microsporidium (protozoan) was found infecting several fire ant species in the genus *Solenopsis* in South America. Field studies in Argentina and Florida reported an 83% reduction in black imported fire ant, *Solenopsis richteri*, populations (Briano et al. 1995) and a 63% maximum reduction in red imported fire ant, *S. invicta*, populations (Oi and Williams, in press). Laboratory studies have shown that *T. solenopsae* reduces the queen's egg production and causes her premature death (Williams et al. 1999). This objective is a continuation of an effort to assess the potential of *T. solenopsae* as an alternative control for pest ants of almonds. If *T. solenopsae* does not infect pavement and southern fire ants, this study will help define its host range. This would be important for the potential use of *T. solenopsae* in California for the control of red imported fire ants, which have been found in California almond orchards.

Summary of progress in 2001:

Attempts to collect southern and pavement ant colonies with viable queens were made in April and October 2001 by USDA-ARS CMAVE personnel with the assistance of Walt Bentley

(UCCE/KAC). Ten colonies of southern fire ants with queens were collected in April 2001, and 51 groups of pavements ant were collected in April and October 2001. Twenty-five (of the 51) groups of pavement ants were shipped to CMAVE, however none of these groups had viable queens. Since we were unsuccessful at collecting pavement colonies with queens, bait studies were conducted on groups of adult worker caste ants. Additional bait testing on lab colonies of the southern fire ant colonies will be initiated this spring.

Obj. 1) Effects of imported fire ant baits on pavement ants

a) Bait Acceptance Study.

Methods: Pavement ant foraging on four imported fire ant baits was compared in a laboratory choice test to assess the palatability of these baits. Groups of pavement ants consisting of approximately 400-800 adult workers plus a minimal amount of brood (<1 ml or no brood) were provided simultaneous access to about 0.5 grams each of the following fire ant baits: Amdro, Clinch, Distance (Esteem), and Extinguish. Ants were held without food for 3 consecutive nights prior to the introduction of baits. The percentage of foraging ants on each bait was determined from the sum of counts recorded at 10, 20, 30, 60, and 120 minutes, and 24 hours for 3 replicates. Percentages were compared by analysis of variance and Tukey's HSD.

Results: The imported fire ant baits Amdro, Clinch, Distance (Esteem), and Extinguish were all foraged upon by pavement ants, with Clinch and Distance having significantly more ants (Table 1). These imported fire ant baits may be useful for pavement ant control if active ingredients are effective against this species.

Table 1. Average percentage of foraging pavement ants on imported fire ant baits. Averages followed by the same letter are not significantly different (P<0.05) by Tukey's HSD.

Bait	Clinch	Distance/Esteem	Extinguish	Amdro
% Ants Foraging:	37.4a	36.8a	15.9b	9.9b

b) Delayed Mortality Assessment.

Methods: Ant baits utilize the natural behavior of ants to collect food and share it with other members of the colony. Thus, it is crucial for any active ingredient, or toxicant, used in these baits to allow enough time for the ants to forage upon and spread the toxicant before dying. We conducted a adopted a standard technique used to evaluate toxicants for fire ant baits (Williams 1983) to assess the delay in death of pavements ants, southern fire ants, and red imported fire ants that were fed commercially available imported fire ant baits. We used imported fire ant baits that contained a metabolic inhibiting toxicant, hydramethylnon (Amdro), and abamectin (Clinch), which can sterilize queens and also cause adult mortality. Baits containing insect growth regulating active ingredients, such as fenoxycarb, pyriproxyfen, or methoprene, were not tested because they generally do not affect adult worker caste ants.

Twenty adult worker caste ants were placed in a plastic portion cup (1 fl. oz.) that had approximately 1/3 of the cup filled with a dental casting plaster (Castone). Castone bottoms were kept damp and the sides of the cups were dusted with talc and covered with a lid to prevent ants from escaping. Ants were starved for 4 days, test baits placed into each cup, after 24 hours,

baits were removed, and the normal diet was added the next day. Thus ants were provided 24 hours without bait or food after treatment exposure to facilitate any bait transfer among the test ants. The number of dead ants was recorded on 1, 2, 3, 6, 8, 10, 13, and 14 days after bait access. Cumulative mortality responses were averaged from 5 replicates.

Results: Delayed mortality was observed with all baits and ant species with the exception of southern fire ants exposed to Amdro (Table 2). In general, mortality of less than 15% for the first day, coupled with a greater than 85% mortality after 14 days has been a desirable response for metabolic inhibiting toxicants (e.g. hydramethylnon in Amdro). Clinch exhibited a very gradual increase in mortality, and because it also acts as a queen sterilizer in red imported fire ants this response seemed sufficient to allow distribution of the active ingredient through a colony. Results from 2000 (Almond Board Project 2000-DO-00) showed significant reductions in southern fire ant lab colonies given access to Clinch. The 54% mortality 1 day after exposure to Amdro may preclude thorough bait spread in southern fire ant colonies, thus allowing queens to survive and colony recovery.

Based on the results from the bait acceptance and delayed mortality assessments, The imported fire ant bait Clinch seems to posses the desired characteristics for the control of pavement ants. However laboratory colony tests and field trials are needed to confirm these conclusions.

Table 2. Average percent cumulative mortality over 14 days of 20 adult worker caste pavement ants, southern fire ants, and red imported fire ants after 24-hour access to imported fire ant baits.

		Average Percent Cumulative Mortality and Std. Errors (N=5)							
Ant Species	**Bait**	**Day1**	**Day2**	**Day3**	**Day6**	**Day8**	**Day10**	**Day13**	**Day14**
Pavement	Amdro	4.0	30.0	49.0	73.6	80.4	87.2	90.1	92.1
	±SE	2.4	12.5	19.3	16.4	12.1	8.2	7.7	5.8
Pavement	Clinch	15.0	22.0	41.0	76.0	82.0	84.0	89.0	89.0
	±SE	7.4	11.5	14.4	15.8	15.6	13.6	9.8	9.8
Pavement	Control	1.1	2.1	2.1	2.1	3.1	5.1	15.3	18.4
	±SE	1.1	1.3	1.3	1.3	2.1	2.3	5.6	6.7
Southern Fire	Amdro	54.0	88.0	97.0	100.0	100.0	100.0	100.0	100.0
	±SE	9.5	4.6	2.0	0.0	0.0	0.0	0.0	0.0
Southern Fire	Clinch	5.1	6.1	7.2	11.3	18.5	28.7	37.9	41.1
	±SE	3.9	4.0	3.8	5.6	5.9	9.9	14.5	15.0
Southern Fire	Control	1.1	5.2	6.2	10.3	11.4	18.5	23.6	24.6
	±SE	1.1	1.7	1.9	2.9	3.5	3.7	4.3	4.3
Red Imp. Fire	Amdro	16.0	44.0	54.0	95.0	100.0	100.0	100.0	100.0
	±SE	14.8	16.1	15.1	2.2	0.0	0.0	0.0	0.0
Red Imp. Fire	Clinch	2.0	9.0	17.0	32.0	47.0	58.0	71.0	71.0
	±SE	2.0	5.3	6.4	10.6	13.1	14.4	12.8	12.8
Red Imp. Fire	Control	2.0	3.0	5.0	11.0	13.0	16.0	19.0	20.0
	±SE	2.0	3.0	5.0	8.6	10.6	11.0	11.7	11.4

Obj. 2a) Inoculation of southern fire ant colonies with *T. solenopsae*

Methods: Collected colonies were allowed to establish and grow in artificial nests for a minimum of 8 weeks. Eight southern fire ant colonies were inoculated with the pathogen by placing 0.5 or 0.75 grams of *T. solenopsae* infected red imported fire ant brood near each colony. Eight other colonies were not inoculated and served as controls. Infection levels of inoculums were determined by examining individual geimsa stained smears of larvae and prepupae samples for vegetative stages of *T. solenopsae* using phase contrast microscopy. Inoculum infection levels were 70% for two replicates, 90% for another two replicates, and 100% for the remaining four replicates. To detect infection, adult, pupal, and larval ant smears were examined for *T. solenopsae* spores and vegetative stages at 12-13, 19-20, and 24 weeks after inoculations. Colony impact was assessed at 2 or 4-week intervals by estimating adult worker populations and the amount of brood using standard fire ant visual rating scales. To confirm that the inoculum could initiate infections, eight laboratory-reared red imported fire ant colonies were inoculated using the same procedures described for the southern fire ants. Eight other red imported fire ant colonies were not inoculated and used as controls. Colony impact observations were terminated after 24-32 weeks because substantial declines of infected red imported fire ant colonies have occurred in other studies within 6 months (Williams et al. 1999). For 2 of the 8 replicates impact observations were discontinued after 19 weeks because infections were not detected. Data were obtained from tests initiated in 1999 - 2001.

Results: *T. solenopsae* infections were not detected in any of the southern fire ant colonies. Adult southern fire ant populations increased an average of 37.4% (±119.1 SD) for the inoculated colonies, and 14.3% (±79.8) for the controls. Brood volumes declined an average of 8.8% (±59.8 SD) and 27.1% (±24.0) for the inoculated and control colonies, respectively. Percent changes in adult ant populations and brood volume from pre-inoculation levels in the inoculated and control southern fire ant colonies were not significantly different over the duration of the study (t-value=0.70, df=14, P=0.4974 for adults; t-value=0.48, df=14, P=0.6415 for brood). All queens remained alive throughout the study.

T. solenopsae infection was detected in 4 of the 8 inoculated red imported fire ant colonies and confirmed that infections could be initiated under the study conditions. In the infected colonies, average number of adults increased 131.0% (±183.6 SD) and brood declined 19.6% (±65.8). The increase in adult populations was not unusual as *T. solenopsae* has minimal effect on adult worker ants. These populations will decline when the queen oviposition rate declines to zero and adults are not replaced after they die naturally. The small reduction in brood reported here is probably indicative of an initial infection in queens. In contrast the control red imported fire ant colonies had increases of 183.4% (±296.1 SD) and 25.4% (±82.4) for adults and brood, respectively.

In summary, *T. solenopsae* infections were not detected in any of the inoculated southern fire ant colonies. These colonies also did not exhibit significant reductions in adult populations or brood. Thus, the southern fire ant does not appear to be a host of *T. solenopsae* based on the data reported here.

Obj. 2b) Inoculation of pavement ant colonies with *T. solenopsae*

Methods: Although we were not able to collect pavement ant colonies with viable queens from California, we were able to obtain a few newly-mated pavement ant queens from a colleague in Tennessee. Three small colonies, each containing 500-700 adult, 10-12 ml of brood, and a

queen, were raised in the laboratory from these queens. Two of these colonies were inoculated with 0.85 grams *T. solenopsae* infected red imported fire ant larvae (no pupae) that had a 90% infection rate using the same methods described for the southern fire ant. The remaining pavement ant colony was used as a control. Two red imported fire ant colonies were also inoculated and another two colonies were used as controls.

Results: After 6 weeks no infections were detected in the pavement ant colonies and there has been an average increase of 87% and 100% in adults and brood. Infections were detected in both of the inoculated red imported fire ant colonies. These colonies also grew since there was insufficient time for infections to reduce queen oviposition rates. Thus far, it seems that *T. solenopsae* will not infect pavement ants, however more data needs to be collected.

Summary of Results:

Objective 1. There were significantly higher numbers of pavement ants that foraged upon the imported fire ant baits Clinch and Distance than Extinguish and Amdro in a laboratory choice test. However pavement ants did forage on all of the baits, thus none of the baits were completely repellent.

Delayed mortality was observed in pavement ants that were given access to Clinch and Amdro, and to southern fire ants exposed to Clinch. Delayed mortality was much less apparent in southern fire ants exposed to Amdro. A delay in ant mortality provides time for ants to distribute bait throughout a colony.

Objective 2). *Thelohania solenopsae* infections were not detected in inoculated southern fire ant colonies, thus indicating this ant species is probably not a host of the entomopathogen. Pavement ant inoculation studies are in progress.

References Cited:

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