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MANAGEMENT OF ANTS IN ALMONDS, CITRUS, AND TABLE GRAPES

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ABSTRACT

The major ant pest on almonds is the southern fire ant, and most of the research has been aimed at this species. A newly developed ant bait, Tahara, consisting of the toxicant hydramethylnon incorporated into freeze-dried and pulverized silkworm pupae, is highly effective in killing southern fire ants. This material, being developed by American Cyanamid Corporation, is similar to an earlier experimental product from the same corporation, known as Amdro (hydramethylnon incorporated into a mixture of corngrits and soybean oil). Tahara generally outperforms Amdro. This comparison makes sense, considering the life style of southern fire ants, which are predators on other insects. In conjunction with Dr. Rust at UC Riverside, we have examined the relative acceptability of a number of insect and animal-derived bait materials. We consistently find that baits prepared from freeze-dried American cockroaches are more readily accepted by southern fire ants than are baits made from a number of other animal materials such as anchovies, mealworms, and crickets. This work is continuing, with the goal of developing a bait that is preferred over most other food materials that the ants might find in their environments. Tahara applied in both May and July prevented reestablishment of worker southern fire ants longer than did a single July application. Although we had earlier assumed that baits might be most easily found by ants if they are placed in discrete piles in the orchards, Tahara uniformly broadcast on the orchard floor seems to give as effective southern fire ant control as does Tahara placed in piles. Research is also being conducted to find a naturally occurring repellent that can keep foraging workers of ants away from nuts in almond orchards or exclude the workers from minisprinklers and other irrigation emitters.

OBJECTIVES

1. For each ant species, on each crop of interest, and in each different climatic or geographic area where the ants present a problem on those crops, develop and evaluate a wide variety of bait formulations of each potential toxicant, to determine chemical, moisture, consistency, granule size, and other characteristics that maximize their acceptability.
2. For each ant species, on each crop of interest, and in each different climatic or geographic area where the ants present a problem on those crops, establish replicated plots of candidate ant-control chemicals, using the most effective baiting procedures found in Objective 1; determine the critical amounts and timings of application that are needed for most effective control.

3. Develop repellents to prevent foraging of worker fire ants in almond orchards or to exclude the ants from irrigation emitters.
4. Establish relations between irrigation strategies, cover crops, and ant infestations, and manipulate irrigation strategies to lessen ant infestations.
5. Determine requirements of federal and state regulatory agencies with regard to registration of the experimental ant control chemicals and establish a practical plan for obtaining this registration.

PROCEDURES

1. For each ant species, on each crop of interest, and in each different climatic or geographic area where the ants present a problem on those crops, develop and evaluate a wide variety of bait formulations of each potential toxicant, to determine chemical, moisture, consistency, granule size, and other characteristics that maximize their acceptability. Work during 1995 emphasized this aspect of comparing the attractive components of baits. The most effective materials found in that testing, against southern fire ants, were from animal products, especially American cockroaches, mealworms, anchovies, and crickets. This work was continued during 1996, comparing these four materials, which had been formulated into blank baits (baits without toxicants) in the laboratory of M. K. Rust, UC Riverside. The blank baits were offered to southern fire ant workers in choice arenas described in our 1995 report. All the work was done in the laboratory at the Kearney Agricultural Center, with the ant colonies being connected to feeding arenas by means of short lengths of Tygon tubing, through which the foraging ants passed. Materials were offered in preweighed piles comprising about 1 g of bait each. Following a several hour foraging period, the baits were reweighed, and amounts that had been removed to the ant colonies were calculated from the differences. Results are given in Table 1.

2. For each ant species, on each crop of interest, and in each different climatic or geographic area where the ants present a problem on those crops, establish replicated plots of candidate ant-control chemicals, using the most effective baiting procedures found in Objective 1; determine the critical amounts and timings of application that are needed for most effective control.

Several experiments have been conducted to compare toxicants incorporated into baits with more conventional spray applications for southern fire ant control. Ant populations in the plots were assessed through a procedure that we call "hot-dogging". Ten flip-cap plastic vials, each containing a slice of hot dog, were placed on their sides adjacent to the two tree rows closest to the center of each plot. After two hours, the investigator picked up the vials, closing the caps on those that now contained ants. The vials were brought to the laboratory, where the ants were identified and counted.

In experiment 1, in Kern County, duration of toxic activity of the bait Tahara, the bait Amdro (consisting of hydramethylnon incorporated onto corngrits plus soybean oil), and the spray-insecticide, Lorsban, was studied following their application as one-time-only applications in mid-May or mid-July, or as a two-time application on both of the above

dates (Table 2). First applications were made May 17, 1996 and second applications on July 12. The crop was sprinkler irrigated. Plot size was 1/4 acre and four replications were set up for each treatment. Lorsban was broadcast-sprayed on the total soil surface of the plots in 50 gallons water per acre. Amdro and Tahara were applied in small piles of material at 10x10-foot intervals, giving about 400 discrete piles of material per acre. Results of hot-dog sampling for both southern fire ants and pyramid ants on various dates following application are given in Table 2.

In experiment 2, in Kern County, Tahara was applied to the soil surface on September 10, 1996. The experiment was arranged as randomized complete blocks with 4 replications. Plot size was 100 x 100 feet, giving individual plots of 1/4 acre area. Tahara was applied at 2 pounds per acre. One method of application was "broadcast", with the materials being administered by a hand-operated "cyclone"-type spreader. The applicator moved through the plots, overlapping the areas of delivery sufficiently to ensure uniform distribution. The other method was the placing of discrete piles of materials in the plots at about 8-foot separations in each direction, giving about 125 piles of material per plot or about 500 piles per acre. Materials were applied on September 10, 1996. Evaluations of efficacy in controlling ants were made by hot dogging (Table 3).

3. Develop repellents to prevent foraging of worker fire ants in almond orchards or to exclude the ants from irrigation emitters. With matching funds from the UC Statewide IPM Project, we have been determining the possibility of using naturally occurring, powerful repellents of foraging ants to prevent them from foraging in almond orchards or to prevent them from entering irrigation emitters, where they frequently cause stoppage of lines by their activities during the periods between irrigations. Using an arena bioassay in the laboratory, we have evaluated a series of candidate repellents against southern fire ants. Each ant colony is connected to a foraging arena by Tygon tubing. The arena is an aluminum-foil-covered table with a band of Stickem applied to the outer edge to prevent ants from escaping. Beeswax is melted at 115 degrees C on a hot plate and mixed with test chemicals or plant macerates to provide concentration series for assays. Beeswax-chemical mixtures are scribed with a Nalgene pipette onto cardboard squares covered with aluminum foil, as 10-cm-diam circles. The resulting circular barriers are 3-mm wide and contain 0.2 g of beeswax/chemical mix. Barriers are used in bioassays within one hr of creation, except during studies of the loss of disruptive activity to foraging ants due to aging.

For each assay, six barriers are tested simultaneously on the arena, with three control cards of beeswax alone being alternated around the arena periphery with three cards having a specified chemical concentration. Food bait is placed inside each wax barrier. A colony of ants is connected to the arena, and ants are allowed to forage there for 1 hr. Then the number of ants crossing the barrier on each card during a 2-min period is recorded. Additional assays are run for each tested chemical at each specified concentration, and each tested chemical/concentration is replicated 4 times.

The arena bioassay is being used to assay the ant-repellent action of 35 naturally occurring chemicals, mixed as 2% solutions in liquefied beeswax. These chemicals were selected following review of the literature with regard to animal- or plant-produced allomones that are highly repellent to ants or other insects. This work is presently being continued to gain more replication (Table 4).

Following our 1995 investigation of a series of plants that produce pungent odors or tastes (to humans), we selected five which were most olfactorily repellent to ants for further testing. They are all known spices; they were pulverized with mortar and pestle, extracted with methylene chloride, incorporated as 2% and 20% concentrations in beeswax, and tested for repellent activity against all three ant species in the arena bioassay (Table 5).

Other research is in process to determine loss of inhibitory activity over time of promising repellent chemicals and plant material extracts when formulated in beeswax. Those that gave better than 80% inhibition of foraging when tested the day they were prepared are retested after being aged for 1, 2, 3, ... and up to 28 days - with the duration of testing being determined by the length of time required for inhibitory activity to fall below 50% (Table 6).

RESULTS AND CONCLUSIONS

The comparison of attractiveness of blank baits (Table 1) shows that, although the results are highly variable (and the test is still continuing to gain more replication), American cockroaches tend to be a most attractive food source for southern fire ants. The ready acceptance of insect-based baits by this species gives promise that Tahara, which is an experimental product containing the toxicant, hydramethylnon formulated on freeze-dried silkworm pupae, may be readily taken by this species.

Field experiments have demonstrated the usefulness of Tahara as a bait against southern fire ants. As single applications, neither Amdro, Tahara, or Lorsban caused reductions in numbers of foraging southern fire ants for longer than 4 to 6 weeks, after which the ants were back in as large numbers as in untreated controls (Table 2). We interpret this rapid reestablishment of ants to indicate that none of these materials kill queens or large numbers of immature ants in nests below ground. Instead, they appear to kill all worker ants they contact; reestablishment after a month must represent the time required to produce a new generation of workers from surviving queens and immature stages. When two applications were made at two-month intervals from each other, Lorsban did not have any more effect in reducing reestablishment of ants than it did when only single applications were made. On the other hand, Tahara applied in both May and July continued to reduce numbers of reestablishing workers in August much better than did a single July application. Amdro was intermediate in this enduring effect of multiple applications, between Tahara and Lorsban. A similar result (Tahara giving the most enduring control of worker ants) was found for pyramid ants, which also were present in the experimental orchard.

Both Tahara and Amdro are being studied at application rates of 2 pounds per acre - a dose that has already been established by American Cyanamid Corporation in pursuing registration of these materials. In most of our experiments, we have applied the materials as small, discrete piles of bait material, equally spaced at about 10-foot intervals. We have inferred that the placing of piles of material may enhance its efficient carriage back to the nests by southern fire ants, which construct trails to such food sources and guide their fellow workers to the food. However, a broadcast procedure for dispensing bait might be easier than dispensing it in piles. We compared the efficacy of these two application methods, using Tahara; the trial was applied in early September, and through the month of October, when the ants stopped most activity for the year, both methods gave 100% control of foraging ants (Table 3).

Study of repellents may lead us to a method for broadcasting on the soil surface chemicals that are non toxic and only function to exclude foraging ants. It is interesting to note that the 5 most repellent plant materials that we have tested come from the reproductive organs (buds, seed pods and seeds) of plants that we use as spices; it makes sense that evolution would have incorporated the most repellent (to phytophagous animals) chemicals in association with these organs.

Further, three of these plants are characterized by humans as having an anise odor and flavor. To follow the lead that anise may be a particularly strong inhibitor of ant foraging, we obtained nine synthetic chemicals that occur in varying proportions in plants characterized by this odor. These are all benzene derivatives, being 2-, 3-, and 4-methoxybenzoic acids, methoxybenzyl alcohols, and methoxybenzaldehydes. In fact, the chemicals, out of all we tested, that show the most universal repellency against all three ant species are the three methoxybenzaldehydes (Table 4). However, these materials appear to lose activity more rapidly, inhibiting foraging of fewer than 50% of southern fire ants by 4 days after the beeswax barriers were prepared (Table 6). [Please note that we are conducting all of this experimentation on a broad front, and none of the data sets are yet complete or statistically analyzed.]

The most ant-repellent semiochemicals tested fall into two classes - benzene derivatives (alcohols, aldehydes, and esters), and long-chain alcohols, aldehydes, and esters. Other highly repellent benzene derivatives in addition to the anise components mentioned have been found. Especially, methyl eugenol had high activity. However, inhibitory activity of this material in beeswax also may be of short duration, not persisting longer than six days for southern fire ants.

In relation to functional groups, when acid, alcohol, aldehyde, and ester groupings were attached to identical benzene ring configurations or long-chain hydrocarbon configurations, the acids tended to be the least active in promoting inhibition of foraging. For southern fire ants only one long-chain chemical (n-dodecyl alcohol) provided more than 80% inhibition of foraging, while eight benzene derivatives were this active.

Table 1. Amounts of various bait materials removed by foraging ants of three species. 1996.

	Mean amounts (mg) removed from indicated bait			
	Anchovy	Mealworm	Am. roach	Cricket
Southern fire ants (5)	212	360	461	336

Table 2. Comparing single vs double applications of Lorsban, Tahara, and Amdro for control of southern fire ants and pyramid ants. 1996.

Treatment	Rate	Number of applications	Time of application	Mean ants per hot-dog sampling vial				
				5/26	6/19	7/12	7/19	8/7
<i>Southern fire ants</i>								
Lorsban	4 pts/A	1	mid May	0.0	8.0	16.1	6.7	21.6b
		1	mid July	---	---	---	0.0	17.5b
		2	above 2 dates	0.0	15.1	16.9	0.0	17.5b
Tahara	2 #/A	1	mid May	0.0	2.0	6.2	10.9	11.1b
		1	mid July	---	---	---	0.0	2.3a
		2	above 2 dates	0.0	0.0	7.0	0.0	0.0a
Amdro	2 #/A	1	mid May	0.0	0.1	7.4	2.1	14.1b
		1	mid July	---	---	---	10.0	21.4b
		2	above 2 dates	0.0	1.1	3.6	0.7	2.4a
Untreated				8.0	82.5	32.7	36.6	42.6c
<i>Pyramid ants</i>								
Lorsban	4 pts/A	1	mid May	---	---	1.2	0.2	2.9
		1	mid July	---	---	---	0.1	0.4
		2	above 2 dates	---	---	2.8	0.6	1.2
Tahara	2 #/A	1	mid May	---	---	1.7	1.0	2.1
		1	mid July	---	---	---	0.0	2.5
		2	above 2 dates	---	---	0.5	0.0	0.0
Amdro	2 #/A	1	mid May	---	---	0.4	0.6	1.2
		1	mid July	---	---	---	0.6	2.5
		2	above 2 dates	---	---	1.9	1.3	3.2
Untreated				---	---	3.6	0.8	4.6

Table 3. Comparing discrete piles of Tahara vs broadcast application of the bait material for control of southern fire ants and pyramid ants. 1996

<u>App. Method</u>	<u>Mean numbers of ants per vial after indicated weeks</u>					
	<u>Fire ants</u>			<u>Pyramid ants</u>		
	<u>3</u>	<u>5</u>	<u>8</u>	<u>3</u>	<u>5</u>	<u>8</u>
Brdst	0.0 a	0.0 a	0.0 a	0.0	0.0	0.0
Mounds	0.1 a	0.0 a	0.0 a	0.0	0.0	0.0
Control	61.0 b	41.0 b	0.6 b	0.8	0.0	0.1

Table 4. Activity of a variety of synthetic semiochemicals and chemicals related to semiochemicals in inhibiting the foraging behavior of ants of three species when incorporated at given concentrations into beeswax barriers.

Semiochemical	Mean percent. inhibition of foraging of southern fire ant workers ¹	
<i>Benzene derivatives</i>		
2-Methoxybenzoic acid (<i>o</i> -Anisic acid)	2%	10 (2)
3-Methoxybenzoic acid (<i>m</i> -Anisic acid)	2%	58 (2)
4-Methoxybenzoic acid (<i>p</i> -Anisic acid)	2%	39
2-Methoxybenzyl alcohol	2%	93
	0.2%	37
3-Methoxybenzyl alcohol	2%	81 (2)
	0.2%	11
4-Methoxybenzyl alcohol	2%	56 (2)
2-Methoxybenzaldehyde (<i>o</i> -Anisaldehyde)	2%	97
	0.2%	44 (3)
3-Methoxybenzaldehyde (<i>m</i> -Anisaldehyde)	2%	92
	0.2%	8
4-Methoxybenzaldehyde (<i>p</i> -Anisaldehyde)	2%	82
	0.2%	58 (2)
2-Aminobenzoic acid (Anthranilic acid)	2%	23 (3)
Methyl 2-Aminobenzoate (Methyl anthranilate)	2%	84 (4)
	0.2%	-9
2-Hydroxybenzoic acid (Salicylic acid)	2%	56 (2)
2-Methoxybenzoic acid (Methyl salicylate)	2%	84 (3)
4-Allyl-2-methoxybenzyl alcohol (Eugenol)	2%	79 (3)
4-Allyl-1,2-dimethoxybenzene (Methyl eugenol)	2%	94 (2)
	0.2%	-16
4-Allyl-1,2-(methylenedioxy) benzene (Safrole)	2%	72 (2)
	0.2%	57
Methyl benzyl ether (Anisole)	2%	22 (2)
<i>Long-chain acids, alcohols, and esters</i>		
3,7-Dimethyl-2,6-octadienal (Citral) (C8)	2%	79 (2)
2,6-Dimethyl-2,7-octadien-6-ol (Linalool) (C8)	2%	3
n-Decyl alcohol (C10)	2%	42
n-Dodecyl acid (Lauric acid) (C12)	2%	38
n-Dodecyl alcohol (Lauryl alcohol) (C12)	2%	85

¹ Number of replicates is given in parentheses. If no number is given, there is 1 replicate. Numbers highlighted in "Bold" are means greater than 80%, most of which we presume, based on visual analysis, to be significantly (5%) greater than 0% inhibition.

Mean percent. inhibition of foraging of
southern fire ant workers

Semiochemical

3,7,11-Trimethyl-2,6,10-dodecatrienol (Farnesol) (C12)	2%	52 (2)
Dodecyl acrylate (Lauryl acrylate) (C12)	2%	81
n-Tetradecyl acid (Myristic acid) (C14)	2%	-12
n-Tetradecyl alcohol (Myristyl alcohol) (C14)	2%	63
n-Methyl tetradecanoate (Methyl myristate) (C14)	2%	11 (4)
n-Hexadecyl acid (Palmitic acid) (C16)	2%	25 (2)
Z-9-Hexadecenoic acid (Palmitoleic acid) (C16)	2%	54
n-Hexadecyl alcohol (Cetyl alcohol) (C16)	2%	-4
n-Hexadecyl acetate (Palmityl acetate) (C16)	2%	21
n-Methyl hexadecanoate (Methyl palmitate) (C16)	2%	11 (3)
n-Octadecyl acid (Stearic acid) (C18)	2%	40
9,10-Octadecenoic acid (Oleic acid) (C18)	2%	74
Z,Z-9,12-Octadecadienoic acid (Linoleic acid) (C18)	2%	38

Table 5. Activity of a variety of macerated natural plant materials when incorporated into beeswax barriers in inhibiting the foraging behavior of ants of three species. These results are based on the first replicate only of a continuing experiment which will be composed of four replicates when complete.

		Mean percent. inhibition of foraging of <u>of southern fire ant workers</u>
<u>Plant material</u>	<u>% in beeswax</u>	
Anise seeds	2	3
	20	55
Star anise seed pods	2	59
	20	94
Fennel seeds	2	57
	20	98 ²
Cloves flower buds	2	32
	20	77
All spice seed pods	2	8
	20	43

²This material was assayed at a concentration of 25%.

Table 6. Loss of inhibitory activity with aging, of barriers containing specified percentages of semiochemicals or of plant extracts.

	Southern fire ants											
	<u>% inhibition after indicated number of days of barrier aging</u>											
<u>Chemicals, all 2%</u>	<u>0</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>6</u>	<u>8</u>	<u>12</u>	<u>16</u>	<u>20</u>	<u>24</u>	<u>28</u>
2-Methoxybenzyl alcohol	82	90	86	75		80						
3-Methoxybenzyl alcohol	93	62	78	47		59	31					
4-Methoxybenzyl alcohol	93	73	67	49								
2-Methoxybenzaldehyde	97	63	72	55	48							
3-Methoxybenzaldehyde	92	73	66	66		46						
4-Methoxybenzaldehyde	82	88	53	84	15							
Methyl 2-aminobenzoate	92	58	72	77		19						
4-Allyl-1,2-dimethoxybenzene	91	63	73			37						
n-Dodecyl alcohol	85	60	48									
<u>Natural plant extracts, all 20%</u>												
Star anise	94	67	40									
Cloves	78	73	81			68	47					
Fennel	99	98	72	64		37	18					