Laboratory Studies with Nine Amidinohydrazones, a Promising New Class of Bait Toxicants for Control of Red Imported Fire Ants[1,2]

D. F. WILLIAMS[3], C. S. LOFGREN[3], W. A. BANKS[4], C. E. STRINGER[4], AND J. K. PLUMLEY[3]
Agric. Res., SEA, USDA

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ABSTRACT

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AC 217,300 (Tetrahydro-5,5-dimethyl-2(1*H*)-pyrimidinone,[3-[4-trifluoromethyl)phenyl]-1-[2-[4-(trifluoromethyl)phenyl]ethenyl]-2-propenylidene]hydrazone) was the most effective of nine amidinohydrazones tested as bait toxicants against workers *Solenopsis invicta* Buren. In screening tests, this chemical dissolved in soybean oil (SBO) showed delayed action over more than a 10-fold dosage range. In secondary tests, this toxicant formulated in granular SBO baits at concentrations of 2.5–10% (in SBO) and fed to entire laboratory colonies (queen, immatures, and 10,000–100,000 workers) either killed the entire colony or killed most of the workers and the colony queen.

The red imported fire ant (RIFA), *Solenopsis invicta* Buren, has been the target of large-scale control programs with mirex bait since the early 1960's (Lofgren et al. 1962, 1963, and 1964). Use of this bait has been the only approved method for control of the ants on large areas since the mid-1960's; however, the discovery of mirex residues and their persistence in nontarget organisms (Ludke et al. 1971, Mehendale et al., 1972, Baetche et al. 1972), the toxicity of mirex to certain estuarine animals (Kaiser 1974, Lowe et al. 1970, 1971), and the possibility of its carcinogenicity (Innes et al. 1969, Mrak 1969, Ulland et al. 1977) led to cancellation of its registrations by the Environmental Protection Agency on June 30, 1978. In an effort to find alternative toxicants, USDA scientists have evaluated more than 5000 chemicals incorporated into food attractants such as soybean oil (SBO), honey-water, or peanut butter (Lofgren et al. 1967, Wojcik et al. 1972, 1973, Levy et al. 1973, 1974, Banks et al. 1977).

Requirements for an effective toxicant were described by Stringer et al. (1964) who showed that to be effective in a bait, a toxicant must exhibit delayed toxicity over a wide range of concentrations and not be repellent when combined with a food attractant.

We report here results of laboratory tests conducted in 1977 and 1978 to determine the toxicity of a promising class of bait toxicants, the amidinohydrazones, on the RIFA.

Materials and Methods

All chemicals submitted for our laboratory evaluation as bait toxicants first undergo primary screening tests. Those chemicals that exhibit delayed toxicity (defined as <15% kill at 1 day but >90% after 14 days) are subjected to secondary screening tests, which involve tests against laboratory colonies containing a queen and workers in all stages of development.

The primary screening procedure for evaluation of bait toxicants was described initially by Stringer et al. (1964); later modifications were described by Lofgren et al. (1967), Levy et al. (1973), Wojcik et al. (1973) and by Banks et al. (1977). Our procedures included further modifications, principally the use of laboratory-reared ants. The method was as follows: 30-ml disposable plastic medicine cups (40 mm ID at the top, tapering to 32 mm ID at the bottom × 38 mm high) were used as test chambers. A 6-mm hole was drilled through the bottom of each cup, and a layer of flesh-tone colored dental labstone (Ransom and Randolph Co., Toledo, OH) was poured over the bottom (15 mm thick). The labstone covered the hole and acted as a wick to absorb water. Because of its hardness, it also prevented ants from escaping through the bottom. The cups were placed on a moist 6.35-mm plastic foam pad in a tray and covered with a sheet of clear Plexiglass® to prevent rapid evaporation of the water and to maintain the high humidity necessary to prevent desiccation of the ants.

Twenty worker ants from laboratory colonies deprived of food for 14 days were placed in each cup ca. 24 h before the test. This pretreatment holding period allowed time for the ants to recover from handling and to orient to the containers. Only those worker ants collected from the inside of rearing cells containing brood were used, since other tests had shown that these younger ants survived longer than foraging ants (Williams and Lofgren, unpublished data).

Candidate chemicals were dissolved directly in once-refined SBO and offered to the ants on a cotton swab placed in a small vial cap that was then placed in a cup. The ants were allowed to feed *ad lib.* on the chemically treated SBO for 24 h, after which the treated SBO was removed. The ants remained without SBO for an additional 24 h, then new vial caps containing cotton swabs saturated with once-refined SBO were placed in the cups and left for the remainder of the test period. In addition to the candidate chemicals, a mirex standard and soybean oil check were also evaluated in each test. Knockdown and mortality counts were made at intervals of 1, 2, 3, 6, 8, 10, and 14 days after initial exposure. Each test consisted of 3 replications at 3 concentrations: 1.0, 0.1, and 0.01%. Room temperature was maintained at 26.7°±2.2°C during the tests.

Nine chemicals were evaluated in these tests (provided by the American Cyanamid Company, Princeton,

[1] Hymenoptera: Formicidae.

[2] Mention of a pesticide, commercial or proprietary product does not constitute an endorsement or recommendation by the USDA. Received for publication Apr. 14, 1980.

[3] Insects Affecting Man and Animals Research Laboratory, AR, SEA, USDA, Gainesville, FL 32604.

[4] Imported Fire Ant Research Laboratory, Agricultural Research, SEA, USDA, Gulfport, MS 39503.

NJ). Their code numbers and names are as follows:

AC 206,848 (AI3-29285) (Octahydro-2*H*-benzimidazol-2-one, [3-(4-chlorophenyl)-1-[2-(4-chlorophenyl)ethenyl]-2-propenylidene]hydrazone);
AC 206,792 (AI3-29283) (Hexahydro-2*H*-1,3-diazepin-2-one, [3-(4-chlorophenyl)-1-[2-(4-chlorophenyl)ethenyl[-2-propenylidene]hydrazone);
AC 206,623 (AI3-29282) (Hexahydro-2*H*-1,3-diazepin-2-one, [3-[4-(trifluoromethyl)phenyl]-1-[2-[4-(trifluoromethyl)phenyl]ethenyl]-2-propenylidene] hydrazone);
AC 201,136 (AI3-29281) (Hexahydro-2*H*-1,3-diazepin-2-one, [3-[4- (trifluoromethyl)phenyl]-1-[2-[4-(trifluoromethyl)phenyl]ethenyl]-2-propenylidene] hydrazone, monohydrochloride);
AC 206,816 (AI3-29284) (4,4-Dimethyl-2-imidazolidinone, [3-(4-chlorophenyl)-1-[2-(4-chlorophenyl) ethenyl]-2-propenylidene]hydrazone, monohydrochloride);
AC 233,382 (AI3-29404) (4-Methyl-2-imidazolidinone, [-3-[4-(trifluoromethyl)-phenyl]-1-[2-[4-(trifluoromethyl)phenyl]ethenyl]-2-propenylidene] hydrazone);
AC 206,859 (AI3-29286) (Tetrahydro-5,5-dimethyl-2(1*H*)-pyrimidinone, [3-(4-chlorophenyl)-1-[2-(4-chlorophenyl)ethenyl]-2-propenylidene]hydrazone);
AC 217,300 (AI3-29349) (Tetrahydro-5,5-dimethyl-2(1*H*)-pyrimidinone, [3-[4-(trifluoromethyl)phenyl]-1-[2-[4-(trifluoromethyl)phenyl]ethenyl]-2-propenylidene]hydrazone); and
AC 222,935 (AI3-29403) (Tetrahydro-2(1*H*)-pyrimidinone, [3-[4-(trifluoromethyl)-phenyl]ethenyl]-2-propenylidene]hydrazone).

In the primary screening tests, we found that the compounds were difficult to solubilize in SBO at concentrations >1%. Thus, before initiating tests with laboratory colonies (secondary screening), we conducted a series of trials to find cosolvents that would permit testing of the chemicals at concentrations >1%. The acceptability of each cosolvent when added to SBO was evaluated in bait acceptance tests as described by Lofgren et al. (1961) against 4 colonies of field-collected RIFA brought into the laboratory in 18.9-liter buckets. A 2.54-cm square of blotter paper on a 5.08-cm square of aluminum foil was impregnated with the cosolvent at various concentrations in SBO, another square was impregnated with the same quantity of neat SBO. The squares were placed on opposite sides in the bucket, and the ants were allowed to orient for 1 min. A 5-min test was then conducted in which numbers of ants were counted on both the cosolvent treated square and the SBO (standard) square (estimated for numbers >100). An acceptance ratio was determined by division of the total number of ants in the 4 colonies feeding on the candidate cosolvent by the total number feeding on the SBO standard.

In the secondary screening tests, the most promising compound was dissolved in SBO at concentrations of 2.5, 5.0, 10.0, and 20.0% and fed to the ants either directly or after impregnation on a granular carrier (extruded corn pellets; Quaker Oats Company, Chicago, IL). The ants were allowed access to the bait for 96 h. The bait was then removed and the colony fed honey water (1:1) and Banks diet (Williams et al. 1980) *ad lib*. General observations on the status of the colony and mortality were recorded weekly. The test was continued until the queen, brood, and 90% or more of the workers were dead, or until the colony recovered and returned to normal. The latter condition was considered to be in effect after the queen resumed egg laying and all stages of brood were present.

In those tests conducted at our Gainesville laboratory, the colonies consisted of 10,000–120,000 workers and 50–60 ml of brood. The chemical was offered to the ants in SBO impregnated on an extruded corn pellet carrier (5 g/colony). In tests conducted at our Gulfport laboratory, the colonies consisted of 10,000–20,000 workers and 10–20 ml of brood. The SBO-toxicant bait was drawn into micropipettes which were then placed on the bottom of the trays holding the colonies (100 μl/colony). The ants readily removed the bait from the corn pellets and micropipettes. Baits with both cosolvents were tested at Gulfport, but only baits with the oleic acid cosolvent were tested at Gainesville.

Further tests were conducted with SBO-toxicant bait formulated on corncob grits (2.5, 5.0, and 10.0% in SBO) and extruded corn pellets (2.5% in SBO). Because of the low absorbency of the corncob grits, these baits contained 15% of the SBO-toxicant bait while the extruded corn pellets contained 30%. Five g of each concentration of bait were offered to each of the colonies, which contained 60,000–120,000 workers.

Results and Discussion

The primary screening results (Table 1) indicated that 7 of the 9 chemicals tested produced delayed toxicity (as defined by Stringer et al. 1964) at at least one concentration; however, only 2 of the chemicals, AC 217,300 and AC 206,859, produced delayed toxicity at 2 levels of concentration (1.0 and 0.1%). Of these 2 compounds, AC 217,300 gave the better results (complete kill), although it was only ca. 1/10 as toxic as the mirex standard.

Results of bait acceptance tests with various types of solvents showed that 2 cosolvents, linoleic acid and oleic acid, increased the solubility of AC 217,300 and permitted concentrations of up to 25% when used in ratios of 1:2 (cosolvent:insecticide). A comparison of acceptance of AC 217,300 in these cosolvents (Table 2) showed good acceptance at 1% of toxicant but a decrease in acceptance at higher concentrations. Solutions with oleic acid were slightly more acceptable than those with linoleic acid except at the 25.0% concentration.

Results of the secondary screening (colony) tests to determine the effectiveness of bait formulations of AC 217,300 (Table 3) showed that concentrations of 2.5, 5.0, and 10.0% killed all colonies. Smaller colonies were killed much faster than larger ones (10–15 days vs. 20–24 weeks). In most cases, the queen was killed before all workers died (1–4 weeks). Worker mortality that appeared to be attributable to the toxicant was complete by 4 weeks, and additional mortality was probably the result of other physiological or environmental causes. Little difference in effectiveness appeared to be attributable to the 2 cosolvents; however, speed of kill was

Table 1.—Percentage knockdown and mortality after indicated number of days posttreatment of red imported fire ants treated with indicated concentrations of candidate chemicals in soybean oil (avg of 3 tests).

Chemical	Concn (%) in Soybean oil	Percentage knockdown and mortality after indicated number of days						
		1	2	3	6	8	10	14
AC 217,300	0.01	2	2	3	7	13	18	20
	0.1	2	2	8	68	93	97	100
	1.0	7	33	67	100			
AC 206,859	0.01	0	0	0	1	1	5	7
	0.1	0	1	26	67	82	94	97
	1.0	9	48	72	96	99	99	99
AC 206,623	0.01	0	0	5	10	12	20	30
	0.1	2	5	42	85	90	90	95
	1.0	42	72	87	92	92	95	95
AC 206,848	0.01	0	2	5	7	7	17	27
	0.1	0	0	45	75	80	95	95
	1.0	37	65	70	77	85	87	87
AC 206,792	0.01	0	0	5	12	12	15	37
	0.1	0	0	2	2	10	10	32
	1.0	0	20	57	72	80	85	90
AC 201,136	0.01	0	0	5	10	22	25	40
	0.1	2	2	5	7	17	25	45
	1.0	0	0	25	60	80	92	100
AC 233,382	0.01	5	7	7	10	10	15	18
	0.1	0	0	0	2	3	3	7
	1.0	0	10	35	65	67	72	92
AC 222,935	0.01	0	1	1	1	1	1	5
	0.1	0	1	1	6	8	19	43
	1.0	0	3	10	30	53	65	76
AC 206,816	0.01	0	2	2	5	10	10	22
	0.1	0	0	0	0	0	2	15
	1.0	0	5	5	12	25	30	42
Mirex standard	0.01	3	3	3	27	62	82	93
	0.1	0	5	75	100			
	1.0	7	97	100				
Soybean oil check		0	0	0	1	2	2	3

Table 2.—Acceptance rates of 2 cosolvents in soybean oil with AC 217,300 against laboratory colonies of red imported fire ants (4 replicates/concentration).

Concn (%) of AC 217,300[a]	Acceptance[b] ratio
Linoleic acid	
1.0	0.84
2.5	0.59
5.0	0.23
10.0	0.33
20.0	0.20
25.0	0.29
Oleic acid	
1.0	1.03
2.5	0.66
5.0	0.46
10.0	0.36
20.0	0.38
25.0	0.09

[a] Concentration of cosolvents was one-half that of AC 217,300.
[b] Acceptance ratio = number of ants feeding on the candidate sample divided by number of ants feeding on the standard (once-refined soybean oil).

inversely correlated with concentration of the toxicant, and no colonies were killed at the highest concentration (20%). Undoubtedly this reflects 2 factors: repellency by AC 217,300 as the concentration is increased and death of many ants that consumed the bait before they could pass it on to their nest-mates.

In laboratory trials (Table 4) with the SBO AC 217,300 bait on corncob grits, all colonies were killed within 24 weeks with mortality of the queens by 4 weeks. Although the initial kill with the extruded corn pellet bait was not as high (20 vs. 31–50% as with the corncob grits) the eventual death of the colony was faster (12 vs. 24 weeks).

The data obtained in our laboratory trials provide evidence AC 217,300 is a very promising toxicant in baits for control of red imported fire ants. In these laboratory trials, mortality of the entire colony was correlated with size and varied from 2 to 24 weeks. This difference in speed is undoubtedly attributable to the amount of bait consumed and the completeness of distribution of the bait to all colony members. Obviously, the larger the colony, the greater the chance that some ants do not receive the toxicant or receive a sublethal amount because of dilution during trophallaxis. Of particular interest is the fact that the colony queen was invariably killed within 4 weeks after the colony received the bait, even though many of the workers were not affected at this time. These results suggest that AC 217,300 is a viable alternative to mirex for the control of imported fire ants.

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Table 3.—Mortality in laboratory colonies of the red imported fire ant treated with indicated concentrations of AC 217,300 in soybean oil baits.[a]

Concn (%) of AC 217,300 in soybean oil	Mortality (%) in each colony at indicated number of weeks posttreatment[b]							
	1	2	3	4	8	16	20	24
			Linoleic acid cosolvent (Gulfport)					
2.5	90	100 QD						
5.0	90	100 QD						
10.0	90	98	100 QD					
20.0	70	70	70	CN				
			Oleic acid cosolvent (Gulfport)					
2.5	90 QD	98	100					
5.0	80	100 QD						
10.0	90	90	100 QD					
20.0	75	75	85	CN				
			Oleic acid cosolvent (Gainesville)					
2.5	58	88	90	93 QD	95	97	98	100
5.0	50	73	75	79 QD	91	95	100	
10.0	30	55	64	66 QD	66	75	80	100
20.0	10	13	18	20 CN				

[a] Tests at Gulfport, Miss. were with colonies with 10,000–20,000 workers and 10–20 ml of brood; tests at Gainesville, Fla., were with colonies with 60,000–120,000 workers and 50–60 ml of brood.
[b] QD = queen dead; CN = colony normal (queen alive with eggs and all stages of brood); colonies receiving only soybean oil and either cosolvent and untreated checks remained normal throughout the test.

Table 4.—Tests with AC 217,300 in baits used against entire laboratory colonies of red imported fire ants.

Type of carrier	Concn (%) in SBO	No. of replications	Avg % mortality after indicated number of weeks[a]						
			1	4	8	12	16	20	24
Corncob grits	2.5	2	50	50 QD	60	80	90	100	
	5.0	8	31	63 QD	73	85	95	98	100
	10.0	2	50	55 QD	65	80	85	85	100
Extruded corn pellets	2.5	4	20	68 QD	89	100			

[a] QD = queen is dead; untreated check mortality averaged 7%.

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