THE DEVELOPMENT OF TOXIC BAITS FOR THE CONTROL OF THE IMPORTED FIRE ANT

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The red imported fire ant (RIFA), *Solenopsis invicta* Buren, and the black imported fire ant, *Solenopsis richteri* Forel were introduced into the United States at Mobile, Alabama, about 40 to 60 years ago. This paper will be limited to the RIFA. The ants spread rapidly from this initial port of entry and now infest all or parts of 9 states and Puerto Rico. Eventually the mound building and stinging habits of the ants caused farmers in infested areas to demand relief and in 1957 the U.S. Congress voted to establish a Federal-State Cooperative Imported Fire Ant Program (Lofgren et al. 1975). A summary of the important events related to the introduction and control of imported fire ants is shown in Table 1. Chemical control of the imported fire ants has involved 3 methods; (1) residual

TABLE 1. A SUMMARY OF THE IMPORTANT EVENTS IN IMPORTED FIRE ANT (*Solenopsis invicta*) CONTROL.

1930-1940	— Accidental introduction of *Solenopsis invicta* into the Mobile, Alabama area.
1949-1953	— Surveys of nurseries showed rapid dissemination of *S. invicta* in the southeast U.S. from shipping infested plant soil from Mobile, Alabama area.
1957	— Cooperative federal-state control program initiated.
1952-1962	— Heptachlor and dieldrin used for long term residual control.
1962	— Mirex replaces heptachlor and dieldrin.
1962-1978	— Over 140 million acres of land were treated with mirex.
1978	— Mirex registration cancelled by the EPA eliminating the only chemical registered for control of fire ants on farmland.
1979	— Experimental use permit issued by EPA for large-scale field testing of American Cyanamid AC 217,300, a new bait toxicant against fire ants.
1980	— Conditional registration approved by EPA of American Cyanamid AC 217,300 (Amdro) for use against fire ants on pasture and range grasses, lawns, turfs and nonagricultural lands.
1981	— Experimental use permit issued by EPA for large scale field tests of Eli Lilly EL-468 (BANT).
Spring 1982	Submit data for full registration of Amdro. Proposed EUP for field testing of avermectin B_1a.
Spring 1983	Possible Conditional Registration of EL 468 (BANT) for use against fire ants.

control in which chlorinated hydrocarbons such as chlordane, heptachlor and dieldrin were applied broadcast as granular formulations, (2) individual mound treatments using numerous chemicals applied by drenching as emulsifiable concentrates mixed with water, and (3) toxic baits such as mirex and Amdro® which consist of a food attractant, a chemical toxicant and a granular carrier broadcast over large areas. Residual treatments are environmentally damaging and too costly. Mound treatments are not economically feasible to treat large numbers of fire ant mounds over a wide area. The most economically feasible and environmentally least damaging treatment for the imported fire ant over large area-wide treatments is the use of the bait toxicant system for control. However, the difficulty in finding a chemical for use as a bait toxicant in control of the RIFA over large areas can be shown by comparing the number of chemicals evaluated over a 23-year period as bait toxicants (7110) vs. the number of chemicals evaluated as single mound treatments or mound drenches (150). Of the 7,110 chemicals only 2 chemicals have been commercially available in baits for RIFA control while of the 150 chemicals tested for single mound treatments more than 50 have become available to the public for use in control of fire ants.

Requirements for an effective toxicant (Stringer et al. 1964) are: (1) give delayed toxicity (<15% mortality after day 1 and >85% mortality after day 14), (2) be effective over a 10-fold dosage range, (3) formulate with carriers easily and (4) be environmentally acceptable. In an effort to discover delayed-action toxicants, USDA scientists have evaluated large numbers of chemicals incorporated into food attractants such as soybean oil, honey-water or peanut butter (Lofgren et al. 1967; Wojcik et al. 1972, 1973; Levy et al. 1973, 1974; Banks et al. 1977). The number of chemicals evaluated as bait toxicants against the red imported fire ant from 1958 through December 1981 by the USDA is shown in Table 2. Eighty-six percent of these chemicals were not effective while 8% gave rapid kill and were, therefore, not also usable as bait toxicants. The remaining chemicals screened were toxicants that exhibited delayed toxicity over a range of concentrations. Of these, 5% showed delayed action over a 1 to 9-fold dosage range, less than 1% over a 10 to 99-fold dosage range and only 1 compound, mirex, over a 100-fold or greater dosage range. An

TABLE 2. NUMBER OF CHEMICALS EVALUATED IN THE LABORATORY AS BAIT TOXICANTS AGAINST THE RED IMPORTED FIRE ANT, *Solenopsis invicta* BUREN, FROM 1958 THROUGH DECEMBER, 1981.

Class	Number of chemicals tested[a]			
	1958-1975	1976-1981	Totals	Percent
I	2,069	2,858	4,927	86
II	340	126	466	8
III	249	58	307	5
IV	19	10	29	<1
V	1	0	1	<0.02
	2,678	3,052	5,730	

[a]Does not include 733 plant extracts and 647 special formulations that were also evaluated. Thus, a sum total of 7,110 chemicals have been evaluated from 1958 through 1981.

interesting point here is that of the total number of chemicals that have been evaluated, only 50 compounds have actually reached the field testing stage. Of this group only 2 have been commercially developed for public sale, mirex and AC 217,300 (tetrahydro-5,5-dimethyl-2(1H)-pyrimidinone, [2-[4-(triflouromethyl)ethenyl]-2-propenylidene]-2-propenylidene]hydrazone). This chemical now is sold commercially by American Cyanamid Company in a bait under the trade name, Amdro®. Two other compounds, EL-468, (N-[2-amino-3-nitro-trifluoromethyl)phenyl]2,2,3,3-tetrafluoropropanamide) produced by Eli Lilly and Company (Williams and Lofgren 1981) and avermectin B_1a, produced by Merck and Company, (Lofgren and Williams 1982) also look very promising as bait toxicants and may be developed commercially. The results of laboratory and field tests with these chemicals will be discussed.

Some other chemicals such as the insect growth regulators also show promise for use in baits; however, they usually do not act as direct toxicants, but rather inhibit larval development. These chemicals will be discussed in the next paper by Mr. W. A. Banks.

PRIMARY SCREENING

All chemicals submitted for evaluation as bait toxicants first must undergo primary screening tests. Williams et al. (1980) described the primary screening procedures for evaluation of bait toxicants as follows: 30 ml disposable plastic medicine cuts (40 mm I.D. at the top tapering to 32 mm I.D. at the bottom X 38 mm high) are used as test chambers. Twenty worker ants from laboratory colonies deprived of food for 14 days are placed in each cup ca. 24 h before the test. This pretreatment holding period allows 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 are used since other tests have shown that these younger ants survive longer than foraging ants (Williams et al. 1980). Candidate chemicals are dissolved in once-refined soybean oil and offered to the ants in the test chambers on a cotton swab placed in a small vial cap. The ants are allowed to feed on the chemically-treated soybean oil for 24 h after which the treated swab is removed. The ants remain without soybean oil for an additional 24 h, then new vial caps containing cotton swabs saturated with once-refined soybean oil are 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 are 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. Preliminary tests with all chemicals were conducted at 1% in soybean oil. Chemicals giving less than 15% kill after day 1 and >85% kill after day 14 were retested at 1%. Chemicals giving >89% kill were retested at 1, 0.1 and 0.01% or until the minimum concentration giving >89% kill was determined.

All candidate bait toxicants tested (Table 2) are classified by effectiveness according to the following system modified from that given by Lofgren et al. (1967). Class I compounds are those that give less than 90% kill at the end of the test period. Class II compounds kill too fast at the higher concentrations but give insufficient kill at the lower concentrations (15% or more kill after 24 h and 90-100% kill at the end of the test period at the higher concentration, but less than 90% kill at the lower concentrations at the end of the test period). Class III compounds are those that show delayed action over a 1 to 9-fold dosage range while Class IV compounds show delayed action over a 10 to 99-fold range of dosages. Class V compounds are compounds that show delayed action over a 100-fold or greater range of dosages.

SECONDARY SCREENING

Chemicals that exhibit delayed toxicity in our primary screening tests are then evaluated at a secondary screening level against laboratory colonies consisting of a queen, eggs, larvae and pupae and more than 40 thousand workers. The test chemical is dissolved in soybean oil and impregnated on a carrier, generally pregel defatted corn grits at a concentration of 30% oil by weight of formulation. Initial tests are usually conducted at 1 to 2% concentration in the total bait using 2 colonies per concentration. Five grams of the bait are made available to each colony for 4 days. The bait is then removed and the colony is fed a standard laboratory diet. Observations on the status of the queen, brood and workers are recorded weekly. The test is continued until the queen, brood and 90% or more of the workers are dead or the colony recovers and returns to normal. Chemicals showing promise are further tested at several concentrations until the best concentration and formulation are available for testing in small field trials against natural populations of IFA.

FIELD EVALUATION

The next level of evaluation for all promising bait toxicants involves small-scale field tests against natural infestations of fire ants. They are formulated usually in baits consisting of the chemical dissolved in soybean oil and applied to a carrier such as pregel defatted corn grits. The tests are conducted on nongrazed permanent pasture, roadsides, airports or other noncropland. Usually, plots are 0.4 hectare (1 acre) in size with a 0.2 hectare (½ acre) evaluation circle in the middle of each plot. Post-treatment evaluations are conducted at 6 to 8 wk intervals after the initial application of the chemical. All baits are applied at this stage with a tractor-mounted auger applicator (Williams et al. 1982).

The evaluation of the field tests involves carefully opening each mound in each evaluation circle and making an estimation of the number of ants per nest and a determination of whether or not brood is present (normal) or absent (abnormal). Toxic effects of the chemical on the queen are signaled by the absence of worker brood. The presence of only sex brood indicates oviposition by unmated queens. The percentage control in field tests is usually determined by the method of Harlan et al. (1981). This method uses a 10 point rating system by which each colony is classified into one of 5 size categories ranging from <100 to >50,000 workers. Colonies without worker brood are then assigned the corresponding point value, i.e., 1 to 5; colonies with worker brood are assigned a double point value (5 to 10). Therefore, in each case categories 1 and 6 (<100), 2 and 7 (100 to 1000), 3 and 8 (1000 to 10,000), 4 and 9 (10,000 to 50,000) and 5 and 10 (>50,000) are the same size, but differ only in the presence or absence of worker brood. The population indices for each plot equals the sum of products obtained by multiplying total nests in each category by the corresponding point value. The difference between the pre- and post-treatment population indices is used to calculate the percentage control for each treatment.

Results and Discussion

The results of primary screening tests comparing EL-468, Amdro and avermectin B_1a are shown in Table 3. Mirex is used as a standard and soybean oil as a check. The data show that AC 217,300, EL-468 and mirex all exhibit delayed toxicity. Avermectin B_1a did not show good delayed toxicity; however, because it was a natural product derived from a soil microorganism (*Streptomyces avermitilis*) we decided to include it in our secondary tests.

The effects of EL-468, AC 217,300 and avermectin B_1a on laboratory colonies of the RIFA are shown in Table 4. EL-468 killed all colonies at the 3 concentrations used. At the low concentrations EL-468 took 8 wks to eliminate the colonies; at the higher concentrations the colonies were killed in a week. The results with AC 217,300 were quite similar to those with EL-468, the only difference being that lower concentrations of AC 217,300 killed the colonies slightly faster. Although the mirex standard was not included in this test, it usually gives delayed kill similar to AC 217,300. The differences are that mirex kills at lower concentrations and AC 217,300 does not always kill all the workers (Williams et al. 1980).

The data show that avermectin B_1a is moderately toxic to individual RIFA workers at concentrations of 0.025% or greater; however, it also causes sterility of the queen at concentrations as low as 0.0025%. These results are amazing and although this chemical did not yield high mortality in workers, the fact that it invariably caused the cessation of reproduction by the queen at such low concentrations makes it a promising chemical for control of RIFA (Lofgren and Williams 1982).

TABLE 3. Results of primary laboratory screening tests against RIFA with new toxicants (avg. of 3 reps with 20 workers/rep).

Toxicant	Concn (%)	Percent mortality on days indicated				
		1	3	6	10	14
EL-468	0.01	1	1	1	16	29
	0.1	1	19	64	89	95
	1.0	22	86	100		
Avermectin B_1a	0.01	3	7	8	22	43
	0.1	3	37	55	75	75
	1.0	65	77	85	93	97
AC 217,300	0.01	2	3	7	13	20
	0.1	2	8	68	93	100
	1.0	7	67	100		
Mirex[a]	0.01	0	1	8	40	65
	0.1	1	18	72	96	100
	1.0	0	84	100		
SBO Check[b]		0	0	2	3	6

[a]Avg. of 12 reps.
[b]Avg. of 15 reps.

TABLE 4. EFFECTS OF EL-468, AVERMECTIN B_1a AND AC 217,300 ON LABORATORY COLONIES OF RIFA (TWO REPS WITH 45,000-120,000 WORKER ANTS PER COLONY).

Concn (%) in soybean oil	Mortality (%) after wks indicated[b]					
	1	4	8	12	16	18
		EL-468				
1.0	97	99	D			
2.5	99	D				
5.0	D					
		Avermectin B_1a.				
0.0025	6	13	15	21	30	QS
0.025	25	34	68	69	71	QS
0.05	48	63	72	90	92	QS
0.1	45	65	83	89	QS	
0.25	30	55	80	90	QS	
0.5	25	35	50	67	88	QS
1.0	51	81	D			
		AC 217,300				
0.25	38	68	D			
0.5	43	D				
1.0	88	D				
2.5	99	D				
Check[a]	0	0	2	2	2	3

[a]Avg. 3 Reps.
[b]Fate of colony indicated at end of each column by D=died and QS=queen sterile.

Our laboratory colony data show that avermectin B_1a exhibits complete sterility of the queen and thus eventual death of the colony at a concentration 1/10th that of mirex, the most effective chemical previously tested against RIFA. For example, unpublished laboratory data (W.A. Banks and C. S. Lofgren) have shown that the lower limit for colony destruction with mirex is about 250 to 500 micrograms/colony while only 37.5 micrograms/colony of avermectin B_1a (0.0025% in soybean oil) produced irreversible effects on the queen (Lofgren and Williams 1982). This low effective concentration also compares favorably with that observed in our field tests.

The results of 2 field tests with avermectin B_1a are shown in Table 5. In the first test observations made 6 wks following application of the bait revealed that at all three application rates, all colonies were without worker brood and the population indices were reduced 90-92%. Further observations of the plots could not be made until September, 1980 because of extremely hot dry weather that forced the ants deep into the soil and made it impossible to accurately search for worker brood. When counts were made in September the plots were reinfested with incipient colonies. Application rates of 0.12, 0.25 and 0.49 grams per hectare were evaluated in a second test initiated in August, 1980 near Valdosta, Georgia in Brooks County. The reduction in population indices at 6 and 12 weeks ranged from 81 to 87%. Of the surviving colonies, 98-100% were without worker

TABLE 5. EFFECTS OF GRANULAR SOYBEAN OIL BAITS CONTAINING AVERMECTIN B_1a ON NATURAL POPULATIONS OF THE RED IMPORTED FIRE ANT IN FLORIDA AND GEORGIA[a].

Toxicant concn (%) in SBO	Application rate g AI/ha	Mean% reduction in population index after wks indicated[b] 6	12
	Jasper, FL April 1980		
1.06	7.41	92 (100)	
0.26	1.85	90 (100)	
0.07	.49	92 (100)	
Check	—	12 (4)	
	Valdosta, GA August 1980		
0.07	0.49	87 (99)	85 (100)
0.035	0.25	84 (99)	81 (99)
0.0175	0.12	84 (96)	85 (98)
Check	—	7 (9)	0 (4)

[a]Bait consisted of 70% pregel defatted corn grits impregnated with 30% of the SBO-toxicant solution. Application rate of formulated bait was 2.24 kg/ha. Avg. of 3 plots except 5 check plots were recorded in the test at Jasper, FL.
[b]Percentage of remaining colonies that did not have worker brood is indicated in parentheses.

brood. Additional studies are reported by Lofgren and Williams (1982) which show avermectin B_1a is active in baits at rates as low as 0.0077 gAI/ha.

The effectiveness of baits containing AC 217,300 (Amdro) against RIFA in Mississippi and Florida after 26 wks are shown in Table 6. Concentrations of 0.75% and 1.5% in the total bait gave the best average results. The results in Mississippi tests show that the 0.75% bait was the most efficacious. The results of field tests with baits containing AC 217,300 were published by Banks et al. (1981) and Harlan et al. (1981).

TABLE. 6. EFFECTIVENESS OF BAITS CONTAINING AC 217,300 FOR CONTROL OF RIFA IN FIELD TESTS IN MISSISSIPPI AND FLORIDA. FALL 1978.

Formulation	Application rate: Bait(kg/ha)	AI(g/ha)	Percent control after 26 wks in each state[a]: Mississippi	Florida
0.375%	1.4	5.25	98	59(90)
	2.8	10.5	81	96(100)
0.75%	1.4	10.5	95	81(97)
	2.8	21.0	88	85(100)
1.5%	1.4	21.0	91	84(100)
	2.8	42.0	69	97(100)
10-5 Mirex bait (standard)	1.37	1.37	90	88(92)

[a]Data corrected for check mortality with Abbott's formula.
[b]Figures in parentheses indicate control if queenless colonies died.

EL-468 (Table 7) gave good control at both concentrations tested and after 12 wks population reductions compared favorably with those obtained with the Amdro standard.

SUMMARY

Efforts to control the RIFA in the Southeastern United States probably began soon after their introduction when individuals came in contact with their painful sting. The rapid spread of these ants, their mound-building habits and their aggressive reaction to anything that disturbs their mounds have caused most individuals to seek relief from the ants. Almost every conceivable method of control has been tried by landowners and this has included burning nests with gasoline, physically removing the mounds, and treatment with an assortment of products found in the home. Chemical control of fire ants before 1957 was usually carried out by treating individual mounds with residual insecticides (Arant and Eden 1949) and from 1957 to early 1960 the use of the chlorinated hydrocarbons, heptachlor, dieldrin and chlordane were recommended for control (Arant et al. 1958). The development of a new method of control using a bait-toxicant system revolutionized not only the type of control, but also the type of chemicals that could be used in such a system. With the discovery of mirex bait in 1961 (Lofgren et al. 1962) and its adoption as the standard treatment for fire ant control in 1962, control of the RIFA was considered to be only a matter of application. Generally between 1962-1970, research was conducted primarily on the improvement of the mirex bait, although a limited amount of research was performed on screening new chemicals as bait-toxicants. The possibility that mirex was a carcinogen (Mrak 1969, Innes et al. 1969), its persistence in the environment (Baetcke et al. 1972, Borthwick et al. 1973, 1974) and its effect on certain estuarine organisms (Lowe et al. 1970, 1971) spelled the beginning of the end of its use as a toxicant in large-scale bait applications. All registrations for products containing mirex were cancelled by the EPA in 1978. Around 1971 a crash program by the USDA was undertaken to find alternative methods of chemical or biological control and to carry out more intensive research on the biology and ecology of the RIFA. Although our knowledge has been increased a

TABLE 7. CONTROL OF RIFA WITH EL-468 IN FIELD TESTS IN HAMILTON CO., FL NOVEMBER, 1979 (30% SOYBEAN OIL ON PREGEL DEFATTED CORN GRITS: AVG. OF THREE 0.4 HECTARE PLOTS).

Chemical	Concn (%) in oil	Application rate		Percent control after indicated wk[a]	
		kg/ha	g AI/ha	6	12
EL-468	2.5	2.8	3.4	75	86
EL-468	5.0	2.8	6.7	77	91
AC 217,300 (standard)	2.5	1.4	1.7	66	88
Check	—	—	—	7	11

[a]Avg. 3 replications.

great deal concerning this insect, new noninsecticidal control techniques and strategies have not been developed as yet. However, despite the difficulty in finding delayed-action toxicants, within a very short time after mirex was no longer available, a new fire ant toxicant was discovered, tested, and made available under the trade name, Amdro®, for use by the public against the RIFA. In addition, two other chemicals EL-468 and avermectin B_1a have shown promise and the former is near the commercial development stage. Also, research in developing bait-toxicants which will be species specific is actively being pursued and although progress is slow the future looks promising in this area.

Few insects have stirred as much controversy and created differences of opinion as the RIFA. With few exceptions, most landowners, homeowners and residents in infested areas want to be rid of the ants, at least from their property and places of activity. Therefore, control of the ants in these areas will be necessary and should be undertaken. I am frequently asked the question, "Is the fire ant *really* a problem?" My first response is always the question, "Where do you live?" The individuals usually say they live outside of the infested area of the U.S. or live in a large urban or suburban area covered with concrete, or are a graduate student or professor who rarely leaves his or her laboratory to venture outdoors, or all of the above.

Anyone who has inadvertently stepped in a fire ant mound has a problem. For a one-time occurrence, its an unforgettable experience. To be subjected to it frequently because of large numbers of mounds around your home and work place is not only unbearable, but should not have to be tolerated.

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This paper reports the results of research only. Mention of a commercial or proprietary product does not constitute an endorsement of this product by the U.S. Department of Agriculture.

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THE EFFECTS OF INSECT GROWTH REGULATORS AND THEIR POTENTIAL AS CONTROL AGENTS FOR IMPORTED FIRE ANTS (HYMENOPTERA: FORMICIDAE)

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Increasing restrictions on the use of mirex in the early to middle 1970's and the ultimate discontinuance in 1978 of registrations for its use (Holden 1976, Johnson 1976) prompted increased investigations of alternate chemicals that might be used to control the red and black imported fire ants (IFA) *Solenopsis invicta* Buren and *S. richteri* Forel. These investigations included evaluation of the potential of some of the insect growth regulators (IGR) as control agents. Several laboratory studies (Banks et al. 1978, Cupp and O'Neal 1973, Robeau and Vinson 1976, Troisi and Riddiford 1974, Vinson et al. 1974, and Vinson and Robeau 1974) demonstrated that IGRs are active against IFA. Effects of the chemicals, however are rather subtle. The IGRs, as a general rule, are not lethal to the worker ants nor to the queen, and death of the colony, if it occurs, usually comes several weeks to months after treatment. Death occurs because of the lack of worker replacement coupled with natural mortality. Worker replacement fails to occur because the IGRs induce deformities in and cause death of developing larvae; they also will shift caste differentiation from worker to sexual forms, and in some cases, reduce or stop egg production by the queen. The IGRs are stored in the colony in the gastral crop of the largest major workers (repletes) and are recirculated via trophallaxis through the communal stomach of the colony and thus exert their effects over several months. Studies (Banks et al. unpublished, Bigley and Vinson 1979, Wendel and Vinson 1978) have shown that in some instances IFAs are able to metabolize and/or excrete the IGRs and recover from their effects. Because of the long period usually required for colony mortality and the fact that some colonies can overcome the effects of IGRs, these chemicals have not been considered as prime control agents for IFA. As a consequence, no IGRs have been registered to date for fire ant control and only 4 have advanced beyond laboratory testing. Nonetheless, the severe disruption of normal colony development and resultant death of many colonies after administration of certain of these chemicals indicated that IGRs have excellent potential for use in control and pest management programs against IFA. We present here a review of our studies with some of the more promising materials.

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