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Avermectin B_1a: Highly Potent Inhibitor of Reproduction by Queens of the Red Imported Fire Ant (Hymenoptera: Formicidae)[1]

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ABSTRACT

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Avermectin B_1a, a natural product derived from the soil actinomycete *Streptomyces avermitilis*, inhibited reproduction of queens of *Solenopsis invicta* Buren when fed to laboratory colonies at concentrations as low as 0.0025% in the soybean oil bait (37.5 μg of AI available). Some worker mortality occurred at concentrations of 0.025% or greater. Good results were obtained in field tests also. Worker brood was found in only 8 of 928 colonies that fed on the baits which were applied at rates of AI ranging from 0.0077 to 7.41 g/ha. Death of the treated colonies, especially at the lower application rates, was very slow, since the primary effect of avermectin B_1a was on the reproductive capacity of the queen rather than acute toxicity for workers. Therefore, overall effects of avermectin B_1a baits were determined with a population index method that gave a greater numerical value to colonies with functioning queens, as evidenced by the presence of worker brood. With this method, the average population indices in the field plots treated with 0.0077 g or more per ha were reduced 81 to 93%.

Toxic baits have been used since 1962 for control of the red and black imported fire ants, *Solenopsis invicta* Buren and *S. richteri* Forel, respectively (Lofgren et al. 1975). The baits are composed of a liquid food attractant, a soluble toxicant, and a granular carrier. Their effectiveness is dependent upon the toxicant having sufficient delayed toxicity to allow time for the worker ants to harvest the bait and distribute it via trophallaxis to other colony members (Stringer et al. 1964). Death of the colony, however, is contingent upon death of the queen. If not killed, she may rebuild the colony again with the aid of only a few workers (Stringer et al. 1976).

In the past, the search for slow-acting toxicants has centered on the classical inorganic and organochlorine or organophosphate insecticides. The earliest ant baits contained thallium sulfate or sodium arsenate (Mallis 1969). The first highly effective toxicant discovered was mirex (Lofgren et al. 1963, 1964, Stringer et al. 1964), which has been used successfully for control of many pest ants. However, in 1978, registrations of baits containing this toxicant for imported fire ant control in the United States were discontinued because of environmental residues and suspected carcinogenicity (Johnson 1976).

The USDA has been searching for delayed-action toxicants since 1958 (Banks et al. 1977) and over 6,000 compounds have been tested. Williams et al. (1980a) reported recently on a promising new class of toxicants, the amidinohydrazones. The most promising of these, AC 217300 (tetrahydro-5,5-dimethyl-2(1H)-pyrimidinone[3-[4-trifluoromethyl)phenyl]-1-[2-[4-trifluoromethyl)phenyl]ethenyl-2-propenylidene]hydrazone) was granted conditional registration in August 1980 by the Environmental Protection Agency under the trade name Amdro. AC 217300 is a particularly interesting compound because of its specific toxic effect on the queen.

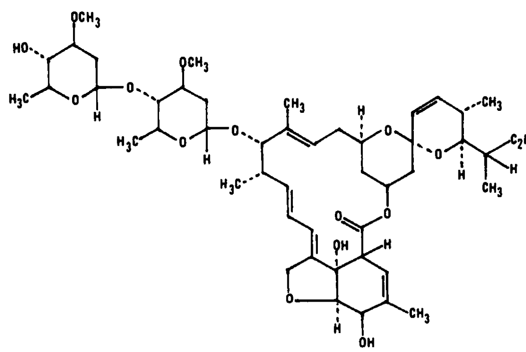

FIG. 1.—Chemical structure of avermectin B_1a.

Williams and Lofgren (1981) reported on another new chemical (a phenyledediamine) that exhibited delayed toxicity. The compound, Eli Lilly, EL-468 (N-[2-amino-3-nitro-5-(trifluoromethyl) phenyl] -2,2,3,3-tetrafluoropropanamide) was shown to be effective in laboratory and field tests against red imported fire ants (RIFA).

Although standard toxicants provide a direct means of control by affecting all members of the colony, another more subtle approach, with possibly fewer environmental hazards, is the use of chemicals that inhibit reproduction. Any compound that inhibits larval development or kills or destroys the queen's reproductive capacity automatically kills the colony. Unlike honey bees, the worker ants are incapable of rearing replacement queens. The practicality of this concept for controlling natural infestations of ants has been demonstrated. Edwards (1977) and Hrdy et al. (1977) obtained control of populations of the pharoah ant, *Monomorium pharaonis* (L.), in buildings with two or more applications of baits containing the juvenile hormone analogues, methoprene or hydroprene. Banks et al. (1978), in tests with the RIFA, obtained 67 to 75% kill of laboratory colonies

[1]Mention of a pesticide or a commercial proprietary product does not constitute an endorsement or recommendation by the USDA, nor does it imply registration under FIFRA as amended. Received for publication 9 September 1981.

with two juvenile hormone analogues (E)-1-[7-ethoxy-3,7-dimethyl-2-octenyl)oxy]-4-ethylbenzene (JH-25) and 1- (8-methoxy-4, 8-dimethyl-nonyl) -4- (1-methylethyl) benzene (Stauffer MV-678). In addition, Banks and Schwartz (1980) obtained a reduction of 76% in one field test with a soybean oil bait of Stauffer MV-678; most of the surviving colonies appeared abnormal after 26 to 40 weeks, as evidenced by the lack of worker brood.

The juvenile hormone analogues exert their effect by interfering with larval development; thus, the most apparent symptom of their effect is the absence of worker brood, even though the queen appears to be healthy and, in fact, can in some cases produce normal progeny when placed with untreated workers.

Screening of compounds for inhibition of reproduction with RIFA has been facilitated in recent years with the development of methods for rearing queen-right colonies (Williams et al. 1980b, Banks et al. 1981). Thus, we expanded our research program to include tests with compounds that produce effects other than acute toxicity. As a part of our testing program with colonies, we discovered an experimental chemical that inhibits reproduction by RIFA queens at extremely low dosage levels. This compound is one of a series of eight macrocyclic lactone glycosides isolated from the soil microorganism *Streptomyces avermitilis* (Putter et al. 1981). Reported here are results of studies conducted with one of these compounds, avermectin B_1a.

Materials and Methods

Figure 1 gives the structure of avermectin B_1a. The initial screening tests were conducted with procedures described by Williams et al. (1980a). Basically, the method involves holding groups of 20 worker ants in small humidity chambers in which they are allowed to feed on soybean oil containing the test chemical or neat soybean oil (checks) over a 24-h period. Then, bait is removed, ants are supplied with uncontaminated food, and mortality counts are made over a 2-week period. Concentrations of 0.01, 0.1, and 1.0% were evaluated.

Laboratory queen-right colonies were used for the secondary tests. The colonies were 10 to 20 months old, with ca. 30,000 to 60,000 workers and 5 to 10 ml of brood. They were reared by procedures described by Banks et al. (1981), with the exception that they were fed a diet of honey-water, boiled eggs, and moth pupae. The avermectin B_1a was offered to the colonies in a bait consisting of 30% once-refined soybean oil (SBO) and 70% pregel defatted corn grits (Lauhoff Grain Co., Danville, Ill.). The bait was prepared by dissolving the toxicant in the soybean oil and then pouring this solution over the grits as they were mixed in a food blender. Each colony was allowed to feed ad lib. on 5 g of bait for 4 days, unless it was consumed sooner. With the exception of the 4th instar, larval and adult ants ingest only the liquid portion of the bait; thus, each colony had access to 1.5 g of food (SBO). Weekly observations were made of worker mortality, amount of eggs and brood present, and general condition of the queen. The test was continued until the queen, brood, and 75% or more of the workers were dead, or until the colony recovered and returned to normal, i.e., the queen resumed oviposition and all stages of brood were present. Check colonies were fed the same bait without the avermectin B_1a.

Formulated baits were field tested on sites in nongrazed pastures or along roadsides (Jasper, Fla., and Valdosta, Quitman, and Statenville, Ga.). The baits were prepared as described for the secondary laboratory tests and applied with a tractor-mounted auger applicator (Williams and Lofgren, unpublished data). Table 3 shows various application rates of AI. All plots were 0.4 ha in size. Pre- and posttreatment counts of active nests were made on 0.2-ha circles in the center of the pasture plots and on 0.06-ha (Quitman) or 0.13-ha (Statenville) rectangular plots adjacent to the road in the roadside plots. Each nest was opened with a shovel, and observations were made for the presence of worker ants and brood. Effect of the chemicals on the queen was signalled by the absence of worker brood. The presence of only sex brood indicates oviposition by unmated queens.

Evaluation of chemicals that express their effect only on reproduction is difficult. Permanent inhibition of reproduction by the queen implies eventual death of the colony even though some worker ants can survive, depending upon caste (minor, major, replete) from a few weeks to a year. This poses a question as to whether death of the colony occurs when the queen dies or ceases to reproduce, or when the last worker has died. RIFA workers are incapable of rearing replacement queens, but there is some evidence that orphaned workers may adopt newly mated queens after nuptial flights (Banks et al. 1981, Tschinkel and Howard 1980). However,

Table 1.—Standardized weighting system for colonies of RIFA

	Colonies			
	Without brood (abnormal)		With brood (normal)	
No. of worker ants	Class	Weighting factor	Class	Weighting factor
<100	A	1	AA	5
100–1,000	B	2	BB	10
1,000–10,000	C	3	CC	15
10,000–50,000	D	4	DD	20
>50,000	E	5	EE	25

Table 3.—Effect of avermectin B_1a on laboratory colonies of the red imported fire ant when fed in soybean oil bait[a]

Avermectin B_1a concn (%)	No. of replications	% reduction in workers or brood after the following wk								Fate of queens[b]
		Workers				Brood				
		1	4	8	16	1	4	8	16	
1.0	6	41	64	72	100	5	88	100		5 QD; 1QS
0.5	2	25	35	50	88	5	85	100		2 QS
0.25	2	30	55	80	90	15	95	100		1 QD; 1QS
0.1	2	45	65	80	100	23	100			1 QD; 1 QS
0.025	4	39	73	96	100	4	100			4 QD
0.0025	4	6	13	15	30	0	99	100		4 QS
0.00025	4	0	3	5	8	5	25	10	1	4 QN
–(Ck)	4	0	1	3	11	0	0	2	4	4 QN

[a]Five grams of bait offered to each colony; bait consisted of 1.5 g of soybean oil and toxicant impregnated on 3.5 g of pregel defatted corn grits.
[b]QD, Queen dead; QS, queen sterile; QN, queen normal.

and April 1981), both of which showed the population indices had been reduced 80 to 90%. However, we also noted reductions in the population indices of the checks. This was undoubtedly attributable to a faulty test design that involved treatment of plots on opposite sides of the road with different application rates. State Road 33 is a paved, two-lane highway, and apparently some of the RIFA colonies built foraging tunnels under the highway and were partially affected by the different treatment on the opposite side of the road. This included the check colonies, all of which were opposite plots treated at the highest (0.12 g/ha) application rate. However, despite this problem, even the lowest application rate (0.015 g/ha) produced excellent results, with 100% of surviving colonies without worker brood compared with 17% for the checks. The lack of 100% effect on the colonies exposed to the high rate of application (0.12 g/ha) could reflect reinvasion from the check plots located on the opposite side of the road. In addition, we observed colonies of RIFA ca. 1.5 km from the test site at the same time as the 18-week posttreatment evaluations. Of 63 mounds examined, 11 or 17% were without worker brood. The average mound population index was 18.86 compared with 13.15 for the internal checks and a range of 2.32 to 3.26 for the treated plots. Thus, despite a faulty experimental design, effects of the avermectin B_1a were apparent. No further observations of these plots could be made because of road-building activities.

The fourth test was conducted along Georgia State Road 94, west of Statenville, Ga., with application rates of 0.0019 to 0.12 g/ha. The population indices were reduced by 95 and 88%, respectively, at the two highest application rates, 0.12 and 0.03 g/ha, and all surviving colonies were without brood. Some decrease in effectiveness was noted at 0.0077 g/ha (91% of surviving colonies without brood), and at 0.0019 g/ha the effects declined dramatically (25% of colonies without brood).

Discussion

The avermectins are a new class of natural products produced by *S. avermitilis* (Burg et al. 1979, Miller et al. 1979). They were discovered originally in a screening program for anthelmintic agents (Egerton et al. 1979). Putter et al. (1981) described their activity against mites and several insect pests and made reference to our preliminary data of RIFA. Studies indicate that avermectin B_1a is a gamma-aminobutyric acid (GABA) agonist. It eliminates GABA-mediated inhibitory postsynaptic potentials but has no effect on cholinergic nervous systems (Fritz et al. 1979).

Observations (unpublished data) of the ovaries of queens exposed to 0.0025% avermectin B_1a by one of our co-workers (B. M. Glancey) have shown abnormalities in the developing eggs and nurse cells. These queens oviposited, but the eggs were infertile.

Our laboratory colony data show that avermectin B_1a is an extremely potent chemical that inhibits reproduction of RIFA queens. The compound exhibits effects at a concentration one-tenth that of mirex, the most effective chemical we have tested previously against RIFA. For example, laboratory data (W. A. Banks and C. S. Lofgren, unpublished data) have shown that the lower limit for colony destruction with mirex is ca. 250 to 500 μg per colony, whereas 37.5 μg of avermectin B_1a (0.0025% in SBO) per colony produced irreversible effects on the queen. This low concentration of effectiveness also compares favorably with that observed in our field tests. The minimum effective application rate provided ca. 7,700 μg of avermectin B_1a per ha. The average mound density on the plots in these tests (Statenville) was 18. Since each plot was 0.13 ha, the average mound density per ha was 138, and the available avermectin B_1a was 56 μg per mound (colony). Another illustration of the effectiveness of avermectin B_1a is the fact that only 8 of the 928 (0.9%) colonies exposed to application rates of 0.0077 g or more per ha contained worker brood at the time of our final posttreatment evaluation.

Although the avermectin B_1a baits are highly effective inhibitors of reproduction against RIFA queens, they do not, as we described previously, provide rapid elimination of the total colony. Consequently, early observations of treatment sites might suggest the baits have failed to control the RIFA. On a practical basis, this slow death of RIFA colonies might not be satisfactory

Table 4.—Effects of granular soybean oil baits containing avermectin B_1a on populations of the red imported fire ant in Florida and Georgia[a]

Location	Toxicant concn (%) in SBO	Application rate (g of AI/ha)	Avg no. of mounds/plot	Avg mound population index	Mean % reduction in population index after wk indicated[b]			
					6	12	14	18
Jasper, Fla.; Apr. 1980	1.06	7.41	21	21.4	92 (100)a			
	0.26	1.85	22	20.7	90 (100)a			
	0.07	0.49	25	21.0	92 (100)a			
	–(Ck)	—	27	21.4	12 (4)b			
Valdosta, Ga.; Aug. 1980	0.070	0.49	35	21.8	87 (99)a	85 (100)a		
	0.035	0.25	21	20.7	84 (99)a	81 (99)a		
	0.0175	0.12	26	21.6	84 (96)a	85 (98)a		
	–(Ck)	—	33	21.5	7 (9)b	0 (4)b		
Quitman, Ga.; Nov. 1980	0.0175	0.12	20	19.8	—	—	91 (100)a	88 (92)a
	0.0088	0.06	19	20.8	—	—	90 (100)a	93 (100)a
	0.0044	0.03	17	21.1	—	—	85 (100)a	89 (100)a
	0.0022	0.015	24	21.3	—	—	89(100)a	93 (100)a
	–(Ck)	—	16	19.4	—	—	53 (31)b	51 (17)b
Statenville, Ga.; June 1981	0.037	0.12	20	18.9	96 (100)a			
	0.009	0.03	19	21.0	91 (100)a			
	0.0023	0.0077	17	19.2	85 (91)a			
	0.0006	0.0019	17	19.4	56 (25)b			
	–(Ck)	—	14	20.8	22 (0)c			

[a]Bait consisted of 70% pregel defatted corn grits impregnated with 30% of the soybean oil-toxicant solution. Application rate of formulated bait was 2.24 kg/ha at first three sites and 1.12 kg/ha at the Statenville site. Averages of three plots except five untreated check plots were recorded in the test at Jasper. Means not followed by the same letter are significantly different at the 5% level of confidence, based on Duncan's multiple range test.

[b]Percentage of remaining colonies that did not have worker brood is indicated in parentheses.

Table 2.—Toxicity of avermectin B_1a, to red imported fire ants when fed in soybean oil bait (average of six replications)

Chemical	Concn (%) in soybean oil	Mean % mortality after indicated no. of days						
		1	2	3	6	8	10	14
Avermectin B_1a	0.01	6	7	11	17	27	34	48
	0.1	8	32	49	61	67	74	75
	1.0	51	71	78	84	86	88	90
Mirex (std)	0.01	0	0	1	4	9	17	53
	0.1	0	0	1	48	67	88	98
	1.0	0	9	68	100			
Soybean oil (ck)		0	0	1	1	1	1	2

even this is dependent upon release of the queen recognition pheromone which new queens do not produce until they are 7 to 10 days old (Glancey et al. 1981). Also, it is complicated further by new laboratory data that show that some colonies tend their queen (or her abdomen) after death from a few days to several months (Williams et al. 1981). During this time, replacement queens are not accepted.

In an effort to give credit for effects other than complete colony kill, Harlan et al. (1981) developed a colony rating system for tests with AC 217,300, a toxicant that often kills the queen without eliminating the entire colony. They classified colonies into categories based on the estimated numbers of workers per colony and whether or not worker brood was present. Those colonies without worker brood were given ratings of 1 to 5 and those with brood 6 to 10. These numerical ratings represented the population index for each colony. Then a population index for each plot was calculated by multiplying the number of colonies in each category by the numerical rating (= weighting factor) of that category. An apparent flaw in this system was that the weighting factor of colonies of different sizes with and without brood was not consistent. For example, the weighting factors for the smallest colonies with and without worker brood were 6 and 1, a difference of sixfold. In contrast, the weighting factors for the largest colonies with or without worker brood were 10 and 5, a difference of twofold. Obviously, no system can be developed that is flawless; however, in an effort to standardize the weighting system of Harlan et al. (1980) we modified it by giving all colonies with worker brood (normal) fivefold the weight of those without brood (abnormal) as shown in Table 1.

Results

Avermectin B_1a is moderately toxic to individual RIFA workers (see Table 2), and its action is slow relative to most other insecticides. Based on the classification system devised by Lofgren et al. (1967), avermectin B_1a is not a good bait toxicant (Class IIb), since more than 15% of the ants were dead after 24 h. However, because of the unique source and chemical structure of avermectin B_1a , we decided to test for other types of toxic activity by feeding it in a bait to queen-right colonies. The results of these tests (Table 3) show that: concentrations of 0.025% or greater in SBO caused death of large numbers of worker ants and brood, and death or sterility of the queen; a concentration of 0.0025% caused little mortality of workers, but loss of all brood by 4 weeks and queen sterility; and a concentration of 0.00025% caused no worker mortality, a small decline in brood production, and a return to normalcy by 16 weeks.

Because of the promising effects of avermectin B_1a on the reproductive capacity of the queen, we conducted four field tests to evaluate baits containing various concentrations of the chemical (see Table 4). In our first test (Jasper, Fla., April 1980) observations made 6 weeks after application of the bait revealed that all three application rates (0.49 to 7.41 g/ha) all colonies were without worker brood, and the population indices were reduced 90 to 92%. Further observations of the plots could not be made until September 1980 because of extremely hot, dry weather that forced the ants deeper 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. However, the colony size profile gave a good indication that a high percentage of the colonies present at the time of treatment had been killed. For example, mature queen-right colonies more than 1 year old should have worker brood and >10,000 workers (classes DD and EE; see above), whereas new colonies 3 to 5 months old should contain brood with <10,000 workers (classes AA, BB, and CC). Data from our treated plots showed that 82% of the colonies were rated DD or CC before treatment, but only 14% after 30 weeks. In comparison, the number of colonies rated DD or CC in the check plots increased from 75 to 89% during the same time period.

Application rates of 0.12, 0.25, and 0.47 g/ha were evaluated in the second test initiated in August 1980 near Valdosta, Ga., in Brooks County. The population indices at 6 and 12 weeks were reduced from 81 to 87% of the pretreatment value, and 98 to 100% of the surviving colonies were without worker brood.

The plots for the third test were located along Georgia State Road 33, south of Quitman, Ga. Four application rates ranging from 0.015 to 0.12 g/ha were tested. The bait was applied in November 1980, but no evaluations were made until March 1981 because of the cold winter temperatures, which decreased production of brood and made it impossible to determine accurately if reproduction had ceased. Two evaluations were made (February in urban areas where rapid elimination of a colony from a lawn or playground is needed, but could be completely acceptable in pastures, cropland, and nonagricultural sites. It should be noted that colonies without brood or a fertile queen are in a continuous state of decline, and they do not maintain their nests or require large amounts of food; thus, they would not be as great a problem on farms. Also, human contact with RIFA occurs primarily while they are foraging or when the nest is disturbed, so non-reproductive colonies would be less of a threat to public health.

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