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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 unpublished, Bigley and Vinson 1979, Wendel and Vinson 1978) have shown that in some instances IFAs are able to metabolize and/or excrete the IGRs and recover from their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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LABORATORY STUDIES

Our early laboratory tests (Banks et al. 1978) with IGRs showed that 14 of 26 compounds administered in peanut butter baits were active against IFA but only 3 were sufficiently active to disrupt caste differentiation and inhibit egg production and worker replacement severely enough to kill a large percentage (65-75%) of the treated colonies.

Test procedures were modified in 1977 to test all IGR in soybean oil solution rather than peanut butter. The oil solutions were offered in micropipets from which the ants fed. Each concentration of a candidate IGR was initially tested against 3 laboratory colonies, reared from newly-mated queens by standard techniques (Banks et al. 1981), that were composed of a queen, 10-20 ml brood (eggs, larvae, and pupae), and 40-60 thousand worker ants. Check colonies of comparable size were each given an equivalent volume of neat soybean oil. After treatment the colonies were returned to the normal diet (Banks et al. 1981) and held in the laboratory at 27± 2° C. Each colony was examined biweekly through 16 weeks posttreatment and monthly thereafter until the colony died or recovered from obvious effects of the IGR. At each examination the status of the queen, type and quantity of brood, estimated number of worker ants, and any obvious morphological anomalies were recorded.

After the modification of test procedure an additional 29 IGRs were tested against laboratory colonies with the result that the following 4 additional IGRs were discovered to be equal or superior in activity to those previously reported (Banks et al. 1978):

Company Designation	Chemical Name
Eli Lilly L-794	*N*-[[[5-(4-bromophenyl)-6-methyl-2-pyrazinyl] amino]carbonyl]-2,6-dichlorobenzamide
CIBA-GEIGY CGA-38531	1-(3-ethoxybutoxy)-4-phenoxybenzene
Maag Agrochemicals Ro 13-5223	Ethyl[2-(*p*-phenoxyphenoxy)ethyl]carbamate
Montedison JH-286	1,[(5-chloro-pent-4-ynyl)-oxy]-4-phenoxybenzene (1)

The first compound is an antimolting compound that interferes with cuticle formation and molting in many insects. In the fire ant, the production of eggs by the colony queen was totally suppressed in some colonies and partially so in others. However, the mechanism by which this occurred has not been explored. In any case, most colonies were able to overcome effects of the treatment and the queens resumed egg production. Only 1 of 5 colonies treated at 5 mg died, and none of those treated at 10 mg died. CGA-38531, Ro 13-5223, and JH-286 are juvenile hormone (JH) mimics that appear to affect the IFA colonies in the same manner described previously (Banks and Harlan 1982, Banks et al. 1978, Banks and Schwarz 1980). Of course not all JH mimics are active against IFA and the effective dosage varies among those that are active. No detailed physiological studies have been done with treated colonies but the following changes have been readily observable. The production of worker ants ceased because of a shift in caste differentiation from worker forms to reproductive forms (winged males or females or both in any given colony). The disappearance of worker brood from the colonies coincided with the appearance of abnormal numbers of large reproductive larvae at 4-8 weeks posttreatment. Many winged sexuals eclosed in some treated colonies; however, the numbers varied with IGR and dosage. The majority of those that did eclose had deformed wings and dealated very soon after eclosion. Other studies (Texas Agricultural Experiment Station 1980) have shown that the females in IGR-treated colonies also have malformed ovaries and are sterile. The 3 JH mimics cited above were very effective in preventing maturation of the developing reproductives. An estimated 90+% of the reproductives died or were killed by the workers in the immature stages and were discarded by the workers in the colony refuse pile.

All 4 of the IGRs demonstrated activity against the ants at all dosages tested (Table 1); however, CGA-38531 was the only compound that was very effective in causing long-term suppression of brood production and inducing colony death. CGA-38531 caused all colonies treated at 1 and 2 mg to die. Only 50% of the colonies treated at 5.0 mg/colony died, however, our observations suggested that the difference could be related to colony composition rather than differential response to dosage. Colony composition plays a very important role in long-term effect of IGR since we (Banks and Schwarz 1980) have found that the larger workers in the colony (repletes) store the oil solutions of IGR in the gastral crop and recirculate them via trophallaxis through the communal stomach of the colony. Our studies indicate that death of IFA colonies as a result of IGR ingestion requires that the chemical be retained and redistributed over several months. Obviously, colonies with few repletes or with repletes whose storage capacity was already filled at time of treatment would retain less IGR and would thus be better able to overcome effects of the treatment. JH-286 at 10.0 mg/colony and Ro 13-5223 at 5.0 mg/colony were slightly less effective than CGA-38531 against laboratory colonies. Both chemicals caused the death of 67% of colonies treated at the indicated dosage. Ro 13-5223 caused total suppression of brood production in all treated colonies through 16 wk posttreatment at 5.0 mg/colony and total suppression through 8 wk with continued high level (85.7%) suppression through 32 wk at 10.0 mg/colony. JH-286 was not highly effective in suppression of brood production, there being only 1 observation (8 wk) when all treated colonies were devoid of worker brood.

The untreated check colonies did not exhibit the effects noted in the treated colonies. With the exception of 1 colony in which the queen died from unknown causes shortly after initiation of the test, worker brood was present throughout the test and the colonies increased in size. Sexual forms were produced in some colonies but not to the exclusion of worker larvae and no morphological deformities were seen in any of the eclosed sexuals.

Field Studies

Although our laboratory studies have given us some insights into the basics of developmental biology and caste determination in the ants, the

TABLE 1. EFFECTS OF INSECT GROWTH REGULATORS ON LABORATORY COLONIES OF RED IMPORTED FIRE ANTS.

Chemical	Dosage mg/colony	Avg. percent reduction in workers and brood after indicated weeks: Workers 2	Workers 4	Workers 8	Workers 16	Workers 32	Workers 52	Brood 2	Brood 4	Brood 8	Brood 16	Brood 32	Brood 52	% of colonies killed
L-794	5.0	0	0	7.1	17.9	21.4	21.4	20.0	60.0	77.3	67.7	52.0	20.0	20
	10.0	0	0	9.1	45.5	45.4	18.2	—	93.3	93.3	93.3	66.7	16.7	0
CGA-38531	1.0	0	0	0	30.0	50.0	60.0	50.0	100	100	100	100	100	100
	2.0	0	0	28.1	62.5	71.9	93.8	57.1	90.5	100	100	100	100	100
	5.0	0	4.8	28.6	52.4	52.4	42.9	72.7	100	100	100	63.6	63.6	50
Ro 13-5223	5.0	—	12.5	25.0	43.8	50.0	62.5	—	100	100	100	77.8	55.6	67
	10.0	0	5.9	17.6	29.4	52.9	52.9	42.9	100	100	85.7	25.7	57.1	33
JH-286	10.0	0	0	12.5	31.3	50.0	62.5	85.7	71.4	100	71.4	28.6	28.6	67
Check	—	0	0	4.0[1]	4.0[1]	4.0	6.0	3.8[1]	7.7	15.4	30.8	19.2	38.5	11

[1]These figures represent increases in worker numbers or brood quantity.

basic objective of our studies with IGR has been to assess the potential of these chemicals as control or population management tools. Through the course of these studies we have identified 7 IGRs that have demonstrated sufficient activity against laboratory colonies to warrant field testing. Because sufficient quantities of some materials are not available or other problems however, only 4 of the chemicals have been field tested. The four compounds are Stauffer MV-678 (1-(8-methoxy-4,8 dimethylnonyl)-4-(methylethyl)benzene); and CIBA-GEIGY CGA 38531, Maag Ro 13-5223, and Montedison JH-286, previously referred to in the laboratory tests reported in this paper. The Stauffer compound, determined to be effective in our earlier studies (Banks et al. 1978), is a JH analogue that was synthesized in the USDA-ARS Biologically Active Natural Products Laboratory, Beltsville, MD by Dr. Meyer Schwarz and initially tested under the USDA designation AI3-36206. The material was subsequently acquired by Stauffer Chemical Co. and is under development under the MV-678 designation.

In initial small plots tests MV-678, (Banks and Schwarz 1980) killed up to 76% of active field colonies and left most of the surviving colonies abnormal, as evidenced by lack of worker brood, after 26-40 wk following broadcast application of MV-678 bait at an equivalent rate of 4.75 g active/ha (36.2 mg/mound). Similar results were obtained in additional small plot tests (unpublished); however, rapid reinfestation of the small plots prevented accurate evaluation of the test and indicated that an assessment of the real potential of MV-678 would require tests on larger areas. Large area tests conducted from 1979 to 1981 will be reported in detail elsewhere but are summarized in Table 2.

Because activity of the other 3 IGRs was discovered only relatively recently and considerable time is required to complete tests, only small plot tests have been conducted with those materials. Baits for the small plot tests and also for the large area tests with MV-678 were formulated at the Gulfport laboratory. The candidate IGR was incorporated into once-refined soybean oil and the solution was sprayed at 30% by weight of the formulation onto 8-30 mesh pregel defatted corn grits (Lauhoff Grain Co., Danville, IL). The grits were tumbled in a small cement mixer during application of the oil and for 5-10 minutes thereafter to assure proper distribution of the oil and chemical throughout the formulation. Stauffer MV-678 was formulated as a 2.4% bait and CGA-38531, Ro 13-5223, and JH-286 were formulated as 1.0 and 2.0% baits.

Plots that ranged in size from 0.25 to 1.0 ha for the small plot tests to 40 to 350 ha for the large area tests with MV-678 were established and treated in nongrazed permanent pasture and on grass-sod military stage fields. Circular subplots (0.1 to 0.2 ha) were established within each larger plot for pre- and posttreatment evaluation of ant populations. Since the IGRs are essentially nontoxic to the worker ants and the queen and express their effects primarily through suppression of brood production, evaluation of these effects sometimes becomes very difficult. Similar problems, however, were encountered with some of the other materials evaluated for IFA control in recent years and an evaluation system that considered effects other than direct toxicity was necessary. In order to give credit for effects other than acute toxicity Harlan et al. (1981) developed a colony or nest index and population index method for work with AC-217,300 (Amdro®)

TABLE 2. EFFECTS OF THE INSECT GROWTH REGULATOR STAUFFER MV-678 ON FIELD POPULATIONS OF RED IMPORTED FIRE ANTS, FT. STEWART, GA. 1979-81.

Treatment	Pretreatment		Percent reduction in[1,2]							
	Number active nests	Pop'n index	Number active nests				Population index[3]			
			Apr '80	Jul '80	Oct '80	Apr '81	Apr '80	Jul '80	Oct '80	Apr '81
Single application 11.86 g/ha AI October 1979	160	3684	0	31.2	7.6	34.3	26.6	65.5	10.7	35.2
Dual application 11.86 g/ha AI each Oct '79 & Apr '80	152	2794	9.6	84.4	89.5	61.3	62.0	95.8	88.9	80.6
Dual application 11.86 g/ha AI each Oct '79 & Oct '80	149	3479	23.9[4]	6.9	10.4	30.0	24.7	47.5	19.0	44.0
Triple application 11.86 g/ha AI each Oct '79, Apr '80 & Oct '80	154	3133	9.4	69.8	64.9	56.5	57.6	92.8	63.1	76.8
No treatment (check)	202	4590	2.5	1.0[4]	37.1	20.8[4]	6.8	4.2[4]	35.1	3.4[4]

[1]Data corrected with Abbott's formula for changes in untreated check.
[2]Apr '80—24 wk after initial treatment
July '80—37 wk after initial treatment, 13 wk after 2nd treatment
Oct '80—51 wk after initial treatment, 27 wk after 2nd treatment
Apr '81—76 wk after initial treatment, 51 wk after 2nd treatment, 26 wk after 2nd treatment
[3]Population index is used to denote effects of compounds on reproduction by absence of brood. Population index is derived by summation of the products of the number of colonies in each nest index category X value of categorgy (see text for nest indices).
[4]Figures represent increases, all others are reductions.

that was subsequently modified by Lofgren and Williams (1982) for work with avermectin. In this system as modified, the entire area within each subplot was carefully searched before treatment and at predetermined intervals posttreatment and each active nest found was opened with a spade and the contents carefully examined. Each nest was then assigned a rating in the field of from 1 to 10 as indicated in the following table based on the estimated number of worker ants and the presence or absence of worker brood. Based on the field rating each nest was then assigned a nest index as follows:

Index of ant nests of indicated size and brood status

Number of worker ants	Without worker brood		With worker brood	
	field rating	nest index	field rating	nest index
<100	1	1	6	5
100-1000	2	2	7	10
1000-10000	3	3	8	15
10000-50000	4	4	9	20
>50000	5	5	10	25

The interaction of the number of nests on a subplot and the nest index was then used to derive a population index for each subplot that can be expressed mathematically as follows:

$$\text{Population index (PI)} = \sum_{K=1}^{25} K(N_k)$$

where N_k = the number of ant colonies on a given subplot with a nest index of K, where $(25 \geq K \geq 1)$.

The population indices for plots or treatments were obtained by summation of the indices for all subplots within the unit. Effectiveness of the treatments was determined by comparison of the pre- and posttreatment population indices and also by comparison of the reductions in number of active nests.

The baits were applied to the small plots with a tractor-mounted granular applicator and the large plots with fixed-wing aircraft. Flagmen were used at both ends of all plots to maintain proper swath spacing in all applications. The IGR baits were tested at the following rates:

Chemical	Formulation	Rate of Application	
		Bait (kg/ha)	IGR (g/ha)
MV-678	2.4% bait	0.49	11.86
CGA-38531	1.0% bait	0.63	4.76
		1.26	9.44
	2.0% bait	0.75	15.0
Ro 13-5223	1.0% bait	0.62	6.2
		0.92	9.18
		1.17	11.74
	2.0% bait	1.01	20.25
		1.06	21.17
JH-286	1.0% bait	1.13	11.27
	2.0% bait	1.01	20.28

All 4 IGRs exhibited good activity against field populations of the ants. Two applications separated by a 6 month interval provided the most effective control with MV-678. That treatment eliminated 89.5% of the active nests and reduced the population index by 95.8% (Table 2). Three applications 6 months apart did not appear to be more effective than 2; however, this may be a false assumption. Two of the treated blocks and the untreated check showed a substantial increase in the number of active nests between October 1980, when the third application of bait was made, and the final evaluation in April 1981. This fact suggests that mating flights occurred during that interval to reinfest the plots. It is unknown how many of the colonies on the plots treated 3 times were survivors and how many were new colonies that arose from mated queens that flew into the area. Three treatments theoretically should give a higher level of control, however, determination of that fact will require additional tests on large areas. A single application of MV-678 or 2 applications separated by 1 year were essentially ineffective.

The other 3 IGR effectively reduced ant populations on the small plots (Table 3). JH-286 was the most effective causing reductions of 90.1 and 98.4% respectively in the number of active nests and the population index with the 1.0% bait. The 2.0% JH-286 bait was somewhat less effective, possibly due to repellency of the higher concentration of chemical in the oil. CGA-38531 eliminated from 61.6 to 82.1% of the active nests and reduced the population indices by 81.3 to 96.2%. Ro 13-5223 eliminated 49.8 to 84.8% of the active nests and reduced the population indices by 82.4 to 97.8%. The reduction in the population indices is the best indicator of activity of the IGR in these tests since the posttreatment evaluations were made at 13 weeks and colony mortality with IGR is usually very long term. In most cases reinfestation of the small test plots occurs before kill is complete thus confusing final evaluation.

The effects of the IGR on imported fire ants in both laboratory and field have been every dramatic and suggest that these chemicals may be effectively utilized in control and population management programs against the ants. Available information indicates that the IGR are non-persistent and should be free of the undesirable effects on the environment encountered with materials previously used.

Information derived from our field studies to this point indicates that the most effective use of IGR will be in broadcast application over pastures, cropland, and industrial areas. Because of the long period required for colony mortality, they do not appear to be viable materials for individual mound treatments or for area treatment of dooryards, lawns, playgrounds, or other areas frequently used by people. However, this potential needs further study.

The demonstrated ability of the ants to metabolize or excrete certain of the IGR (Bigley and Vinson 1979, Wendel and Vinson 1979) and the possible effect that this ability may have on use of IGR in ant control needs to be studied. Research is also needed to identify other IGR that may be more effective against IFA and to develop the best formulations to achieve maximum effectiveness against the ants in the field.

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TABLE 3. EFFECTS OF THE IGR CIBA-GEIGY CGA-38531, MAAG RO 13-5223, MONTEDISON JH-286 ON FIELD POPULATIONS OF RED IMPORTED FIRE ANTS.

Application rate		Number active nests		Population index[2]		Mean percentage reduction[3]	
Bait kg/ha	AI g/ha	Pre-treatment	Post-treatment[1]	Pre-treatment	Post-treatment	Number nests	Population index
				CGA-38531			
0.63	4.76	60	17	1258	165	61.6	81.3
1.26	9.44	39	9	823	22	68.7	96.2
0.75	15.0	53	7	1179	57	82.1	93.1
Check	—	65	48	1433	1008	26.1	29.7
				Ro 13-5223			
0.62	6.2	59	14	1138	96	70.6	89.1
0.92	9.18	61	15	1211	165	69.5	82.4
1.17	11.74	41	10	825	14	69.8	97.8
1.01	20.25	37	15	865	36	49.8	94.6
1.06	21.17	49	6	1070	19	84.8	97.7
Check	—	62	50	1277	990	19.4	21.5
				JH-286			
1.13	11.27	46	4	1005	8	90.1	98.4
1.01	20.28	42	10	872	17	72.9	97.8
Check	—	58	51	1121	972	12.1	13.3

[1]Posttreatment observations made at 13 wk after treatment.
[2]Population index is used to denote effects of compounds on reproduction as evidenced by absence of brood. Population index is derived by summation of the products of the number of colonies in each nest index category X value of category (see text for nest indices).
[3]Data corrected with Abbott's formula for changes in untreated check.

typing the manuscript and the various chemical companies for supplying the IGR.

This paper reports the results of research only. Mention of a pesticide does not constitute a recommendation for use by the United States Department of Agriculture nor does it imply registration under FIFRA as amended.

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