THE EFFECTS OF INSECT GROWTH REGULATORS ON LABORATORY AND FIELD COLONIES OF RED IMPORTED FIRE ANTS[1,2]

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Organized large-scale control programs have been conducted since 1957 against the red and black imported fire ants, *Solenopsis invicta* Buren and *Solenopsis richteri* Forel, respectively. Between 1957 and 1961, the chlorinated hydrocarbons heptachlor (1,4,5,6,7,8,8-heptachloro-3*a*, 4,7,7*a*-tetra-hydro-4,7-methanoindene) and dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1,4,-*endo-exo*-5,8-dimethanonaphthalene, 85% minimum), were applied as broadcast residual contact treatments. After 1962 a toxic bait containing mirex (dodecachloroocta-hydro-1,3,4-metheno-1*H*-cyclobuta [*cd*] pentalene) (Lofgren et al. 1964) was aerially applied to millions of acres annually. However, in the early 1970's reports of the accumulation of residues of mirex by nontarget organisms (Baetcke et al. 1972, Borthwick et al. 1973, 1974, Markin et al. 1972a, 1972b), of high level sensitivity of certain estuarine organisms to mirex (Lowe et al. 1970, 1971), and of increased tumor incidence in mice and rats fed mirex in their diets (Mrak 1969, Innes et al. 1969) led to severe restrictions on its use. All application ceased June 30, 1978 (Holden 1976, Johnson 1976).

For some years, researchers have been investigating alternate methods of control. One possibility is a class of materials, analogues of the juvenile hormones, collectively referred to as insect growth regulators. In laboratory studies, Cupp and O'Neal (1973) found that 2 such compounds, methoprene (isopropyl (*E*,*E*)-11-methoxy-3,7,11-trimethyl-24,-dodecadienoate) and hydroprene (ethyl (*E*,*E*)-3,7,11-trimethyl-2,4-dodecadienoate) caused deformities in the ants and increased mortality. Troisi and Riddiford (1974) reported that the same 2 compounds interfered with egg production, embryonic development, and metamorphosis in *S. invicta*. Vinson et al.

[1] Hymenoptera: Formicidae

[2] Mention of a pesticide or a proprietary product does not constitute a recommendation of this product by the U.S. Department of Agriculture.

(1974) found that topical application of insect growth regulators to pharate reproductive pupae of *S. invicta* reduced the number of adults eclosing and prevented the development of normal pigmentation in those adults that did eclose. In other tests, Vinson and Robeau (1974) found that insect growth regulators administered to small colonies (3-4,000 workers) of fire ants by feeding or through contact and fumigation caused delayed cessation of egg-laying by the queen, initiation of sexual brood production and ultimate death of the colony.

The Imported Fire Ant Research Laboratories, Federal Research/SEA at Gainesville, Florida, and Gulfport, Mississippi, have conducted laboratory and field studies since late 1974 to evaluate the effect of insect growth regulators on imported fire ants and to determine their potential for controlling these pests. This paper presents a review of these studies.

Laboratory Studies

In tests in the laboratory with 26 insect growth regulators (Banks et al. 1978), we found that certain of these compounds caused severe disruption in caste differentiation, reproduction, and social organization of large colonies (30-50 thousand workers) of imported fire ants. The 3 most effective insect growth regulators we tested, 1-(8-methoxy-4,8-dimethylnonyl)-4-(1-ethylethyl) benzene (AI3-36206), 1-(8-ethoxy-4,8-dimethylnonyl)-(1-methylethyl)benzene (AI⅜-36093, and (*E*)-1-[(7-ethoxy-3,7-dimethyl-2-octenyl)oxy]-4-ethylbenzene (also known as (E)-7-ethoxy-1-(*p*-ethylphenoxy)-3,7-dimethyl-2-octene) (USDA, JH-25) caused 75, 75, and 67 percent, respectively of the treated colonies to die. The first 2 of these chemicals were synthesized by Dr. Meyer Schwarz of the Biologically Active Natural Products Laboratory, Federal Research, SEA, USDA, and the 3rd by the Organic Chemicals Synthesis Laboratory, Federal Research, SEA, USDA.

Because it was better accepted by the ants and had slightly more effect, we have devoted most of our efforts to studies with the first of these 3 compounds, AI3-36206. The results of the laboratory studies with this compound are summarized in Table 1.

Concentrations of as much as 5 percent of AI3-36206, when incorporated into peanut butter or into peanut or soybean oil, were readily accepted by the ants. Single doses of 2 to 100 mg/colony produced the same basic effect when they were administered in the diet. We were unable to discern any particular advantage of increasing the dosage above 2 mg; however, theore-

tically, dilution of the administered concentration to ineffective levels should proceed more slowly when the higher dose is used. Repeat treatments after 60 days did not appear to increase effectiveness of the chemical.

The first obvious effect of the insect growth regulator was the appearance of an abnormal number of large sexual larvae at 2 to 3 weeks after treatment. Closer examination at that time revealed that no worker larvae were present; also the pharate workers present, instead of the normal dark reddish-brown pigmentation, were light yellowish-red to only slightly lighter than normal. By 6 to 10 weeks after treatment, all immature worker forms had disappeared, and large numbers of adult sexuals and sexual larvae and pupae were present. Some colonies produced males, some females, and some both, but most (62 percent) produced only females.

We found that the appearance of the abnormal numbers of sexuals resulted from a shift in caste differentiation; larvae that would normally develop into worker ants became sexual adults. However, the insect growth regulator seems to affect only the 1st- and possibly the 2nd-instar larvae, not the queen or the eggs she lays. When we removed a queen from a treated colony (40 mg AI3-36206), that was producing only sexual larvae and placed her with untreated pharate workers, she laid eggs that produced normal worker ants, though eggs remaining with the treated colony produced female sexuals.

There may, of course, be a chronic effect on the queen. Egg production by the queens in treated colonies has been substantially reduced after prolonged exposure of the queen to insect growth regulator (through continued trophallaxis from the workers), but this reduction may reflect lack of proper food and attention because of a general breakdown of a colony organization, not a direct effect. Some differences have been noted in ovariole development in treated queens and also in the contents of the postpharyngeal glands (B. M. Glancey — personal communication). The relationship of these changes to the presence of the insect growth regulator and their significance in queen health, egg production, and ultimate colony survival is under investigation.

The lack of effect on the eggs was verified in further studies in which we removed eggs from treated and untreated colonies and divided them equally between treated and untreated workers. Eggs from both treated and untreated colonies produced only sexual forms when tended by insect growth regulator treated workers but produced normal worker ants when tended by untreated workers.

We found that the majority of adult females to emerge from the first bloom of sexual larvae after treatment with AI3-36206 (by ingestion) dealated shortly after eclosion. This effect was also noted by Kearney et al. (1977) who made topical applications of the naturally occurring JH's I, II, and III to virgin female fire ants. Thus dealation of the female sexuals may also be hormonally controlled.

The effect of all 3 of the effective insect growth regulators on caste differentiation was similar to that reported by Robeau and Vinson (1976) for JH-25. They found that JH-25 administered orally at 5 mg/colony to red imported fire ants stimulated production of alate females, major workers, and some intercastes. The doses we administered were apparently massive enough to shift differentiation to sexuals only. When we did notice an increase in major workers, it was in colonies that ultimately survived the treatment and it only occurred several weeks after treatment when the levels of insect growth regulator had been reduced through trophallaxis, metabolism, excretion, etc. We did not observe intercastes in the laboratory when we treated with AI3-36206, though we did subsequently find some in treated field colonies and in colonies treated in the laboratory with other insect growth regulators.

We, therefore, agree with the conclusions of Robeau and Vinson (1976) that juvenile hormone is important in caste differentiation. Our findings suggest that a fertilized egg becomes a female sexual or remains a worker because of the level of hormone in the early larval instar(s). We postulate that every fertilized egg laid by the fire ant queen is a potential female sexual but that some intervening factor arrests full development, so the individual larva becomes a worker. Whether this intervening factor is juvenile hormone (or the lack of it) and what the controlling mechanism is remain to be determined. More definitive information concerning hormonal control of caste differentiation, worker size, egg production, etc., may be forthcoming from work now in progress in our laboratories and in those of Dr. Murray Blum at the University of Georgia and Dr. Bradleigh Vinson at Texas A&M.

Field Studies

The underlying objective of our laboratory studies was, of course, to determine whether any of the insect growth regulators were effective enough against the imported fire ants to warrant field testing against natural infestations. The severe effects we observed in laboratory colonies tre-

ated with AI3-36206 indicated that the material had good potential for control of the ants in the field. We, therefore, made 2 series of field tests with AI3-36206 formulated to prevent degradation and deliver as much material as possible to the ants. For all formulations, technical AI3-36206 was incorporated into once-refined soybean oil at rates of 2, 3, or 5 percent by weight and was then microencapsulated in gelatin-plastic capsules (177-840 μ) by the Capsular Products Division of NCR Corporation. These rates in the oil produced 1.56, 2.34 and 3.9 percent formulations, respectively. The microcapsules were composed of ca. 78 percent core material (insect growth regulator + soybean oil) and 22 percent capsular wall.

Test 1. — Two blocks, 1.4 and 1.8 ha, in permanent pasture in Clay County, Florida were used in the first test initiated in August 1975. Within each block, 3 rectangular plots (55 × 36.5 m) were established for pre- and posttreatment observations of active ant nests. Also, 3 plots of the same size were established in an adjacent pasture as untreated checks.

The 1.4-ha block was treated with the 2.34 percent encapsulated formulation at a rate of 10.5 g/ha (80 mg/nest) active ingredient; the 1.8-ha block was treated with the 1.56 percent encapsulated formulation at a rate of 4.75 g/ha (36.2 mg/nest) active ingredient. We applied the microencapsulated baits in the first test with a CO_2 aspirator system similar to that described by Markin et al. (1969). Effects of the insect growth regulator on the colonies was determined by using a spade to open all nests on the observation plots at predetermined intervals after treatment. Each nest was carefully examined to determine general condition of the colony, presence or absence of sexual brood, and relative numbers of each life stage compared with untreated colonies.

The effects of the treatments on the field colonies of red imported fire ants are shown in Table 2. They were similar to the effects observed in laboratory colonies but were usually not as pronounced, perhaps because the larger colony size in the field resulted in more rapid dilution of the hormone.

In this test, both dosages produced comparable effects. With the lower dose (36 mg/colony), 5 and 1 percent of the colonies appeared normal at time of the 13 and 26 week examinations, respectively; none of the colonies treated with the higher dose (80 mg/colony) appeared normal through the 40th week after treatment.

However, both treatments caused an initial increase in the number of mounds due to fragmentation of some colonies. These fragments then gradually died out, so by 1 year posttreatment the number of active nests was

substantially reduced, 76 percent for the lower dose and 64 percent for the higher dose. We did not follow the colonies longer than 1 year posttreatment, but if the colonies that appeared abnormal at that time eventually died the reductions would be 92 and 74 percent, respectively.

Colonies on the untreated check plots appeared normal throughout the test. However, there was a reduction of 4 percent in the number of colonies on these plots at the end of the year.

Test 2. — Two blocks of ca. 15 ha each in permanent pasture in Sumter County, Georgia, were used in the test initiated in September 1976. Six 0.4 ha circular plots were established in each block for pre- and posttreatment observations of colonies. Plots were established in an adjacent untreated pasture as checks.

Both blocks were treated with the 2.34 percent microencapsulated AI3-36206, however, difficulties in calibration resulted in a heavier than desired dose; block 1 received 4.80 g/ha (66.9 mg/nest) and block 2 received 8.25 g/ha (77.7 mg/nest). In April, 1977, block 2 was retreated with 3.9 percent microencapsulated A13-36206 at a rate of 28 g/ha. The microencapsulated baits in the second test were applied with a Vibra-Flow Volumetric Feeder Machine modified for pesticide application (Banks et al., unpublished).

Effects of the insect growth regulator on the colonies were determined by the same methods used in test 1. Results of this test are shown in Table 2. The first posttreatment observation was made at 26 weeks, just before the second treatment was applied to block 2. At that time there had been an increase in the 2 blocks in the total number of active nests; however, only 31 percent of those present could be classified as normal, and 64 percent had no brood of any type.

By 40 weeks after the beginning of the test there was a 23 percent reduction in the number of active nests on block 1, and only 18 percent of the remaining colonies appeared normal. On block 2, which had received the second treatment 12 weeks earlier, there was a 54 percent reduction in the percent number of active nests, and only 3 percent of the colonies appeared normal. Numerous very small new colonies were present on both blocks but these were not considered in calculating effects of the insect growth regulator.

The reductions in number of active nests had increased to 28 and 81 percent, respectively, for the single and dual treatments by 1 year. However, the reduction on the untreated check was 62 percent because of the extremely dry spring and summer immediately preceding, thus it is im-

possible to determine what the true effectiveness of AI3-36206 was in test 2.

Discussion. — The results of the two field studies show that natural populations of the ants can be severely affected by application of insect growth regulator. However, the true efficacy is difficult to assess because of the length of time (9-12 months) required for the material to have exerted its full effect. Also, with small area treatment, reinfestation usually occurs within 6 months so that it becomes increasingly difficult and sometimes impossible to determine whether one is dealing with colonies present at treatment or with new ones.

We found that effectiveness of insect growth regulator on the colonies seemed to vary greatly from colony to colony. The exact reason is unknown; but there are 2 factors that appear to be important (1) the number of very large workers in the colony at the time of treatment and (2) the quality and quantity of food available to the colony just before treatment. Glancey et al. (1973) found that major workers serve as repletes and thus would be reservoirs of insect growth regulator which could be fed to developing larvae over an extended period. The greater the number of such workers, the larger the quantity of insect growth regulator that can be stored. Also, availability of natural food sources is important because if a particularly desirable food has been readily available to the colony, the storage capacity of the repletes will have been taken up, and the insect growth regulator will pass through the colony relatively quickly and so have less impact than if it is retained and distributed over a longer interval. No doubt there are numerous other factors that may be as or more important in determining activity of the growth regulating compounds on imported fire ants.

Determination of the nature and function of hormones in the imported fire ants will require challenging research. There are some real possibilities that such materials can be used to control or at least reduce the impact of the ants on people and their possessions in the Southeast.

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Table 1. Effects of the insect growth regulator AI3-36206 on laboratory colonies of *Solenopsis invicta*.[a] (Table adapted from Banks et al. 1978.)

Dosage (mg/colony)	No. wk. after treatment to			Type of sexuals produced
	DWB	RWB	DC	
2	6	NR	32	F
	6	10	68	M
10	6	13	52	F
	6	10	52	F
20	6	15	48	M & F
	4	NR	15	M
40	3	13	23	M
	4	10	S	M
	5	NR	16	D
	4	12	S	F
	4	10	S	M & F
60	4	NR	15	M
	6	12	68	F
80	6	NR	32	M
	6	NR	36	F
[b]	6	NR	34	F
[b]	6	20	S	M
100	6	10	45	F
120[c]	4	NR	14	F
[c]	4	24	37	M & F

[a] Abbreviations used in table — DWB = Disappearance of worker brood; RWB = reappearance of worker brood; DC = death of colony; F = female; M = male; D = died before sex was determined; S = survived; NR = No recovery.

[b] Administered as two 40 mg doses 60 days apart.

[c] Administered as two 60 mg doses 60 days apart.

Table 2.—Effects of insect growth regulator AI3-36206 on field colonies of *S. invicta*.

Examination intervals	No. active nests	Percent of colonies as indicated			
		Normal	Abnormal		
			Sex brood and workers	Sex brood only	Workers only
		Test I			
		(Avg. 3 plots — 36.2 mg/colony)			
Pretreatment	92	100			
Posttreatment					
8 wk	88	0	29	56	15
13 wk	108	5	34	35	26
26 wk	103	1		8	91
40 wk	64	0	53	28	19
52 wk	22	68		18	14
		(Avg. 3 plots — 80 mg/colony)			
Pretreatment	77	100			
Posttreatment					
8 wk	98	0	32	58	10
13 wk	106	0	26	50	24
26 wk	109	0	4	4	92
40 wk	65	0	62	3	15
52 wk	28	71	4	7	18

Table 2. — Continued

Examination intervals	No. active nests	Percent of colonies as indicated: Normal	Abnormal: Sex brood and workers	Abnormal: Sex brood only	Abnormal: Workers only
		Test II			
		(Avg. 6 plots — 66.9 mg/colony)			
Pretreatment	175	100			
Posttreatment					
26 wk	233	40	0.5	1	58.5
40 wk	135	18	38	33	11
52 wk	96[a]	98	0	0	2
		(Avg. 6 plots — 77.7 mg/colony)			
Pretreatment	258	100			
Posttreatment					
26 wk	245	23	0.5	8	68.5
		Retreatment			
Posttreatment (1st application)					
40 wk	116	3	26	39	32
52 wk	40[a]	95	0	0	5

[a] 52 wk counts based on 5 plots with original pretreatment counts of 133 and 206 active nests, respectively, for blocks 1 and 2.