Red Imported Fire Ants:[1] Effects of Insect Growth Regulators on Caste Formation and Colony Growth and Survival

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ABSTRACT

Fourteen of 26 insect growth regulators (IGRs) administered in peanut butter bait to large laboratory colonies of *Solenopsis invicta* Buren caused a shift in caste differentiation with the result that worker brood production ceased and production of alate forms, most with deformed wings, increased. Also, 8 of the compounds caused one or more colonies to die: AI3-36206 (1-(8-methoxy-4,8-dimethylnonyl)-4-(1-ethylethyl) benzene) caused 15 of 20 treated colonies to die, and AI3-36093 (1-(8-ethoxy-4,8-dimethylnonyl)-4-(1-methylethyl) benzene) and MI3 35477 (JH-25) ((*E*)-1-[(7-ethoxy-3,7-dimethyl-2-octenyl) oxy]-4-ethylbenzene or (*E*)-1-[(7-ethoxy-3,7-dimethyl-2-octenyl) oxy]-4-ethylbenzene) caused 3 of 4 and 6 of 9 colonies, respectively, to die.

Increasing restrictions on the use of baits containing mirex for control of the red and black imported fire ants (IFAs), *Solenopsis invicta* Buren and *Solenopsis richteri* Forel, respectively, have necessitated studies into alternate methods of control. Since the insect growth regulators (IGRs) have shown promise against a number of insects (Staal 1975) they have been included in these studies against the fire ants.

Cupp and O'Neal (1973) found that methoprene and hydroprene caused deformities in the ants. Troisi and Riddiford (1974) reported that the same 2 compounds interfered with egg production, embryonic development, and metamorphosis in the red IFA. Vinson et al. (1974) treated pharate reproductive pupae of the red IFA with acetone solutions of IGRs and found that certain juvenile hormone (JH) analogues substantially reduced eclosion and prevented normal pigmentation in the adults that did eclose. Also, Vinson and Robeau (1974) evaluated the effects on small laboratory colonies of IFAs when IGRs were administered by feeding or through contact and fumigation. The most effective compound they tested, AI3-35477 (JH-25) ((*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), caused egg production by the queens to cease at 28–45 days after levels of 0.5–50 mg/colony were fed, caused initiation of production of sexual brood, and ultimately after 45–60 days caused the colony to die. The IGR was ca. 10-fold more effective by contact and fumigation than by feeding. Robeau and Vinson (1976) found that JH analogues stimulated production of major workers, intercastes, and alate females in fire ant colonies. Their results suggested that caste determination in the fire ants is influenced by juvenile hormone just as in termites (Luscher 1972, Hrdy 1973) and in honeybees, *Apis mellifera* L., (Wirtz and Beetsma 1972).

We report the results of laboratory studies made between 1974 and 1976 to evaluate effects of IGRs on large colonies of IFA and to determine if IGRs might be effective as control agents for field use against the ants.

MATERIALS AND METHODS.—Laboratory colonies consisting of a queen, ca. 2000 immatures, and 20–30,000 workers of all sizes were used in the tests. These colonies were begun in the laboratory by newly-mated queens collected after mating flights in the field and were a minimum of 7 mo old; most were at least 1 yr old when tested. The colonies were housed in Plexiglas® ant nests (Wilson 1962) in plastic trays (38×79×15 cm or 61×76×10 cm). All colonies were fed 3 times weekly on an artificial medium composed of macerated fly pupae, pureed beef, whole hen egg, and multiple vitamins in an agar base. The laboratory was maintained at 28°± 2°C.

Each IGR was initially tested by admixing it with 2 g peanut butter (2% by wt; 40 mg/colony), and allowing the colony to feed ad lib. for 24 h. Any bait remaining was removed and the colony was returned to the regular diet. IGRs that were repellent at this level were tested with lower concentrations and the more effective compounds were tested at both higher and lower concentrations and as dual applications. Control colonies were exposed to peanut butter bait containing no IGR and were then handled in the same manner as the test colonies.

The following IGRs were tested at least at 1 concentration:

AI3-No.	Chemical Name	Other Designations
27995	1-methyl-2-propynyl (4-chlorophenyl) carbamate	Hercules 24108
27906	1-methyl-2-propynyl (3,4-dichlorophenyl) carbamate	Hercules 24734
35477	(*E*)-1-[(7-ethoxy-3,7-dimethyl-2-octenyl) oxy]-4-ethylbenzene, or (*E*)-7-ethoxy-1-(*p*-ethylphenoxy)-3,7-dimethyl-2-octene	USDA (OCSL) JH-25
35531	(*E*)-1-[(7-ethoxy-3,7-dimethyl-2-octenyl) oxy]-4-(1-methylethyl) benzene	USDA (OCSL) RS-H-281.1

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AI3 No.	Chemical Name	Other Designations
35540	(*E*) -1-ethoxy-4-[(7-ethoxy-3,7-dimethyl-2-octenyl) oxy]benzene	USDA (OCSL) RS-II-289.2
35542	(*E*) -1-[7-butoxy-3,7-dimethyl-2-octenyl) oxy]-4-ethylbenzene	USDA (OCSL) RS-II-288.1
35543	(*E*) -1-ethyl-4-[(3,7-dimethyl-7 propoxy-2-octenyl) oxy]benzene	USDA (OCSL) RS-II-287.1
35838	3 [6 (4-ethylphenyl) -3-methylhexyl]-2,2-dimethyloxirane	USDA (BANPL) MS-9-158
35851	1-(8-ethoxy-4,8-dimethyl-nonyl) -4 ethylbenzene	USDA (BANPL) MS-9-160-2
35853	1-ethyl-4- (8-methoxy-4,8-dimethylnonyl) benzene	USDA (BANPL) MS-10-126-2
36003	1-(8-ethoxy-4,8-dimethyl nonyl) -4- (1-methylethyl) benzene	USDA (BANPL) MS-10-164
36206	1-(8 methoxy-4,8-dimethyl-nonyl) -4- (1-methylethyl) benzene	USDA (BANPL) MS-10-160b
36241	2,2-dimethyl-3-[3 methyl-6-[4-(1-methylethyl) phenyl]hexyl]oxirane	USDA (BANPL) MS-11-50
70221	(*E*) -3-[5- (4-ethylphenoxy) -3-methyl-3-pentenyl]-2,2-dimethyloxirane, or (*E*) -6,7-epoxy-1- (*p*-ethylphenoxy) -3,7-dimethyl-2-octene	Stauffer R-20458
70348	ethyl (*E*) -7,11 dichloro-3,7,11-trimethyl-2-dodecenoate	Hoffman-LaRoche RO-6-8415
70349	methyl 4-[(1,5-dimethyl-hexyl) oxy]benzoate	Hoffman-LaRoche RO-8-3627
70350	ethyl 10,11-epoxy-3,7,10,11-tetramethyl-2,6-dodecadienoate	Hoffman-LaRoche RO 8-4314
70351	2,2-dimethyl-3-[3-methyl-5 (2-propynyloxy) -3-hexenyl]oxirane	Hoffman-LaRoche RO-8-5496
70357	(*E*) -4-[6,7-epoxy-3,7-dimethyl-2-nonenyl) oxy]-1,2- (methylenedioxy) benzene	Hoffman-LaRoche RO 20-3600
70536	(*E*) -5-[(3,7 dimethyl-2,6 octadienyl) oxy] 2-methylpyridine	Stauffer HS-2
70598	*S*- (phenylmethyl) 3,5-bis (1,1-dimethylethyl) -4 hydroxybenzenecarbothioate	Chevron Ortho 17565
70599	*S*- (1,1-dimethylethyl) 3,5-bis (1,1-dimethylethyl) -4-hydroxybenzenecarbothioate	Chevron Ortho 18286
70613	(*E*) -5-[(3,7-dimethyl-2,6-octadienyl) oxy]-2-ethylpyridine	Stauffer HS-103
70649	ethyl 3-methyl 4-[4-(phenylmethyl) phenoxy]-2 butenoate	Ciba-Geigy CGA-19353
70670	6-azido-*N* cyclopropyl *N'*-ethyl-1,3,5-triazine 2,4-diamine	Ciba-Geigy CGA-19255
70681	*S*-cyclopentyl [2-[[(cyclopentylthio) carbonyl] amino]ethyl] ethylcarbamothioate	Stauffer R-38898

Each colony was examined at 2-wk intervals through 4 mo after treatment and at monthly intervals thereafter until the colony died or recovered from any obvious effects of the IGR. Colonies that never exhibited any effects of the treatment were removed from test at 6 mo posttreatment. At each examination, we noted general condition of the colony, presence or absence of worker brood, increased production of major workers or sexual forms, lack of pigmentation, deformities in any insects, and colony mortality.

RESULTS AND DISCUSSION. — No discernible effects were observed in ants treated with AI3-70670, 70599, 70598, 35542, 70351, 70221, 70350, 70349, 27996, and 35543. The ineffectiveness of 7 of these compounds probably reflects an observed repellency to the ants; they were unacceptable or poorly acceptable at any dose. However, acceptance of AI3-70221 and 70599 was fair and that of 70598 was good; thus, the lack of effectiveness of these compounds must result from factors other than repellency.

Fourteen of the IGRs caused worker brood to disappear from the colonies (Table 1). AI-70357 and 70643 caused a reduction in the amount of worker brood but did not cause it to disappear. Worker brood continued to be produced in colonies treated with AI3-70348 and 27995 for 24 and 25 wk posttreatment, respectively, so cessation of production in these colonies may not have resulted from direct effects of the IGRs. Apparently the IGRs caused a shift in caste development from worker ants to sexuals and did not have a direct effect on the queen or on the eggs she produced.

The lack of direct effect on the queen or the eggs was demonstrated in a supplemental study we made with one of the most active IGRs, AI3-36206. Eggs were removed from a colony treated with 40 mg of AI3-36206 (only sexual larvae were being produced) and divided ca. equally between groups of IGR-treated and untreated worker ants. Eggs placed with untreated workers produced only normal workers; those placed with treated workers and those remaining in the colony produced only sexual larvae. Also, the queen was removed from a 2nd colony treated at the same dose, (only sexual larvae were being produced at 2 wk posttreatment) and placed in a nest with pharate workers from an untreated colony. Within 1 wk, workers had eclosed and were tending eggs laid by the queen; the eggs produced only normal worker ants. Presumably the effect of other IGRs on the eggs and the queen would be similar to that of AI3-36206, however, this has not been studied.

In most cases, there was a reduction in the number of eggs produced by the queen after IGR treatment, but if the queen survived, some eggs were laid because sexual larvae continued to appear. The de-

Table 1.—Effects of insect growth regulators on colonies of the red imported fire ant.[a]

IGR (AI3-)	Dosage (mg/colony)	No. wk after treatment to			Type of sexuals produced
		DWB	RWB	DC	
27995	40	25	NR	51	F
35477	20	5	11	S	F
		6	NR	24	F
		6	NR	24	F
		4	15	50	F
		4	8	S	M
	60	4	NR	24	N
		6	NR	34	M
	120[b]	6	36	S	M
	[b]	6	NR	42	F
35534	18	6	12	S	M
35540	40	7	12	S	M & F
35838	40	6	9	S	M
35851	40	5	9	S	D
35853	40	7	9	S	D
36093	40	6	NR	26	F
		4	NR	32	D
		4	NR	21	D
		7	13	S	M
36206	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
	[c]	6	NR	34	F
	[c]	6	20	S	M
	100	6	10	45	F
	120[b]	4	NR	14	F
	[b]	4	24	37	M & F
36241	40	6	NR	41	F
70348	40	21	NR	53	F
70536	20	6	NR	26	F
		7	10	S	D
70649	20	5	11	S	D
70681	20	4	9	S	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 60-mg doses 60 days apart.
[c] Administered as two 40-mg doses 60 days apart.

velopment of many female sexuals, that quickly delated in most colonies, made determination of presence of the colony queen extremely difficult and in some cases, impossible. Our studies indicate that the attending worker ants feed the IGR by trophallaxis to the larvae, but the larvae seem to be susceptible to the IGR only in the 1st, and possibly 2nd, instar. Older larvae present in the nest at the time of treatment continued to develop normally and produce worker ants. However, young larvae present at the time of treatment, and those hatched after treatment, developed only into sexuals until the IGR was eliminated from the colony or was diluted through trophallaxis to ineffective levels.

Except for the colonies treated with AI3-70348 and 27995, disappearance of worker brood occurred within 4–6 wk after treatment with most compounds and dosages, although it was slightly earlier or later in a few cases. Colonies that recovered from the effects of the IGR and resumed worker brood production usually did so within 9–12 wk after a single treatment or within 26 wk after 2 treatments. The longest period of worker brood suppression among colonies that survived treatment occurred in a colony that received 2 treatments 60 days apart of 60 mg each of AI3-35477 (JH-25). No worker brood was present in this colony from the 6th through the 35th wk after the initial treatment. However, a colony that received a single treatment (60 mg of JH-25) produced no worker brood after 6 wk even though the queen survived through 26 wk posttreatment, and male and female sexual brood were occasionally produced through 22 wk. The colony died at 34 wk posttreatment.

Production of sexual brood was initiated in all colonies treated with the 14 effective compounds, apparently, as noted, because of a shift in caste differentiation during the early larval instars, not because of IGR effect on the eggs. Other researchers also have observed initiation of sexual brood production after IGR treatment. Troisi and Riddiford (1974) found that the sexuals that eclosed were predominantly males; Vinson and Robeau (1974) found that their colonies produced female sexuals. We found that some colonies treated with IGR produced female sexuals, some produced males, and some produced both simultaneously (Table 1). However, the number that produced females was ca. 60% greater than the number that produced males. (In some colonies, large numbers of sexual larvae were produced, but all died or were killed by the workers before they reached the stage where sex could be determined easily.)

The shift from worker ants to female sexuals is easily understood because worker ants are female. If we assume that workers are all potential female sexuals and that their development is normally arrested by variation in hormonal level, nutrition, or some other factor, then we can see that the addition of hormone (IGR) could cause them to continue to develop to female sexuals. The production of male sexuals, however, is not as easily explained. If the male fire ants develop from unfertilized eggs, as is generally accepted, then production of males in a colony that has been producing only workers would indicate some phenomenon other than just a shift in caste differentiation. Troisi and Riddiford (1974) suggested that the IGR acted upon the queen so that some unfertilized eggs were laid. This may very well occur but our testing has not yet been adequate to demonstrate this effect. Obviously, additional study is needed to more clearly delineate how an IGR influences caste differentiation in fire ants.

As some colonies treated with AI3-35838 and 35477

began to recover from the effects of the IGRs, they produced excessive numbers of major workers. Probably the IGR had been diluted through trophallaxis until it was no longer effective enough to cause the shift to alates but was still sufficiently strong to cause a shift to major workers.

Changes in pigmentation in some of the eclosing adults were noted after treatment with most of the 14 effective IGRs. Usually only 15–20% of the ants were affected, but in some colonies treated with AI3-35477 and 36206 almost all remained light in color. The effect on pigmentation varied between individual insects from very light yellowish red to an almost imperceptible lightening of the normal color.

We did not observe the deformities reported by Cupp and O'Neal (1973) in any of our treated colonies. If they occurred, the ants died or were killed by the workers and were discarded without being noticed. We did note a high incidence of deformities in the wings of the eclosing sexuals, particularly in those colonies treated with AI3-35477, 70536, 36093, and 36206.

One or more colonies died of those treated with AI3 36241, 36093, 35477, 70348-X, 36206, 33972, 27995, and 70536 (Table 1), but the period from treatment to death was usually considerably longer than that reported by Vinson and Robeau (1974). The difference probably reflects the fact that we used much larger colonies and that we considered our colonies to be alive until the worker force dropped below 25 or until a producing queen was no longer present. The shortest period from treatment to death of a colony found by us was 14 wk after the initial treatment in a 2-treatment regimen of 60 mg each of AI3-32606 at a 60-day interval. However, no correlation was apparent between dose and lethal effect for those compounds for which we obtained sufficient data to make such a determination. Sufficient testing has not been done to determine whether any of the 8 IGRs are more lethal than others to IFA colonies.

The method by which the IGRs cause death of IFA colonies is not completely defined. However, in all colonies, the number of workers declined because of lack of replacement and natural mortality and in some, die-off of the workers was accelerated. Then, as the worker force diminished, food gathering, brood tending, and general colony maintenance declined so that presumably the queen eventually died. Nevertheless, in some cases the queen died within a relatively short time after treatment, whether from direct effects of the IGR or not is unknown. As we have previously indicated, however, the presence of numerous de-alated virgin females made it impossible to determine the fate of the queen in most colonies.

None of the effects observed in the colonies treated with IGRs was noted in the controls. Control colonies produced worker brood throughout the test period. In some, male and female sexuals were observed, but they were not as numerous as in the treated colonies and were not produced to the exclusion of workers. None of the control colonies died during the test.

AI3-36206 was the most promising of the IGRs since levels as low as 2 mg/colony showed good activity, and it was consistently well accepted by the ants in the bait at concentrations as high as 5% by weight. Also, only 5 of 20 colonies treated with 36206 survived treatment. AI3-35477 (JH-25) and 36093 were slightly less acceptable to the ants but were ca. equal in overall effectiveness: only 3 of 9 colonies treated with JH-25 and 1 of 4 treated with 36093 survived. Most other IGRs were somewhat repellent to the ants and thus far less acceptable to them.

Our results showed that imported fire ants vary in reaction to IGRs, but that some of these compounds can severely disrupt reproduction, caste formation, and social organization of the colony. Troisi and Riddiford (1974) concluded that IGRs were not suitable control agents for IFA. However, the fact that some of these compounds cause a large percentage of the treated colonies to die suggests that they might be very effective in suppressing natural populations of the ants in the field.

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