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Insect Growth Regulators for Control of the Imported Fire Ant

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Studies with insect growth regulators (IGR) and the red imported fire ant (RIFA), *Solenopsis invicta* Buren, began in the early 1970s when Cupp and O'Neal (1973) and Troisi and Riddiford (1974) found that methoprene and hydroprene (ingested or by contact) interfered with maturation of developing larvae, normal metamorphosis, and caused worker mortality. However, Troisi and Riddiford (1974) suggested that, because of lack of persistence in a colony, hormonal growth regulators did not appear to be suitable for control of RIFAs.

Subsequently, Vinson et al. (1974) demonstrated that a number of IGRs were active against pharate reproductive pupae of RIFAs at 0.1 ug/ant or less when topically applied. In other tests, small laboratory colonies fed soybean oil or egg yolk baits containing certain IGRs decreased or stopped egg production, reduced or stopped larval and pupal production, and ultimately died from effects of the chemical (Vinson and Robeau 1974). The same IGRs were more active by contact or fumigation than by ingestion. In these and other studies (Robeau and Vinson 1976), IGRs were shown to be strongly active in shifting caste differentiation in RIFAs. IGR introduction into a colony producing only minor workers stimulated production of major workers, intercastes, and alate queens.

Further studies (Texas Agricultural Experiment Station 1980) showed that although soybean oil baits containing an IGR were very effective in eliminating laboratory colonies of RIFAs, they gave very erratic results against field populations. This was attributed in part to the fact that the ants very rapidly metabolized and/or excreted two of the IGRs found to be most effective, methoprene and Stauffer R-20458 (1-(4'-ethylphenoxy)-6,7-epoxy-3,7-dimethyl-2-octene) (Wendel and Vinson 1978; Bigley and Vinson 1979). They concluded that for effective control the IGR must be more resistant to breakdown or it must be formulated in a way that prevented or retarded breakdown. Also, new techniques were needed to introduce

the chemicals into the colonies (Texas Agricultural Experiment Station 1980). Subsequently, Bigley and Vinson (1979) demonstrated that piperonyl butoxide or DEF (S,S,S-tributyl phosphorothioate) retarded breakdown of methoprene by RIFAs.

Banks et al. (1978) demonstrated in laboratory tests that 3 of 26 IGRs administered in peanut butter baits were highly active, eliminating 65 to 75% of the treated colonies. After modification of test procedures in 1977, an additional 29 IGRs were tested in soybean oil bait and 4 additional IGRs were equal or superior in activity to the 3 found in earlier tests (Banks et al. 1983).

In field tests, it was found that one of the more active IGRs, Stauffer MV-678 (1-(8-methoxy-4,8-dimethylnonyl)-4-(methylethyl)benzene), killed up to 76% of treated field colonies in small plots and left most of the surviving colonies without worker brood 26 to 40 weeks following treatment with 4.75 g AI/ha. On larger plots, two applications of a granular soybean oil bait (11.85 g AI/ha) applied with aircraft at 6-month intervals eliminated 89.5% of active colonies and reduced the population index by 95.8% (Banks et al. 1983). Small plot tests with CIBA-GEIGY CGA-38531 (1-(3-ethoxybutoxy)-4-phenoxybenzene), MAAG Agrochemicals RO 13-5223 (ethyl[2-(p-phenoxyphenoxy)ethyl]carbamate), and Montedison JH-286 (1,[5-chloropent-4-ynyl]oxy)-4-phenoxybenzene (1)) showed that these chemicals killed 61.6 to 82.1, 49.8 to 84.8, and 72.9 to 90.1% of active nests and reduced population indexes by 81.3 to 96.2, 82.4 to 97.8, and 97.8 to 98.4%, respectively (Banks and Harlan 1982; Banks et al. 1983).

Continuing laboratory and field studies have identified additional IGRs with excellent activity against RIFAs and substantiated the results of earlier IGR tests. The results of some of this work are reported here.

MATERIALS AND METHODS

Laboratory Studies

Procedures for laboratory evaluation of IGRs were standardized about 1977. The candidate chemicals are dissolved in once-refined soybean oil and the solutions are fed to the test colonies in micropipets. Tests are conducted against laboratory-reared RIFA colonies (Banks et al. 1981) that consist of a queen, 10 to 30 ml of brood (eggs, larvae, and pupae), and 40 to 60 thousand worker ants. All chemicals are initially tested against three colonies at 10 mg/colony (0.5 ml of 2.0% solution). Check colonies are given an equivalent volume of neat soybean oil. After treatment, all colonies are returned to the normal diet and held in the laboratory at $28 \pm 2^\circ\text{C}$ for observation. Each colony is examined biweekly through 16 weeks post-treatment and monthly thereafter until the colony dies

or recovers from obvious IGR effects. Each colony is rated pretreatment and at each post-treatment interval based on the estimated number of worker ants and quantity of worker brood according to the following colony index scale:

Estimated number of worker ants			Estimated quantity of worker brood (ml)		
	Rating	Value		Rating	Value
< 100	1	1	0	A	1
101-5000	2	2	1-5	B	5
5001-20000	3	3	5-10	C	10
20001-35000	4	4	10-20	D	15
35001-50000	5	5	20-30	E	20
>50000	6	6	>30	F	25

The colony index is derived for each colony by multiplication of the assigned numerical value for worker numbers times the numerical value for quantity of brood; e.g., a colony with a rating of 6E would have a colony index of 120 (6 x 20). The effectiveness of a chemical against a colony is determined by comparison of the pre- and post-treatment colony indices.

Field Studies

Those chemicals that cause death of laboratory colonies are formulated into granular baits and tested against natural infestations of the RIFA according to procedures described by Banks et al. (1983). Baits are prepared by dissolving the IGR in once-refined soybean oil and spraying the solution (30% by weight) onto 8 to 30 mesh pregel defatted corn grits as they are tumbled in a mixer. The baits are applied with a tractor-mounted granular applicator to small plots (0.25 to 1.0 ha) or with fixed-wing aircraft to large plots (40 to 350 ha) located in nongrazed pasture and on grass-sod military stage fields and airports. Circular subplots (0.1 to 0.2 ha) are established within each larger plot for pre- and post-treatment evaluation of the 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 is sometimes very difficult. Similar problems were experienced with some of the other materials evaluated for RIFA control, and a population index system was devised for evaluation of these materials. The population index system developed by Harlan et al. (1981) for work with American Cyanamid AC-217,300 (Amdro®) and modified by Lofgren and Williams (1982) for work with avermectin was also adopted for evaluation of IGRs. With this method the entire area within each subplot is searched carefully before treatment and at predetermined

intervals post-treatment, and each active nest found is opened with a spade and the contents carefully examined. Each nest is then assigned a rating of from 1 to 10 based on the estimated number of worker ants and the presence or absence of worker brood. This rating is then used to calculate a population index using the following scale:

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 population index for each subplot 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 index for all plots or treatments is obtained by summation of the indices for all subplots within the unit. Effectiveness of the treatment is determined by comparison of the pre- and post-treatment population indices.

RESULTS AND DISCUSSIONS

Laboratory Studies

Twenty-six IGRs in addition to those reported by Banks et al. (1978) and Banks et al. (1983) were evaluated in the laboratory from 1982 to 1984. Two chemicals (Table 1) were highly effective in suppressing worker brood production, Sumitomo S-4496 (propionaldehyde oxime O-2-(4-phenoxyphenoxy) ethyl ether) and S-4624 (propionaldehyde oxime O-2-(4-phenoxyphenoxy) propyl ether). The effects of S-4624 were slightly slower than those of S-4496; however, both reduced colony indices by greater than 95% within 8 weeks after treatment and all colonies had died by 48 weeks after treatment. The effects of these compounds on laboratory colonies were similar to those noted with other compounds we have found to be effective.

TABLE 1. Effects of Sumitomo S-4496 and S-4624 on laboratory colonies of red imported fire ants.

Chemical and dosage (AI)	Pretreatment colony index ^a	Percent reduction in colony index after indicated weeks					
		4	8	12	16	20	24
<u>Test 1</u>							
S-4496 10 mg	104.2±42.7	95.5±1.5	96.2±1.0	98.0±1.1	98.2±1.0	99.3±0.8	99.8±0.4
S-4624 10 mg	129.2±24.6	67.5±20.9	96.5±0.8	97.7±0.8	98.0±0.6	98.7±0.5	97.3±3.6
Check ^b	114.2±32.3	33.3±26.6	7.7±22.5	-6.5±50.3	14.7±61.4	28.0±54.0	32.0±60.6

^aSee text for method of calculating colony index; mean and standard deviation (n=6).

^bThe large standard deviation from weeks 12 to 24 was due to the death of the queen in two of the four colonies.

Figure 1 outlines the general effects observed in all of our tests with active IGRs. Although the two compounds involved in these studies were accepted very well by the ant colonies, some chemicals are repellent to the ants and are either totally rejected or consumed in ineffective quantities. Even if an IGR is readily accepted by the ants, it may produce no effect or only transient effects.

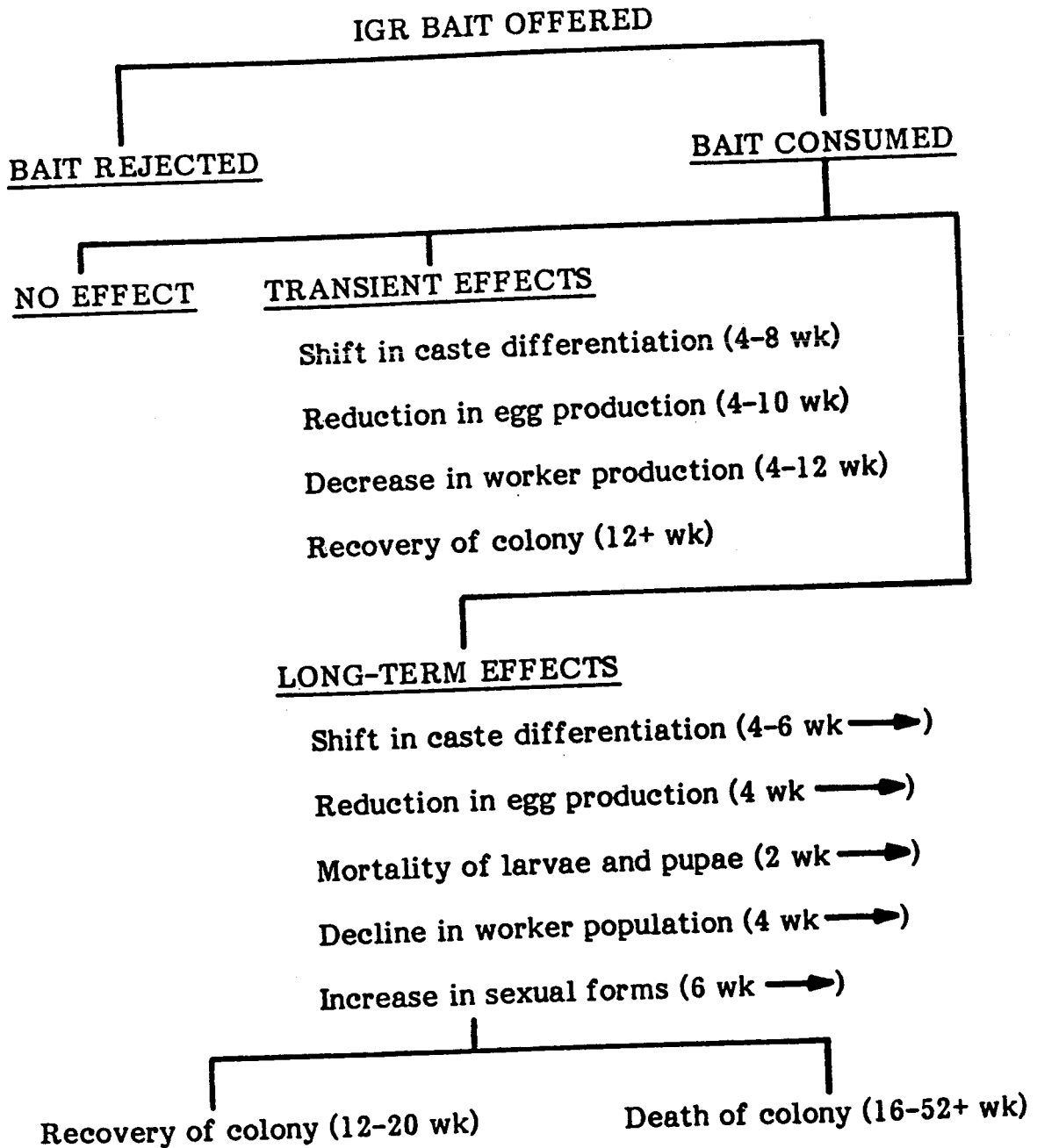


FIGURE 1. Effects of insect growth regulators administered to imported fire ant colonies in oil-based baits.

No detailed physiological studies have been done to determine the mode of action of any IGRs on RIFAs; however, the following changes have been consistently observed with all the active IGRs. As a general rule, they are not lethal to the queen or worker ants, although death of either or both may be accelerated because of general breakdown in maintenance and social organization of the colony. The most active IGRs are effective in (1) shifting caste differentiation from worker to sexual form, (2) causing deformities and death of many developing larvae, and (3) reducing or stopping egg production by the queen. The net result is that no new workers are produced and the colony declines in size and vigor as the existing worker force ages and dies. This decline may continue to the death of the entire colony or it may be reversed if the IGR is eliminated by metabolism and/or excretion before the colony declines below a critical point that has not yet been determined.

Dosage level does not appear to be a critical factor in the ability of a colony to overcome the effects of an IGR. We found that 1 of 2 colonies treated with 120 mg each of JH-25 (E-1-[(7-ethoxy-3,7-dimethyl-2-octenyl)oxy]-4-ethylbenzene) survived treatment even though 3 of 5 treated at 20 mg each died (Banks et al. 1978). Similarly, 2 of 4 colonies treated at 5 mg each with CIBA-GEIGY CGA-38531 survived although 2 of 2 treated at 1 mg each and 6 of 6 treated at 2 mg each died (Banks and Harlan 1982). We have postulated (Banks and Schwarz 1980; Banks and Harlan 1982) that colony makeup and quality and quantity of food available to the colony just prior to treatment may strongly influence long-term effects of an IGR on the ant colony.

Glancey et al. (1973) found that large workers in RIFA colonies serve as repletes, retaining oily solutions in the gastral crop for up to 18 months. We found that red dye used as a tracer in oil solutions of IGRs was retained by some workers for several months after treatment. Traces of dye appeared in sexual larvae 3 to 5 months after treatment. This supported the hypothesis that IGRs were retained for extended periods of time by some workers and slowly released into the colony food supply. Longevity of such stored material, theoretically, would be proportional to the number of repletes and their capacity for storage at the time of treatment. The long-term retention concept is further supported by the fact that queens removed from treated colonies at 12 weeks post-treatment resumed worker brood production when placed with untreated workers even though they had produced no workers since the fourth week post-treatment. Sister test colonies in which the queens remained with treated workers did not produce worker brood to the time of colony death (20 to 24 weeks post-treatment).

Retention of IGRs does not, however, appear to be as simplistic as we originally envisioned. As we noted in the introduction to this paper, Wendel and Vinson (1978) and Bigley and Vinson (1979)

showed, respectively, that Stauffer R-20458 and methoprene were very rapidly metabolized and excreted by RIFA colonies. Preliminary data suggests that MAAG RO 13-5223 may also be metabolized relatively quickly by RIFAs. The level of parent compound declined by about 45% and the level of metabolites rose 1 to 72 hours after the ants fed on oil bait containing the compound. In companion studies, we found that radioactivity in a colony dosed with ^{14}C labelled RO 13-5223 declined from an average 2220 dpm/ant on day 2 to background levels by day 56. Highly radioactive detritus removed from the rearing tray during the first week after treatment contained only metabolites of the IGR. Obviously further studies are needed to determine if the IGR is indeed stored in the RIFA colony and, if so, in what form, by whom, and for how long.

Field Studies

Field studies with S-4496 and S-4624 were conducted on 0.3-ha plots and with MAAG RO 13-5223 on aerially-treated 85-ha plots. In the small plot tests, Sumitomo S-4496 was more effective than S-4624, causing population reductions of 99.6 and 99.3% and eliminating 94.3 and 91.7% of active nests at 9.06 and 18.12 g AI/ha, respectively. S-4624 was slightly less effective, causing population reductions of 90.8 and 97.4% and eliminating 82.4 and 82.1% of active nests at 9.13 and 18.26 g AI/ha respectively (Table 2).

In the large area tests, MAAG RO 13-5223 formulated as a pregel defatted corn grit bait eliminated 94.0% of the active nests and reduced the population index by 99.5% after 12 weeks post-treatment (Table 3). The corncob grit formulation was less effective providing 89.1% elimination of active nests and 98.0% reduction in the population index.

The reduction in population indices has proven to be the best indicator of IGR effectiveness in field studies, since colony mortality is usually long-term. However, confounding reinfestation of small to medium plots sometimes occurs before kill is complete.

The effects of IGRs on RIFAs in both laboratory and field have been dramatic and show that these chemicals can be effectively used in RIFA population management programs. Available information indicates that IGRs are nonpersistent, which eliminates one of the environmental problems encountered with previous RIFA control methods. Development and registration of formulations containing IGRs for RIFA control have been relatively slow. The U.S. Environmental Protection Agency (EPA) granted registration in 1983 to a pregel defatted corn grit formulation containing Stauffer MV-678 (Prodrone). However, it has not gained wide acceptance because of its erratic performance. EPA registration of a pregel defatted corn grit bait containing MAAG RO 13-5223 (Logic®) is pending. Further studies with CIBA-GEIGY CGA-38531 and

TABLE 2. Effects of Sumitomo S-4496 and S-4624 on field populations of red imported fire ants.

Formulation	Application rate		Pretreatment		Percent reduction after 12 wks	
	Bait (kg/ha)	AI (g/ha)	Number active nests	Population index ^a	Number active nests	Population index
S-4496 1.0% Bait	0.905	9.05	53	1090	94.3	99.6
	1.81	18.12	48	1030	91.7	99.3
S-4624 1.0% Bait	0.913	9.13	51	1122	82.4	90.8
	1.83	18.26	56	1164	82.1	97.4
Untreated Check	—	—	47	980	14.9	21.9

^aPopulation index determined by modification of system of Lofgren and Williams (1982). See text for description of system.

TABLE 3. Effectiveness of the IGR MAAG RO 13-5223 against populations of red imported fire ants in large field plots. Brunswick, GA - 1983.

Application rate Bait kg/ha	AI g/ha	Number active nests		Population index		Mean percentage reduction	
		Pre- treatment	Post- treatment ^a	Pre- treatment	Post- treatment	Number nests	Population index
<u>Pregel defatted corn grit bait^b</u>							
1.12	11.2	265	16	6074	29	94.0	99.5
<u>Expanded corncob grit bait^b</u>							
1.12	11.2	274	30	5743	112	87.9	98.0
<u>Untreated Check</u>							
—	—	260	202	5665	4030	22.3	28.9

^a Post-treatment evaluations made at 12 weeks.

^b Formulations comprised of 70% pregel defatted corn grit, 29% once-refined soybean oil, 1.0% RO 13-5223 or 75% expanded corncob grits, 24% once-refined soybean oil, 1.0% RO 13-5223.

Montedison JH-286 are waiting decisions of the manufacturers on future development and registration of these materials. Studies are progressing with the Sumitomo compounds and they may eventually receive EPA registration.

Research is continuing to identify other IGRs that may be effective against the RIFAs and to develop improved formulations or application techniques for maximum field results.

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