THE EFFECT OF FENOXYCARB ON ALATES OF THE RED IMPORTED FIRE ANT

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ABSTRACT

Female alates of the red imported fire ant, *Solenopsis invicta* Buren did not develop ovaries when exposed to dosages as low as 0.125, 0.25, 0.5, or 1.0 mg per colony of the insect growth regulator, fenoxycarb [ethyl 2-(4-phenoxyphenoxy) ethyl] carbamate. Development of the bursa, spermatheca, and oviducts was normal. Alate females from treated colonies were about three-fourths to one-half the size of those from control colonies, but were capable of histolyzing their wing muscles and forming an esophageal crop. A large number of intercastes were produced at 8-12 weeks after treatment. Males were not affected by the fenoxycarb treatment.

Key Words: Red imported fire ant, *Solenopsis invicta*, virgin queens, male fire ants, fenoxycarb, insect growth regulator, ovaries.

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INTRODUCTION

The granular bait Logic®, containing the active ingredient fenoxycarb (ethyl [2-(4-phenoxyphenoxy) ethyl] carbamate) (Maag Agrochemicals RO 13-5223) is presently used to control the red and black imported fire ants, *Solenopsis invicta* Buren (RIFA) and *S. richteri* Forel (BIFA). Fenoxycarb mimics the effects of juvenile hormone and ingestion of the compound causes complete disruption of RIFA colonies through (1) low level toxicity to the brood, (2) the reduction or cessation of egg production by the colony queen, and (3) a shift in caste differentiation so that any eggs laid produce only sexual forms (Banks et al. 1988). Recently, Glancey and Banks (1988) showed that fenoxycarb has a direct effect upon the reproductive system of the colony queen, causing retrogression of the ovaries of mated queens by completely suppressing growth of the follicular epithelium, nurse cells, and cytoplasm. In addition, most developing eggs are resorbed; no worker ants and only a few sexuals are produced in treated colonies. The queen's reproductive system degenerates, by eight weeks after treatment, to only thin strands of tissues that produce oögonia, but do not support their growth and development.

Since one of the primary effects of the chemical on the colony is the shift in caste from workers to sexuals, an initial reaction from people engaged in control of the ants was that production of large numbers of virgin queens might be counter-productive. Consequently we conducted the study reported herein to assess the effects of fenoxycarb on these virgin sexuals.

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MATERIALS AND METHODS

Fifteen laboratory-reared colonies of red imported fire ants, each consisting of 20-30 ml of immatures (brood), 40,000-60,000 worker ants, and a queen, were selected for the tests. Three colonies each were offered fenoxycarb at dosages of 0.125, 0.25, 0.5, or 1.0 mg per colony in 0.5 ml of once-refined soybean oil (ORSBO). Three control colonies were given an equal volume of neat ORSBO. The oil solutions were offered in micropipets from which the ants drank and after ingestion of the oil solution was complete, usually within 24 h, the ants were returned to the normal laboratory diet (Banks et al. 1981). The colonies were maintained in the laboratory at $27 \pm 2°C$.

All alates were removed weekly from each of the treated and control colonies beginning two weeks after treatment. Since the shift in caste differentiation due to IGR ingestion occurs in the first larval instar, later instar worker larvae or pupae are not affected and pupate and eclose normally for up to 4-5 weeks after treatment (Banks et al. 1978). Thus, to insure that the adults to be examined had received fenoxycarb in the early larval instars all sexuals that eclosed before day 35 were discarded.

Sample male and female alates were randomly chosen each week from those removed from the colonies during weeks 6-12 after treatment and examined for effects on the reproductive system. Each week the ovaries of 3-5 virgin queens from each of the treated and control colonies were removed, examined, and fixed in Kahle's fixative (Whiting 1950). The fixed ovaries were embedded in paraffin (mp:57°C), sectioned at 5-um, and stained with Harris's hematoxylin and eosin. The stained sections were examined under phase microscopy and photographed. Each week the sperm of 3-4 males from each of the treated and control colonies was examined for motility. The males were then dissected in Ringer's solution and their testes examined for any abnormalities.

Four to six third and fourth instar sexual larvae, as described by Petralia and Vinson (1979), and pupae were separated each week from treated and control colonies and prepared as described above to study the effect of fenoxycarb on the immature stages.

RESULTS

The ultimate effects of fenoxycarb on the reproductive system of the fire ant female are shown in Fig. 1. Line drawings were used because the photographs did not adequately illustrate the effects due to the high degree of transparency of the tissues. Comparative descriptions are given of the reproductive system in larvae, pupae, and adults from control and fenoxycarb-treated colonies. The only published description of the reproductive system in immatures of RIFA is for 4th-instar larvae (Petralia and Vinson 1980), thus descriptions for untreated 3rd and pupae in this work are based on previously unpublished studies of several hundred specimens by the senior author. Descriptions of the reproductive system in adult females are based on Hermann and Blum (1965).

The observed effects of fenoxycarb were independent of the dosage, thus the following descriptions of effects of the 0.125 mg/colony dosage are applicable for all treatments.

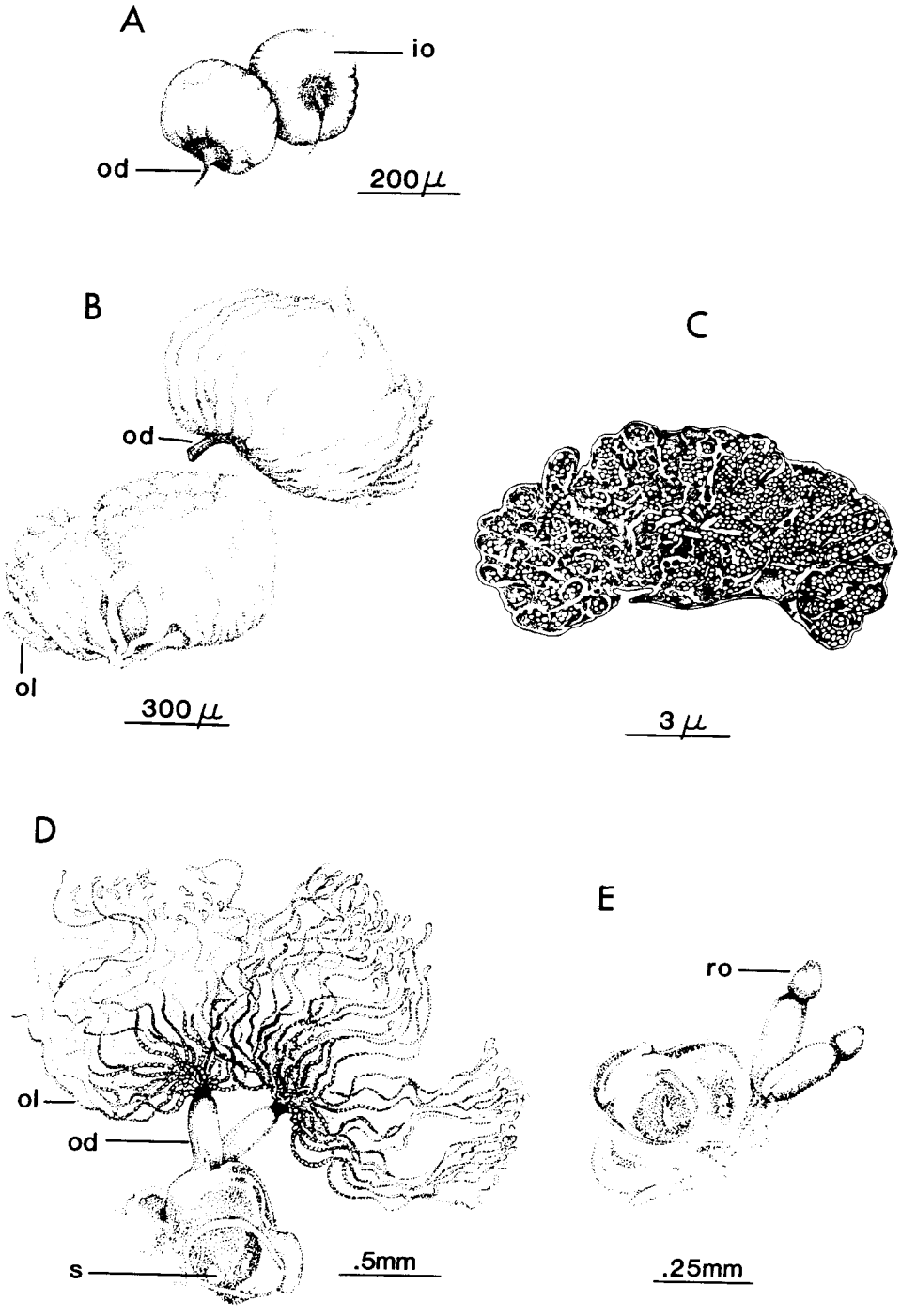

Fig. 1. A. Immature ovaries of 3rd instar female reproductive fire ant larva. B. ovaries from a normal 4th instar reproductive fire ant larva. C. x-section of ovary from 4th instar reproductive fire ant larva treated with fenoxycarb. D. Reproductive system of a normal newly-eclosed female alate fire ant. E. Reproductive system of a newly-eclosed female alate fire ant treated with fenoxycarb while in the larval stage. (io = immature ovary; od = oviduct; s = spermatheca; ro = remnant of ovary).

Normal Female Larvae

Normal 3rd-instar reproductive larvae possess bowl-shaped ovaries located dorsoposteriorly (Fig. 1, A). The ovaries are visible without magnification and can be easily dissected. In the 4th-instar larvae, the individual ovarioles are separate (Fig. 1, B). Microscopic examination of cross-sections of these organs show that tissue differentiation is becoming well-defined with limited production of nurse cells and follicular epithelium.

Treated Female Larvae

The ovaries of fenoxycarb-treated 3rd-instar larvae could not be discerned and those of 4th-instar larvae only with considerable magnification. Microscopic examination of sections of 4th-instar larvae showed that the ovaries were present, but were extremely small. The ovaries (Fig. 1, C) were still encapsulated in a sheath and the number and size of ovarioles was reduced. There was no separation of individual ovarioles and no nurse cells or follicular epithelium could be observed.

Normal Female Pupae

The complete reproductive system, i.e. the ovaries, oviducts, spermatheca, and bursa, is displayed by the untreated female pupa. The system is quite large, visible without magnification, and easily dissected. Serial sections of the pupal ovaries show that tissue differentiation is becoming well-defined with the production of öogonia, follicular epithelium, nurse cells, and cytoplasm.

Treated Female Pupae

The bursa, spermatheca, and two oviducts are present in the treated pupae, but no ovaries are evident. The only ovarian development is an enlargement of the apical end of the oviducts that is shown by serial sections to consist only of a small patch of undifferentiated tissue.

Normal Virgin Female Alate Upon Eclosion

The reproductive system of a virgin alate upon eclosion is nearly complete (Fig. 1, D) and is functional. Each ovariole contains well-defined germarial and vitellogenic tissues, and trophic eggs at various stages of development, including yolk and a chorion, are present. Development and size of the oviducts, spermetheca, and bursa are normal.

Treated Virgin Female Alates Upon Eclosion

The oviducts, spermatheca, and bursa are normal, but the only evidence of ovarial development is the enlargement of the apical end of the oviducts (Fig. 1, E). Serial sections show that this consists of the same type of undifferentiated tissue observed in the pupae. Functional ovarioles are absent.

All of the female alates that we examined (n = 70) from the treated colonies during weeks 6-12 were devoid of ovaries. In a companion study (Obin et al. 1988) some ovarian development was noted in ca. 53% of the females examined (n = 17) from colonies treated with 0.125 or 0.25 mg, however, these females were all more than 12 weeks posttreatment. Whether or not these females would be capable of mating and founding colonies is unknown. By contrast, the ovaries were well-developed in all female alates from control colonies.

Males

Fenoxycarb did not produce any detectable effect on the testes or the motility of the sperm of RIFA males at any dosage. The size and shape and the genitalia of all adult males was normal.

Other Effects

Fenoxycarb produced other physical effects on the ants in addition to the direct effect on the ovarian tissues. The mean weight of alates that eclosed in the treated colonies during the first 5-6 weeks of the experiment was comparable to those from the controls and from untreated monogynous field colonies (i.e. 12 mg, n = 56) whereas the size and weight of those eclosing later was reduced. The mean weight of alates that eclosed during weeks 6 and 12 after treatment was 7 (n = 16) and 4 (n = 12) mg respectively. The comparative size of a normal and a 4-mg alate is shown in Fig. 2.

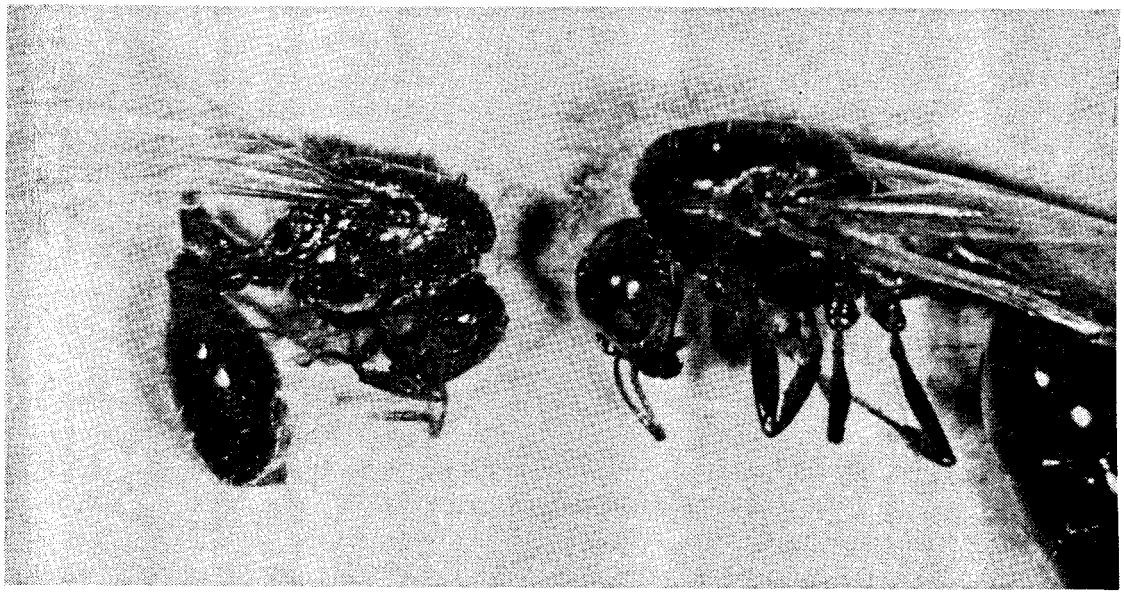

Fig. 2. Normal alate (NA) and 4 mg alate from fenoxycarb treated colony (FA).

Examination of the thorax of a number of the small alates showed that all possessed alary muscles and that some of them histolyzed those muscles when they were isolated individually in vials for two weeks. Behavioral tests to determine if the small alates produced pheromones are reported elsewhere (Obin et al. 1988).

Another observed effect of fenoxycarb treatment was the production of intercastes. Although apparently normal colonies will occasionally produce a few intercastes (Glancey 1980; Hung et al. 1975), there are never as many as were found in the treated colonies. We have observed that intercastes are also produced in colonies treated with other IGR (Banks and Schwarz 1980), however, they do

not usually appear until 8-12 weeks after treatment. We hypothesize that intercastes occur in IGR-treated colonies because some individuals receive less than the minimal dosage necessary to induce a full shift in caste differentiation as the titre of chemical in the colony is reduced through trophallaxis, metabolism, and excretion. Those individuals that became intercastes in the fenoxycarb-treated colonies exhibited morphological characteristics that ranged from worker-like to queen-like, apparently dependent upon the amount ingested. The intercastes exhibited various stages of differentiation; ocelli ranged from 0 to 2 or 3, wings ranged from only buds to complete but smaller than normal wings. A spermatheca was sometimes present, but developed ovaries were not observed in any intercaste.

DISCUSSION

Although the initial studies on fire ants showed that IGR interfered with the maturation of developing larvae, prevented pupation, caused deformities, and resulted in some worker mortality, hormonal growth regulators were not considered suitable for control of RIFA (Cupp and O'Neal 1973; Troisi and Riddiford 1974). The effects of IGR on the internal morphology of alate reproductives produced in treated colonies (Robeau and Vinson 1976; Banks et al. 1978) were not investigated.

The potential of IGR, such as fenoxycarb, to control and manage populations of RIFA has been demonstrated (Banks et al 1983). Although IGR are generally not lethal to the worker ants or to the queen, colony death occurs because workers that die of natural causes are not replaced due to ovarian regression and atrophy (Glancey and Banks 1988) that reduces or prevents egg-laying, and to the shift in caste differentiation. Fenoxycarb produces excellent control of established infestations by these modes (Banks et al. 1988). The ability of fenoxycarb to prevent ovarian development in most developing females lends an added dimension as a control agent not found in other IGR or chemicals that do not produce this effect. New colonies of RIFA are founded by newly-mated queens descending from the nuptial flight. We know from alate trap studies (Banks et al. - unpublished data) that although the numbers are reduced by about 85% as compared to untreated colonies, females from fenoxycarb-treated colonies do participate in mating flights, however, whether they actually mate is unknown. Even if they do mate the lack of ovaries in a large percentage of these females would, greatly reduce, if not eliminate, colony-founding.

The defeminizing effect of fenoxycarb has not been observed in other insects although other research indicates that gonads that are produced in the larval stage are susceptible to the effects of JH or JH-mimics. Masner (1969) found that topical application of dichloride of methyl farnesoate to larvae of the bug *Pyrrhocoris apterus* (L.) caused limited differentiation of the ovarian follicular epithelium and prevented formation of active secretory tissue. The effect carried over into the adult stages. No effect was seen on the testes. Vogel et al. (1976) showed that larvae of the citrus mealy bug *Planococcus citri* Risso respond differentially to the IGR epofenonane depending on the instar and the dosage. Few activated oocytes were produced with high dosages but many, some of which differentiated and became yolked, were produced with low dosages. We found that no differentiation occurred in fire ant larvae fed fenoxycarb. Fenoxycarb will control the pharaoh's ant, *Monomorium pharaonis* (L.), but does not cause intercaste formation and defeminization of alates. The effects upon the pharaoh ant queen

are almost identical to those reported by Glancey and Banks (1988) on the RIFA queen (personal communication - J. P. Edwards, Pest Infestation Control Laboratory, Ministry of Agriculture, Fisheries and Food, Slough, England).

The reason for the production of very small female alates in our study was unclear. They may have occurred because of the reduced number of workers to provide food and brood care or they may have been due to the ovarian regression in the fenoxycarb-treated queen (Glancey and Banks 1988) that resulted in eggs with less yolk to support initial larval development.

Obin et al. (1988) demonstrated that fenoxycarb also affects ability of both inseminated queens and virgin female alates to produce the queen recognition and dealation inhibition pheromones. Worker response to the queen and virgin alates from fenoxycarb-treated colonies was similar and indicated that the queen recognition pheromone was present, but reduced in amount. Tests with virgin females showed that the fenoxycarb-treated queen or virgin alates produced significantly less dealation inhibitor pheromone than untreated. Their results indicate that production of the queen recognition pheromone is independent of ovarian development, but the production and distribution of the inhibitory pheromone is dependent on functional ovaries. The lack of effect of fenoxycarb on males appears consistent with published data.

Field colonies of RIFA exhibit the same effects of fenoxycarb treatment as laboratory colonies (Banks et al. - unpublished data). Effects in field colonies are usually more pronounced, possibly due to the higher dosages available to field colonies. Logic bait is applied at the rate of 1.12 kg/ha (11.2 g fenoxycarb), thus even with a heavy infestation of 250 nests per ha there would be ca. 45 mg fenoxycarb available for each nest. The caste shift occurs in treated field colonies and small virgin alates are produced that, as indicated previously, participate in mating flights. All virgin females examined from fenoxycarb-treated field colonies were devoid of ovaries, whereas the males were normal and produced motile sperm. Most treated colonies in the field died within 12-13 wk.

Some important questions, such as the ability of the small virgin alates from treated colonies to mate and found colonies, the effects of subminimal defeminizing doses on the ability of alates to found colonies, and the effects on developing brood of any residual fenoxycarb retained by a newly-mated queen from a treated colony, remain unanswered. These are all of considerable importance in assessing the total effect of fenoxycarb on RIFA colonies.

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