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### Effect of the Insect Growth Regulator Fenoxycarb on the Ovaries of Queens of the Red Imported Fire Ant (Hymenoptera: Formicidae)<sup>1</sup>

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**ABSTRACT** The insect growth regulator, fenoxycarb, ethyl [2-(4-phenoxyphenoxy)ethyl] carbamate, causes retrogression of the ovaries of mated queens of the red imported fire ant, *Solenopsis invicta* Buren. Fenoxycarb completely suppresses growth of the follicular epithelium, nurse cells, and cytoplasm/yolkplasm. Most developing eggs are resorbed and no new workers are produced. Thus, prolonged exposure of the queen to the compound causes death of the entire colony. The ovaries return to normal if the queen is removed from exposure to the chemical.

KEY WORDS Insecta, Solenopsis invicta, fenoxycarb, ovaries

IN LATE 1985 the U.S. Environmental Protection Agency granted registration to Logic, a new bait for control of the red and black imported fire ants, Solenopsis invicta Buren and Solenopsis richteri Forel. The active ingredient in Logic is fenoxycarb, ethyl [2-(4-phenoxyphenoxy)ethyl] carbamate (RO 13-5223, Maag Agrochemicals, Vero Beach, Fla.), which is one of a group of carbamates that mimics the effects of juvenile hormone (JH) on a variety of insects (Dorn et al. 1980, Masner et al. 1981), including the red imported fire ant (Banks et al. 1983, 1988). Three effects of the compound have been observed on fire ants: direct toxicity to larvae and pupae, a reduction or cessation of egg production by the colony queen, and a shift in caste differentiation from worker to sexual forms (Banks et al. 1988). The exact mode of action of fenoxycarb on fire ants is unknown.

In this study we investigated if suppression of egg production results from direct effects of fenoxycarb on the reproductive system of the queen. We also investigated the ability of queens to recover from the effects of fenoxycarb.

#### **Materials and Methods**

Twenty-seven queenright laboratory colonies of red imported fire ants, each consisting of 20–30 ml of immatures, 40,000–60,000 worker ants, and a colony queen were fed 0.5 ml of once-refined soybean oil containing 2.0% fenoxycarb (10 mg [AI] per colony). An equal number of colonies of similar size was given an equal volume of once-refined soybean oil as controls. The oil solution was administered in micropipets from which the ants drank. Ingestion of the oil solution was complete within 24 h, after which the ants were returned to the normal laboratory diet (Banks et al. 1981). The colonies were maintained in the laboratory at  $27 \pm 2^{\circ}$ C.

At weekly intervals for 8 wk, the ovaries of the queens from each of three treated and three untreated colonies were removed, examined, and fixed in Kahle's fixative (Whiting 1950). The fixed ovaries were embedded in paraffin (melting point 57°C), sectioned at 5  $\mu$ m, and stained with Harris's hematoxylin and eosin. The stained sections were examined under phase microscopy and photographed.

The ability of queens to recover from the effects of fenoxycarb was studied by dividing each of 21 queenright colonies into two approximately equal parts. The portion of each colony that contained the queen was then treated with fenoxycarb. Six weeks after exposure to the fenoxycarb (2% solution), each queen was transferred from the treated to the untreated portion of her respective colony. Thereafter, at weekly intervals for 7 wk, three queens were chosen randomly and isolated for 48 h to determine their egg-laying capacity. Their ovaries were then removed, examined, and prepared as described above.

#### Results

**Control Queens.** The ovarioles of queens of the red imported fire ant, described by Hermann & Blum (1965), are meroistic (=polytrophic); each follicle contains one oögonium and several nurse cells. The nurse cells and oöcyte are generally derived from one oögonium. The ovarioles (60+) are tapered proximally and all are attached by the terminal filament to the body wall.

The germarium (Fig. 1) contains the oögonia, which differentiate into the primary oöcytes and

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<sup>&</sup>lt;sup>1</sup> This article represents the results of research only. Mention of a proprietary product does not constitute an endorsement or recommendation for its use by USDA.

nurse cells. These areas of the ovarioles are packed with heavily stained nuclei. Nurse cells are attached to the apical end of the older developing oöcytes, and the entire mass is surrounded by the follicular epithelium.

As the oöcytes move down the ovarioles (Fig. 2), nurse cells continually deposit cytoplasm within them and they become larger and larger as they move distally (Fig. 3). Spheres of yolk are added to the cytoplasm as the eggs enter the lower vitellogenic zone. Having transferred their cytoplasm to the oöcytes, the nurse cells begin to degenerate, eventually disappearing completely (Fig. 4).

In the lower vitellogenic zone where the chorion is laid down, up to 10 very large heavily yolked eggs may occur. Another 10–20 smaller eggs with some yolk spheres occur above the yolked eggs. Each ovariole may contain 70–80 eggs in various stages of development.

Under gross examination, the ovaries appear as a group of pearls, each ovariole containing eggs of various sizes. The ovaries are quite large and fill the entire abdominal cavity.

**Treated Queens.** The ovaries of queens exposed to fenoxycarb for only 1 wk do not show any gross or microscopic signs of pathology. The nurse cells, zones of differentiation with cytoplasm, and lower vitellogenic zones with yolk are quite obvious. Many eggs are ready for deposition.

At 2 wk after treatment, the proximal ends of the ovarioles lack a zone of differentiation. Very little cytoplasm surrounds the oöcyte, and only a small amount of cytoplasm occurs within the nurse cells. Few yolked eggs are ready for deposition and few oöcytes are moving down the ovariole. Although some eggs are in the ovarioles, the eggs are surrounded by few or no nurse cells. Some portions of the ovarioles appear vacuolated and lack developing eggs. These conditions are not apparent in all ovarioles at the same time or in all of the examined ovaries. Damage is evident in some ovarioles, whereas others appear quite normal.

Three weeks after treatment, individual ovarioles contain fewer eggs, and the proximal ends look like thin strands of tissue. The ovaries are much reduced in size and do not fill the abdominal cavity. Microscopically, about five eggs in each ovariole contain yolk, but at a much reduced level. In the zone of differentiation, the sequence of nurse cells, oöcyte, nurse cells, oöcyte, etc., is ill-defined with no definite zones. Only a series of egg nuclei without cytoplasm is present, without the accompanving nurse cells.

By the fourth week, only 1 or 2 extremely small eggs with yolk are present in each of the ovarioles. The zone of differentiation is nearly absent, only egg nuclei are present, and the follicular epithelial cells lack cytoplasm.

The number of eggs with yolk in the ovarioles continues to decline from weeks 5 through 8 until only one or two small eggs remain in the entire ovary. The shrinkage of the ovarioles reduces the overall size of the ovaries so that both ovaries occupy just one abdominal segment. Some ovarioles have been reduced to hollow tubes, while others are merely strands of tissue. Some ovarioles show the chorion present, but no eggs. Examples of the effect at 8 wk are seen in Fig. 5–11.

**Recovery.** In newly mated fire ant queens, the entire abdomen is lined with fat body that penetrates the primordial ovaries. The newly mated queen contains only a few eggs, and further oögenesis is associated with the histolysis of the fat body. Consequently, the size of the body is reduced in mature queens. The recovery of the ovaries from treatment with fenoxycarb begins within 2 wk, during which time the queens reconstitute the fat body lining the posterior abdomen. Three or four ovarioles each contain a small egg with yolk, and a few nurse cells occur adjacent to some of the oöcytes. Although the ovaries are still small (Fig. 12), they have begun to grow and occupy about half the volume of the abdomen.

The effects of the compound are still evident microscopically at 2 wk. Most ovarioles are still vacuolated, the follicular epithelium is lacking, and a general lack of organization is apparent. The queens from the 2-wk recovery period deposited no eggs in 48 h.

Recovery is well underway by 3 wk. The ovaries occupy about three-fourths of the abdomen and the fat body is reduced, indicating that it is used for yolk production. Each ovariole has one to three normal eggs with yolks and five to six developing eggs. Microscopically, the ovarioles resemble the untreated condition, with good organization of tissue and nurse cells and follicular epithelium. Although several eggs of normal size may be present, together with some developing eggs, the entire ovariole is still not fully recovered. About 10% of the ovarioles show the effect of the compound. The treated queens laid an average of 120 eggs during a 24-h period.

After 4 wk of recovery, the queens have returned to a nearly normal condition. The ovaries fill the abdominal cavity and each queen has two to three mature eggs of normal size ready for deposition. The fat body has become reduced as in a maturecolony queen. Microscopically, everything appears normal, and oögenesis extends to the tip of each ovariole. As many as 23 developing eggs can be seen and another 20–25 young oöcytes surrounded by nurse cells and follicular epithelium can be counted. No pathological condition can be detected at this time. Three of the queens deposited an average of 302 eggs per day before they were dissected.

The same level of recovery is apparent in queens at 5, 6, and 7 wk as at 4 wk.

#### Discussion

Death of fire ant colonies treated with fenoxycarb can be attributed to a number of interrelated



**Fig. 1–4.** Sections through some ovarioles of an untreated red imported fire ant queen. Germarium (1) with oögonia (og), nurse cells (nc) and developing oöcyte (ocy); region of differentiation (2) with nucleus of oöcyte (en) surrounded by cytoplasm (cy); and lower end of vitellogenic zone (3–4) showing addition of yolk (yo) to eggs.



Fig. 5–8. Sections through some ovarioles of queens 8 wk after treatment with fenoxycarb showing loss of activity in the germarium (5), lack of development of nurse cells and follicular epithelium (6); and lower vitellogenic zone (7–8) showing the high degree of vacuolation (vac) and production of very small eggs (e).



**Fig. 9-12.** Section of lower end of ovariole of fenoxycarb treated fire ant queen (9-11) showing reduced size of eggs (9) and resorbtion of eggs (10) with remaining chorion. Reduction of the ovary can be so great as to be almost total (11). Recovery from the fenoxycarb treatment can be seen in (12) with large ovaries (bottom) from an untreated queen compared with the upper smaller ovaries from a queen 2 wk after recovery from the fenoxycarb treatment.

causes. Of primary importance is the lack of worker brood production; this lack results eventually in a greatly reduced worker population. Concomitant with this is a lower rate of food gathering, queen tending, and colony maintenance. This syndrome can lead to the death of the queen and eventually to the death of the colony itself. Fenoxycarb successfully controls the fire ant probably because the replete workers store it in the crop and the postpharyngeal glands and thus serve as a reservoir for the constant introduction of the material into the colony (Banks et al. 1983). As long as a certain titer of the material remains in the colony, the queen is subjected to its effects.

Fenoxycarb is not the only material which has an effect on the reproductive tissues of the fire ant queen. Glancey et al. (1982) found that avermectin  $B_t$  caused irreversible cell and tissue damage to the ovaries of fire ant queens. The damage includes hypertrophy of the squamous epithelium and pycnosis of the nurse cell nuclei. No reduction in the size of the ovaries or the elimination of the nurse cells was observed. Queens treated with this compound were rendered completely sterile.

The morphological effects of fenoxycarb on the reproductive system of the fire ant queen as shown in this study are well defined. Instead of a normal area of differentiation of cells in the ovarioles into oöcytes, trophocytes, and follicular epithelial cells, differentiation is completely lacking. The nurse cells fail to develop, so that the oöcytes are not nourished with cytoplasm and later the necessary yolk protein globules are not added.

The effects produced by fenoxycarb are very similar to those reported for methoprene (a JH analog) on the ovaries of the Pharaoh ant, Monomorium pharaonis (L.). Gross examination of the ovaries showed ovarioles reduced in size and containing no developing oöcytes or trophocytes (Edwards 1975). No microscopic examination of the ovarioles was made during the experiment (30 wk) so the pathology of the two compounds cannot be compared. The effects of fenoxycarb on the ant queens are similar also to those produced by two other JH analogs, dichloride of methyl farnesoate and epofenonane, on the morphogenesis and function of the ovaries in a bug Pyrrhocoris apterus (L.), and a mealy bug Planococcus citri Risso, respectively (Masner 1969, Vogel et al. 1976).

Our limited data allow few firm conclusions to be drawn as to the mode of action of fenoxycarb upon the ovaries of the queen. However, two mechanisms seem to offer some explanation of the chemical effects. The first is a direct effect upon the ovaries. The lack of follicular epithelium, nurse cells, and cytoplasm-yolk spheres indicates that a process of differentiation was impaired. This lack of effect of the compound upon the germ tissue and the temporary inhibition of the nutritive cells seem to point to an effect caused by JH overloading. Such a morphogenetic action of JH overloading was demonstrated by Rohdendorf & Sehnal (1972). The administration of JH isolated from *Hyalophora cecropia* (L.) to the firebrat adult female (*Thermobia domestica* (Packard)) led to severe disorders in the development and function of the ovaries. The treatment resulted in derangements of oöcytes and follicular cells, with reduction in the germarium, lack of oöcytes and well-formed egg chambers, and failure of the ovarioles to initiate yolk deposition. In some cases, the ovariole was reduced to a very thin strand of tissue. In the fire ant queen, this condition can be reversed by the removal of the queen from the JH overload. This is well demonstrated by the reappearance of the fat body and the recovery of the egg-laying ability of the queen.

A second mechanism that might account for the pathological condition is interference with the normal output of hormones by the corpora allata. Because control of differentiation is assumed to be in the corpora allata (CA) (Wigglesworth 1973, 653–656), it could be inferred that one of the modes of action might be the reduction in the output of hormones by the CA. The effect upon the nurse cells also seems to point to the CA, the neurosecretory cells, or both (Engelmann 1970). Additionally, the control of yolk synthesis is indirectly under the control of the CA (Englemann 1970), and therefore the effect of fenoxycarb might be upon the hormonal release program.

The time sequence of the effects as well as the course of recovery indicate strongly that the most important effect of fenoxycarb on the ovaries is the inhibition of differentiation, primarily within the germarium.

In social insects pheromones are important in governing the behavior of the colony and its individual members. The production and dissemination of certain pheromones may be closely related to the development and function of the queen's ovaries, so that the effect of compounds such as fenoxycarb may have more serious implications than researchers thought. For example, with the reduction in size of the ovaries, does it follow that there will be a reduction in the ability of the queen to produce the queen recognition pheromone that governs worker behavior toward her? If the queen is affected, how are the young virgin queen alates affected? Are males affected in any way? Do the workers experience any behavior changes as a result of the presence of the compound in the colony? It is clear that fenoxycarb offers not only a viable control measure for the imported fire ant, but also offers a chance to investigate the relationships between the reproductive system of the queen and the pheromonal control of the colony.

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