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# Applied Myrmecology

## A World Perspective

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Westview Press
BOULDER, SAN FRANCISCO, & OXFORD

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# Effects of IGR Fenoxycarb and Sumitomo S-31183 on the Queens of Two Myrmicine Ant Species

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## INTRODUCTION

After the U.S. Environmental Protection Agency (EPA) banned mirex and other chlorinated hydrocarbons, an intensive search began for other materials to control pest ants. Insect growth regulators (IGR) are generally non toxic to adult worker ants but provided effective control of certain problem species because they stopped queen egg production. Banks et al. (1983, 1988) showed that the IGR fenoxycarb (Maag Agro-chemicals, Vero Beach, Fl) was effective against *Solenopsis invicta* Buren (see Banks this volume). In recent years, Amdro has become the most popular toxic bait for control of fire ants. However, polygyne *S. invicta* (multiple nest queens), which is becoming more prevalent, is more difficult to control with Amdro than monogyne *S. invicta* (one nest queen) (Glancey et al. 1987). Recent field studies show that fenoxycarb effectively controls the polygyne form (Banks et al. unpublished). It kills *S. invicta* larvae and pupae, and - most importantly - inhibits queen egg production and shifts caste differentiation from worker to sexual forms (Banks et al. 1988). Histological studies of the queen's reproductive system reveal that fenoxycarb causes regression of the ovaries while completely suppressing the growth of the nurse cells, cytoplasm and follicular epithelium; developing eggs were reabsorbed (Glancey and Banks 1988).

Fenoxycarb's effectiveness against polygyne *S. invicta* colonies led us to test it and Sumitomo S-31183 (Sumitomo America Inc., New York, NY), a compound with similar IGR action, against another polygyne species, the big-headed ant, *Pheidole megacephala* F. This latter ant is a well known pest of domestic and institutional premises and may be a care-taker of mealybugs that transmit wilt in Hawaiian and Costa Rican pineapples. This mutualism enhances mealybug survival and creates dense mealybug populations. High mealybug populations promote the spread of wilt through the fields. Without *P. megacephala*, mealybug populations do not become established and wilt is not a problem. In Hawaii, *P. megacephala* also tend the green scale (*Coccus viridis* [Green]) in coffee and aphids in taro, chew on drip irrigation tubing in sugarcane (see Chang and Ota this Section) and cause quarantine and nuisance problems in Protea (a cut flower crop) where it occurs in flower heads (Rohrbach et al. 1988). The IGR methoprene effectively controlled *P.*

*megacephala* in hospitals (Edwards et al. 1981) and, although its effect on the *P. megacephala* queen or workers was not determined, methoprene may have impaired the queen's reproductive capacity.

Although distantly related, *S. invicta* and *P. megacephala* belong to the same subfamily (Myrmecine); therefore, treatment with fenoxycarb or Sumitomo S-31183 might produce similar pathological effects in both species. Herein we report the effects of the two IGRs on *S. invicta* and *P. megacephala* queens.

## METHODS AND RESULTS

### Fenoxycarb Effects

*Solenopsis*. The effects of fenoxycarb on *S.invicta colony queens* have been previously reported (Glancey and Banks 1988) and that research is summarized in this paper's introduction. Earlier studies also showed that after ingesting the compound queens and virgin dealates were unable to inhibit dealation in virgin queens (Obin et al. 1988). However, neither the queen nor the virgin dealates lost the ability to induce queen recognition.

In the present study, fenoxycarb also affected the maternal instinct of virgin queens. Ten days after eclosion, groups of untreated and fenoxycarb-treated virgin dealate queens histolyzed their alary muscles, produced an esophageal crop and began to produce the queen recognition pheromone. Thirty queens from each group were isolated in one ounce plastic cups with castone bottoms and given eggs produced by the queen from a laboratory colony. The untreated virgin queens tended the eggs immediately, whereas treated queens either ignored or ate the eggs. This behavior pattern remained consistent throughout a week of observation. In contrast, untreated queens exhibited typical egg-tending behavior and, by the end of the week, microlarvae were evident in the small colonies. *S. invicta* colonies treated with fenoxycarb produced virgin females that lacked ovaries (Glancey et al. 1989). The failure of the treated virgin queens to tend eggs suggests that maternal instinct (i.e., egg-tending) is mediated by a material produced by the ovaries, or the corpora allata with a release related to ovary development, and is thus absent in queens without ovaries.

*Pheidole*. Thirty-six *P. megacephala* colonies in hollow pineapple stumps were collected from 0.2 ha of a ratoon pineapple field on the island of Oahu, Hawaii. The ants, taken to a laboratory at a University of Hawaii at Manoa laboratory, were placed in plastic boxes 26.5 X 33 X 9 cm. To remove the ants from the stumps, they were offered a small black vial which contained moist tissue paper. The negatively phototrophic queens quickly moved into the darkened vials; these were then transferred to 15 X 29 X 9 cm plastic shoe boxes along with a number of workers. Each colony was provided with water, a modified Banks diet (Banks et al. 1981) and a modified Bishop nest (Bishop et al. 1980). Reduced to a single queen and comparable numbers of brood, workers and soldiers, the colony was allowed to acclimatize in the laboratory. Afterward, the colonies were counted; each consisted of one queen, 20-50 eggs, 1-2 ml brood, 200-400 workers and 20-40 soldiers.

Following four days of access only to water, 12 colonies were fed 0.5


FIGURE 1. Section through the ovary of a normal *P. megacephala*. The germarium is filled with dark stained packed öogonia (og); an egg nucleus (en) is shown surrounded by the follicular epithelium (fe) with supporting nurse cells (nc), all within the zone of differentiation. A mature egg with yolkplasm (yo) is pictured along with the lateral oviduct (lov). A sperm bundle (sb) is in the spermatheca. Bar= 70u.

ml of 2.0% fenoxycarb in soybean oil and 12 colonies were fed neat soybean oil as controls. Any soybean oil not eaten was removed after 24 hours and the colonies returned to their normal diet. Every two weeks, the number of eggs, workers and soldiers and the volume of brood were measured. Ovaries of queens, removed from each of three treated and control colonies at weeks five, six, seven and eight, were fixed in Kahle's solution for 24 hours and subsequently held in 70% ethanol for embedding.

Fixed ovaries were embedded in paraffin (m.p. 57°C), sectioned at 5 um and stained with Harris's hematoxylin and eosin solution. Stained sections were examined under phase microscopy and photographed.

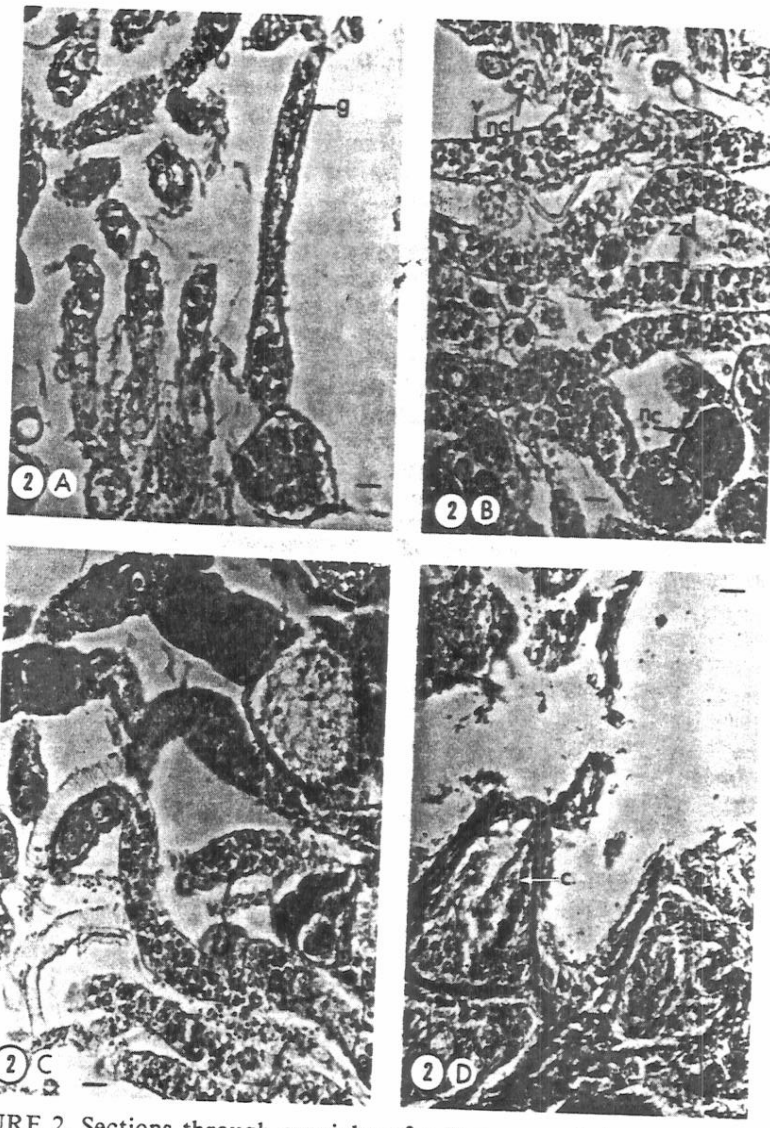


FIGURE 2. Sections through ovarioles of a *P. megacephala* queen 8 weeks after treatment with fenoxycarb. The figure shows loss of activity in the germarium (g: 2A); lack of development of nurse cells (ncl) and follicular epithelium in zone of differentiation (zd: 2B); vacuolation of lower vitellogenic zone (v: 2C) ; and remnants of the chorions of resorbed eggs (c: 2D). Bar= 40u.

Ovaries of untreated *P. megacephala* queens have fewer ovarioles but otherwise resemble the ovaries of the fire ant queens (see Glancey and Banks 1988 for a detailed discussion of the *S. invicta* ovary). Both species' ovaries

nurse cells. Fenoxycarb's effects on the ovaries of Solenopsis queens are visible within two weeks after treatment. However, no effects were observed on the ovaries of *P. megacephala* queens until six weeks after treatment. This delay may have been caused by the repellent effect of fenoxycarb on *P. megacephala* workers. After six weeks, fenoxycarb affected *P. megacephala* in ways similar to *S. invicta* (Glancey and Banks 1988). Eggs laid were about one fourth normal size and few contained yolk. Resorption of many eggs left only the chorion visible. In the region of öocyte differentiation, ovarioles were highly vacuolated; nurse cells were lacking and the follicular epithelial cells lacked cytoplasm. Some ovarioles contained only a series of egg nuclei without cytoplasm and nurse cells. After eight weeks, most ovarioles had shrunk so much that the entire ovary was only one fourth its original size. Only one or two eggs with yolk could be found and they were further reduced in size. Fig. 1 shows a cross-section of a *P. megacephala* control queen's ovary. Fig. 2, A-D, shows the effect of fenoxycarb on the ovary after eight weeks.

Fenoxycarb's ultimate effect on *P. megacephala* was similar to that observed in *S. invicta* colonies (Banks et al. 1988) and is indicative of an IGR overload, which resulted in a decline in colony size and ultimately led to its death. We have previously discussed the effect of an IGR on germinal tissues: inhibition of the nutritive cells and impairment of the differentiation process (Glancey and Banks 1988). Even though *P. megacephala* and *S. invicta* are unrelated, the effects of the IGR on both species are similar. The ovarian tissues of *S. invicta* and *P. megacephala* are affected in similar ways by fenoxycarb treatment, but the effect varies as to time and degree.

Sumitomo Effects

*Solenopsis*. The effects of Sumitomo S-31183 on laboratory and field colonies of *S. invicta* will be reported elsewhere (Banks et al. - ms in preparation). The compound produces many of the same "symptoms" as fenoxycarb, including a disappearance of worker brood due to low level toxicity, suppression of egg production and a shift in caste differentiation so that only sexual forms are produced. Treated laboratory colonies died within 24 weeks.

We studied the effect of S-31183 on *S. invicta* queens by feeding 0.5 ml of once-refined soybean oil containing 2.0% active ingredient to each of six queenright laboratory colonies. These colonies consisted of 20-30 ml of immatures and 40,000-60,000 workers. Six similar colonies were fed neat soybean oil as controls. Ants ingested the oil solution from micropipets within 24 hours and then returned to their normal laboratory diet (Banks et al. 1981).

Five and six weeks after treatment, ovaries were removed from queens of three treated and three control colonies. The ovaries were examined, fixed in Kahle's solution, embedded in paraffin (m.p. 57° C), sectioned at 5um and stained with Harris's hematoxylin and eosin solution. Stained sections, examined under phase microscopy, were photographed.

Ovaries showed no size reduction after five weeks; however, the normal complement of yolked eggs fell from seven to three; each was approximately one half normal size. Microscopic examination of serial sections revealed some ovarioles were empty tubes, while others contained a series of egg nuclei

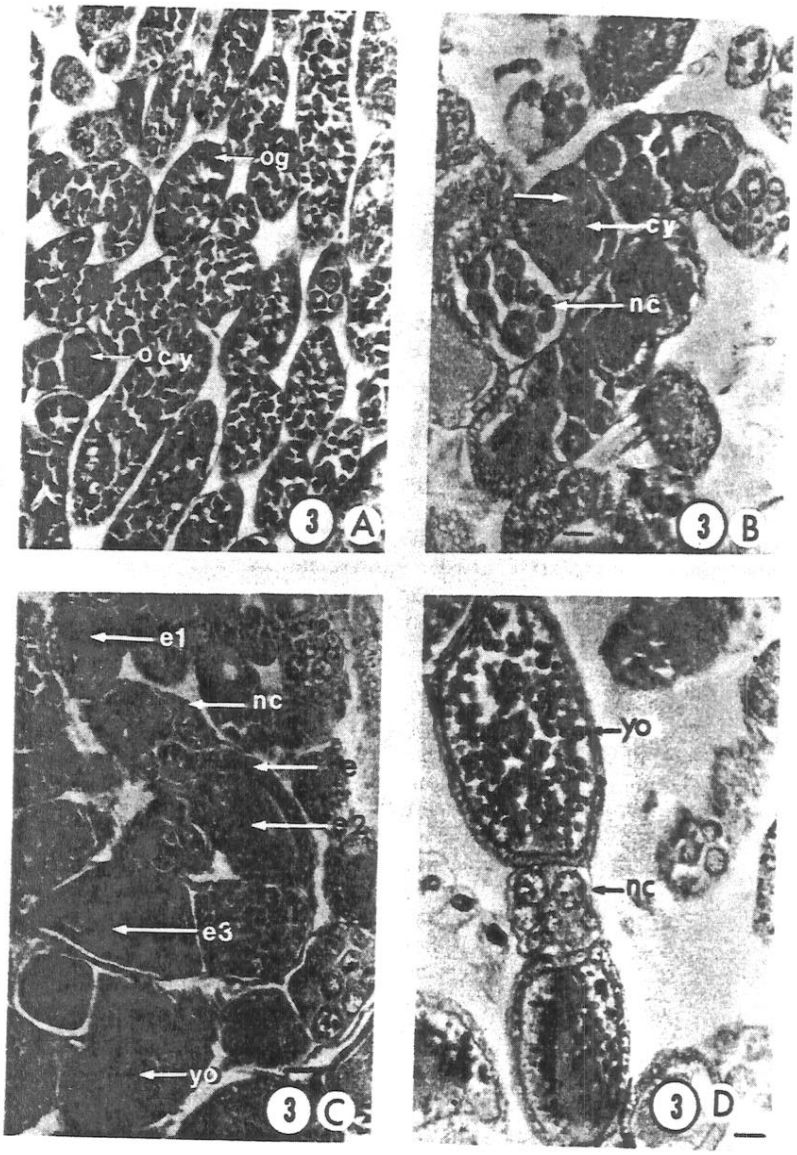


FIGURE 3. Sections through ovarioles of an untreated *S. invicta* queen (from Glancey and Banks 1988). The germarium (3A) is filled with dark stained densely packed öogonia (og) and the zone of differentiation shows differentiated nurse cells (nc) and öocytes (ocy). An egg nucleus (en) surrounded by cytoplasm (cy) is in 3B. Lower end of vitellogenic zone (3C and 3D) showing addition of yolk (yo) to eggs. Bar = 35u.

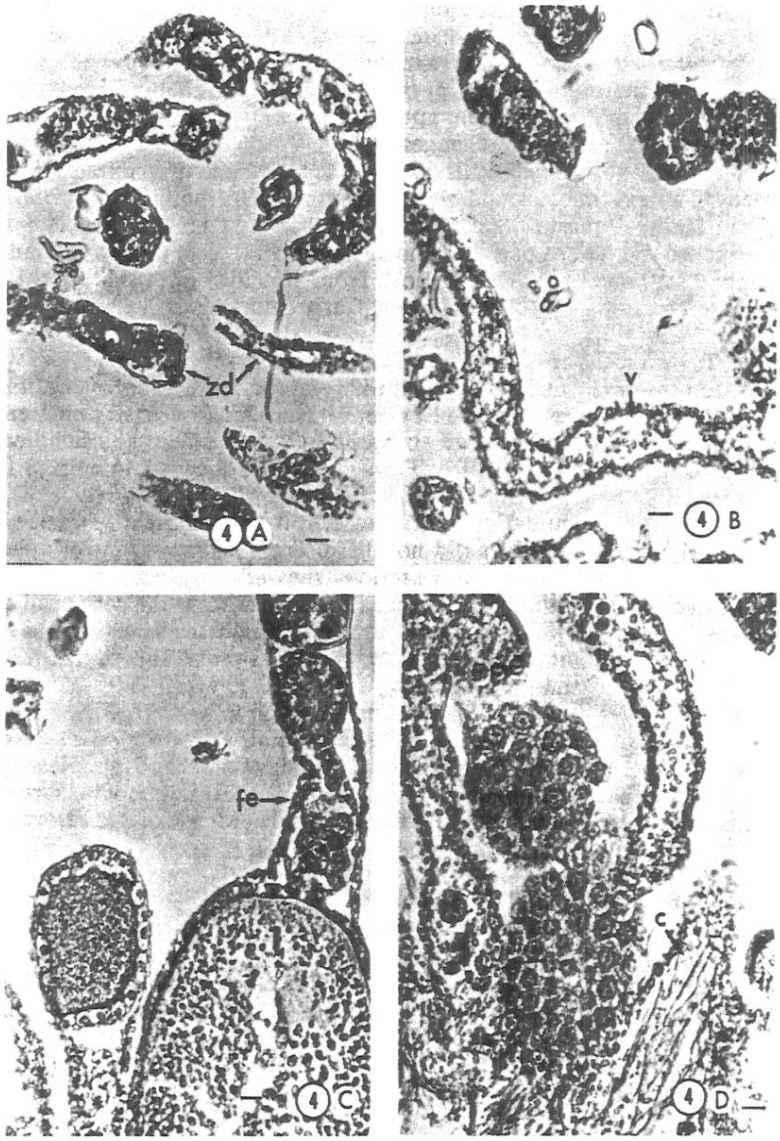


FIGURE 4. Sections through ovarioles of *S. invicta* queens 6 weeks after treatment with Sumitomo S-31183. Effects at germarial level include partial lack of zone of differentiation (4A); vacuolation of ovarioles (4B); increased thickness of follicular epithelium with failure of nurse cells to deposit cytoplasm (4C); and failure to produce large viable eggs in lower end of ovariole because of resorption of the yolk leaving the chorion (4D). Bar= 20u.

without nurse cells. The walls (tunica propria) of most ovarioles were thicker. Egg resorption left only the chorion in 5-10% of the ovarioles.

Although the ovaries in treated queens equaled the size of those in control queens six weeks after treatment, most individual ovarioles were simply tubes, somewhat enlarged at the distal end and containing one small yolked egg; other ovarioles were empty. Microscopic examination of ovarian sections revealed extensive changes. Over 90% of the ovarioles were vacuolated, with thickened walls in most cases. The differentiation zone consisted of an egg nucleus and a few nurse cells. In those instances where cytoplasm and yolkplasm were added, resorption had occurred. The few formed yolked eggs were one third normal size. Fig. 3, A-D (from Glancey and Banks 1988) shows ovarian sections from a control queen. Fig. 4, A-D, shows the effects of S-31183 six weeks after treatment.

*Pheidole*. Techniques for treating *P. megacephala* colonies with S-31183 were identical to those previously described for fenoxycarb. Effects of treatment on the colonies will be reported elsewhere. Ovaries of queens from treated colonies were removed at five and six weeks after treatment and handled as described for *S. invicta* queens. No *P. megacephala* queens laid eggs at five or six weeks.

Under gross examination - five weeks after treatment - ovaries of treated *P. megacephala* queens did not differ from those of control queens. Microscopic examination of serial sections showed mostly normal ovarian tissues, with a small amount of disorganization occurring at the germarial end. Gross examination six weeks after treatment revealed the same effects seen in *S. invicta* (i.e., intact ovaries), but with simple tubes instead of differentiated tissues and only one, very small yolked egg in a few ovarioles. Histological changes caused by the treatment are shown in Fig. 5, A-D. Tissue differentiation was almost absent in the ovariole. Many ovarioles were completely vacuolated; most nurse cells were undeveloped and cytoplasm was missing in those that were developed. The follicular epithelium was disrupted and the tunica propria thickened. Choria served as evidence of egg resorption.

## DISCUSSION

The purpose of these studies was to determine if the IGR that controlled polygynous *S. invicta* colonies would also control the polygynous pest ant, *Pheidole megacephala*. Based on our histological studies, the tested compounds affect both species in the same manner and with the same results. However, the repellency of fenoxycarb to *P. megacephala* has proved a problem. Studies are presently being conducted in both the field and laboratory to determine if lower dosages can be successful.

We proposed in this paper that fenoxycarb affects the ants by overloading the hormonal system. S-31183 appears to be of a different modality. Fenoxycarb causes retrogression of ovarian tissues, whereas one primary effect of S-31183 is egg resorption. The effects of S-31183 on queen ovaries, particularly with respect to the thickening of the tunica propria, approximate those produced by avermectin (Glancey et al. 1982). IGRs control *S. invicta* and *P. megacephala* because they combine morphogenic effects in developing brood with activity that adversely controls egg nutrition.

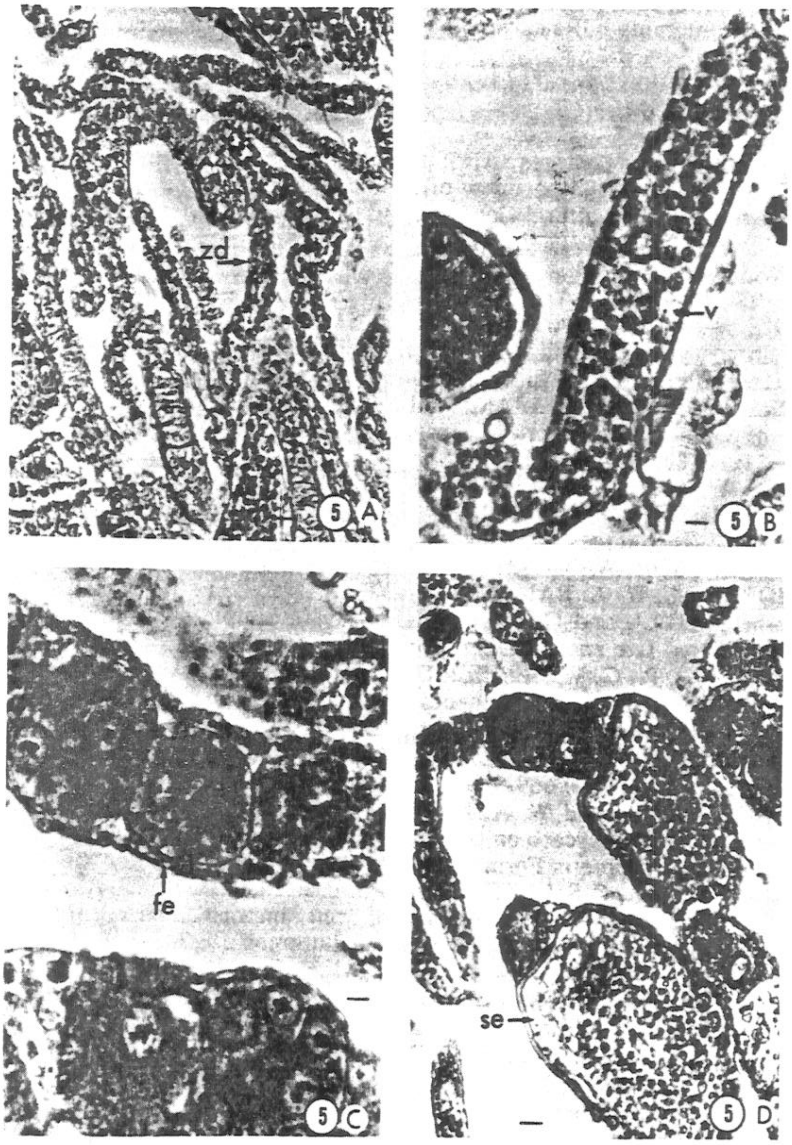


FIGURE 5. Section through ovarioles of *P. megacephala* queens 6 weeks after treatment with Sumnitomo S-3l183. Effects, including small eggs (se), are the same as in *S. invicta* queen (Fig 4 A-D). Bar= 40u.

We lack the direct experimental evidence which would tell us if the action site is the corpora allata's neurosecretory cells or the ovary's cells. In any event, the queen's sterility that results from treatment with either fenoxycarb

or S-31183 provides excellent control of the ants. Present evidence suggests that baits containing IGRs may be the treatment of choice to control polygyne ants.

## ACKNOWLEDGMENTS

We thank T. Krueger, ARS, for his technical assistance and Dr. J. Beardsley, Chairman, Department of Entomology, University of Hawaii and The Governors Agricultural Coordinating Committee for generous support.

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