

Influences of Pyriproxyfen on Fecundity and Reproduction of the Pharaoh Ant (Hymenoptera: Formicidae)

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ABSTRACT The effects of the juvenile hormone analog pyriproxyfen (at concentrations of 0.1, 0.5, and 1%) on egg production, number of ovarioles, and length of oocytes were examined in queens of the Pharaoh ant *Monomorium pharaonis* (L.). Pyriproxyfen significantly reduced egg production in queens from week 3 onwards. Queens that were exposed to 1% pyriproxyfen stopped producing eggs at week 8. After 8 wk, ovaries were dissected from all queens, and the number of ovarioles and the length of the largest oocytes were recorded. The ovaries of queens in treated colonies were smaller than those in untreated queens, and the number of ovarioles in the ovaries was significantly lower in all pyriproxyfen-treated queens. Queens treated with the highest concentrations of pyriproxyfen tended to have significantly shorter oocytes than untreated queens. Histological studies of the ovaries revealed that pyriproxyfen caused vacuolation in the ovarioles, thickening of the tunica propria, development of small eggs, and underdevelopment of nurse cells and the follicular epithelium. Exposure to pyriproxyfen reduced egg production and induced severe morphological changes in the ovaries of queens, and the effects increased with increased concentration of pyriproxyfen.

KEY WORDS *Monomorium pharaonis*, juvenile hormone analog, pyriproxyfen, egg production, queen reproduction

The Pharaoh ant, *Monomorium pharaonis* (L.) (Hymenoptera: Formicidae), is a pest ant species globally (Yap and Lee 1994). Pharaoh ant infestations have been reported in hospitals and health care centers throughout the world (Beatson 1972, Edwards and Baker 1981, Wetterer 2010). They are considered to be tramp ants and are characterized as being highly polygynous, polydomous, unicolonial, and not territorial (Passera 1994, Buczkowski and Bennett 2009). Management of this species has relied heavily on the use of insecticides bait; however, use of residual insecticidal sprays has been unsuccessful owing to the ability of this species to bud and establish new colonies (also known as sociotomy; Lee et al. 1999).

Pyriproxyfen (2-[1-methyl-2-(4-phenoxyphenoxy)ethoxy] pyridine) is a juvenile hormone analog (JHA) reported to be effective in the management of various pests such as mosquitoes (Nayar et al. 2002), houseflies (Kawada et al. 1987), and cockroaches (Koehler and Patterson 1991). It mimics JH activities by competing for binding sites on JH receptors and thus acts on the endocrine system in insects (Sullivan and Goh 2008). As a result, larvae are not able to undergo normal metamorphosis. In ants, pyriproxyfen interferes with brood development, resulting in pupal mortality and the death of the colony as the workers age (Vail and Williams 1995,

Vail et al. 1996, Oi et al. 2000, Lim and Lee 2005). Previous studies showed that pyriproxyfen can be used to control large Pharaoh ant colonies with multiple nest sites in the field (Vail et al. 1996) as well as colonies of the red imported fire ant *Solenopsis invicta* Buren (Hwang 2009). Because the application of pyriproxyfen has no effect on foraging workers, it has a good chance of being distributed throughout the entire colony, including to the queens and brood, via trophallaxis by the workers (Vail et al. 1996, Oi et al. 2000, Lim and Lee 2005).

Queen fertility is important for the Pharaoh ant because workers lack ovaries (Børgesen 1989). In addition to its physiological effects on larval morphogenesis, pyriproxyfen can also target and disrupt the reproductive abilities of the colony queens. Studies have shown that application of pyriproxyfen reduces egg production in queens of *Pheidole megacephala* (F.) (Reimer et al. 1991) and *M. pharaonis* (Kao and Su 1995). In addition, Lim and Lee (2005) and Vail and Williams (1995) described physiological abnormalities and morphogenetic deformations, such as wing deformation and reduction in melanization, in queens of Pharaoh ant laboratory colonies on exposure to pyriproxyfen. Another insect growth regulator, fenoxycarb, was found to interfere with differentiation of the ovaries in queens of *S. invicta* (Glancey and Banks 1988, Glancey et al. 1989) and *P. megacephala*. (Glancey et al. 1990).

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Hsieh and Su (2000) reported that abnormalities such as depletion of yolk and cytoplasm in the oocytes occurred in the ovaries of queens treated with pyriproxyfen. However, it remains unclear whether these abnormalities are the sole and direct cause of the reduction in egg production reported by Kao and Su (1995) for pyriproxyfen-treated Pharaoh ant queens because reduction in larvae could also influence the egg production (Børgesen 1989, Børgesen and Jensen 1995). The influence of pyriproxyfen on the ovaries and its relationship with the reproductive capabilities of the queen remains unknown. In the present study, we evaluated the effects of pyriproxyfen on the egg production and histology of ovaries in Pharaoh ant queens with a steady supply of larvae in the laboratory. We also report the relationship between the ovariole number and egg production.

Materials and Methods

Ant Colonies. The experimental Pharaoh ant colonies were obtained from established ant cultures maintained since 2000 at the Urban Entomology Laboratory, Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia. The colonies were maintained under environmental conditions of $26 \pm 1^\circ\text{C}$, $60 \pm 5\%$ relative humidity, and a photoperiod of 12:12 (L:D) h.

Experimental Design. Each colony was reared in a polyethylene container (130 by 115 by 60 mm) with its sides and inner surface coated with a thin layer of Fluon (polytetrafluoroethylene suspension; BioQuip, Rancho Dominguez, CA) to prevent ants from escaping. A petri dish (55 mm in diameter and 17 mm in height), with three small entry holes on the side, and containing folded corrugated paper (80 by 30 mm), served as harborage. Water and 10% sucrose solution were provided *ad libitum*.

Each colony consisted of 1 queen, 800 workers, and 0.3 g of brood. Fertile 5- to 6-wk-old queens were moved from the established colony to a new colony on their emergence. Each queen was isolated in a container 1 d before the experiment began to confirm that it was mated and it could lay eggs. The brood component (larvae and pupae in an estimated ratio of 2:1) was weighed to the nearest 0.01 mg using a digital analytical balance (BP 190 S Sartorius AG, Goettingen, Germany). All colonies were acclimatized for 3 d to the rearing conditions and then starved for 1 d before pyriproxyfen was introduced. Pyriproxyfen (Sumitomo Chemical Co., Ltd., Takarazuka, Japan) was diluted into an ethanol:peanut oil mixture (vol:vol) to yield three different final concentrations (0.1, 0.5, or 1.0%). Each colony was fed with peanut oil (0.1 ml) (Sime Darby Edible Products Ltd., Jurong Town, Singapore) impregnated with pyriproxyfen on a Whatman No. 1 filter paper (20 by 20 mm). Control colonies were fed with peanut oil in ethanol without pyriproxyfen. Peanut oil containing pyriproxyfen was removed after 1 wk, and all colonies were returned to their normal diet of freshly killed late instars of lobster cockroach (*Nauphoeta cinerea* (Olivier)), canned

tuna fish (TC Boy Marketing Sdn. Bhd., Shah Alam, Malaysia), and hard-boiled egg yolk, which was changed weekly. The colonies were maintained under the environmental conditions specified above. New larvae and pupae from the established colony were added weekly to the experimental colonies when their numbers were found to be reduced owing to the effects of pyriproxyfen. The rationale for this procedure was to avoid reduction of liquid nourishment from larvae to queens, which has been suggested to occur in this species via trophallaxis by workers (Børgesen 1989, Børgesen and Jensen 1995).

Four replicates were performed for each concentration. Because Pharaoh ant queens do not lay eggs daily but instead lay them in batches (Børgesen 1989), the number of eggs produced by queens was counted under a stereo microscope at weekly intervals for 8 wk. The eggs were removed from the tested colony weekly after being counted. At the end of the experiment, queens were killed in the freezer and dissected immediately under a stereo microscope. The total number of ovarioles in both ovaries for each queen and the length of the largest oocyte for each queen were measured based on the pictures taken with a CCD camera system attached to a stereo microscope (45 \times). The ovaries were fixed in Kahle's fixative, embedded in paraffin (melting point 57°C), and sectioned at 5 μm . The sections were mounted on slides, stained with haematoxylin and eosin, and examined and photographed under a light microscope (400 \times) equipped with a CCD camera system.

Statistical Analysis. A one-way ANOVA and Tukey's HSD test were used to compare the mean egg production among the pyriproxyfen treatments at each week. The number of ovarioles and the length of the largest oocyte among the treatments were compared by one-way ANOVA and Tukey's HSD test. All analyses were performed using SPSS version 11.5 (SPSS Inc 2002). The level of significance was set at $P = 0.05$.

Results

Egg Production. Egg production at week 1 and 2 did not differ significantly between treated and untreated colonies (week 1, $F = 1.22$, $df = 3, 12$, $P = 0.35$; week 2, $F = 2.75$, $df = 3, 12$, $P = 0.09$). From week 3 onwards, however, queens of the pyriproxyfen-treated colonies laid significantly fewer eggs than the untreated colonies, except for colonies subjected to 0.1 and 1% pyriproxyfen at week 4 (Fig. 1). From week 6 onwards, queens treated with 1% pyriproxyfen produced fewer eggs than the queens in the 0.1 and 0.5% pyriproxyfen treatments, and at week 8 the four queens treated with 1% pyriproxyfen completely ceased egg production. In contrast, the mean egg production was 11.8 ± 1.8 and 3.2 ± 0.6 , respectively, in the colonies subjected to 0.1 and 0.5% pyriproxyfen (Fig. 1). Mean egg production in the untreated colonies remained high throughout the experimental period (Fig. 1).

Effects of Pyriproxyfen on Ovary Morphology. The female reproductive system of the Pharaoh ant con-

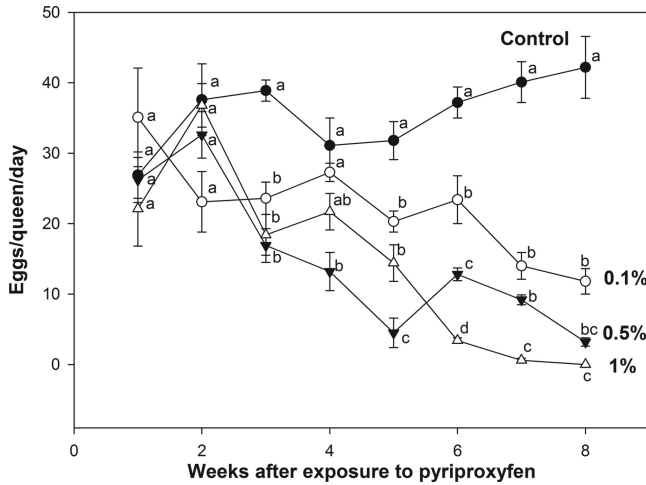


Fig. 1. Egg production of *M. pharaonis* (mean \pm SE) after exposure to different concentrations of pyriproxyfen. (●) control, (○) 0.1%, (▼) 0.5%, and (△) 1% pyriproxyfen; data are means of four replicates. Vertical bars indicate standard error of the means. For each week, symbols labeled with the same letters are not significantly different at $P = 0.05$ (Tukey's HSD).

sists of a common oviduct, lateral oviducts, a spermatheca, and a pair of ovaries, each of which consists of a number of ovarioles. The ovaries were larger in the queens of untreated colonies compared with the treated colonies. In untreated colonies, ovaries were

fully developed, each ovariole contained one large, mature oocyte near the oviduct that was ready for deposition together with a few smaller oocytes (Fig. 2). In contrast, the ovarioles of pyriproxyfen-treated queens were thin, reduced in size (Fig. 2), and filled

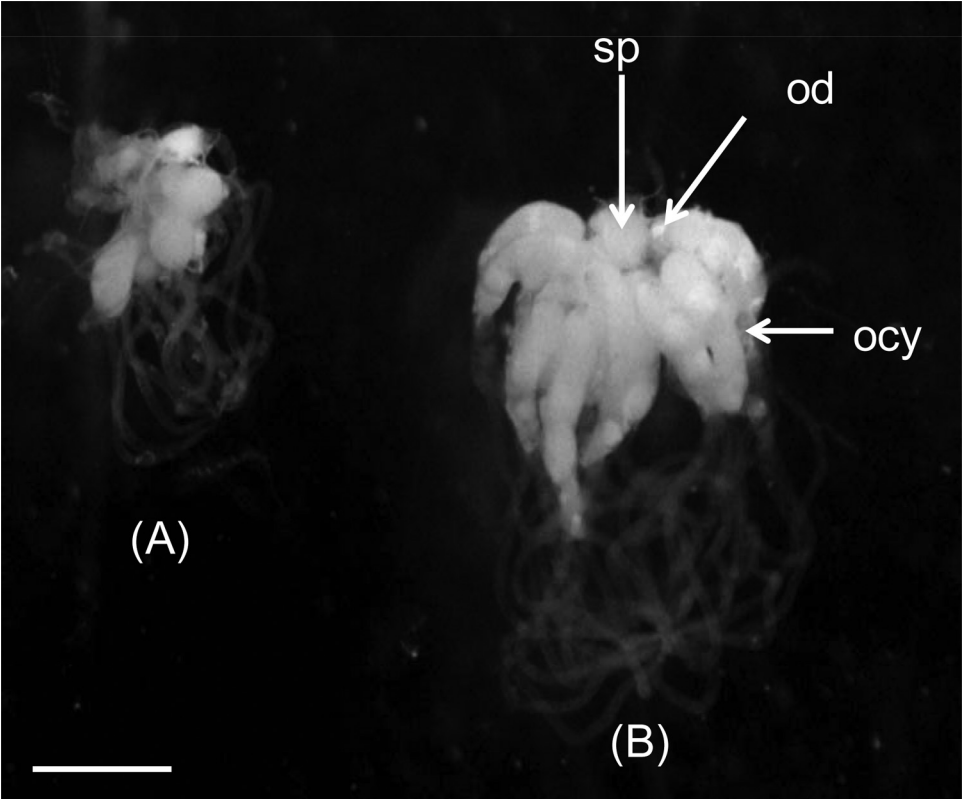


Fig. 2. Ovaries dissected from a (A) treated and (B) untreated queen of *M. pharaonis*. Abbreviations: ocy, oocyte; od, oviduct; sp, spermatheca. Scale bar = 1 mm.

Table 1. Ovary development of *M. pharaonis* after exposure to different concentrations of pyriproxyfen

Treatment (%)	Mean \pm SE	
	Number of ovarioles per queen	Length of the largest oocyte (mm)
Control	18.25 \pm 0.63a	0.37 \pm 0.01a
0.1	8.75 \pm 1.25b	0.35 \pm 0.01a
0.5	10.00 \pm 0.91b	0.34 \pm 0.01a
1	7.25 \pm 1.31b	0.28 \pm 0.02b

Data are mean of four replicates. Means with the same letter within a column are not significantly different at $P = 0.05$ (Tukey's HSD).

only the last segment of the abdomen. Most ovaries from the treated queens did not contain large oocytes. In some cases, both ovaries contained a few ovarioles but only one oocyte was found. Pyriproxyfen led to reduction in the number of ovarioles in the ovaries: A mean of 18.3 ± 0.6 ovarioles per queen was found in the untreated colonies, whereas 8.8 ± 1.3 , 10.0 ± 0.9 , and 7.3 ± 1.3 were found in colonies subjected to 0.1, 0.5, and 1% pyriproxyfen, respectively. Significant differences were found between the treated and untreated colonies regardless of the pyriproxyfen concentrations ($P < 0.05$), whereas no significant difference was observed among the treated colonies ($P > 0.05$; Table 1). As the concentration of pyriproxyfen increased, the average length of the largest oocyte in the queens tended to become smaller (0.37 ± 0.01 mm in the untreated colonies compared with 0.35 ± 0.01 , 0.34 ± 0.01 , and 0.28 ± 0.02 mm in colonies exposed to 0.1, 0.5, and 1% pyriproxyfen, respectively). However, this value differed significantly ($P < 0.05$) from that of the untreated colonies only for the 1% pyriproxyfen treatment (Table 1).

Histological Observation of Ovaries After Treatment With Pyriproxyfen. Although egg production decreased with increasing concentration of pyriproxyfen toward the end of the study (Fig. 1), the histological observation of the queen ovaries revealed severe microscopic signs of abnormality that were similar regardless of the concentration of pyriproxyfen. Longitudinal sections of the untreated ovaries showed normal follicular epithelium, nurse cells, cytoplasm, and differentiation of ovarioles into oocytes, and cross-sections of untreated ovarioles showed that the nucleus of the oocyte was surrounded by cytoplasm and that a few nurse cells were located adjacent to the oocyte (Fig. 3A and C). In contrast, instead of having the full complement of an oocyte within the ovarioles, the ovaries of queens in the treated colonies were underdeveloped. The ovarioles became vacuolated and lacked nurse cells, and oocytes lacked a nucleus (Fig. 3B and D). Exposure to pyriproxyfen induced deterioration of the follicular epithelium, nurse cells, and cytoplasm in the ovarioles. Thickening of the tunica propria and development of small eggs was also observed in the ovarioles (Fig. 4A–D).

Discussion

The current experimental results and observations suggest that pyriproxyfen in peanut oil was not repellent to Pharaoh ants; thus, it was distributed to the colony, including queens that usually remained inside the harborage. Although dead larvae and pupae were replaced with new larvae and pupae weekly, the egg production in the treated colonies began to decrease from week 3 onwards irrespective of the concentration of pyriproxyfen and finally stopped at week 8 in the 1% pyriproxyfen treatment. Reimer et al. (1991) reported a similar reduction in egg production within 3 wk in pyriproxyfen-treated colonies of *P. megacephala*. Topical application of pyriproxyfen has also been reported to inhibit reproduction in the German cockroach, *Blattella germanica* (L.) (Kawada et al. 1989). The effects of pyriproxyfen can be explained by its disruptive effects on the ovary in queens. Only 5–12 ovarioles were found in the pyriproxyfen-treated queens, whereas 17–20 ovarioles were present in the ovaries of the untreated queens. However, the effects of pyriproxyfen on the length of the largest oocyte were only detected in the colonies exposed to the highest concentration (1%) of pyriproxyfen. Thus, decreased egg production, which was also reported by Kao and Su (1995) in pyriproxyfen-treated Pharaoh ant queens, likely is attributable largely to the reduced ovariole number. Tschinkel (1987) pointed out that the reproductive parameters in ants, such as egg production, sperm production, and number and length of the ovarioles in the ovaries, probably are linked to one another.

The observed abnormalities in Pharaoh ant ovarian morphology caused by exposure to pyriproxyfen were similar to those caused by fenoxycarb in *P. megacephala* (Glancey et al. 1990) and *S. invicta* (Glancey and Banks 1988), and s-methoprene in *M. pharaonis* (Edwards 1975). Avermectins, which are macrocyclic lactones, also had distinctive effects on ovary development in *S. invicta* (Glancey et al. 1982) and *Acromyrmex subterraneus subterraneus* (Forel) (Antunes et al. 2000). Pyriproxyfen, which mimics JH activity, was stored in the crop of ants, causing hormonal disturbance and thereby inhibiting egg production on various pests (Ishaaya et al. 1994, Schneider et al. 2008, Sullivan and Goh 2008). Because JHs are secreted by neurosecretory cells and are responsible for vitellogenesis and reproductive activities in various insects, pyriproxyfen is thought to disrupt vitellogenin synthesis (Wigglesworth 1973), which is necessary for the late stage of oogenesis in insects (Hartfelder 2000). The proposed action of insect growth regulators is to cause abnormal hormonal output of neurosecretory cells, which in turn inhibits vitellogenesis in the oocyte (Glancey et al. 1982, 1990). Insufficient concentrations of vitellogenin may then prevent protein synthesis and yolk deposition in the developing oocyte, resulting in severe deformities in the ovaries (Chapman 1969, Glancey et al. 1982). Because reductions in larvae and pupae could affect queen fecundity as reported in previous studies of *M. pharaonis*

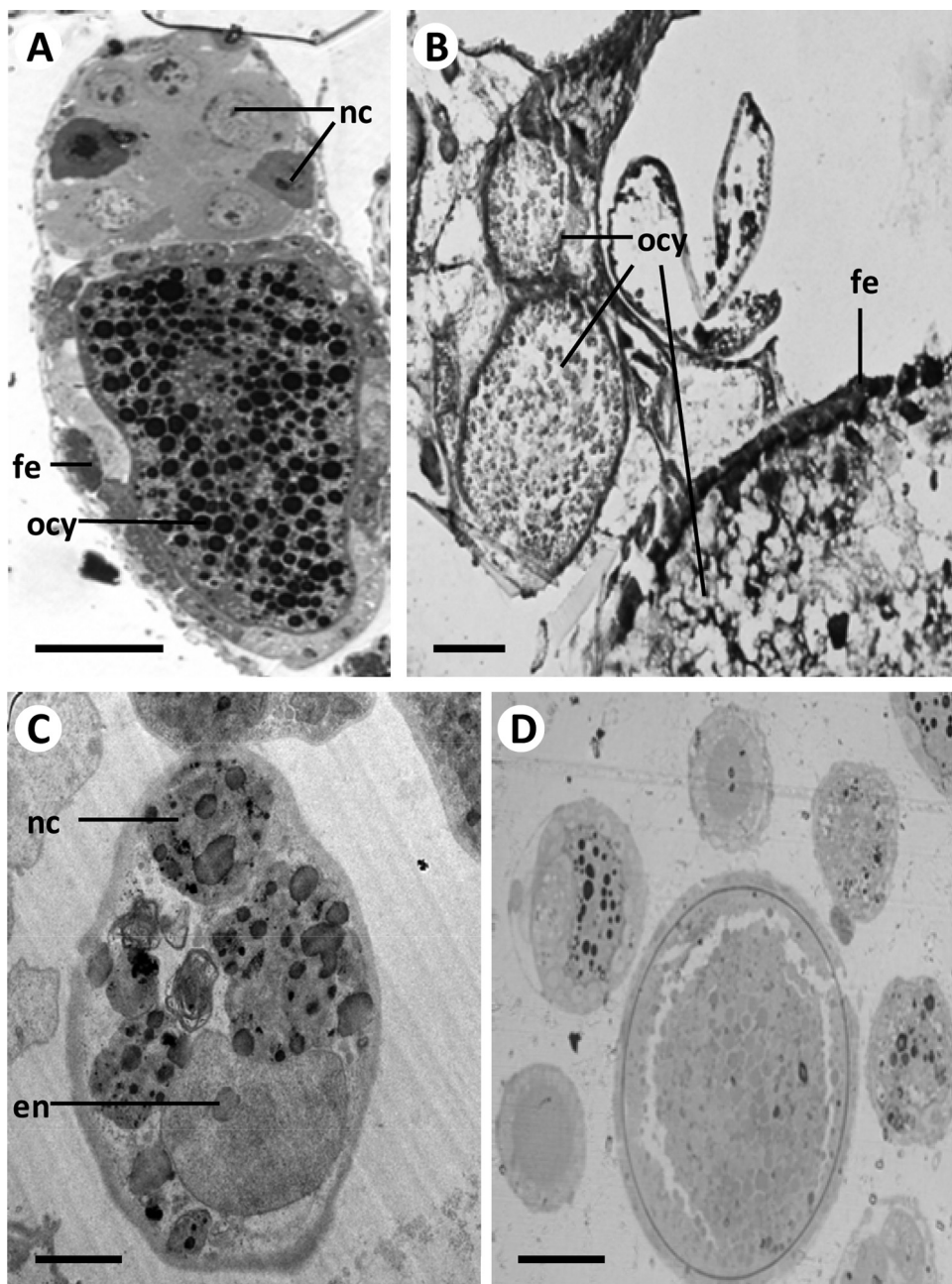


Fig. 3. (A) Longitudinal section of an untreated ovariole showing a mature oocyte with a nurse cell. (B) Longitudinal section of a treated ovariole showing oocytes lacking a nurse cell. (C) Cross-section of an untreated ovariole showing an oocyte with a nurse cell and nucleus. (D) Cross-section of a treated ovariole showing an oocyte without a nurse cell and nucleus. Abbreviations: en, nucleus of oocyte; fe, follicular epithelium; nc, nurse cell; ocy, oocyte. Scale bar = 20 μ m.

(Børgesen 1989, Børgesen and Jensen 1995) and *S. invicta* (Tschinkel 1995), untreated larvae and pupae were added weekly when needed to the treated colonies. So, we could confirm that the reduction in egg production was not owing to decreased nutrient availability to the queens caused by lack of larvae and pupae. Thus, the reduction of egg production, ovariole number, and length of the largest oocyte and the

observed microscopic abnormalities in the ovaries likely are owing to the direct effect of pyriproxyfen. The similarities in the ovarian abnormalities observed for fenoxycarb (Glancey and Banks 1988), avermectins (Glancey et al. 1982), and pyriproxyfen (this study) suggest a common mechanism of action among these chemicals. However, the reduction in the number of ovarioles and the length of the largest oocyte

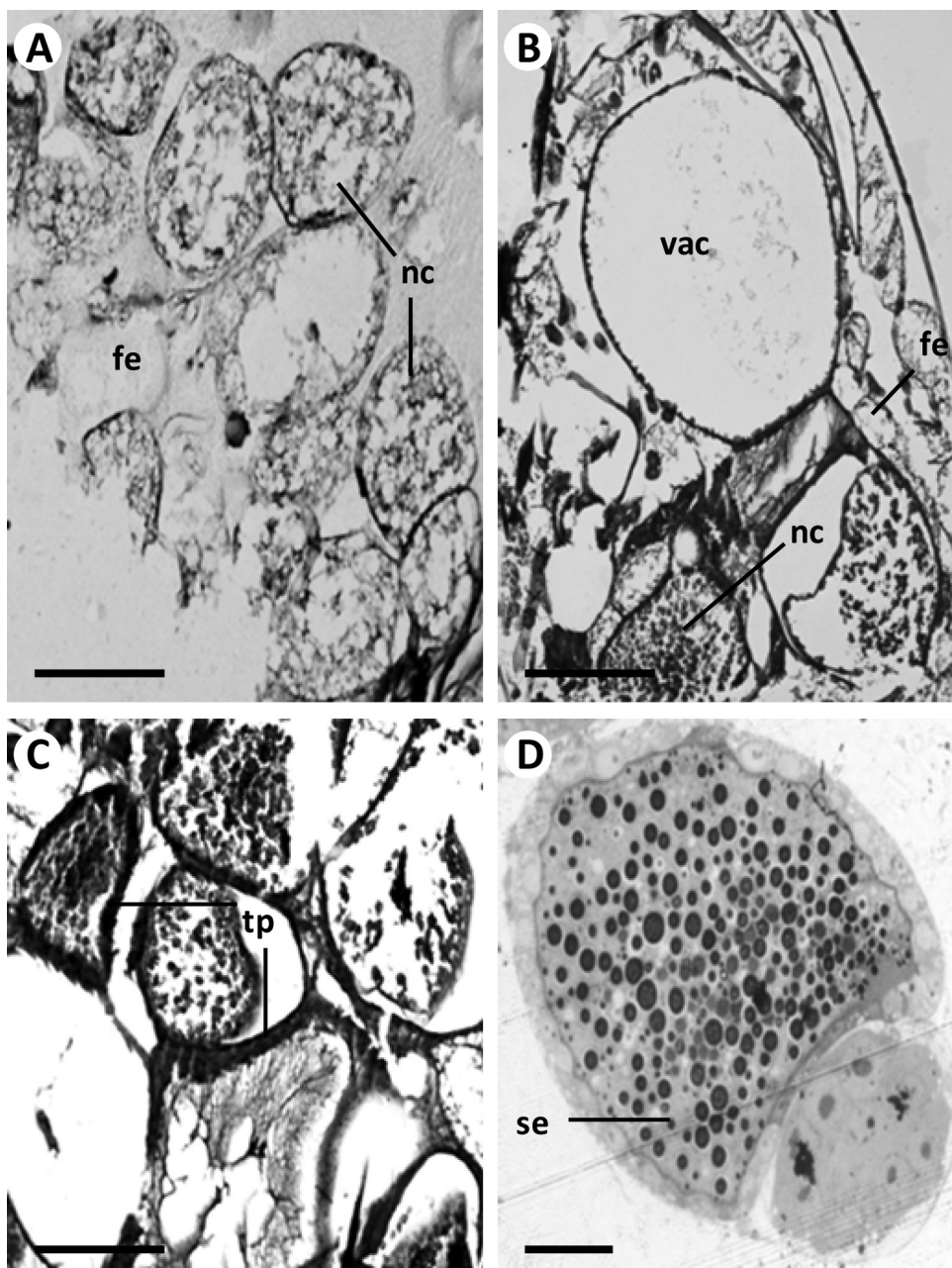


Fig. 4. Sections of some ovarioles in a treated queen showing (A) follicular epithelium lacking cytoplasm, (B) vacuolation of ovarioles, (C) increased thickness of the tunica propria, and (D) development of small eggs. Abbreviations: fe, follicular epithelium; nc, nurse cell; SE, small eggs; tp, tunica propria; vac, vacuole. Scale bar = 20 μ m.

have not been reported in other insect species and thus warrants further study to elucidate the underlying mechanism.

There is no evidence showing that JHA causes mortality of the worker and queen caste (Banks et al. 1983). No queen mortality and queen production was recorded during the experimental period ($n = 16$), and no abnormalities were detected in already emerged workers. Worker populations were found to

decline slowly when queens ceased egg production and when workers reached the end of their life span (Vail and Williams 1995, Vail et al. 1996). Vail and Williams (1995) documented 100% queen mortality in laboratory colonies treated with 0.5% pyriproxyfen in peanut oil at 16 wk after treatment. Thus, complete elimination of Pharaoh ant colonies likely would be seen with a longer duration of observation. Because Pharaoh ant queens are known to be short lived

(Petersen-Braun 1975, Keller 1998), the queen's sterility in treated colonies would provide excellent control of Pharaoh ant colonies (Vail and Williams 1995). The application of peanut oil impregnated with pyriproxyfen provided effective control during the experimental period, especially for the 1% pyriproxyfen treatment from week 6 onwards. Kao and Su (1995) also reported that 1% pyriproxyfen more effectively reduced egg production toward day 78 as compared with 0.1 and 0.5% pyriproxyfen. However, the effects of pyriproxyfen appeared to be reversible at 24 wk posttreatment in a previous study of *S. invicta* (Banks and Lofgren 1991). Thus, repeated application of bait is necessary to prevent recovery of the reproductive capabilities of the queens for long-term control.

The results of the current study demonstrated that use of food impregnated with slow-acting toxicants such as pyriproxyfen acted as a reproductive disturbing agent with no effect on sterile workers. Pyriproxyfen affected the reproductive capabilities of the queen, causing cessation of egg production by week 8. As Pharaoh ant workers are sterile, queen fertility is important for colony survival. Pyriproxyfen can disrupt or completely inhibit ovary development, as histological changes were observed in the ovary sections of the treated Pharaoh ant queens at week 8. The 1% pyriproxyfen treatment reduced the number of ova-rioles, the size of the largest oocyte, and most importantly, inhibited the egg production of queens. Without a fertile queen, individuals cannot be replaced, thus the colony is suppressed. These results provide further support that pyriproxyfen has potential for use in Pharaoh ant control in Integrated Pest Management programs.

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