*Journal of the American Mosquito Control Association*, 18(4):321–328, 2002


# LARVICIDAL EFFECT OF THE JUVENILE HORMONE MIMIC PYRIPROXYFEN ON *CULEX PIPIENS*

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ABSTRACT. The larvicidal activity of the juvenile hormone mimic pyriproxyfen was evaluated against the 4th larval instar of *Culex pipiens* under 5 constant temperatures in the laboratory. Toxicity of this insect compound increased with temperature. The 50% lethal concentrations ranged between 0.00111 ppm at 20°C and 0.00013 ppm at 32°C. A similar trend was observed for the 90% lethal concentrations, which varied from 0.00379 ppm to 0.00024 ppm at the 2 temperatures, respectively. Some effects of the compound were observed on the ultrastructure of the 4th-stage larval integument, where electron micrographs revealed the destruction of procuticle lamellae, formation of cuticular vacuoles, deformed mitochondria, and destruction of nuclear envelopes and the epidermal layer, in addition to an increase in electron-dense lysosomelike bodies.

**KEY WORDS** Insect growth regulators, juvenile hormone mimics, pyriproxyfen, larvicidal effect, *Culex pipiens*, integument ultrastructure

## INTRODUCTION

The medical importance of *Culex pipiens* L. is well documented (e.g., Mitchell et al. 1980, Johnson 1990, Kettle 1995). Rift Valley fever, which occurs only in Africa, normally is transmitted by mosquitoes. In 1977–78, a widespread epizootic and epidemic that involved an estimated 18,000 human cases and 598 deaths occurred in Egypt (Johnson et al. 1978). The most common and widespread mosquitoes were members of *Cx. pipiens* complex, from which Rift Valley fever virus was isolated, and laboratory transmission of the disease by *Cx. pipiens* implicated it as a major vector in Egypt (Hoogstraal et al. 1979).

The overproduction of detoxifying mechanisms of chemical insecticides has been reported for *Cx. pipiens* (Raymond et al. 1987, 1992; Severini et al. 1993). On the other hand, some mosquito species have developed high levels of resistance to microbial control agents (Rao et al. 1995). One alternative approach is the use of insect growth regulators; for instance, the insect growth regulator methoprene has been successfully used to control some species of mosquitoes (Ross et al. 1994a, 1994b; Ritchie 1997; Pinkney et al. 2000).

The juvenile hormone mimic pyriproxyfen (Sumilarv–Public Health) is an insect growth regulator that has been developed comparatively recently. Insect juvenile hormone is 1st produced in the late embryo and sems to be important for normal dorsal closure, formation of the larval cuticle, and differentiation of the midgut. The corpora allata continue to produce the hormone throughout the larval life until the final instar. Intermolt juvenile hormone influences maintenance of larval-specific organs and behavior, and the production of the prothoracicotropic hormone (Vennard et al. 1998). Application of natural juvenile hormones or juvenile hormone mimics at appropriate times can result in disruption of normal development (Menn et al. 1989). The objectives of this study were to determine the efficacy, through laboratory bioassays, of pyriproxyfen against the larvae of *Cx. pipiens* under constant temperatures ranging between 20 and 32°C, and to determine some ultrastructural changes induced in the larval integument after the application of a sublethal dosage of this juvenile hormone mimic.

## MATERIALS AND METHODS

*Laboratory bioassays:* Laboratory bioassays were used to determine the effectiveness of pyriproxyfen as a larvicide against *Cx. pipiens* at constant temperatures of 20, 23, 26, 29, and 32°C. Egg rafts were obtained from colonies maintained at the Medical Entomology Research Institute (Dokki, Egypt), and a laboratory colony of the mosquito larvae was established in the Entomology Department, Faculty of Science, Cairo University, by following common standard mosquito rearing techniques. Larval bioassays were conducted on 1-day-old, 4th-stage larvae.

Pyriproxyfen (technical grade, 99.5%, Sumilarv, Sumitomo, Chuo-ku, Osaka, Japan) was dissolved in water to prepare a 1% stock emulsion and 8 dilutions (20-fold dilutions) for each temperature. Three replicates, each consisting of 20 larvae, were used for each concentration. Each group of experimental insects was placed in a capped 250-ml glass jar. All concentrations were prepared in 150 ml of water and kept at a photoperiod of 14:10 h light:dark. Similar control groups were used at each temperature.

The jars were examined daily and mortality was recorded until adult emergence was completed in the control jars. The mortality was corrected by Abbott's formula (Abbott 1925). Probit analysis (Finney 1971) was used to determine the median lethal concentration ($LC_{50}$) and 90% lethal concentration ($LC_{90}$) at all the tested temperatures.

The effect of temperature on the inhibition of

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Table 1. Toxicity of pyriproxyfen against laboratory-reared[1] 4th-stage larvae of *Culex pipiens* at 5 constant temperatures.

| Temperature (°C) | Lethal concentration (ppm)[2] | | | | Slope |
|---|---|---|---|---|---|
| | $LC_{50}$ | 95% CL | $LC_{90}$ | 95% CL | |
| 20 | 0.00111 | 0.00074–0.00143 | 0.00379 | 0.00256–0.00571 | 2.42 |
| 23 | 0.00087 | 0.00069–0.00103 | 0.00287 | 0.00211–0.00390 | 2.81 |
| 26 | 0.00042 | 0.00027–0.00055 | 0.00155 | 0.00130–0.00191 | 2.28 |
| 29 | 0.00037 | 0.00023–0.00057 | 0.00113 | 0.00068–0.00263 | 2.63 |
| 32 | 0.00013 | 0.000098–0.00016 | 0.00024 | 0.00011–0.00038 | 3.91 |

[1] Egg rafts were obtained from a colony maintained for more than 10 years in the Medical Entomology Research Institute–Egypt.
[2] $LC_{50}$, median lethal concentration; CL, confidence limits; $LC_{90}$, 90% lethal concentration.

adult emergence by pyriproxyfen was determined by the use of the $LC_{50}$ at each of the tested temperatures. One hundred larvae were placed in capped 1-liter glass jars containing 600 ml of water in which the desired volume of pyriproxyfen for each temperature was mixed. Control groups were set up for each temperature in a similar fashion. The number of emerged adults was recorded.

*Effect of a sublethal dosage on the larval integument:* The effect of a sublethal dosage ($LC_{50}$ at 23°C) on the integument was examined by the use of electron micrographs of normal and treated 4th-stage larvae. Samples were prepared 72 h after treatment. The larvae were processed for electron microscopy and examined according to the procedure described by Locke and Huie (1980) with some modification. Control and treated larvae were fixed in 2.5% glutaraldehyde (0.1 M Sörensen phosphate buffer, pH 7.2) for 2 h at 4°C. They were rinsed overnight in 0.1 M phosphate buffer–sucrose 12.5% (1:1) and postfixed in 1% osmium tetroxide (in 0.1 M phosphate buffer, pH 7.2). Larvae were dehydrated by passage through ethanol and propylene oxide and then embedded in Spurr resin. The ultrathin sections were examined in a Philips 201 electron microscope. Electron microscopic studies were conducted in the Electron Microscopy Unit, Ain Shams University Hospitals, Cairo, Egypt.

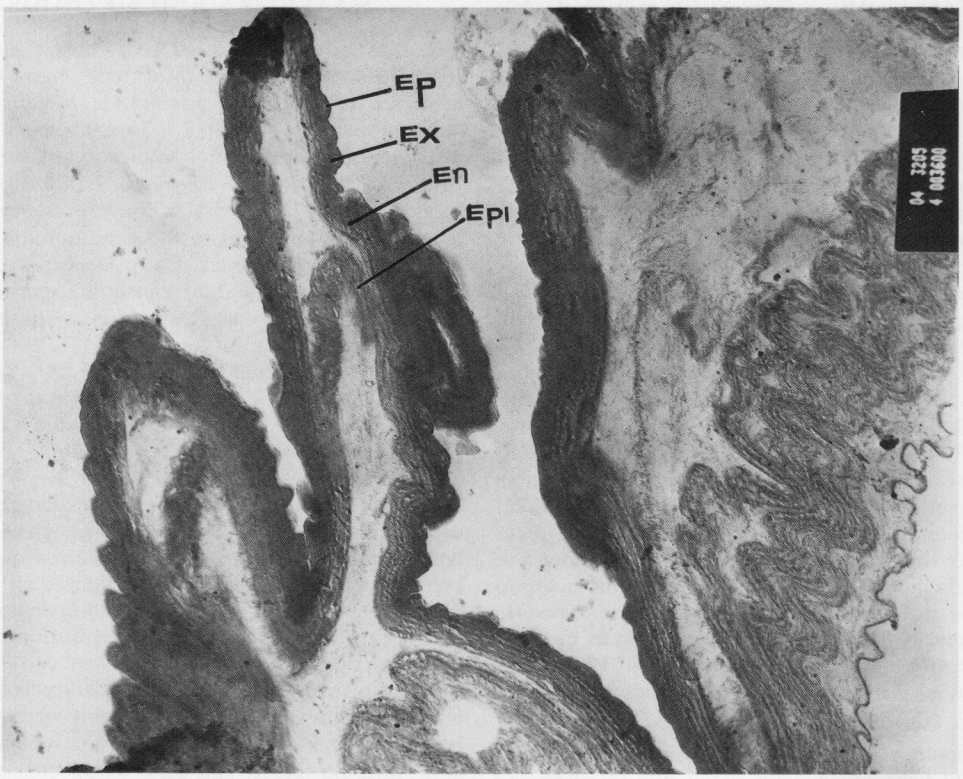


Fig. 1. Electron micrograph of a normal 3-day-old 4th-stage larva of *Culex pipiens* showing the fine structure of normal integument. Ep, epicuticle; Ex, exocuticle; En, endocuticle; Epl, epidermis. 3,600×.

Table 2. Emergence inhibition of *Culex pipiens* by median lethal concentrations ($LC_{50}$s[1]) of pyriproxyfen at constant temperatures.

| Temperature (°C) | Percent (%) emergence | |
|---|---|---|
| | Control | Treated |
| 20 | 95 | 11 |
| 23 | 94 | 13 |
| 26 | 95 | 9 |
| 29 | 97 | 7 |
| 32 | 93 | 6 |

[1] $LC_{50}$s are given in Table 1.

## RESULTS AND DISCUSSION

### Bioassays

Larvae of *Cx. pipiens* were susceptible to the juvenile hormone mimic pyriproxyfen at all the tested temperatures. However, the sensitivity of the insects was positively correlated to temperature. Thus, the $LC_{50}$ was 0.00013 ppm at 32°C and increased with the decrease in temperature to reach 0.00111 ppm at 20°C. A significant difference ($P < 0.05$) was found between the $LC_{50}$ obtained at each tested temperature vs. that obtained at 32°C, as indicated by the absence of overlapping (or nonoverlapping) of the respective 95% confidence limits (Table 1). According to our results, we suggest that this compound can be used to control *Cx. pipiens* over a wide range of temperatures. The efficacy of pyriproxyfen against mosquitoes seems to be relatively high compared to that of other juvenile hormone mimics. When working with *Aedes albopictus,* Ali et al. (1995) cited that this juvenile hormone mimic was 2.23 times and 21.5 times more active than diflubenzuron and methoprene, respectively, where the $LC_{50}$ of pyriproxyfen was 0.00011 ppm to the 3rd- and early 4th-stage larvae.

Our laboratory data on emergence inhibition of adult *Cx. pipiens* by pyriproxyfen are presented in Table 2, which shows that the emergence inhibition from the treatment of survivors with $LC_{50}$s generally increased with temperature. When using this juvenile hormone mimic, Kawada (1993) reported 50% emergence inhibition of *Ae. albopictus* at 0.024 ppb. Ali et al. (1995) reported 50% emergence inhibition of the same species at 0.11 ppb.

### Effect of pyriproxyfen on the ultrastructure of the integument

The cuticle of the 4th-stage larva is characterized by the presence of projections or papillae bounded by the epicuticle (Figs. 1 and 2). An amorphous

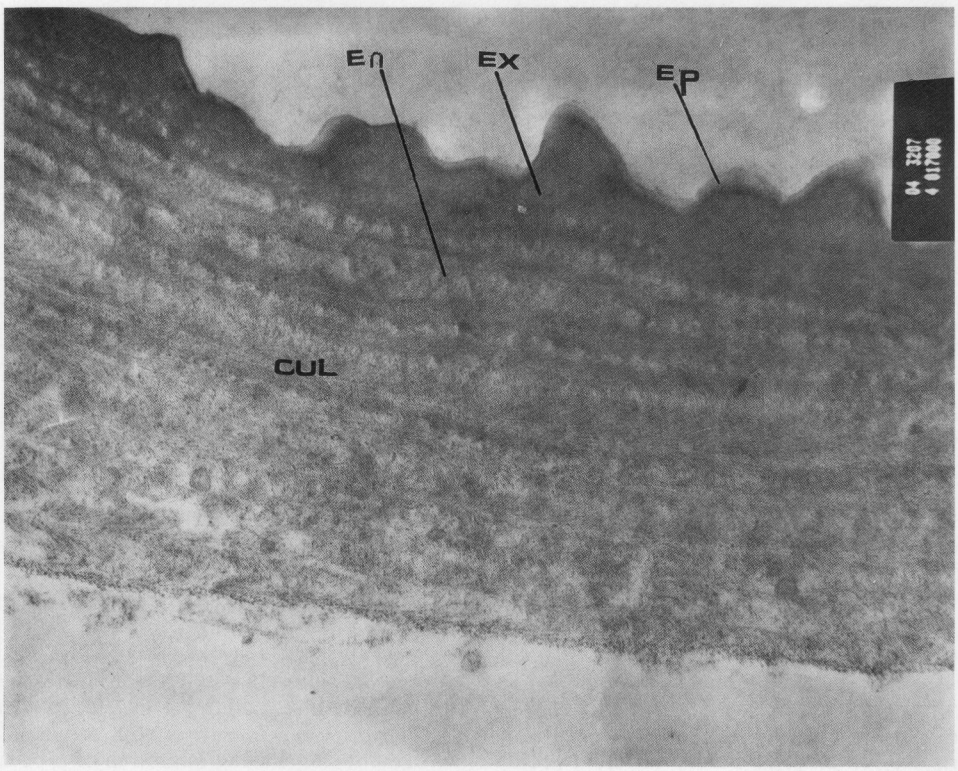


Fig. 2. Electron micrograph of a normal 3-day-old 4th-stage larva of *Culex pipiens* showing the normal compositions of its integument, with higher magnification. Ep, epicuticle; Ex, exocuticle; En, endocuticle; Cul, procuticle lamellae. 17,000×.

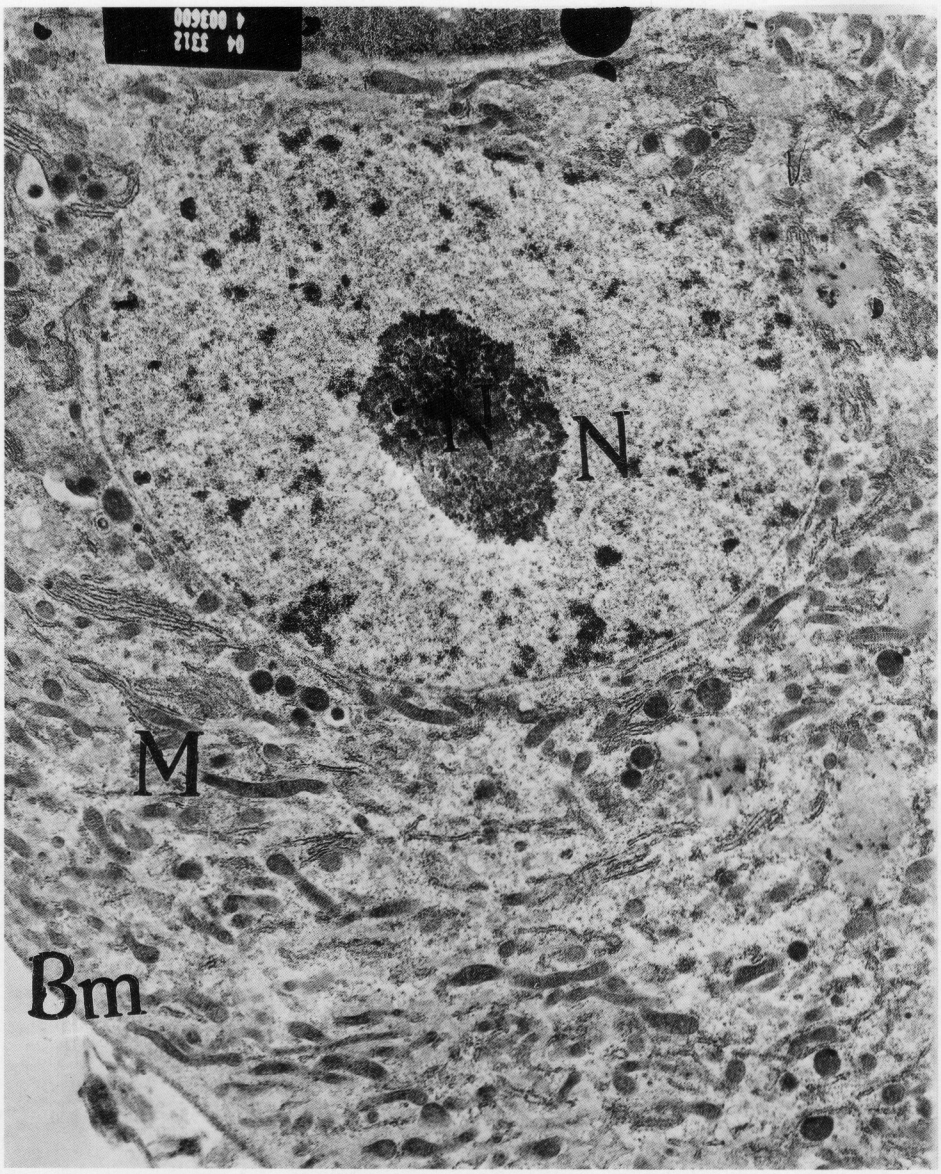


Fig. 3. Electron micrograph of epidermal layer of normal 3-day-old 4th-stage larva of *Culex pipiens* showing the mitochondria (M), basement membrane (Bm), and nucleus (N). 13,000×.

(nonlamellate) exocuticle is found below the epicuticle (Figs. 1 and 2). The endocuticle forms the bulk of the cuticle, with a group of procuticle lamellae (Fig. 2). As in other insects, the multilayered cuticle overlays a single layer of epidermal cells with relatively large nuclei and mitochondria that are scattered through the cytoplasm (Fig. 3). The cuticle of 4th-stage larvae of *Cx. pipiens* treated with pyriproxyfen presents a very different profile from that of normal cuticle. The endocuticle appeared devoid of lamellae and some vacuoles appeared under the epicuticle (Figs. 4 and 5).

Although pyriproxyfen is a juvenile hormone mimic, its effect on the cuticle (Figs. 4 and 5) somewhat resembles the effect of chitin synthesis inhibitors such as diflubenzuron. Thus, the integument of pyriproxyfen-treated larvae of *Cx. pipiens* exhibits an amorphous cuticular region instead of normal lamellate cuticle. This evidence indicates that pyriproxyfen interferes with the deposition of normal cuticle, causing inhibition of procuticle formation. The same effect was observed with benzoylphenyl ureas such as diflubenzuron, hexafluron, and teflubenzourn on *Lucilia cuprina* Wied. (Dip-

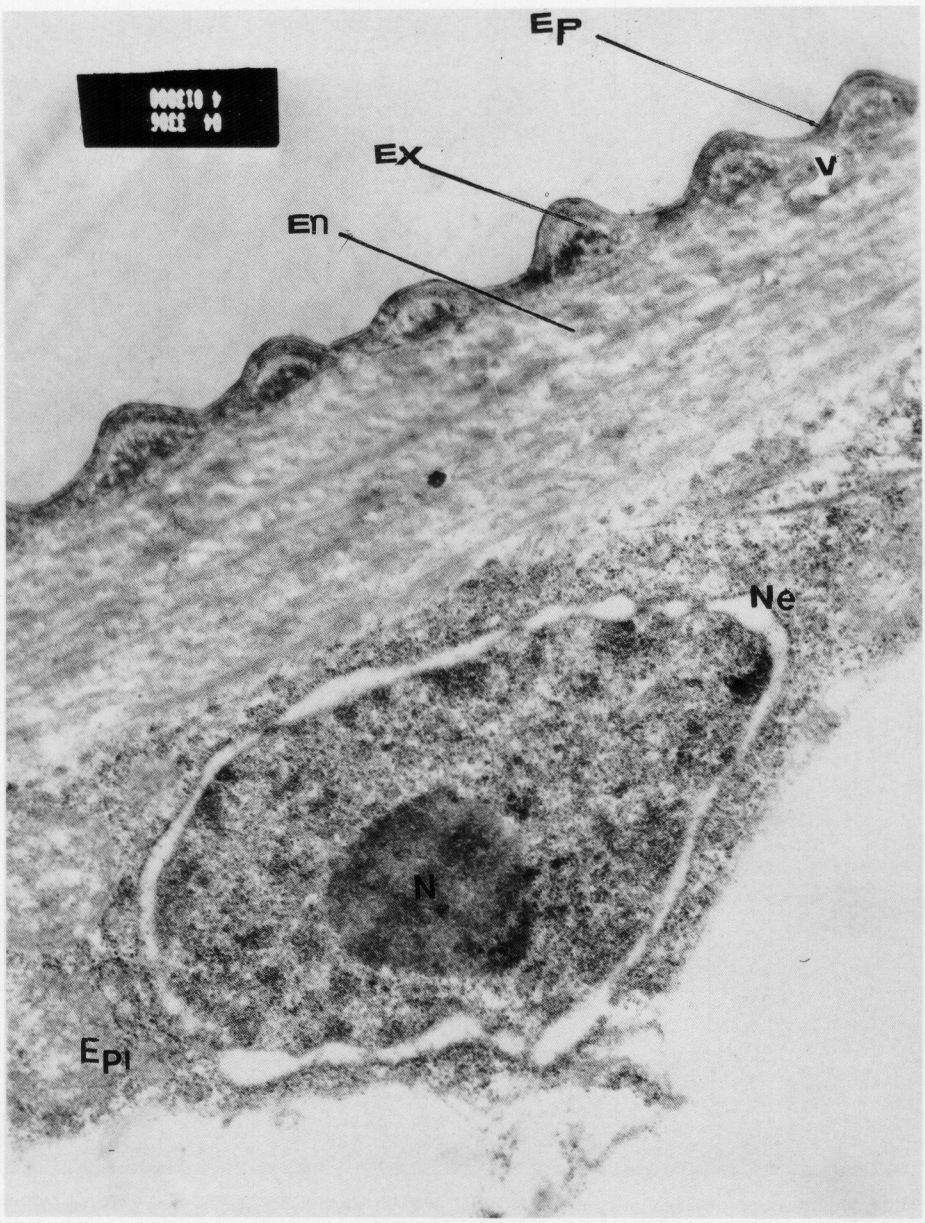


Fig. 4. Electron micrograph of the integument of 3-day-old 4th-stage larva of *Culex pipens* treated with pyriproxyfen showing presence of vacuoles (V), destruction of nuclear envelope (Ne), and epidermal layer (Ep1). Ep, epicuticle; Ex, exocuticle; En, endocuticle; N, nucleus. 13,000×.

tera: Calliphoridae) (Binnington 1985) and *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) (Hegazy et al. 1989a, 1989b; Degheele 1990).

On the other hand, pyriproxyfen caused drastic effects on the epidermal cells, where the nuclei shrank, nuclear membranes ruptured (Fig. 4), and vacuoles appeared in the cells. The mitochondria appeared swollen, enlarged, or even completely destroyed (Fig. 6). Similarly, hexafluron and teflubenzuron caused drastic effects on the mitochondria of larvae of *Spodoptera litoralis* (Bosid), in which case, many vacuoles and enlarged mitochondria could be seen (Hegazy 1990). The appearance of vacuoles can be considered to be an immune response stimulated by the exposure of larvae to the compound. Epidermal phagic vacuoles and vacuoles induced by cuticle abrasions have been document by Locke (1998).

After treating larvae of *Cx. pipiens* with pyriproxyfen, an increase generally occurred in the electron density of all the ultrastructural compo-

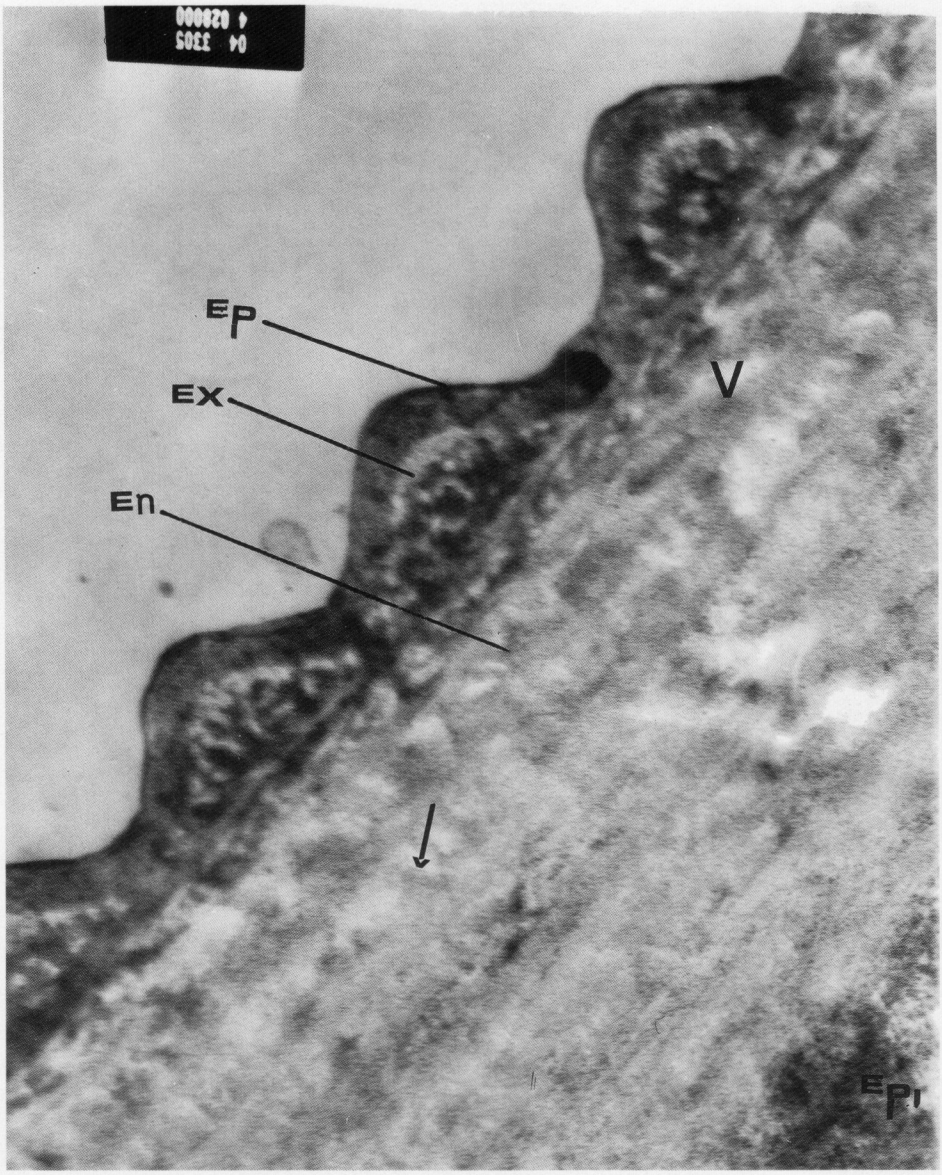


Fig. 5. High magnification of larval integument of 3-day-old 4th-stage larva of *Culex pipiens* treated with pyriproxyfen showing destruction of procuticle lamellae (arrow) and presence of vacuoles (V). Ep, epicuticle; Ex, exocuticle; En, endocuticle. 28,000×.

nents in the cytoplasm in addition to an increase in the number of lysosomelike bodies, which became more electron-dense (Fig. 6). Juvenile hormone III induces a similar effect on the hypopharyngeal glands of *Apis mellifera* (L.) Liu (1989); this author suggested that juvenile hormone activated the lysozyme system, which caused cytolysis. Cytolysis could eventually lead to degeneration of the epidermal layer. Feyereisen et al. (1981) concluded that precocenes trigger an extensive alkylation of the macromolecules in the corpora allata, and ultimately cause their destruction. The cells of the treated corpora allata show an aggregation of smooth endoplasmic reticulum and an increase in lytic structures (Cassier 1998).

The use of insect growth regulators as larvicides is a proven strategy in the control of some mosquito species. For instance Ali et al. (1995) demonstrated that the insect growth regulators diflubenzuron, methoprene, and pyriproxyfen have superior larvicidal activity against *Ae. albopictus,* as indicated by the low $LC_{90}$s in parts per billion. In addition, vector control programs in southeastern Queensland, Australia, showed great success against *Aedes vi-*

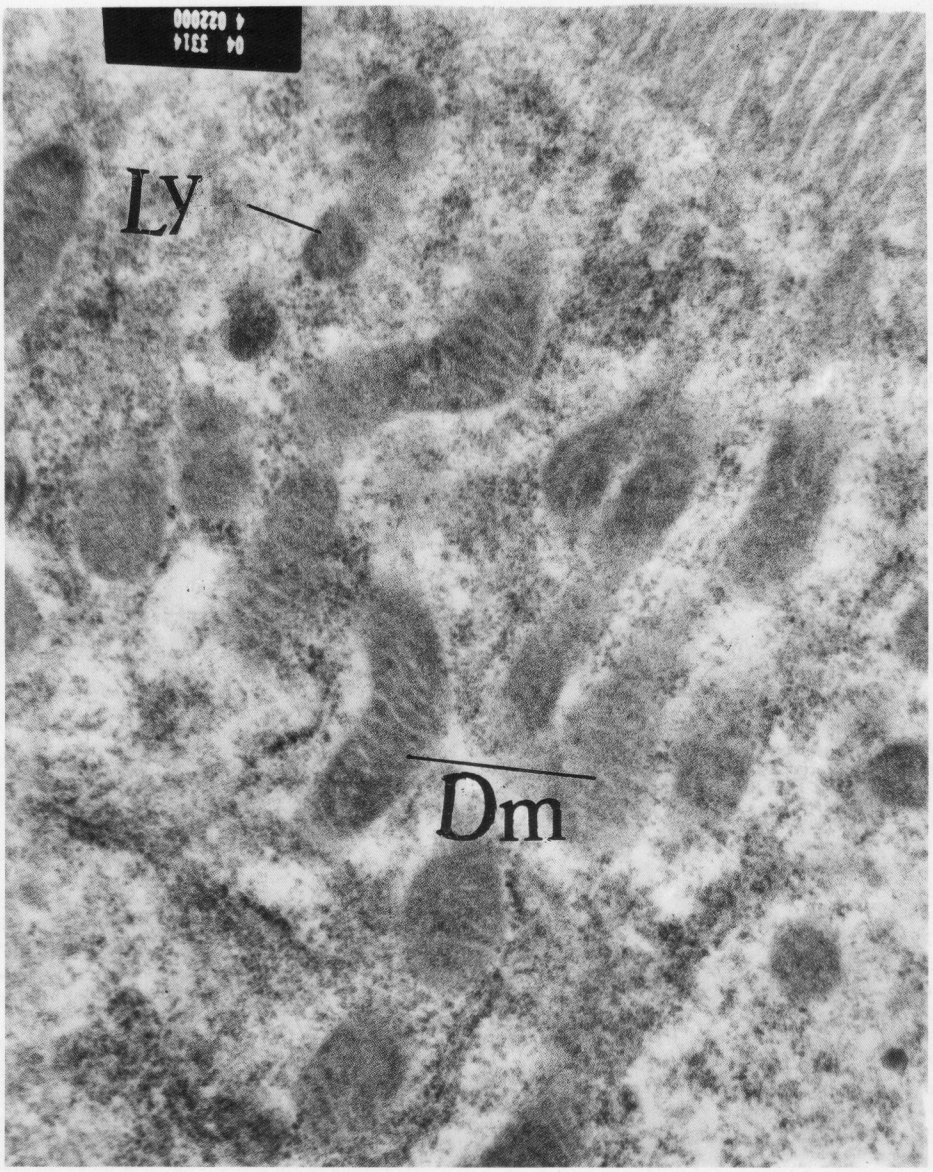


Fig. 6. High magnification of epidermal layer of pyriproxyfen-treated 3-day-old 4th-stage larva of *Culex pipiens* showing deformed mitochondria (Dm) and lysosomelike bodies (Ly). 22,000×.

*gilax* (Skuse) with *S*-methoprene (Ritchie et al. 1997). Finally, 2 conclusions can be reached from this investigation. First, comparison of our results with those obtained with chitin synthesis inhibitors indicated that treatment with pyriproxyfen results, at least partly, in similar symptoms. Second, the juvenile hormone mimic pyriproxyfen can be used as a larvicidal agent against *Cx. pipiens* over a wide range of temperatures.

## ACKNOWLEDGMENT

Grateful thanks are due to E. H. Shaurub (Professor of Insect Control, Entomology Department, Faculty of Science, Cairo University) for his technical assistance in the present work.

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