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Sterility and retardation of oocyte growth by penfluron in soapnut bug *Leptocoris coimbatorensis* (Gross) (Hemiptera, Coreidae)

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Abstract

Topical treatment of penfluron to the adults of *Leptocoris coimbatorensis* induced 52, 64 and 100 % sterility at 0.01, 0.1, and 1 % respectively. At 3 % concentration remarkable retardation of the reproductive system was observed and fecundity became nil. During early vitellogenic stages disorganisation of oocytes started and ovarioles became smaller than control ovarioles. By late vitellogenic stages the oocytes got resorbed in all ovarioles. In certain cases one or two gigantic

oocytes grew, but these eggs were retained in the body. Oviposition was not observed and there was no indication of any further development of second oocyte cycle.

1 Introduction

The discovery of the selective properties of certain N-phenyl-N-benzoyl ureas initiated new ways of insect suppression by interfering with moulting and reproduction. Most of the efforts on these compounds were concentrated on their larvicidal effects and much less efforts were devoted to their sterilizing effects on adults and utilization of this property for population control. Pest management could be best achieved by combining the toxic effects in immature stages with reproductive inhibition in adults.

Among the benzoylphenyl urea derivatives, diflubenzuron captured much attention as the most promising insect control agent. Later, an analogue of diflubenzuron, 1 (2-6-diflubenzoyl)-3-(4[trifluoromethyl]phenyl) urea known as, penfluron was reported to be more effective than diflubenzuron as a boll weevil sterilant (OLIVER et al. 1977), but only slightly more effective than diflubenzuron in the impregnated filter paper test and less effective in the surface treated petridish test on faceflies (PICKENS and DEMILO 1977). CHANG and BORKOVEC (1980) subsequently reported by using ^{14}C -labelled diflubenzuron and penfluron that penfluron was 1.8 times as active as diflubenzuron in preventing egg hatch of houseflies. Variable differences between sterilizing effect of penfluron and diflubenzuron were found in the azukibean weevil (NAGASAWA et al. 1978), southern pine beetle (RICHMOND et al. 1978) tsetsefly (JORDAN et al. 1979) and milkweed bug (REDFERN et al. 1980). Both diflubenzuron and penfluron apparently did not produce any physiological effect on the adults, but the biological effect of interfering with chitin deposition during moulting, appears to manifest itself in the early embryonic stages and causes failure of egg hatching. In the present study penfluron was selected to see its effect on the reproduction of soapnut bug, *Leptocoris coimbatorensis* (Gross).

2 Materials and methods

The sample of penfluron was obtained as a gift from Dr. A. B. BORKOVEC, Chief, Insect Reproduction Laboratory, USA. Different concentrations of penfluron solutions were prepared with acetone. The test insect *Leptocoris coimbatorensis* (Gross) (Hemiptera, Coreidae) was reared in the Laboratory at $28^\circ\text{C} \pm 2^\circ\text{C}$ in glass troughs. The mouths of the troughs were covered with a muslin cloth. Soaked soapnuts in petridishes were provided as food. Cages with late fifth instars were checked for adult emergence. Adults were removed in the evening and the cage was kept ready to receive the adults emerging overnight. The freshly moulted female insects were treated topically with penfluron ventrally on the abdominal region by an agla all-glass syringe attached to a micrometer. A control batch of females received solvent acetone. The applied volume was always kept constant as $1\ \mu\text{l}/\text{insect}$ in all cases. Treated insects were provided with food and allowed to mate with untreated, unmated males of the same age. Similarly control solvent-treated females were also caged with unmated normal males. The eggs from both treated and untreated females were collected in batches and kept separately for hatchability studies. The experiment continued for twenty days. For histopathological studies insects which received a higher dose like 3% were withdrawn from treated cages every 24 h/post treatment, ovaries dissected, stained and whole mounts made according to Gray's method (GRAY 1954). Normal untreated insects of the same age were also dissected for control studies.

3 Results and discussion

Leptocoris coimbatorensis possesses 7 ovarioles in each telotrophic ovary. Vitellogenesis is completed by 4th day of adult life and 1st batch of eggs are laid by 5th day. A female is capable of laying nearly 100 eggs in its life time.

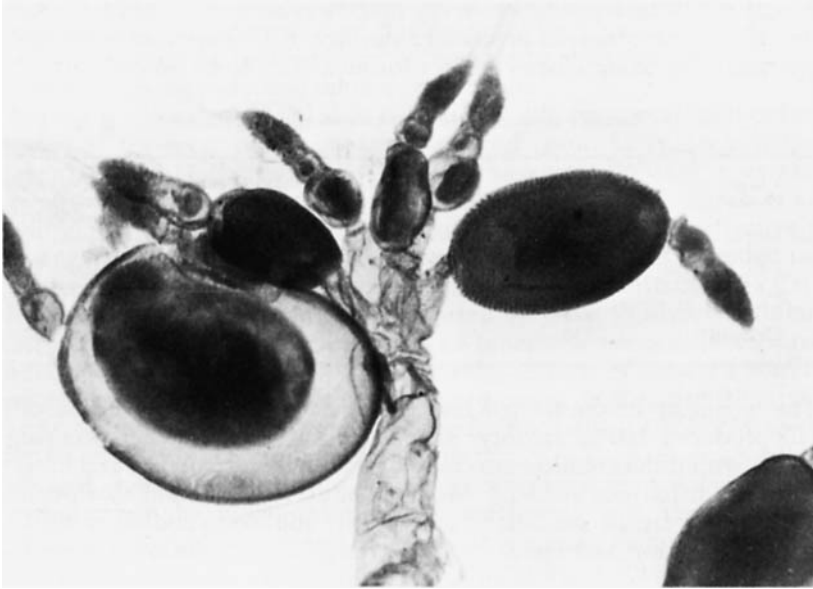
When fresh females received penfluron treatment, it was noticed that a concentration of 0.01 % induced 52 % sterility and with the increase of dosage the sterility also showed a rise. At a dosage of 0.1 % the sterility produced was 64 % and at 1 % total inhibition in egg hatch was achieved. The data presented in the table is the observed percentage sterility in *L. coimbatorensis* subjected to correction by using Chamberlain's formula (CHAMBERLAIN 1962).

Sterility induced in females treated with Penfluron

Dose (in conc.)	No. of eggs collected	No. of eggs hatched	Observed sterility (%)	Corrected sterility (CHAMBERLAIN'S formula) (%)
1 %	503	0	100	100
0.1 %	524	172	67.17	63.92
0.01 %	275	120	56.36	52.04
Control	530	482	9	-

The fecundity of the treated insect did not get affected at a dose of 1 % which produced 100 % sterility. KUNZ and BAY (1977) while working on hornflies with diflubenzuron also found no change in fecundity and longevity but the egg hatch was reduced. MOFFITT et al. (1983) found that penfluron acted only as a female sterilant when topically applied to codling moth *Cydia pomonella*. PICKENS and DEMILO (1977) reported that penfluron when fed to faceflies inhibited egg hatch but fecundity was unaffected. Pronounced sterilizing effect was manifested and reduction in egg production was observed in *Spodoptera littoralis* by RADWAN et al. (1978) after topical treatment with diflubenzuron. SARASUA et al. (1983) reported that ingestion of diflubenzuron by females of *Ceratitis capitata* resulted in a reduction in fecundity. The action was irreversible and this reduction was presumably directly related to interference with endocuticular deposition. SAXENA and MATHUR (1981) while working on eggs of *Tribolium castaneum* with benzoylphenyl urea compound, found that although the egg shell splits, the larvae failed to come out of the egg. The death of the offspring may be due to a lower degree of rigidity in cuticle which fails to resist the muscular traction during hatching thereby resulting in the death of larvae within the egg. The benzoylphenyl urea derivatives in general apparently show no physiological effects on the treated adult insect but their activity depended on their ability to infiltrate the eggs and interfere with embryogenesis (IVIE and WRIGHT 1978). Electron microscopic observation of embryos of *Leptinotarsa* contaminated with diflubenzuron via the female showed disturbed cuticle formation in the embryo resulting in an amorphous cuticular region instead of normal lamellate deposition patterns (GROSSCURT 1978). When the dose affecting egg hatch did not affect fecundity and longevity in *Leptocoris coimbatorensis*, we increased the dose to understand the changes which might be induced in the reproductive system. At a dose of 3 %, the insect did not lay eggs at all and still the longevity and mating behaviour was

not much affected. This dose was selected for further study on oocyte growth. Females were treated on the day of emergence and kept separately to observe the progressive changes in the ovary. It was observable from the dissection of the ovary made every 24 h post-treatment that disorganisation started during early vitellogenic stages and the ovarioles appeared much shorter and compressed when compared to control ovarioles. As vitellogenesis progressed, the oocytes were getting resorbed and in certain cases one or two eggs, gigantic in growth were observed (figure) and it appeared that all the physiological efforts



Abnormal oocyte growth in the ovary of 4th day treated adult

of the insect was concentrated to save these few eggs, at the expense of other eggs. But at no time of the life of the female these eggs were oviposited. In solvent treated control insects vitellogenesis was completed by 4th day and eggs were laid soon after. When the abnormal oocyte was studied under higher magnification in comparison with a control oocyte, it appeared that there were disturbances in the embryonic growth pattern. LIM and LEE (1982) found in grasshopper females when fed with diflubenzuron retardation of ovarian development causing an increase in terminal oocyte resorption. An aspect noticeable in *Leptocoris* was that at higher doses the females failed to oviposit. It appears that penfluron at higher doses apart from interfering with oocyte growth also prevents oviposition but not the mating behaviour. Penfluron produced pronounced ovarian inhibition in *D. cingulatus* at higher doses and caused sterility at lower doses without affecting mating behaviour (SATYANARAYANA et al. 1985). Diflubenzuron when topically applied to fresh fifth instars of *D. similis* produced ovarioles of unequal length with reduced oocytes or without oocyte development (ARUNAKUMARI et al. 1982).

The characteristic action of benzoylphenyl ureas on interfering with chitin deposition during metamorphosis appears to get extended to the early stages of embryonic development thus resulting in the failure of egg hatch. The success

of the compounds depends on their absolute activity in interfering with embryogenesis apart from larvicidal effects, an additional property which enhances its usefulness as a pest control agent. As penfluron acts as an insect sterilitant too, it can be rated as a chemical with promise in future.

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Zusammenfassung

Sterilität und Verzögerung des Oozyten-Wachstums durch Penfluron bei der Seifennuß-Wanze Leptocoris coimbatorensis (Gross) (Hemipt., Coreidae)

Topikale Applikation von Penfluron bei den Adulten von *L. coimbatorensis* verursachte 52, 64 und 100 % Sterilität bei 0,001, 0,1 und 1,0 % Konzentration. Bei einer Anwendung von 3 % wurde eine wesentliche Verzögerung der Entwicklung des Reproduktions-Systems und ein Absinken der Fruchtbarkeit auf 0 beobachtet. Während der frühen vitellogenen Stadien begann eine Desorganisation von Oozyten, wobei die Ovariolen kleiner als bei Kontrolltieren blieben. Bei späteren vitellogenen Stadien wurden die Oozyten in allen Ovariolen resorbiert. In einigen Fällen wuchsen 1 oder 2 Riesen-Oozyten heran, doch wurden sie im Körper zurückgehalten. Eine Eiablage wurde nicht beobachtet, auch gab es keine Anzeichen der Entwicklung eines sekundären Eizyklus.

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Forest Lepidoptera attracted to six synthetic pheromones in Czechoslovakia

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Abstract

The attractiveness of six synthetic pheromones to forest Lepidoptera was studied at two locations in Czechoslovakia during 1983. The synthetic attractants tested included pheromones for *Choristoneura fumiferana* (Clem.) (Tortricidae), *Eucosma sonomana* Kearf. (Olethreutidae), *Lymantria dispar* (L.) (Lymantriidae), *Orgyia pseudotsugata* (McD.) (Lymantr.), *Rhyacionia frustrana* (Comst.) (Olethr.) and *Rhyacionia buoliana* (Schiff.) (Olethr.). The attractant for *R. buoliana* was prepared in Czechoslovakia for use with European species but the other five materials are of North American origin and intended for use with species there. Sticky traps baited with these pheromones captured some 32 species in 26 genera with significant numbers of species as follows: Traps with the *C. fumiferana* attractant contained *Acleris sparsana* (Den. et Schiff.) (Tortr.) and *Cnephasia stephensiana* (Doubl.) (Tortr.). Those with the *E. sonomana* attractant contained *Enarmonia formosana* (Scop.) (Tortr.) and *Caradrina morpheus* (Hufn.) (Noctuidae). The *L. dispar* material attracted *L. monacha* (L.) and *L. dispar* (L.). Traps with the *O. pseudotsugata* contained *O. antiqua* (L.) and those with the *R. frustrana* pheromone contained *Cnephasia interjectana* (Haw.) and *Dichrorampha simpliciana* (Haw.) (Olethr.). Traps with the *R. buoliana* attractant contained *Gypsonoma oppressana* (Treit.) (Olethr.). Our results indicate that substantial species, genus and family cross attraction occurs with some of the synthetic sex attractants tested.

1 Introduction

Synthetically produced pheromones of specific Lepidoptera are often attractive to a number of species, and sometimes genera, other than the target organism. For example, the sex pheromone of the Douglas-fir tussock moth *Orgyia pseudotsugata* (McD), identified as (Z)-6-heneicosen-11-one, has been shown to be attractive to males of seven species of tussock moths, in two genera (GRANT and FRECH 1980). Also, STEVENS et al. (1985) reported that moths of 29 nontarget species of Tortricidae, representing 13 genera, were lured to synthetic pheromones of moths of the genera *Eucosma* and *Rhyacionia*.

A single compound also may attract different species at different geographic locations. For example, traps containing disparlure (*cis*,-7, 8-epoxy-2, methyl octadecane) synthesized from *Lymantria dispar* (L.) in the United States (BIERL et al. 1970) are attractive to the Nun Moth, *Lymantria monochoa* (L.) in Europe (SCHÖNHER 1972; SKUHRAVÝ et al. 1974), to *Zanclagnatha lunaria*