# AVERMECTINS: EFFECT OF SUBLETHAL DOSES ON OOCYTE DEGENERATION IN FEMALE *CADRA CAUTELLA* WALKER[1] (LEPIDOPTERA: PYRALIDAE)

P. T. M. Lum and W. R. Halliday
U. S. Department of Agriculture
Agricultural Research Service
Stored-Product Insects Research and Development Laboratory
P. O. Box 22909
Savannah, GA
(Accepted for publication 26 April 1988)

## ABSTRACT

Oocyte degeneration in *Cadra cautella* was reduced by 40 to 70% following injection with sublethal doses of avermectin $B_1$a and ivermectin. When the reproductive tract was dissected following treatment with avermectin, however, muscular contractions of the bulla seminalis were visually unaffected. This suggests that the inhibition of oocyte degeneration occurs not in the bulla seminalis, the site of oocyte degeneration, but elsewhere in the reproductive system, possibly in the ovaries or the oviducts. The differential response of the organs in the reproductive tract suggests that these organs may have potential in evaluating toxins affecting the visceral muscles.

Key Words: *Cadra cautella*, avermectin $B_1$a, ivermectin, sublethal doses, oocyte degeneration.

J. Entomol. Sci. 24(1): 21-25 (January 1989)

## INTRODUCTION

Avermectins, a new family of chemicals isolated from a soil microorganism, affect a number of nematode and arthropod parasites of animals (Campbell et al., 1983). Two compounds of this family, avermectin $B_1$a and ivermectin, also are promising in controlling infestations of non-parasitic insects. Beeman and Speirs (1983) found avermectin $B_1$a more effective than malathion in controlling *Tribolium* spp. Their observations over a period of time showed that suppression of progeny was one of the noticeable effects. Avermectin $B_1$a reduces fecundity in the red imported fire ant (RIFA), *Solenopsis invicta* Buren, by inhibiting ovarian development (Lofgren & Williams 1982; Glancey et al. 1982). Injections of avermectin $B_1$a or ivermectin into ixodid ticks reduced the viability of their eggs (Kaufman et al. 1986). Thus, the avermectins affect reproduction in several ways.

In some lepidopteran species, several organs in the female are involved in reproduction. Three, the bursa copulatrix, the bulla seminalis, and the ovaries with their oviducts, are surrounded by muscular sheaths consisting of circular and/or longitudinal muscles. Contractions of these muscles enable the organs to perform various functions. For example, the bulla seminalis is used to translocate sperm from the bursa copulatrix to the spermatheca, and also to break down oocytes during egg resorption (Lum 1979). The movement of oocytes during ovulation,

[1] Mention of a chemical does not constitute an endorsement or recommendation for its use by the U. S. Department of Agriculture. Accepted for publication 26 April 1988.

oviposition, and egg resorption are controlled by muscular contractions of the oviducts. In arthropods, ivermectin inhibits signal transmission at the neuromuscular junction apparently by increasing chloride ion permeability in the muscle membrane (Chalmers et al. 1986). The result is a paralysis that leads to death. This paper investigates the effects of sublethal doses of avermectin $B_1a$ and ivermectin and how they affect the bulla seminalis and oviducts which are involved in oocyte degeneration in the almond moth, *Cadra cautella* Walker.

## MATERIALS AND METHODS

Almond moths were reared in laboratory conditions at 30 ± 1°C, 60% RH, and alternating 12-h light and dark periods. Female moths were collected as they eclosed and isolated singly in plastic vials (29 × 80 mm).

Sublethal doses of avermectin $B_1a$ and ivermectin, 'Ivomec'(R), (Merck and Co., Rahway, NJ) were determined empirically by injecting 24-h old females with doses ranging from 0.015 pg to 2.5 ng in 0.5 microliter of solution that allowed 70-90% (LD 10-30) of the females to survive 6 days post injection. Two doses were then selected. For avermectin $B_1a$ 250 pg and 500 pg were selected and for ivermectin it was necessary to select lower doses of 1.5 pg and 0.15 pg. Avermectin $B_1a$ was dissolved in ethanol and diluted in insect saline to various concentrations for injection. Ivermectin formulated as 'Ivomec' in glycerol formal was diluted with glycerol formal instead of saline. Controls were injected with either an ethanol-saline mixture or glycerol formal. The technique for injection was suggested by A. O. Lea (personal communication). A fine glass needle which was drawn from capillary tubing was attached to one end of a length of rubber tubing. A small mouthpiece was attached to the other end of the tubing. The volume required for injection was dispensed by a microapplicator and transferred to the glass needle before each injection. Each insect was placed on the stage of a dissecting microscope and under a magnification of 20-30X, injected at the ventro-lateral margin of the second abdominal segment. The pressure to expel the fluid from the tip of the needle was provided orally through the mouthpiece. Each female was immobilized with cold five minutes before injection. Each injection was considered a success when the insect recovered within 10 minutes after injection and survived the six days post injection.

Oocyte degeneration was measured in the treated and control insects 6 days post injection. The bulla seminalis was removed from each female and its contents recorded in numbers of whole oocytes and/or shells.

To determine the effect of avermectin on sperm translocation (passage of sperm from the bursa copulatrix to the spermatheca), mated females were injected with 250 or 500 pg within 30 minutes after mating. Five hours later (time normally required for sperm translocation) each female was examined to determine whether sperm had reached the spermatheca. Only females with spermatophores in the bursa were scored as mated.

## RESULTS AND DISCUSSION

Virgin females injected with sublethal doses of avermectin experienced a 40-70% decrease in oocyte degeneration as measured by the number of shells and/or whole oocytes in the bulla seminalis (Table 1). Ivermectin, formulated as 'Ivomec',

was more effective. The concentration required for comparable reduction decreased by a factor of ca 3000. In females injected with 250 or 500 pg of avermectin, the number of degenerated oocytes/female was 2.3 and 3.2, respectively, significantly lower than the 8.7 oocytes/female in the control. Ivermectin decreased the oocyte count from 9.3 oocytes/female in the untreated control to 5.3 and 4.4 when treated with 0.15 and 1.5 pg/female, respectively. It appears that in this assay, avermectin had a delayed action effect. In almond moths oocyte degeneration usually begins about 24 hours post eclosion (Lum 1983) or at the time of injection. The few empty shells recovered from treated females showed that the action of avermectin was not immediate.

Table 1. Oocyte degeneration in the bulla seminalis of female *Cadra cautella* injected with sublethal doses of avermectin $B_1$a or ivermectin.

| | | Mean Number ± S.E.* | | | |
|---|---|---|---|---|---|
| Treatment | Dose (pg) | Shells | Whole Oocytes | Total (Shell/Oocytes) | Total Per Female |
| Avermectin$B_1$a | 250 | 19.2±3.7c | 3.0±0.5b | 22.2±6.8d | 2.2±1.4 |
| | 500 | 24.2±3.7c | 8.5±1.3ba | 32.7±7.2dc | 3.3±1.4 |
| Control | 0.0 | 78.0±3.3a | 9.7±1.1a | 87.7±5.8a | 8.8±1.2 |
| Ivermectin | 0.15 | 47.0±1.3b | 5.5±0.3b | 52.5±2.2b | 5.3±0.5 |
| | 1.5 | 40.5±1.6b | 3.7±0.5b | 44.2±2.9bc | 4.4±0.6 |
| Control | 0.0 | 80.5±3.0a | 12.5±2.4a | 93.0±9.8a | 9.3±1.8 |

* Mean ± S.E. of 4 replicates (10 ♀/replicate). Mean ± S.E. in each column followed by the same letter are not significantly different ($P \geq 0.05$, Duncan's multiple range test).

Although the muscular contractions of the bulla seminalis are instrumental in the breakdown of oocytes, there are reasons to suggest that the reduced rate of oocyte degeneration is not due to the bulla seminalis being affected. The presence of only the shells of a few oocytes (Table 1) in the bulla of treated females indicated that contractions of the bulla was not affected. Observations of the active bulla from treated females supported this. Placed in saline, the organ contracted vigorously and continuously, and the contractions were similar to those of the control females. In females injected with 250 - 500 pg of avermectin 30 minutes after mating, translocation of sperm from the bursa copulatrix to the spermatheca occurred in the normal time of 3 - 5 hours (250 pg: 93% translocation, n = 28; 500 pg: 96%, n = 29; control: 96%, n = 29). Thus, successful transfer of sperm was another sign that the bulla was unaffected, at least during the first few hours after injection, since contractions of the bulla govern the movement of sperm from the bursa copulatrix to the spermatheca. Also, had the muscular function of the bulla been inhibited, there would have been an accumulation of only whole oocytes in the bulla. None of the treated females showed this condition.

The reduced rate of oocyte degeneration in the treated females was more likely due to the toxic effect of avermectin on the ovaries or oviducts which interrupted the flow of oocytes from the ovaries to the bulla seminalis. This probably accounts for the lower number of whole oocytes found in most of the treated females (Table 1). Except for the group of females treated with 500 pg of avermectin $B_1$a, the numbers of whole oocytes in treated females were significantly lower than those of the controls. Observations of the muscular activity of oviducts isolated in saline

from a dozen treated and control females showed that oviducts from treated females (injected with 500 pg of avermectin) contracted slowly and irregularly, compared to rapid, continuous contractions of oviducts from control females during a 20 min period.

However, until the stimulus and mechanism which initiate and control the flow of oocytes from the ovaries to the bulla seminalis are determined, observations of isolated tissues in saline are at best only superficial and insufficient for one to draw any conclusion regarding the effect of avermectins on the muscles of the oviducts.

In insects, sublethal doses of avermectin inhibit feeding, locomotion, molting, and various aspects of reproduction such as fertilization and oviposition (Strong and Brown 1987). On the cellular level, one of the effects of avermectin is that it amplifies GABA action at the neuromuscular juctions (Wright 1986). Thus, in inhibiting reproduction, avermectin may be affecting the muscles of the ovaries and oviducts. The reduced oocyte degeneration that was observed in this study strongly suggests that the visceral muscles of the oviducts may be a target of avermectin. Oocyte degeneration, which depends on the muscular activity of several organs, may be a means of evaluating toxins that affect specific visceral muscles. The selective action of toxins on different muscles is not unprecedented since toxin from *Bracon hebetor* Say paralyzes somatic muscles but allows the visceral muscles of the heart and digestive system in the prey to function (Beard 1952).

## ACKNOWLEDGMENTS

The authors thank J. L. Robertson, Biological Laboratory Technician at this laboratory, for her technical assistance; Dr. K. Burnham for his assistance in statistical analysis; Drs. U. E. Brady and D. V. Hagan for critical reviews of manuscript.

## LITERATURE CITED

Beard, R. L. 1952. The toxicology of *Habrobracon* venom: A study of a natural insecticide. Conn. Agric. Exp. Sta. Bul. 562: 1-27.

Beeman, R. W. and W. E. Speirs. 1983. Toxicity, persistence, and antagonism of Avermectin $B_1$a against stored - product insects. Proc. 3rd Intern. Conf. Stored - Product Entomol. p. 246-55.

Campbell, W. C., M. H. Fisher, E. O. Stapley, G. Albers - Schonberg and T. A. Jacob. 1983. Ivermectin: A potent new antiparasitic agent. Science 221: 823-28.

Chalmers, A. E., T. A. Miller, and R. W. Olsen. 1986. The actions of avermectin on crayfish nerve and muscle. Eur. J. Pharmocol. 129: 371-74.

Glancey, B. M., C. S. Lofgren, and D. F. Williams. 1982. Avermectin $B_1$a: Effects on the ovaries of red imported fire ant queens (Hymenoptera: Formicidae). J. Med. Entomol. 19: 743-47.

Kaufman, W. R., S. G. Ungarian, and A. E. Noga. 1986. The effect of avermectins on feeding, salivary fluid secretion, and fecundity in some Ixodid ticks. Exp. & Appl. Acarology 2: 1-18.

Lofgren C. S., and D. F. Williams. 1982. Avermectin $B_1$a: A highly potent inhibitor of reproduction by queens of the red imported fire ant (Hymenoptera: formicidae). J. Econ. Entomol. 75: 798-803.

Lum, P. T. M. 1979. Degeneration of ova in the bulla seminalis of Lepidoptera. J. Insect. Physiol. 25: 595-99.

Lum, P. T. M. 1983. Oocyte degeneration in *Plodia interpunctella* Hubner and *Cadra cautella* (Walker) (Lepidoptera: Pyralidae): Influence of temperature and humidity. Environ. Entomol. 12: 1539-41.

Strong, L. and T. A. Brown. 1987. Avermectins in insect control and biology: a review. Bull. Entomol. Res. 77: 357-89.

Wright, D. E. 1986. Biological activity and mode of action of avermectins. pp. 174-202 in Ford, M. G., Lunt, G. G., Deay, R. C., and Usherwood, D. N. R. (Eds.). Neuropharmacology and Pesticide Action. 512 pp. Chichester, Ellis Horwood.