TARGET SITE RESISTANCE TO \PYRETHROIDS IN LARVAL AND ADULT TOBACCO BUDWORMS, HELIOTHIS VIRESCENS

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Abstract

Reduced neuronal sensitivity (= target site resistance) to the pyrethroid, allethrin was detected in tobacco budworms collected from the Red River (RR) and Macon Ridge (MRS) field stations in northern Louisiana during the 1995 cotton season. Expression of this resistance mechanism was similar between male and female larvae but differed between sexes in adults from the MRS location. No differences in expression were measured between male and female adults collected at the RR site. In addition, patterns of expression were compared between larvae and adults collected during the early- and late-season at the MRS location. Although expression of target site resistance in larvae was greater in the late- than early-season collection, frequencies of expression in adults were similar for the two collections. Finally, expression of this mechanism was compared between adults that were collected at the same location from either pheromone traps or with sweep nets. Differences in frequency of expression of target site resistance were found between pheromone-trapped and sweep-netted adults from MRS but not RR, suggesting potential differences in toxicological attributes of insects collected by these two methods. The significance of these findings with respect to the utility of the adult vial test as a monitoring tool is discussed.

Introduction

A major factor contributing to the pest status of the tobacco budworm, Heliothis virescens (F.) (Lepidoptera: Noctuidae) is its resistance to all modern groups of insecticides (Elzen et al., 1992; Sparks et al., 1993). Whereas the synthetic pyrethroids remain the most widely used insecticides for the control of cotton pests, pyrethroid resistance in H. virescens has developed throughout the Americas (Ernst and Dittrich, 1992) and threatens the economical production of cotton.

Since 1987, monitoring of pyrethroid resistance in U. S. populations of H. virescens has been based on mortality of pheromone-trapped male moths as measured in insecticide-coated glass vials (Adult Vial Test; AVT)(Plapp et al., 1986). An assumption of this widely-adopted test is that expression of resistance (and associated resistance mechanisms) in pheromone-trapped moths is representative of that in larvae, the developmental stage responsible for crop damage (Plapp et al., 1990). In addition, the utility of the AVT as a monitoring tool is based on the assumption that response to pheromone is equivalent between insecticide-susceptible and -resistant moths, a premise that has been questioned (McCutchen et al., 1989) but not rigorously tested.

Physiological and biochemical mechanisms of resistance to pyrethroids have been studied in larval H. virescens and include delayed penetration of the insecticide, enhanced metabolism by mixed function oxidase and esterase enzymes and reduced neuronal sensitivity resulting from altered target sites (McCaffery et al., 1991; Ottea et al., 1995a). The expression of these resistance mechanisms in U. S. field-collected H. virescens appears to be dynamic (Ottea et al., 1995a). Reduced neuronal sensitivity was expressed with high frequency in larvae collected during the 1990 and 1991 cotton seasons (Gladwell et al., 1990; McCaffery et al., 1991; McCaffery et al., 1995), but declined in frequency throughout the 1992 and 1993 cotton seasons, a decline which coincided with a concomitant increase in expression of metabolic resistance (Ottea et. al., 1995a). Despite their importance to the validity of the AVT as a monitoring tool, pyrethroid resistance mechanisms in adult tobacco budworms have not been studied until recently (Holloway and McCaffery, 1994; Holloway and McCaffery, in press).

The objective of this research was to measure and compare expression of target site resistance between field-collected larval and adult tobacco budworms. In addition, frequencies of target site resistant individuals were compared between adult insects that were collected from a single location in pheromone traps and sweep nets. Results of this study are discussed with respect to the validity of the AVT as a method for monitoring pyrethroid resistance in populations of tobacco budworms.

Materials and Methods

Insects: Field strains of H. virescens were collected in 1995 during June and September from an unsprayed site at the Macon Ridge (MRS) location of the Northeast Research Station (Winnsboro, LA). In June, larvae were collected as neonates and adults were collected using sweep nets from a cultivated stand of velvet leaf (Abutilon theophrasti) and, in August, eggs and neonate larvae were collected from cotton. During September, immature insects were collected as eggs from cotton, and adults were collected by pheromone trap or sweep net from an adjacent wild stand of Dallas grass (Paspalum notatum). Insects were also collected during August from a single location near the Red River Research Station (RR; Bossier City, LA) as either eggs from cotton foliage, or as adults from pheromone traps or sweep netting. This site received repeated applications of pyrethroid, organophosphate and carbamate insecticides.

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A reference laboratory strain of susceptible (LSU) H. virescens was used throughout this study and has been maintained in the laboratory without exposure to insecticides for 19 years.

Insects were reared in the laboratory as described previously (Leonard et al., 1988) and assays were performed on field strain insects during their first 3 generations in the laboratory. Gender of test insects was determined based on the presence or absence of yellow testicular sacs (for larvae) or the ovipositor (for adults). For experiments in which neuronal sensitivity was compared between developmental stages, collections of immature insects were used as the source for both larval and adult test insects, and comparisons were made within the same generation.

Toxicity measurements: Susceptibility of larvae to cypermethrin (trans/cis mixture, 94% purity) was measured by topical bioassay using a diagnostic dose (1.75 μg/insect) of insecticide. This dose corresponds to 6 times the LD_{80} measured for susceptible, LSU larvae. Cypermethrin was applied in 1 μl of acetone to the thoracic dorsum of larvae and mortality (defined as the inability for coordinated movement within 30 seconds of being prodded with a sharpened pencil) was assessed after 72 hrs. Control insects were treated with acetone alone. Results presented represent means of two determinations, each with 10 larvae.

Neurophysiological assay for neuronal sensitivity: Extracellular recordings of spontaneous activity were made from peripheral nerves from developmentally synchronous adults and fifth instar larvae of each strain. Larvae (150-200 mg) were decapitated, opened dorso-medially and pinned out on a wax dish. The inner surface of the body wall and its associated nervous tissue was exposed by dissection and bathed in saline. Adult moths (<5 days post-emergence) were decapitated and their wings removed before being pinned onto a wax dish. A longitudinal incision was made through the dorsal side of the abdomen and the exposed body wall, reproductive tissues and associated nervous system were bathed in saline. Measurements of spontaneous neuronal activity were made from nerves in the lateral wall of the abdomen using a suction recording electrode. Extracellular activity was amplified and filtered with a high gain, low noise amplifier (Warner Instruments, Hamden CT) before relay to either MacLab-2e or -4 data recording and analysis instruments (Analog Digital Instruments, Milford, MA) interfaced with a Macintosh computer.

Nerve preparations were exposed to a range of allethrin concentrations (0- 100 μM) using a cumulative dose-response assay of Gladwell et al. (1990) as modified by Ottea et. al (1995a, b). The number of action potentials discriminated above a threshold was recorded over 3 min. periods, first with the preparation in bathed in saline alone, followed by a 3 min. control period in saline containing 0.2% ethanol. Nerve preparations were then exposed to successive perfusions of saline containing increasing concentrations of allethrin dissolved in ethanol. The endpoint of the assay was defined as the lowest concentration of allethrin at which the mean frequency of action potentials was over 2 times greater than the mean value during the ethanol control period. Recordings were made from at least 30 larvae or adults from each strain.

Results and Discussion

Cypermethrin Resistance: High levels of resistance were measured in larvae from the three MRS collections (data not shown). In bioassays with MRS-June (F2) insects, only 33% mortality was observed following treatment with the diagnostic dose of cypermethrin. In addition, levels of mortality decreased throughout the growing season to 21.1% (MRS- September F1). This trend was qualitatively similar to that measured from earlier collections at this site (Ottea et al., 1995a; Ibrahim and Ottea, 1995) and reflects a seasonal increase in expression of pyrethroid resistance at this location (Leonard et al., 1991; Graves et al., 1993; Martin et al., 1994).

Target Site Resistance in Larvae and Adults: Levels of target site sensitivity to allethrin were lower in larvae from all field collections than in LSU larvae. Whereas 100% of the LSU larvae tested responded at an allethrin concentration of 0.1 μM (Figure 1), only 70% response was measured at this concentration in tests with MRS-June (F3) insects (Figure 2). In addition, 10% of the larvae sampled from the MRS-June collection did not respond in assays with 100 μM allethrin, the highest concentration of insecticide used in these tests. Target site sensitivity was even lower in late-season, MRS-September (F1) larvae, in which only 50% responded at 0.1 μM allethrin and 13% failed to respond at 100 μM (Figure 3).

Higher concentrations of allethrin were necessary to elicit responses from adults than larvae from the LSU strain (Figure 4). Whereas all LSU larvae were sensitive at concentrations $\leq$ 0.1 μM, treatment with allethrin concentrations of 1- 100 μM was required to measure a full range of responses in adult insects from this strain. This difference between life stages is likely a result of disparities between larval and adult preparations (e.g. size and tissue composition of the insects) rather than intrinsic differences in target site sensitivity. Because 7% of LSU adults responded at 100 μM allethrin, adults from field collections that did not respond at this high concentration were considered to be target site resistant.

Despite this relatively high discriminating concentration, target site resistance was evident in adult tobacco budworms. In the early-season MRS-June insects, a clear shift in target site sensitivity was measured relative to LSU adults (Figure 5). Whereas all LSU adults responded at

concentrations ≤ 100 μM, 10% of MRS-June insects were not sensitive at these concentrations. An additional 27% of MRS-June adults did not respond at concentrations < 100 μM (compared with 7% of LSU adults). Similarly, 10% of MRS-September adults did not respond at allethrin concentrations ≤ 100 μM (Figure 6), but only 10% failed to respond at concentrations < 100 μM. In contrast to results from larval assays, no increase in the frequency of target site resistance was measured between MRS-June and -September adults.

Target Site Resistance in Male and Female Tobacco Budworms: Expression of target site resistance was similar between male and female larvae from the MRS collections (Figure 7). Regardless of the sex, ca. 60% of larvae were sensitive to allethrin at the lowest concentration tested (0.1 μM) and no response at concentrations ≤ 100 μM was measured in 15% and 12% of female and male larvae, respectively. In MRS adults, however, patterns of expression of target site resistance varied somewhat between male and female insects (Figure 8), especially at allethrin concentrations ≥ 10 μM. Of the insects that were highly target site resistant (i.e. did not respond at allethrin concentrations ≥ 100 μM), 11% were female and 16% were male. Fewer dissimilarities were measured between sexes of insects tested from the RR collection (Figure 9), in which similar frequencies of target site resistance were measured between sexes (12% and 13% for female and male adults, respectively).

Target Site Resistance in Pheromone-Trapped and Sweep-Netted Adults:
Expression of target site resistance was compared between adults that were collected from the same location by sweep net and pheromone trap. In tests with adults collected at the RR site (Figure 10), frequency distributions of neuronal sensitivity were similar between the two collections: for both pheromone-trapped and sweep-netted individuals, 13% of the populations failed to respond at concentrations ≤ 100 μM (i.e. were target site resistant). These results are similar to those from a previous experiment at this site (Ottea et al., 1995b) in which target site sensitivity in adults reared from field-collected larvae was similar to that of pheromone-trapped adults. In contrast, results from parallel tests at the MRS site suggest that target site sensitivity of pheromone-trapped adults may not be representative of that in local populations. At the MRS location, only 7% of the pheromone-trapped adults were target site resistant whereas 19% of adults collected with sweep nets from the same location expressed this resistance mechanism (Figure 11). This result suggests that, at the MRS site, populations sampled in pheromone traps may differ from those in the field. A possible explanation for this finding is that target site resistant individuals do not respond to pheromone as readily as susceptible adults (McCutchen et al., 1989). However, results from the RR site (where target site resistance was also expressed in adults) do not support this hypothesis. Alternatively, if the population structure surrounding a pheromone trap is sufficiently heterogeneous, it is possible that a number of different populations are represented by pheromone-trapped individuals whereas only local populations are sampled with the sweep net. Because insecticide selection was not as severe at MRS compared with RR, greater heterogeneity in population structure might be expected at the former location. Given our scant knowledge of the population dynamics of this pest insect, further research is required to explain adequately these findings.

Summary: Target site resistance was in expressed in larval H. virescens collected from the MRS site in northern Louisiana. This resistance mechanism was also expressed in adult insects at this location, but frequencies of expression in early- and late-season collections were dissimilar between larvae and adults. Further, expression of target site resistance differed between sweep-netted and pheromone-trapped adults at this location suggesting differences in toxicological attributes of the populations sampled using the two methods. However, such differences in expression of target site resistance between sampling methods were not evident at a second, more heavily-sprayed site.

Taken together, these results illustrate potential shortcomings associated with the utility of the AVT for resistance monitoring. However, the value of this methodology is not diminished despite suggestions that use of pheromone-trapped individuals may not provide the most accurate portrayal of larval resistance. Reduced neuronal sensitivity is expressed and contributes to resistance in pheromone-trapped adults, and monitoring of this life stage provides a less labor-intensive technology than alternative methods using larvae.

Acknowledgments

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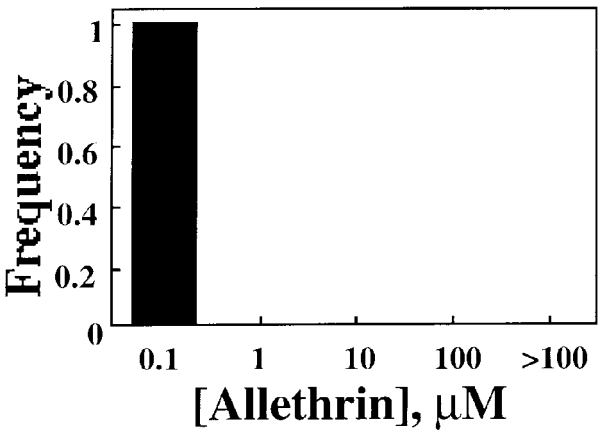

Figure 1: Concentrations of allethrin required to elicit a two-fold increase in frequency of spontaneous firing in larval H. virescens (LSU strain).

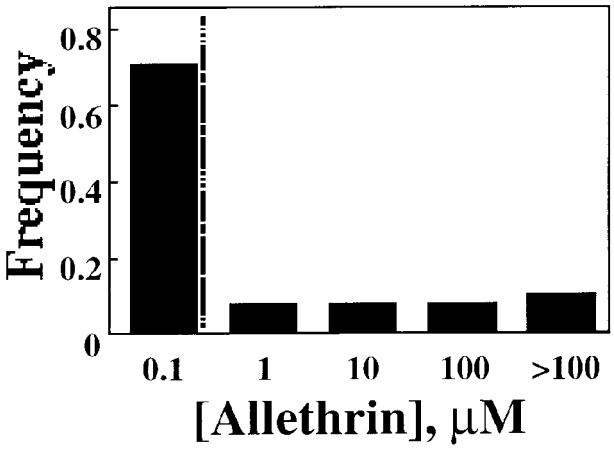

Figure 2: Concentrations of allethrin required to elicit a two-fold increase in frequency of spontaneous firing in larval H. virescens (MRS- June).

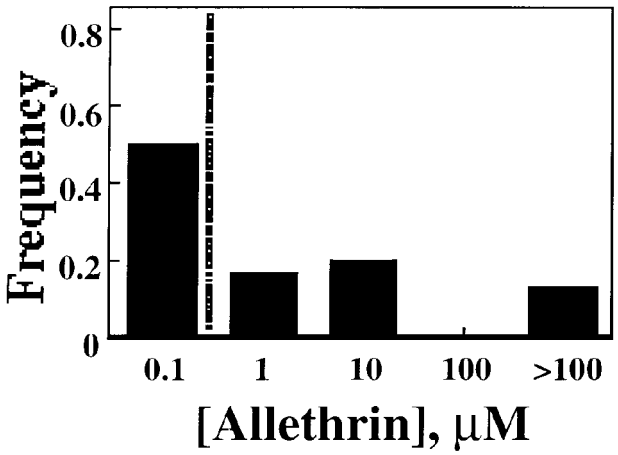

Figure 3: Concentrations of allethrin required to elicit a two-fold increase in frequency of spontaneous firing in larval H. virescens (MRS-September).

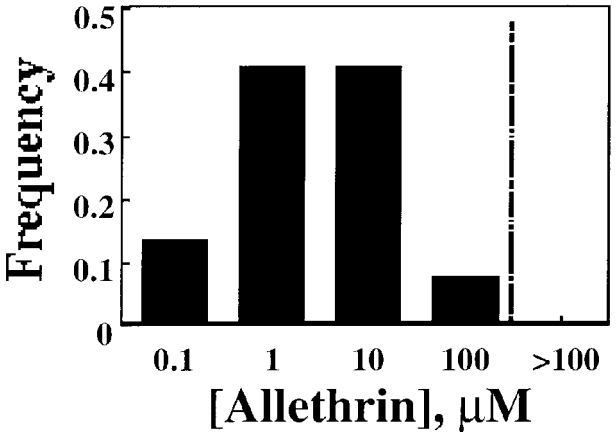

Figure 4: Concentrations of allethrin required to elicit a two-fold increase in frequency of spontaneous firing in adult H. virescens (LSU strain).

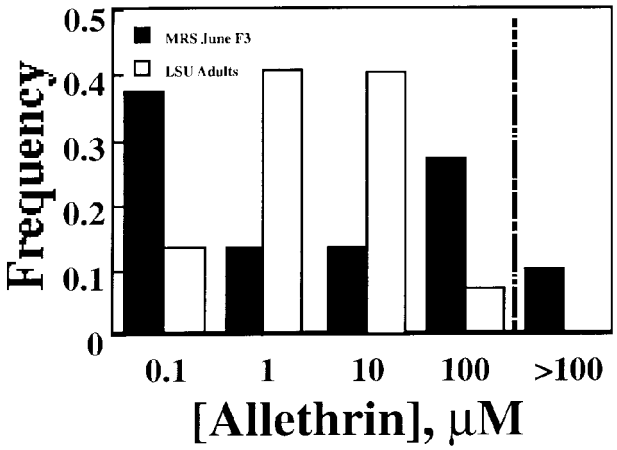

Figure 5: Concentrations of allethrin required to elicit a two-fold increase in frequency of spontaneous firing in adult H. virescens from MRS-June (solid bars) and LSU strains (open bars).

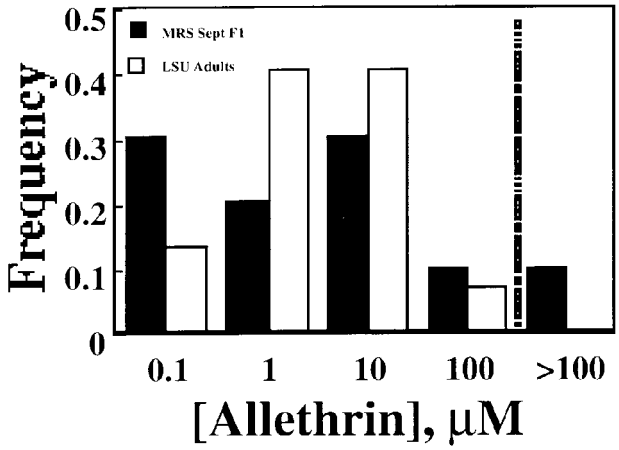

Figure 6: Concentrations of allethrin required to elicit a two-fold increase in frequency of spontaneous firing in adult H. virescens from MRS-September (solid bars) and LSU strains (open bars).

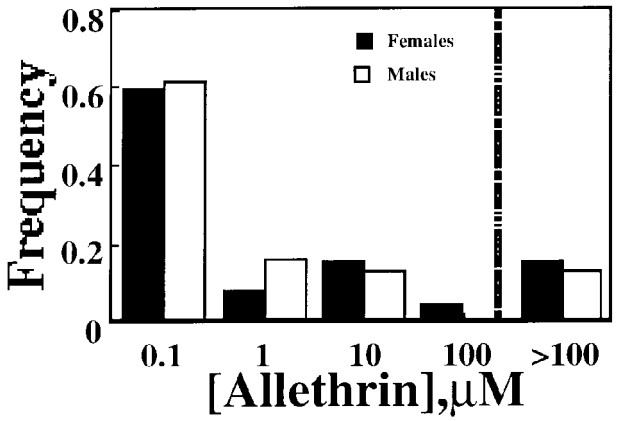

Figure 7: Concentrations of allethrin required to elicit a two-fold increase in frequency of spontaneous firing in larval H. virescens. Results from female (solid bars) and male (open bars) larvae from MRS-June and -September collections were pooled for analysis.

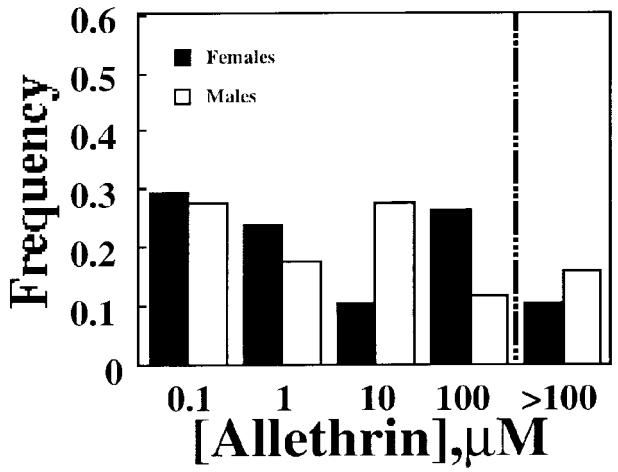

Figure 8: Concentrations of allethrin required to elicit a two-fold increase in frequency of spontaneous firing in adult H. virescens. Results from female (solid bars) and male (open bars) adults from MRS-June and -September collections were pooled for analysis.

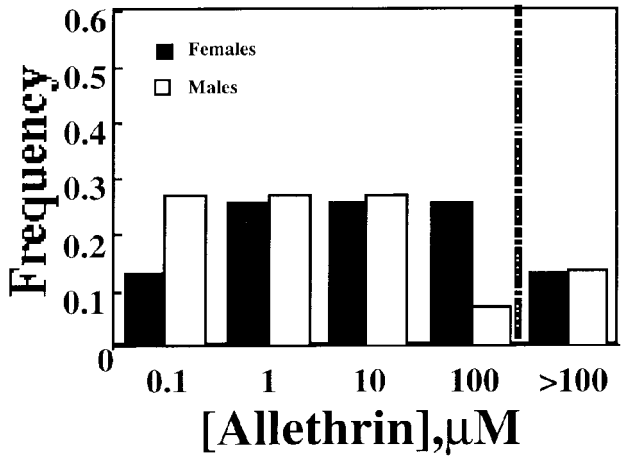

Figure 9: Concentrations of allethrin required to elicit a two-fold increase in frequency of spontaneous firing in adult female (solid bars) and male (open bars) H. virescens from the RR- August strain.

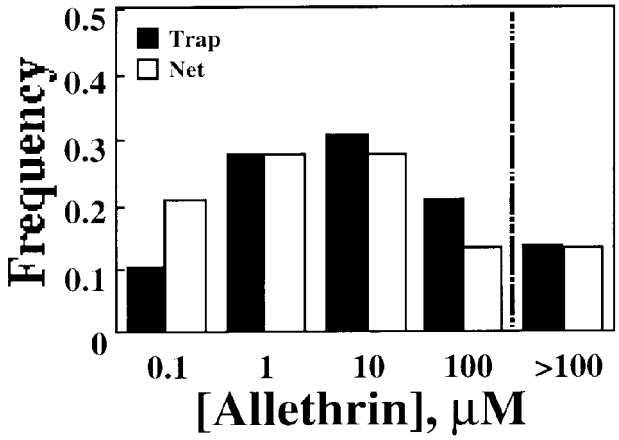

Figure 10: Concentrations of allethrin required to elicit a two-fold increase in frequency of spontaneous firing in H. virescens adults that were pheromone-trapped (solid bars) or sweep-netted (open bars) from the same location (RR- August collection).

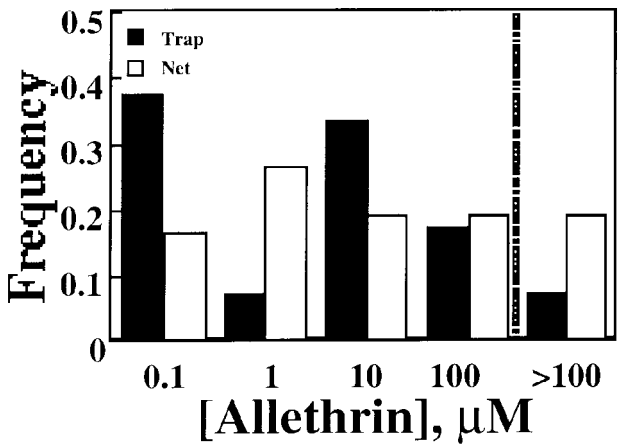

Figure 11: Concentrations of allethrin required to elicit a two-fold increase in frequency of spontaneous firing in H. virescens adults that were pheromone-trapped (solid bars) or sweep-netted (open bars) from the same location (MR-September collection).