Monitoring Permethrin Resistance in Soybean Looper (Lepidoptera: Noctuidae) Adults[1]

J. S. Mink, D. J. Boethel, and B. R. Leonard

Department of Entomology, Louisiana Agricultural Experiment Station
Louisiana State University Agricultural Center
Baton Rouge, LA 70803

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ABSTRACT A glass vial monitoring technique was developed to test for permethrin resistance in soybean looper, *Pseudoplusia includens* (Walker), adults. Male and female moths from laboratory permethrin-resistant and -susceptible soybean looper strains were exposed to permethrin residues in glass vials to estimate concentration-mortality lines. Probit analysis of results indicated that male and female LC_{50} values were not significantly different within each strain. The resistant male and female moths were 13.4 and 12.2-fold more tolerant of permethrin, respectively, when compared to the same sex from the susceptible strain. A discriminating concentration of 2.5 µg permethrin per vial was identified for detection of permethrin resistance in soybean looper adults. Male soybean looper moths were captured during the 1991 growing season at three locations in Louisiana using wire cone traps baited with pheromone. Results of discriminating concentration tests on these moths indicated that all strains were resistant to permethrin, but frequencies of resistance varied within locations over time.

KEY WORDS Insecta, *Pseudoplusia includens,* resistance, soybean looper, permethrin.

The soybean looper, *Pseudoplusia includens* (Walker), is a migratory insect pest of soybean, *Glycine max* (L.) Merrill, in the southeastern United States. Mitchell et al. (1975) suggested that potentially damaging populations of soybean loopers in Georgia and South Carolina resulted from populations that migrated from overwintering sites in southern Florida. Overwintering populations occur in southern Texas, also, but annual immigrations from Central or South America and the islands of the Caribbean are suspected to occur (Herzog 1980). Soybean producers presently rely upon a limited number of insecticides to control the soybean looper. A pyrethroid insecticide, permethrin, has been the choice among growers for controlling this pest because of broad spectrum activity at a relatively low cost. However, wide-spread use of the pyrethroids in cotton-soybean agroecosystems has contributed to the development of permethrin resistance in the soybean looper (Leonard et al. 1990, Felland et al. 1990, Mink and Boethel 1992).

With the onset of permethrin resistance, a southern region soybean looper pheromone trapping project was initiated in 1990 to monitor seasonal activity of soybean looper adults in the region. This information is important in

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understanding the movement of this pest, but it does not provide information on the frequency of permethrin resistance in these populations. Therefore, if a resistance monitoring technique for soybean looper adults could be incorporated into this project, information on resistance frequencies also could be generated. A similar project monitoring pyrethroid resistance in tobacco budworm, *Heliothis virescens* (F.), throughout the cotton growing regions of the U. S. has been successful (Plapp et al. 1990). The objective of the study reported herein was to develop a quick bioassay technique for detecting and monitoring permethrin resistance in soybean looper adults so that information on permethrin resistance frequencies could be obtained throughout the southeastern U. S.

Materials and Methods

The development of a permethrin resistance monitoring technique for soybean looper adults followed methods similar to those developed for *Heliothis virescens* (Plapp et al. 1987, 1990). Analytical grade permethrin (60:40 cis:trans; FMC Corporation, Middleport, NY) was dissolved in acetone and diluted to six concentrations (0.25, 0.5, 1.0, 2.5, 5.0, 10.0 μg per vial) plus an acetone control. The glass vials were prepared by pipeting 0.5 ml of solution into each vial. Vials then were rolled on a mechanical roller (Star Manufacturing Company Model 45, St. Louis, MO) until the acetone evaporated, leaving the permethrin residue evenly distributed on the interior of the vial.

Initial assays were conducted using male or female adults from laboratory strains of permethrin-susceptible and -resistant soybean loopers to establish base-line data and identify a discriminating concentration to be used in subsequent field studies. The permethrin-susceptible adults were obtained from a laboratory strain maintained at the USDA-ARS Southern Field Crops Research Insect Laboratory at Stoneville, MS. This colony was begun in 1981 with moths from a South Carolina laboratory colony, and although it has been supplemented periodically with field-collected larvae, it has been maintained in continuous culture without exposure to insecticides. The permethrin-resistant laboratory strain was begun with soybean looper larvae collected from soybean during the 1990 growing season at Blackville, Barnwell County, SC after a control failure using permethrin (0.17 kg [AI]/ha). Since the initial collection, the colony was maintained in the laboratory without exposure to insecticides, and the adults used in this study were from the F_4 generation. Topical bioassay (Mink and Boethel 1992) results on larvae from the F_4 generation indicated a permethrin LD_{50} = 1.24 μg, approximately 21-fold greater than the LD_{50} for the permethrin-susceptible strain (LD_{50} = 0.06 μg).

Soybean looper larvae were sexed during the prepupal stage. The yellow primordial testes in abdominal segment 7 of male larvae were used to distinguish them from females (Shour and Sparks 1981). Pupae were placed in cardboard cartons (3.8 liters) covered with cotton gauze. Adults were fed a 10% sucrose solution. One day old male or female moths from each strain were exposed to a series of permethrin concentrations by placing a single moth in each vial. Vials then were loosely capped and held at room temperature (23-27°C). Moths were scored after 24 h as normal or knocked down. This criterion is more practical than death because poisoned adults may survive for several days before death

occurs (Plapp et al. 1990). Moths were scored as being knocked down if they were unable to fly or could only fly a short distance (< 3 m) when tossed into the air. Concentration-mortality regressions were estimated for each sex from the vial data by probit analysis using POLO-PC (LeOra Software, Berkely, CA 1987). All data were corrected for control mortality by Abbott's (1925) formula. Differences among strains were considered significant if 95% confidence limits (CL) of the LC_{50} values did not overlap.

The vial method was developed to monitor the resistance frequencies of field collected moths throughout the soybean growing season in Louisiana. Soybean looper male moths were collected from pheromone baited wire cone traps (Hartstack et al. 1979) during the 1990 growing season. The traps were mounted on steel reinforcement rods pushed into the ground with the bottom of the cone approximately 1.5 m above the ground. Traps were baited with a soybean looper pheromone lure comprised of the components described by Linn et al. (1987). The pheromone baits were changed approximately every three weeks.

Traps were monitored at least every other day during periods of peak adult captures. Three traps were placed along soybean field borders at three different sites throughout Louisiana (one trap per location). The south Louisiana location was on the St. Gabriel Research Station, St. Gabriel, Iberville Parish, where soybean looper control with permethrin has remained adequate (although reduced) since the onset of permethrin resistance. Two traps were located in the cotton growing regions of northeast Louisiana (Macon Ridge Research Station, Winnsboro, Franklin Parish and Northeast Research Station, St. Joseph, Tensas Parish) where soybean looper control failures with permethrin have been observed in recent years (Leonard et al. 1990, Mink and Boethel 1992).

The presence of resistance in the field-collected moths was determined by the discriminating dose method. Male moths collected in the traps were exposed to pretreated vials of permethrin (2.5 μg) or acetone (control) either in the field or after return to the laboratory. The 2.5 μg dose had caused 98% mortality in the susceptible laboratory colony, but only 23% mortality in the resistant strain. Only active moths that appeared young and healthy were included in the bioassay. Bioassay procedures followed those described for the laboratory studies. Any moth surviving the discriminating concentration was considered resistant. Mortality data at the discriminating concentration were subjected to Chi-square analysis ($P = 0.05$, Proc Frequency, SAS Institute 1988) after being corrected for control mortality (< 10% for all populations tested) by Abbott's (1925) formula.

Results and Discussion

No significant differences were observed between male and female soybean looper moths within a strain at the LC_{50} (Table 1). Similar results have been observed for tobacco budworm, *H. virescens* (F.), adults (Micinski et al. 1991) and diamondback moths, *Plutella xylostella* (L.), (Tabashnik et al. 1988) using similar techniques. However, McCaffery et al. (1989) reported that female tobacco budworm adults were about twice as tolerant of cypermethrin as male adults; however, because 95% CL were not reported, significance was not determined.

Table 1. Response of male and female soybean looper moths from permethrin-susceptible and -resistant laboratory strains to permethrin using a vial residue bioassay.

Strain	Sex	n	Slope ± SE	LC_{50} (95% CL)*	RR†	LC_{95} (95% CL)*	RR†	$X^{2‡}$
Stoneville	males	250	2.34 ± 0.40	0.39 (0.29 - 0.49)	–	1.95 (1.27 - 4.41)	–	0.75
	females	250	2.46 ± 0.41	0.42 (0.33 - 0.52)	–	1.94 (1.28 - 4.18)	–	0.58
South Carolina	males	135	1.81 ± 0.41	5.22 (3.81 - 8.36)	13.4	42.47 (19.35 - 303)	21.7	1.37
	females	130	1.64 ± 0.49	5.13 (3.85 - 7.56)	12.2	51.04 (19.90 - 1595)	25.6	0.51

* Concentrations reported in µg permethrin per vial.

† Response ratio of each sex relative to Stoneville susceptible strain (LC_{50} South Carolina males or females / LC_{50} Stoneville males or females).

‡ X^2 values for each bioassay were not significant (P = 0.05, POLO-PC, LeOra Software, 1987).

The South Carolina male and female moths were 13.4 and 12.2-fold more tolerant of permethrin, respectively, when compared to the same sex from the Stoneville strain. This was lower than the 21-fold resistance to permethrin observed in topical bioassays of third instar larvae. Riley (1988) compared the adult vial technique to third instar topical bioassays in the 1987 PEG-US tobacco budworm monitoring program. His results also indicated higher levels of resistance using the topical bioassay in six of seven strains tested. Similar results for tobacco budworm also were reported by McCaffery et al. (1989).

Resulting mortalities from the adult vial test (AVT) to soybean looper adult males throughout Louisiana during the 1991 growing season are shown in Table 2. A discriminating concentration of 2.5 μg permethrin per vial was chosen for detecting permethrin resistance in soybean looper adults. This concentration killed 98% of the male moths from the permethrin-susceptible strain and was the approximate $LC_{97.5}$ obtained from the probit analysis. This concentration should be sufficient to detect low levels of resistance by causing high levels of mortality of permethrin-susceptible individuals. This is similar to the rationale used by others in determining discriminating doses (McCutchen et al. 1989, Zettler & Cuperus 1990). Mortalities of all field-collected strains were significantly lower than that observed for the Stoneville susceptible strain and were considered to be resistant to permethrin. These results indicated that permethrin-resistant soybean looper adults were present in Louisiana from July through September. The highest level of mortality was observed during July at St. Gabriel. Adult captures during August and 1-24 September were too low to obtain reliable results. However, a sharp increase in the number of adults captured was observed during 25-30 September, and vial results indicated a dramatic decrease in mortality when compared to mortality observed in July. Mortality observed at St. Gabriel (south LA) from 25-30 September was similar to that observed at St. Joseph (north LA) in August. Data from the National Weather Service (St. Joseph, Tensas Parish, LA) indicated the movement of a cold front into north Louisiana on 20 September which dropped the average high temperature from 33°C (1-19 Sept) to 24°C (20-22 Sept). This weather event could have triggered the southern movement of this pest and help explain the increased trap catches observed at St. Gabriel. Soybean looper larval populations did not develop beyond detectable levels in soybean fields at St. Gabriel during the growing season. Therefore, it is highly unlikely that maturation of local populations resulted in the increase in trap captures at this location. Although return migration in the soybean looper has not been documented, the regional pheromone trapping project during 1990 also indicated higher populations of soybean looper adults in south Louisiana and Texas during Oct than throughout the southeastern U.S. (Hamer and Pitre 1990). Earlier, Mitchell et al. (1975) had suggested return migration in Florida based on data from pheromone-baited blacklight traps.

Adult mortality at St. Joseph decreased slightly from 12-14 August to 20-27 August. During most of the growing season at this location, very low tobacco budworm and bollworm, *Helicoverpa zea* (Boddie), infestations had been observed in cotton adjacent to soybean fields where traps were located. However, during Aug, several applications of insecticides (mainly pyrethroids) were applied to cotton for tobacco budworm and bollworm control. The insecticide

Table 2. Mortality of laboratory and field-collected strains of soybean looper adults exposed to the discriminating concentration of permethrin in glass vials.*

Location	Testing Date	n	% Mortality†
Stoneville, lab	–	50	98
South Carolina, lab	–	40	23*
St. Gabriel, La.	7/8 - 7/29	72	75*
	9/25 - 9/30	87	41*
Winnsboro, La.	8/18 - 8/30	133	60*
	9/9 - 10/1	70	66*
St. Joseph, La.	8/12 - 8/14	150	43*
	8/20 - 8/27	100	32*

* Discriminating concentration = 2.5 µg permethrin per vial.

† Mortalities followed by asterisks are significantly different from that of Stoneville (permethrin susceptible) strain (P = 0.05; Proc Frequency, SAS Institute 1988).

selection pressure in cotton could have been sufficient to cause the increase in permethrin resistance observed in soybean looper moths at this location. It has been documented on several occasions that soybean loopers exhibit greater levels of permethrin resistance in this area of the state where cotton and soybean constitute the predominant crop mix (Leonard et al. 1990, Mink and Boethel 1992), with applications on cotton being one of the suggested causal factors contributing to this phenomenon.

At Winnsboro, few soybean looper moths were captured and tested until 18 August. Mortality at this location was higher than that observed at St. Joseph, with a slight increase in mortality observed from August to September. Identification of captured adults as soybean loopers was not performed prior to testing in the vials at Winnsboro as was performed at the other two locations. However, identification of adults after testing indicated that soybean loopers comprised 91 and 93% of total trap captures during August and September, respectively.

The development of the glass vial bioassay for adult soybean loopers has potential to be incorporated with the ongoing regional soybean looper pheromone monitoring program to further document and monitor resistance in this major soybean pest. One of the major problems of the regional project, however, is the use of a non-specific looper lure. Although the lure used in these studies was different than that used in the regional study and was reported as being specific for soybean looper adults (Linn et al. 1987), other looper species also were captured. However, Linn et al. (1988) reported that trap captures in Louisiana with the five-component lure used in our studies contained 97% soybean loopers. When using a less specific lure, Porter (1990) reported that soybean loopers were the predominant species captured during August and September in Mississippi.

Therefore, before implementing the adult vial technique for monitoring resistance, either a more specific lure needs to be developed or cooperators involved in the project must be trained to identify soybean loopers for testing in the vials. These studies represent another step toward developing a resistance management program for soybean loopers and further studies are needed to realize the full potential of this technique for adult resistance monitoring.

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