PYRETHROID RESISTANCE LEVELS IN SOYBEAN LOOPER (LEPIDOPTERA: NOCTUIDAE) IN MISSISSIPPI

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ABSTRACT

Resistance to permethrin was monitored in soybean looper, *Pseudoplusia includens* (Walker), strains established with larvae collected from soybean and cotton during the growing season in the central Delta area in Mississippi in 1989 and 1991. Dose responses were tested topically using larvae. Populations (=strains) of first generation soybean looper collected on soybean in August in both years had LD_{50}'s [95% confidence limits (CL), expressed as μg/g larval weight] of 1.30 (0.80-2.30) and 0.77 (0.01-2.19) in 1989 and 1991, respectively, which were not significantly different from the LD_{50} (CL) of the susceptible strain [0.63 (0.47-0.87) in 1989 and 0.18 (0.13-0.23) in 1991]. Soybean looper strains (second generation on soybean) collected in September showed 6.8 to 10.8-fold increase in LD_{50} (CL) [4.30 (3.00-6.80) in 1989 and 1.94 (0.31-4.04) in 1991], compared with the susceptible strain. The two strains collected in September showed 3.31 and 2.52-fold increases in resistance levels, respectively, when compared with the strains collected in August 1989 and 1991. Soybean looper strains collected on cotton during September had levels of resistance similar to those collected on soybean in both years. However, a soybean looper strain collected on cotton in October (third generation) in 1989 showed a significantly higher level of resistance than any other strain. Levels of soybean looper resistance to pyrethroid insecticides were low early in the season, but increased as the season progressed.

Key Words: Insecticide resistance, loopers, cotton, soybean.

RESUMEN

La resistencia a permetrina en razas del gusano medidor de la soya, *Pseudoplusia includens* (Walker), establecidas con larvas colectadas en soya y algodón durante la época de cultivo en el área central del Delta en Mississippi durante 1989 y 1991 fué evaluada. Respuestas a dósis aplicadas topicalmente en larvas fueron evaluadas. Poblaciones (=razas) de *P. includens* colectadas en soya en agosto de ambos años tuvieron un DL_{50} [límites fiduciales al 95% (LF), expresado en μg/g peso larval] de 1.30 (0.80-2.30) y 0.77 (0.01-2.19) en 1989 y 1991, respectivamente, los cuales no fueron significativamente diferentes a el de la raza susceptible [0.63 (0.47-0.87) en 1989 y 0.18 (0.13-0.23) en 1991]. Las razas colectadas en septiembre, segunda generación en soya, SB89-2 y SB91-2, mostraron incrementos significativos en el DL_{50} (LF) a razón de 6.8X a 10.8X [4.30 (3.00-6.80) en 1989 y 1.94 (0.31-4.04) en 1991], si se comparan con la raza susceptible de cada año. Las razas SB89-2 y SB91-2 mostraron aumentos de 3.31X y 2.52X en el nivel de resistencia al compararse con las razas SB89-1 y SB91-1, respectivamente. Razas colectadas en algodón durante septiembre tuvieron niveles de resistencia similares a los de las colectadas en soya en ambos años. Sin embargo, una raza colectada en algodón a principios de octubre de 1989, (tercera generación), mostró niveles de resistencia significativamente was altos que cualguiera de las otras razas. Estos datos indican que los niveles de resistencia a insecticidas piretroides en *P. includens* fueron bajos al comienzo de la temporada, pero se aumentaron significativamente a medida que la temporada transcurrió.

Resistance of the soybean looper, *Pseudoplusia includens* (Walker), to pyrethroid insecticides has been documented in areas of the southern United States where soybean, *Glycine max* (L.) Merrill, and cotton, *Gossypium hirsutum* L., are grown in close proximity (Felland et al. 1990, Leonard et al. 1990). It has been speculated that selection for insecticide resistance in the soybean looper can be linked to cotton (Luttrell et al. 1990), which can serve as a host for soybean looper larvae (Canerday & Arant 1966) and provide nectar for adults (Burleigh 1972, Jensen et al. 1974). Burleigh (1972) suggested that the soybean looper can develop on a number of other plant species before they move to soybean plants that are sufficiently developed to be attractive as ovipositional hosts. However, the importance of cotton as a host of the soybean looper was thought to decline as the crop growing season advanced (Felland et al. 1992). This may be related to the decline in nectar availability as the cotton plants mature (Stone et al. 1984).

Soybean loopers in Puerto Rico were observed to be difficult to control with some insecticides used on soybean in the United States (Boethel 1990). If pyrethroid insecticides are used extensively for control of soybean looper on crops grown outside the continental United States, the appearance of migratory pyrethroid-resistant loopers in the southern United States might be explained.

In this study, soybean looper larvae were collected from soybean and cotton in the Delta of Mississippi and monitored for resistance to permethrin insecticide at selected times during the crop growing season.

Materials and Methods

Soybean looper strains, in 1989 and 1991, were established by collecting larvae from natural populations on soybean and cotton. A laboratory (LAB-MS) strain, maintained at the Southern Field Crop Insect Management Laboratory at Stoneville, Mississippi, was the source of the susceptible insects. This colony was started with moths from a South Carolina laboratory colony. Moths from larvae collected on untreated soybean in the Delta in Mississippi were added into the colony in 1981 and 1986. The colony has not been exposed to insecticides in the laboratory.

Field collections of larvae were made based on larval size. Larvae from generations one through three were identified and separated according to instars within sample collections. The first collections were made on 17 August 1989 and 14 August 1991 from soybean (SB89-1, $n>200$ and SB91-1, $n=250$) in a field adjacent to cotton in Holmes County (fields were separated by about five miles). Larvae representing the second generation (or possibly new immigrants) were collected from the same soybean fields and from an adjacent cotton field on 2 September 1989 and 1991 (SB89-2, $n>200$ and SB91-2, $n=320$) and from cotton on 2 September 1989 and 16 September 1991 (C89-2, $n>200$ and C91-2, $n=219$). A third collection of larvae, representing the third generation, was taken from cotton on 2 October 1989 (C89-3, $n>200$). The soybean fields had not been sprayed with pyrethroid insecticide before the insect collections, whereas the cotton fields had received seven pyrethroid insecticide applications at approximately 10-day intervals from 14 July to 9 September 1989, and five pyrethroid insecticide applications at approximately 10-day intervals from 17 July to 6 September 1991.

Larvae and soybean or cotton foliage (depending on larval host) were placed in plastic bags inside a box containing blue ice and transported to a laboratory at the Department of Entomology, Mississippi State University. Larvae were transferred individually to plastic cups (29.6 ml) containing nutri-soy flour/wheat germ diet (Raulston & Shaver 1970). The first laboratory generation for each field strain was obtained by randomly selecting 25 adult males and 25 adult females from each field collection. These were maintained in two 4-liter glass jars [no more than 30 adults (males + females) per jar] and fed a 30% honey water solution. Muslin was hung inside as an oviposition substrate and was also used to cover the jars. Further laboratory generations from each field strain were obtained by rearing at least 300 neonates using the same procedure.

The standard assay procedure for measuring insecticide resistance in *Heliothis* spp. was followed (Anonymous 1970). Neonates in the first and second laboratory generations were randomly selected from each field strain. Early third instars (pooled mean weight 12.66 ± 2.93 mg, mean ± SD) were topically treated on the thoracic dorsum with 1-μl aliquot of acetone (check) or an acetone solution of one of five serial concentrations of technical grade permethrin (FMC Corporation, Philadelphia, PA 19103). Treated larvae were held in the cups with diet and mortality was recorded 48 h after treatment. Total mortality was recorded as the number of dead and moribund larvae.

Dose-mortality regressions and the 95% confidence limits (CL) for the LD_{50} (based on μg of permethrin insecticide per g larval body weight) were obtained by probit analysis using the microcomputer software Polo (Robertson et al. 1980). Differences among strains were considered significant based on the failure of 95% CL to overlap. Resistance ratios (RR) were calculated by dividing the LD_{50} of each field strain by that of the LAB-MS susceptible strain.

Results and Discussion

Resistance levels of soybean looper larvae representing the first field generation were 2.1 and 4.3 times greater than that of the susceptible LAB-MS in 1989 and 1991, respectively (Table 1). However, the LD_{50}s (95% CL) were not significantly different from that of the LAB-MS strain. The results suggest that resistance selection pressure for pyrethroid insecticides at source sites of migratory soybean looper moths arriving in this area of Mississippi may be relatively low. This assumption needs documentation.

TABLE 1. Toxicity of permethrin to several strains of soybean looper larvae collected in the Mississippi Delta (topical bioassays).

Strain	Field Generation[1]	Laboratory Generation[2]	n[3]	Slope ± SE	LD_{50} (95% CL)[4]	RR[5]
1987[6]						
Soybean	2	1	146	1.45 ± 0.27	13.31 (8.92-23.6)	21.2
1989						
Lab-MS	–	1	356	2.27 ± 0.24	0.63 (0.47-0.87)	—
Soybean	1	2	71	1.61 ± 0.33	1.30 (0.80-2.30)	2.1
Soybean	2	2	107	1.63 ± 0.28	4.30 (3.00-6.80)	6.8
Cotton	2	2	130	1.99 ± 0.27	4.60 (2.20-9.50)	7.3
Cotton	3	1	80	2.59 ± 0.51	34.4 (23.4-51.1)	54.6
1991						
Lab-MS	–	1	500	1.55 ± 0.15	0.18 (0.13-0.23)	—
Soybean	1	1	120	1.10 ± 0.36	0.77 (0.01-2.19)	4.3
Soybean	2	1	476	1.24 ± 0.15	1.94 (0.31-4.04)	10.8
Cotton	2	1	375	2.06 ± 0.43	1.79 (0.44-2.90)	9.9

[1]Represents generations of larvae collected as they appear in the field through time.
[2]Represents generations of larvae after transferring to the laboratory.
[3]Number of subjects excluding controls.
[4]Doses reported in μg of insecticide/μg larval weight.
[5]Resistance ratios (RR) = LD_{50} of the field strain/LD_{50} Lab-MS.
[6]From Felland et al. 1990.

The earliest confirmed presence of soybean looper adults in pheromone traps occurred on 12 July in both 1989 (Porter 1990) and 1991 (Southern Regional Soybean Looper Resistance Project, 1991). This indicated that in both years some immigrant moths were exposed to at least one pyrethroid application on cotton. Using the developmental times for soybean looper larvae, pupae and adults on cotton and the pre-oviposition and oviposition periods for females (Mitchell 1967), the number of pyrethroid applications applied after the first immigrant moths arrived can be calculated (using a conservative approach of non-overlapping ages of eggs, larvae and adults). Eggs of the first generation on cotton were exposed to one pyrethroid insecticide application, first generation larvae to two applications, and first generation adults, possibly, to one insecticide application in 1989. Thus, soybean loopers were exposed to 4 and possibly 5 insecticide applications (including the first on immigrant moths) prior to the collection of the SB89-1 strain. In 1991, eggs and larvae of the first generation on cotton were exposed to one pyrethroid insecticide application and the adults of the first generation to another application. This accounted for 3 to 4 applications prior to the collection of the SB91-1 strain. If adults resulting from the first generation of larvae on cotton are responsible for eggs laid on soybean, giving rise to the first generation on soybean, these data would support the idea of low levels of pyrethroid resistance in migratory soybean looper moths arriving in Mississippi. Low levels of resistance were observed in the SB89-1 (RR=2.1) and SB91-1 (RR=4.3) strains even after the populations had been exposed to 3 to 5 pyrethroid insecticide applications. Because of the relatively low population density in the first generation of soybean looper and the possibility that susceptible migratory moths were still arriving in the study area, the observed low levels of insecticide resistance may have been caused by influx of susceptible genes from migratory moths. It is also plausible that moths emerging from other ecosystems mated with those from the first larval generation on cotton, and diluted the resistance allele frequency. Both effects can be negligible in following generations due to explosion in soybean looper population density.

There were significantly higher levels of pyrethroid resistance in the second field generation of soybean looper larvae collected on soybean (SB-2) compared with the first field generation on soybean (SB-1) and the LAB-MS strain in both 1989 and 1991. SB89-2 and SB91-2 strains showed significantly higher levels of resistance (6.8 and 10.8-fold, respectively) as compared with the LAB-MS strains (Table 1). Although SB89-2 and SB91-2 strains showed higher levels of resistance than that observed in SB89-1 and SB91-1 strains (3.31 and 2.52-fold, respectively), the increase was significantly higher only in the SB89-2 strain. The second field generation of soybean looper larvae collected on cotton in 1989 and 1991 also showed significantly higher LD_{50}s as compared with the susceptible strain, with 7.3 and 9.9-fold levels of resistance, respectively (Table 1). LD_{50}s observed in C89-2 and C91-2 strains were not significantly different from levels observed in SB89-2 and SB91-2 strains when comparisons within years were made. Soybean fields had not been treated with pyrethroids in either years. These results support the hypothesis that selection for pyrethroid resistance in soybean looper occurs on cotton. The data also support observations that soybean looper control failures with pyrethroid insecticides on soybean planted in the vicinity of cotton are more frequent than insecticide failures on soybean planted away from cotton (Felland et al. 1990, Leonard et al. 1990).

Soybean loopers collected from soybean (SB-2 strain) and cotton (C-2 strain) during early to mid-September in 1989 were exposed to 6 to 7 pyrethroid applications. Those collected from soybean (SB-2 strain) and cotton (C-2 strain) about the same time in 1991 were exposed to 4 to 5 and 5 to 6 applications, respectively. The increase in exposure of soybean loopers through the second generation to pyrethroid insecticide represents an increase in selection pressure for pyrethroid resistance. These results indicate that selection pressure for resistance to pyrethroid insecticide on migratory and first generation soybean looper may be key to the rapid increase in levels of resistance observed in the second field generation of this insect pest. This increase in resistance in the soybean looper population may be attributed, in part, to the presence of low to moderate levels of resistace in migratory moths. These levels of resistance may not have been detected by our techniques.

The third field generation of soybean looper larvae collected from cotton in 1989 (no third generation collected on cotton in 1991) showed a significantly higher resistance level (LD_{50}) to permethrin as compared with the C89-2 (7.5-fold increase) and the susceptible LAB-MS strain (54.6-fold increase) (Table 1).

Because no additional pyrethoid insecticide applications were made after the collection of the C89-2 strain, this and the C89-3 strain were exposed to the same number of pyrethroid applications before resistance testing. The dramatic increase in levels of pyrethroid resistance observed in the C89-3 strain may be related in great part to inbreeding of resistant moths in the second generation, migration of moths exposed to permethrin insecticide in other fields, and to an exponential increase in resistance after a certain level of resistant allele frequencies is attained (Tabashnik & Croft 1982). Increased levels of insecticide resistance in *H. virescens* during the growing season has been reported (Graves et al. 1991). Resistance was linked with selection pressure on the insect population exposed to increased number of insecticide applications.

Resistance levels of 1989 strains were generally higher, although not all of them were significantly different, than those of 1991 strains (Table 1). Felland et al. (1990) reported resistance in a strain of soybean looper (second generation larvae) collected from soybean in 1987 at the same location as insects used in the present study. The dose-mortality lines of the three strains were compared. There was a trend for a higher level of pyrethroid resistance in the 1987 strain compared with the 1989 and 1991 strains (Fig. 1). The 1987 strain had a significantly higher LD_{50} than the 1989 or 1991 strains

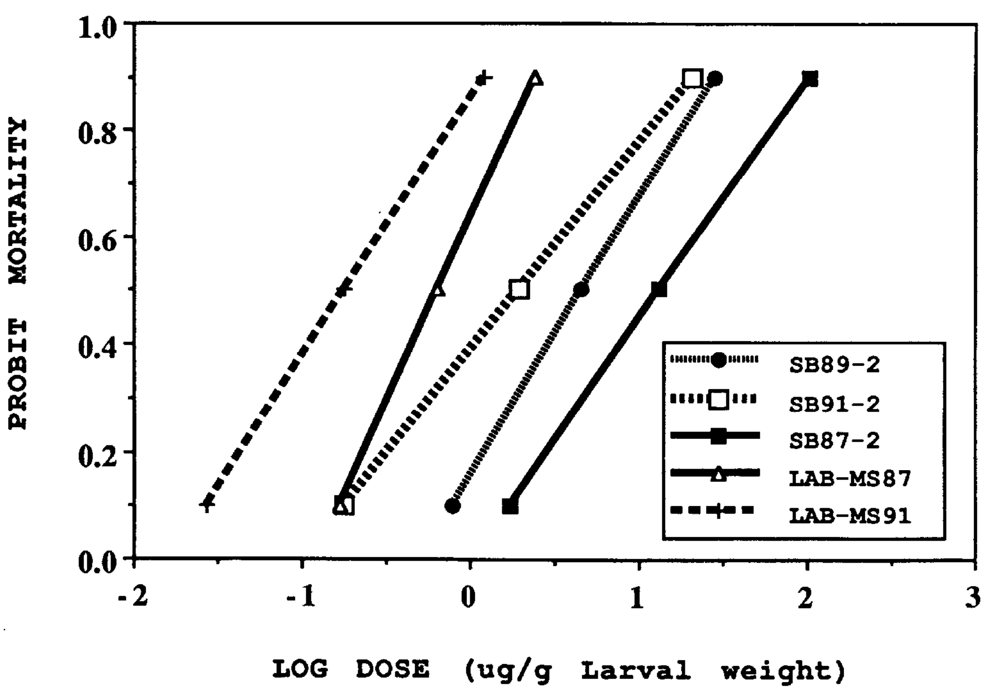

Fig. 1. Dose-mortality lines for permethrin on laboratory and second generation field strains of soybean loopers collected on soybean in the Mississippi Delta in 1987, 1988, and 1991.

(Table 1). The lower number of pyrethroid applications in 1991 than in 1989 may have contributed to the lower levels of insecticide resistance in 1991 compared with 1987 and 1989.

The number of pyrethroid insecticide applications on cotton were closely related to the levels of *H. virescens* infestations on cotton, with 1989 having higher infestations than 1991 (Head 1990, 1992). Although the number of pyrethroid applications on cotton in the study fields in 1987 is unknown, the *H. virescens* infestation levels on cotton in 1987 were higher than in 1989 or 1991. It is reasonable to consider that there were more pyrethroid insecticide applications on cotton in 1987 than in 1989 or 1991 (King et al. 1988, Head 1990, 1992); thus, the selection pressure for pyrethroid resistance in soybean looper was greater in 1987 than in the other two years.

Results of this study indicate increased resistance levels to pyrethroid insecticides in soybean looper as the growing season progressed in 1989. We suggest that the frequent use of pyrethroid insecticides on cotton early in the growing season significantly increases the selection pressure for resistance in soybean looper on cotton. Low levels of pyrethroid resistance were observed early in the 1989 and 1991 crop growing seasons with significant increases in levels of resistance in the populations in 1989 and a 2.52-fold increase in resistance between SB91-1 and SB91-2 strains as the season progressed. A significant increase in the levels of resistance also was observed between the C89-2 and C89-3 strains.

Pyrethroid insecticide resistance management programs for soybean looper on soybean should be viewed in the broad scope of integrated pest management in the crop ecosystem. In areas where the soybean looper is a constraint to soybean production, it can contribute to high levels of defoliation of cotton. Here it is exposed to large amounts of insecticide and initiates insecticide resistance build up. The *Heliothis* pyrethroid resistance management program in the southern United States advises the use of insecticides other than pyrethroids on the earliest infestations of this pest on the cotton crop, thereby delaying selection for *H. virescens* resistance (Anonymous 1986). Soybean looper control on soybean should benefit, as well, from the reduced number of pyrethroid insecticide applications on cotton early in the season, especially when soybean is planted in the proximity of cotton. Additionally, methods for detection of low levels of resistance in migratory soybean looper moths are needed for the effective implementation of insecticide resistance management programs. A glass vial bioassay technique to test for permethrin resistance in adult soybean looper (Mink et al. 1993) can be used to document insecticide resistance in this pest. Identifying low levels of insecticide resistance early in the year could indicate the potential of soybean looper outbreak. Additional work is needed to associate topical LD_{50} responses with estimates of gene frequencies and anticipated levels of control in the field.

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EVALUATION OF LOSS OF (+)-DISPARLURE FROM GYPSY MOTH (LEPIDOPTERA: LYMANTRIIDAE) PHEROMONE DISPENSER TAPES UNDER FIELD CONDITIONS IN FLORIDA

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ABSTRACT

The residual pheromone content of laminated plastic, pheromone-dispensing tapes impregnated with (+)-disparlure, the sex pheromone of the gypsy moth, *Lymantria diapar* (L.), was assessed after exposure to field conditions in Gainesville. As determined by gas chromatography, lure tapes deployed on 26 August 1991 rapidly lost pheromone during the first 2 months in the field. Loss of pheromone was considerably less for the remaining 4-month exposure period (28 October 1991 to 3 March 1992). Lure tapes at 5 locations differed slightly in their rates of pheromone loss, and traps placed on the north side of tree trunks retained more pheromone than traps placed on the south side. These data indicate that a pheromone lure used for monitoring gypsy moth during spring and early summer in north Florida may lose its effectiveness rapidly and may have to be replaced more often than is currently recommmended for other regions of the country.

Key Words: *Lymantria dispar*, sex pheromone, pheromone trap, population monitoring, gas chromatography.

RESUMEN

Se evaluaron después de exposición a las condiciones de campo en Gainesville, Florida, U.S.A., el contenido residual de feromona de plástico laminado, cintas de dispensar feromona impregnadas con (+)-disparlure, la feromona de la polilla gitana, *Lymantria dispar* (L.). Segun análisis por cromotografía de gas, cintas atrayentes col-