Development and Antibiosis of Released Soybean Germplasm Lines Resistant to Mexican Bean Beetle (Coleoptera: Coccinellidae)

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J. Econ. Entomol. 82(1): 259–263 (1989)

ABSTRACT Four soybean (*Glycine max* (L.) Merr.) germplasm lines resistant to Mexican bean beetle, *Epilachna varivestis* Mulsant, were developed and released. These lines, 'HC83-123-9,' 'HC83-46-1,' 'HC83-46-2,' and 'HC83-50-1,' are from the cross 'Pixie' × 'PI 229358' and carry resistance (antibiosis) levels nearly equal to the resistant parent, 'PI 229358.' They were developed by the pedigree breeding procedure, using a systematic laboratory bioassay with Mexican bean beetle larvae to identify resistant plants in each generation. Survival and development of Mexican bean beetle larvae on these lines were similar to that on 'PI 229358.' Larval mortality for each line was 97.3, 90.7, 90.0, and 84.0%, respectively, compared with 27.2 and 98.0% for 'Pixie' (a susceptible control) and 'PI 229358,' respectively. More than half the larval mortality occurred during the first and second stadia. These lines also showed resistance to another soybean defoliator; field screening against defoliation by Japanese beetle, *Popillia japonica* Newman, confirmed the high levels of resistance of these four lines, with defoliation ratings ≤2 for all four lines compared with the susceptible cultivar, 'Pixie,' which had a rating of 5 (scale 0–5). The resistant lines are determinate plant types of mid-to-late group IV maturity. Because of their earlier maturity (compared with the PIs) and high level of resistance (antibiosis), these lines should be a useful source of resistance for the development of high-yielding soybean cultivars with resistance to the Mexican bean beetle and other soybean defoliators.

KEY WORDS Insecta, Japanese beetles, germplasm, soybeans

PLANT BREEDERS AND ENTOMOLOGISTS have been attempting to develop high-yielding, insect-resistant soybean cultivars ever since three, highly insect-resistant sources were first discovered and described ('PI 229358,' 'PI 171451,' and 'PI 227687' [Van Duyn et al. 1971, 1972]). Currently, only one insect-resistant cultivar, 'Shore,' has been released to growers, and that line has met with limited acceptance because of low yield potential. Development and release of resistant germplasm that researchers can use in insect-resistance breeding programs has been more successful. Sullivan (1985) listed nine germplasm releases from various breeding programs (more have been released or are near release since that report), including five lines adapted for northern U.S. conditions. Those five lines, 'L76-0038,' 'L76-0049,' 'L76-0132,' 'L76-0272,' and 'L76-0328' from maturity groups IV and V, were developed from a joint cooperative program of Maryland, Illinois, and Indiana (Elden et al. 1982). Field screening was used for most of the resistance evaluations during their development. Rufener et al. (1986) studied the development of the Mexican bean beetle, *Epilachna varivestis* Mulsant, on these lines and found that the levels of antibiosis resistance varied greatly, with only the resistance level of 'L76-0132' approaching that of the resistant PIs.

Our insect-resistant soybean breeding program began in 1980. A goal (along with developing resistant cultivars to release to growers) was to develop germplasm with resistance levels similar to the resistant PIs and adapted to northern conditions. Having first developed a laboratory antibiosis screening technique (Rufener et al. 1987) that allows successful separation of highly resistant from low and moderately resistant lines (based on the relative development and survival [antibiosis effects] of Mexican bean beetle larvae), we began a series of twice-yearly screening cycles to develop germplasm lines with resistance levels that approached the resistant parents. Here we report the development of four, recently released insect-resistant soybean germplasm lines (Cooper & Hammond 1988) and describe Mexican bean beetle growth on them.

Materials and Methods

Development of Lines. The lines that were evaluated and selected for germplasm release during the intensive screening procedure were selections from the cross of 'Pixie' (a group IV determinate semidwarf cultivar) × 'PI 229358' (a group VII determinate line with a high level of insect resis-

0022-0493/89/0259-0263$02.00/0

tance). Lines were developed by the pedigree breeding procedure. A systematic laboratory antibiosis screening technique with Mexican bean beetle larvae (Rufener et al. 1987) was used to identify resistant plants in each generation. Beginning in the F_2 generation, plants were identified and progeny tested for resistance in the following generation; the cycle was repeated each generation until homozygosity for resistance was reached.

Two plant generations, one with field-grown plants and a second with greenhouse plants, were screened each year (because the screening technique relies on laboratory bioassays, it allows for at least two screening cycles per year). Leaves were collected at the fifth trifoliolate leaf stage (V5; Fehr et al. 1971) and carried to the laboratory where they were placed in Petri dishes on moistened filter paper. Ten neonate Mexican bean beetle larvae (eggs having been obtained from a greenhouse colony) were placed on each leaflet; leaves were replaced with fresh leaflets from the original tagged plants at 4 and 7 d. Scorings on larval growth rate and mortality were taken at 10 d and compared with known standards of larval development and survival on both the susceptible and resistant parents to determine the level of antibiosis resistance in each line tested. (See Rufener et al. [1987] for a detailed description of the technique and its use.) In the F_3 and F_4 generations, 10 plants were screened from each line; in the F_5 and subsequent generations, 5 plants per line were screened. Homozygosity for resistance, determined when all progeny showed similar levels of resistance, was reached in either the F_5 or F_6 generation for all lines tested.

First Evaluation. During the summer of 1985, 65 advanced breeding lines that were homozygous for insect resistance were planted at the Southern Branch of the Ohio Agricultural Research and Development Center (OARDC), Ohio State University, near Ripley, Ohio. This is a southern Ohio location where Japanese beetles, *Popillia japonica* Newman, were in sufficient numbers to obtain data on field resistance; no Mexican bean beetles were present. Seeds were planted in early May (two replications of a single row for each line); ratings of defoliation were taken on 8 August using a system of 0 = no defoliation to 5 = heaviest feeding (that of the susceptible checks). Only those lines that had a rating ≤ 2 in both rows were kept for further evaluation in the following screening cycle. A total of 16 lines met this criterion and were selected forward for further consideration.

Second Evaluation. The 16 lines chosen as being most resistant in the summer of 1985 were planted in the greenhouse along with 'Pixie' (a susceptible control) and 'PI 229358' (the resistant parent) on 20 December 1985. Between 23 January and 11 February 1986, nine trials were done to evaluate the relative resistance among these 16 lines compared with the resistant parent. Leaflets of each line were brought to the laboratory and placed in Petri dishes. Five neonate Mexican bean beetle larvae were placed in each dish; leaflets were replaced as needed. Petri dishes were held in darkness at 23.4°C. Larval development and mortality were monitored daily for 8–20 d, depending on the relative survival in each of the trials. Although developmental and mortality data were recorded, we did not subject these data to statistical analysis. Therefore, no results from this second evaluation are presented. Based on relative larval mortality on these 16 lines during these trials, 8 lines were selected to be further tested in an intensive developmental study in the summer of 1986.

Intensive Evaluation. 'Pixie,' 'PI 229358,' and the eight lines that were selected during the previous winter's evaluation were planted at the OARDC on 23 May and 13 June 1986. The later planting provided plants for screening in late summer. Six trials of an intensive evaluation were done throughout the summer; the first trial began on 30 July and the last trial began on 3 September. Trials were initiated approximately every two weeks during the summer. The growth stage of the plants during screening varied from V5 to R3 depending upon the planting date and time of summer. Trials 1 and 2 began with soybeans in growth stage V5, trials 3 and 4 with plants at stage R1, and trials 5 and 6 with plants in stage R3. 'Pixie' and 'PI 229358' were only included in two of the trials.

Leaves from each line were brought to the laboratory and placed in Petri dishes lined with moist filter paper. Five neonate Mexican bean beetle larvae were placed on each leaflet. Five Petri dishes were established for each line during each trial. Instar development and survival were monitored and recorded daily. The dates of larval pupation and adult emergence also were recorded for each surviving larva. Leaves were changed as needed; Petri dishes were held in a growth chamber at 23.4°C in complete darkness.

The number of larvae that survived each stadium was determined for each trial, and then mortality for each instar during all the trials (expressed as percent) was calculated. Only the number of larvae that completed the previous instar (or those entering the stadia in question) was used to calculate percentage of mortality. Percentage of mortality (transformed before analysis by arcsine$\sqrt{x}$) for each stadium and for overall mortality was analyzed with analysis of variance ($P = 0.05$; df = 7, 35). When significance was so indicated, Duncan's (1955) multiple range test was used to separate treatment means. The experimental design was a randomized complete block of eight treatments (the eight advanced germplasm lines) with six replications (trials). Although susceptible and resistant controls were included in the trials, the analysis was done only on the eight lines in question. Our intent was to select the "most" resistant lines from these eight selections; the controls were included in the trials for comparative purposes only.

Instar development was calculated in a similar fashion; data (days in stadia) for all larvae from

Table 1. Mexican bean beetle larval mortality ($\bar{x}$ percent [SEM]) for selected soybean germplasm lines

Line	Instar mortality					Total mortality
	1st	2nd	1st & 2nd	3rd	4th	
Advanced breeding lines						
HC83-123-9	52.0 (13.6)a	24.0 (8.1)	76.0 (8.9)a	9.3 (4.1)	12.0 (9.0)	97.3 (2.7)a
HC83-46-1	45.3 (10.6)ab	23.3 (5.0)	68.6 (7.8)ab	13.3 (5.3)	7.8 (2.6)	89.7 (6.4)ab
HC83-46-2	35.3 (11.4)bc	27.3 (2.4)	62.6 (12.2)ab	17.3 (9.0)	10.0 (5.3)	89.7 (7.1)ab
HC83-46-3	38.7 (13.8)abc	19.5 (5.8)	58.2 (13.3)abc	14.7 (6.1)	11.3 (5.7)	84.0 (14.5)ab
HC83-46-4	35.3 (9.9)abc	21.3 (5.5)	56.6 (10.6)bc	16.7 (6.0)	14.0 (4.4)	87.3 (9.5)ab
HC83-50-1	33.3 (10.8)abc	18.0 (4.2)	51.3 (10.6)bcd	14.0 (6.6)	20.0 (8.0)	85.3 (10.7)bc
HC83-63-2	20.0 (6.1)c	12.0 (3.4)	32.0 (8.1)d	14.0 (5.4)	22.0 (8.2)	68.0 (11.6)d
HC83-36-4	26.7 (9.5)bc	15.3 (3.2)	42.0 (9.3)cd	16.0 (4.8)	14.0 (6.3)	70.0 (12.3)cd
Check lines						
Pixie	6.4 (2.0)	5.6 (2.7)	12.0 (2.8)	5.6 (3.0)	9.6 (4.5)	27.2 (8.4)
PI 229358[a]	50.0	12.0	62.0	24.0	60.0	98.0

Means within columns followed by the same letter are not significantly different ($P > 0.05$; Duncan's [1955] multiple range test); mortality data transformed to arcsine$\sqrt{x}$.

[a] All larvae of the second replication (out of only two) died during the first stadium; therefore, no SEM could be calculated.

each line within a trial were combined. Developmental data were then analyzed with analysis of variance ($P = 0.05$). The experimental design was a randomized complete block, eight treatments (lines) with six replications (trials). Only data from those larvae actually completing a stadium were included in the analysis. Because 100% mortality occurred before pupation during certain trials for some of the lines, the experimental design was not balanced; therefore, degrees of freedom for each stadium varied.

Results and Discussion

All eight advanced lines caused high larval mortality, ranging from 68.0 to 97.3% (Table 1). Overall mortality of 'Pixie,' a susceptible control, was 27.2% and the mortality of 'PI 229358,' the resistant parent, was 97.3%. Because our intent was to determine the most resistant lines among the eight advanced lines, we chose overall larval mortality as the primary criterion for selection. Analysis of variance of total mortality indicated a significant difference ($F = 5.11$, $P = 0.001$); Duncan's mean separation indicated that lines 'HC83-63-2' and 'HC83-36-4' were not as resistant as the others. Of the remaining lines, 'HC83-123-9' gave the highest resistance score, overall mortality of 97.3%; the only line significantly different from it was 'HC83-50-1' with a mortality of 85.3%.

Analysis of the mortality during individual stadium indicated that significant differences occurred among the eight lines only during the first stadium ($F = 2.66$, $P = 0.03$), with no significant differences being obtained during the second, third, or fourth stadium ($F = 0.95$, $P = 0.51$; $F = 0.36$, $P = 0.92$; and $F = 1.42$, $P = 0.23$, respectively). To aid in the decision of which germplasm lines to release, we chose to combine the mortality of first and second instars and examine the mortality of small larvae (first and second instars). A significant difference among these combined mortalities was obtained (Table 1) ($F = 4.87$, $P = 0.001$). Most of the lines had ≥50% larval mortality in the early (first and second) instars. The two lines ('HC83-63-2' and 'HC83-36-4') that had a relatively low (≤50%) combined mortality for the early instars and the lowest total mortality (68.0 and 70.0%, respectively) were considered to be relatively poor candidates for release and were not considered further. However, these lines are resistant; our purpose was to select only the most resistant lines. We consider high mortality in the early instars as an important criterion for any released germplasm.

Data (Table 2) indicated that all eight lines had larval development times that were numerically greater than 'Pixie' (19.3 d) and less than 'PI 229358' (26.4 d). Analysis of larval development among the lines for the individual instars and for the total larval development period indicated no significant differences: $F = 1.62$, df = 7, 34, $P = 0.16$ for first instars; $F = 1.11$, df = 7, 33, $P = 0.38$ for second instars; $F = 0.85$, df = 7, 30, $P = 0.56$ for third instars; $F = 0.45$, df = 7, 16, $P = 0.85$ for fourth instars; and $F = 0.86$, df = 7, 16, $P = 0.56$ for overall larval development. Although 'HC83-123-9' had the highest mortality ratings (Table 1), the time required for larval development on line 'HC83-123-9' (21.2 d) was numerically less than the larval developmental times on other lines in question. Usually, one finds that the more resistant the line, the greater the larval developmental time. The reason that 'HC83-123-9' had a low larval developmental rate was because only one larva over all the trials (out of 150 larvae) reached adulthood, and thus, was the single contribution to this total overall value and that for the fourth instar (8.1 d) (Table 2). The developmental times for larvae on 'HC83-123-9' during the other three stadia were either the longest or the second longest; thus, the low value for overall development because one larva had a short fourth stadium is misleading. Larval development on 'HC83-123-9' was indeed more similar to the rest of the lines.

Table 2. Mexican bean beetle larval development ($\bar{x}$ days [SEM]) for selected soybean germplasm lines

Line	Instar development				Total
	1st	2nd	3rd	4th	
Advanced breeding lines					
HC83-123-9	4.7 (0.3)	6.0 (0.3)	7.0 (1.2)	8.1[a]	21.2[a]
HC83-46-1	3.4 (0.2)	5.8 (0.3)	6.9 (0.6)	8.2 (0.9)	24.0 (1.7)
HC83-46-2	3.7 (0.1)	6.2 (0.7)	7.4 (0.9)	10.5 (2.3)	27.2 (3.6)
HC83-46-3	4.0 (0.2)	4.9 (0.3)	6.5 (0.6)	8.7 (0.9)	23.8 (3.0)
HC83-46-4	3.9 (0.3)	5.7 (0.6)	6.5 (0.7)	8.9 (0.5)	24.2 (1.5)
HC83-50-1	4.6 (0.5)	5.6 (0.3)	6.4 (0.6)	9.3 (0.6)	25.5 (1.5)
HC83-63-2	3.7 (0.2)	5.1 (0.4)	6.0 (0.5)	9.5 (0.8)	24.1 (1.6)
HC83-36-4	3.9 (0.6)	5.2 (0.4)	6.4 (0.7)	9.0 (0.6)	23.6 (1.5)
Check lines					
Pixie	3.2 (0.1)	3.5 (0.1)	4.0 (0.2)	8.5 (0.8)	19.3 (1.0)
PI 229358	4.5[a]	4.1[a]	6.8[a]	11.0[a]	26.4[a]

[a] Only one larva completed development; therefore, no SEM could be calculated.

Germplasm Selection. Based on the mortality data from Table 1, six lines were considered most resistant, with larval mortality highest on 'HC83-123-9.' 'HC83-46-1,' 'HC83-46-2,' 'HC83-46-3,' and 'HC83-46-4' are related lines, having only split as separate lines relatively late in their development. Because of their closeness, only two of these lines, those with the highest mortality, were selected for germplasm release ('HC83-46-1' and 'HC83-46-2').

Although larval mortality on 'HC83-50-1' was significantly less than that on 'HC83-123-9,' we decided to include 'HC83-50-1' in the germplasm release based on its performance in the field. During the summer screening cycles in 1985 and 1986, data were recorded from two Ohio locations on relative maturity, relative field resistance against Japanese beetle feeding, and yield of all the lines being examined, including the four lines that were released (Table 3). 'HC83-50-1,' although not as resistant as 'HC83-123-9' in the laboratory, matures about 1 wk earlier (and is the earliest of all four lines). Based on the possible usefulness of this earlier maturity in breeding programs, we decided to include 'HC83-50-1' in the release.

The four released lines are adapted to northern U.S. conditions, although they are slightly longer in maturity than their susceptible parent. Relative to 'Pixie,' 'HC83-123-9' matures 10 d later, 'HC83-46-1' and 'HC83-46-2' 6 d later, and 'HC83-50-1' matures 2 d later. Homozygosity for resistance was reached in the F_5 for 'HC83-123-9' and in the F_6 for the other lines. All lines trace back to a single F_4 line ('MBB 82-232') that showed a high frequency of resistant plants in 1982. All four lines are of the determinate growth type, have purple flowers, tawny pubescence, and yellow seed with black hilum, and tend to shatter under hot dry conditions and delayed harvest.

Although none of the lines has yields sufficient for growers' needs, they do possess a sufficient yield potential (Table 3), in combination with a resistance level similar to the resistant parent ('PI 229358') (Table 1), to make these lines of interest to other soybean breeders and entomologists desiring group IV resistant germplasm sources with resistance levels nearing those of the original PIs.

Acknowledgment

Salaries and research support were provided by state and federal funds appropriated to the Ohio Agricultural Research and Development Center, Ohio State University. This is journal article no. 251-87.

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Table 3. Comparison of released, insect-resistant soybean germplasm lines with susceptible cultivars in Ohio

Line	Maturity[a]	Resistance field score[b]	Seed yields					
			1985 (q/ha)[c]			1986 (q/ha)[c]		
			Wooster	Ripley	$\bar{x}$	Wooster	Ripley	$\bar{x}$
HC83-123-9	+10	1.8	22.1	25.8	23.9	22.1	28.4	25.2
HC83-46-1	+6	2.0	19.3	23.1	21.2	22.6	28.6	25.6
HC83-46-2	+6	2.0	18.9	25.6	22.3	23.9	26.4	25.1
HC83-50-1	+2	1.8	21.2	24.6	22.9	22.3	27.2	24.7
Pixie	0	5.0	28.7	30.3	29.5	21.9	30.1	26.0
Ripley	0	—	—	—	—	33.8	35.5	34.7
Elf	−4	5.0	29.4	32.0	30.7	29.0	27.7	28.3

[a] Date of maturity relative to 'Pixie.'
[b] Japanese beetle feeding score at Ripley, Ohio (0 = no feeding to 5 = nearly complete defoliation); taken 8 August 1985.
[c] Quintal/hectare; 1 q = 100 kg.

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Received for publication 4 January 1988; accepted 25 July 1988.