PLANT RESISTANCE

Effect of Soil Moisture and Soybean Growth Stage on Resistance to Mexican Bean Beetle (Coleoptera: Coccinellidae)

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ABSTRACT Although plant breeders and entomologists have been working on the development of insect-resistant soybean, *Glycine max* (Merrill), lines and cultivars over the past 20 yr, only 4 cultivars have been released. Questions have been raised regarding the potential for insect resistance in soybeans. One concern is whether resistance can persist at the desired level under a range of environmental conditions and soybean growth stages. This study sought to elucidate the relationship between the expression of Mexican bean beetle, *Epilachna varivestis* Mulsant, resistance in soybean and high and low soil moisture. In addition, we examined the effect that soybean physiological growth stage has on the expression of resistance. Four soybean genotypes that differed in their insect resistance levels were grown at 3 soil moistures in the greenhouse. Insect bioassays were conducted by rearing Mexican bean beetle on excised leaves and comparing larval mortality, developmental periods, and pupal and adult weights. Bioassays were conducted during the V4–R2 growth stages and the R3–R5 growth stage. Total mortality and larval developmental periods increased on all soybean genotypes as soil moisture decreased. Decreased expression in resistance was observed in plants grown in high soil moisture during the R3–R5 bioassay compared with the V4–R2 growth period. Resistance expression was lost for the moderately resistant line, HC83-193, and decreased for the highly resistant line, HC83-123-9, during the R3–R5 bioassay. Ramifications are discussed for growers concerning lower levels of resistance with increasing soil moisture. These results also are discussed as they relate to explaining problems related to breeding programs and expression of resistance in greenhouse and field grown plants.

KEY WORDS soil moisture, soybeans, Mexican bean beetle, insect resistance

RESISTANCE TO INSECTS has become an important objective of soybean, *Glycine max* (Merrill), cultivar development since the identification of insect resistance to the Mexican bean beetle (*Epilachna varivestis* Mulsant) (Van Duyn et al. 1971, 1972). Researchers have shown that the 3 sources of resistance (PI229358, PI171451, and PI227687) also confer resistance to many other important soybean insect pests including the bean leaf beetle, *Cerotoma trifurcata* (Forster), the striped blister beetle, *Epicauta vittata* (F.), the bollworm, *Helicoverpa zea* (Boddie), the cabbage looper, *Trichoplusia ni* (Hübner), and the soybean looper, *Pseudoplusia includens* (Walker) (Clark et al. 1972, Kilen et al. 1977, Luedders and Dickerson 1977).

Cooperative programs between plant breeders and entomologists have produced insect-resistant germplasm lines derived from these 3 resistant plant introduction lines (Hartwig et al. 1984, Hammond and Cooper 1989, Elden et al. 1992). In addition, 4 insect-resistant cultivars have been released ('Shore' [Elden et al. 1974, Smith et al. 1979], 'Lamar' [Hartwig et al. 1990], 'Crockett' [Bowers 1990], and 'Lyon' [Hartwig et al. 1994]). Despite this success, entomologists and plant breeders have become concerned with limitations in the expression of resistance over the entire season and under different soil moisture conditions (Hammond et al. 1995).

Many environmental factors influence the expression of insect resistance in soybeans. Researchers have shown that resistance decreases as the soybean plants mature (McWilliams and Beland 1977, Reynolds and Smith 1985, Nault et al. 1992). Hammond et al. (1995) also observed lower resistance levels as plants mature and found a significant loss in resistance during periods of above average rainfall. They postulated that high soil moisture caused the lower levels of resistance.

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The effect of low soil moisture and plant water status on soybean insects has been examined in only a few studies. McQuate and Connor (1990a, b) studied the effects of minor water deficits on Mexican bean beetle feeding preference and development on a susceptible soybean cultivar. Larvae preferred feeding on foliage from well-watered plants compared with plants grown under minor water deficits. McQuate and Connor (1990a) noted that free amino acid concentration was higher in leaves from well-watered plants. Mexican bean beetle growth and survival were reduced on susceptible plants that had undergone mild and moderate water deficits (McQuate and Connor 1990b). Lambert and Heatherly (1991) found that larval weights of soybean looper on an insect-susceptible cultivar were lower on water-deficient plants than on well-watered plants.

There is a lack of well defined information on the effect of soil moisture on insect resistance in soybean. Our objective was to determine if a relationship exists between the expression of Mexican bean beetle resistance in soybean and soil moisture, and whether this relationship changes as plants age. This study seeks to corroborate past research on the effect of low soil moisture on resistance and examine the effect that soybean physiological growth stage has on this relationship.

Materials and Methods

The 4 genotypes chosen for this study were 'Pixie', a susceptible, semidwarf, determinate cultivar of maturity group IV; HC83-193, a moderately resistant, semidwarf, determinate line of maturity group IV; HC83-123-9, a highly resistant, semidwarf, determinate line of maturity group IV; and PI229358, a highly resistant indeterminate line of maturity group VII. HC83-123-9 was a selection from the cross Pixie × PI229358 (Cooper and Hammond 1988). HC83-193 was a selection from the cross 'Elf' × D75-10169. D75-10169, which was released by Hartwig et al. (1984), is a maturity group VIII, insect-resistant line that derives its resistance from PI229358. Both HC83-123-9 and HC83-193 were developed cooperatively by USDA–ARS and the Ohio Agricultural Research and Development Center (OARDC), Wooster, OH.

Pixie, HC83-193, and HC83-123-9 were selected for use in this study because of their respective resistance levels and their similar maturity. Genotypes that bloom at approximately the same time were desirable to facilitate initiation of the bioassays on the same day for all genotypes.

The experiment was conducted in the greenhouse. A Medway silt loam topsoil was used as the growing medium. The soil was pulverized and sifted through a 6.5-mm screen to enhance uniformity. Gypsum blocks (soil moisture blocks, Soilmoisture Equipment, Santa Barbara, CA) were used to monitor the soil moisture in each pot. Gypsum blocks provide an indirect measurement of the soil water potential (Campbell and Gee 1986). The soil water potential is inferred from measuring the water potential of the standard medium, gypsum. The electrical resistance of the gypsum block is determined by the water content of the block, and the resistance is correlated with readings on the soil moisture meter.

The gypsum blocks were calibrated by adapting a procedure described by Campbell and Gee (1986). Before the study was started, 5 pots were filled with soil and 2 gypsum blocks were imbedded in each pot. The pots were placed in the greenhouse and watered for 3 wk to simulate experimental conditions. After 3 wk, 10 soil plugs, each containing soil and 1 gypsum block, were removed. The gypsum blocks were then calibrated by equilibrating the blocks and soil at several water potentials using a pressure plate apparatus and taking readings at these soil water potentials. A water release curve also was generated using the pressure plate apparatus. Based on water release data, the 3 soil moisture levels chosen for this experiment were: −75 kPa (low), −32 kPa (medium), and −12 kPa (high). The objective in the selection of soil moisture levels was to provide saturated, control, and water-deficit conditions.

A 4 × 3 factorial arrangement of treatments was established with 4 genotypes and 3 soil moisture levels with 4 blocks (replications) in a randomized complete block design. The experimental unit was a pot. Ten imbibed seeds were planted 29 April 1993 into each pot. Each pot was thinned to 4 plants following emergence. Two bioassays were conducted, the 1st during late vegetative to early flowering soybean stages (V4–R2) and the 2nd during early pod to pod filling soybean stages (R3–R5) (Fehr et al. 1971). A 3 × 3 factorial arrangement with 3 genotypes and 3 soil moisture levels was used during the 2nd (R3–R5) bioassay because PI229358 failed to flower and was not included. Thus, a total of 48 or 36 nursery pots was used depending on the bioassay.

One gypsum block was installed in each pot. A total of 6 water lines with timers was installed. Each line supplied 1 of the 3 soil water treatments to the designated pots in 2 blocks. All pots received the same amount of water for the first 2 wk to allow establishment. Soil moisture levels were brought to target levels after 2 wk, and an automatic watering schedule was programmed to retain the soil moisture at the target levels. Gypsum block readings were taken on each pot every 2 d to monitor the soil moisture levels. The watering regime was adjusted as needed to maintain soil moisture at target levels.

Bioassays were initiated 14 and 44 d after the water regimes were implemented (corresponding to the V4–R2 and R3–R5 stages, respectively). All plants were evaluated for resistance using a larval antibiosis bioassay developed by Hammond and Cooper (1989). Newly oviposited Mexican bean

Table 1. Least squares means ± SEM for Mexican bean beetle mortality and days egg-to-adult during the V4–R2 soybean growth period

Genotype	Soil moisture level			Genotype avg
	High	Medium	Low	
		Mortality, %		
Pixie	65.0 ± 7.4	80.0 ± 2.7	91.7 ± 1.7	78.9 ± 4.1a
HC83-193	78.3 ± 3.2	93.3 ± 4.7	96.7 ± 1.9	89.4 ± 3.0b
HC83-123-9	84.9 ± 5.0	98.3 ± 1.7	100.0 ± 0.0	94.4 ± 2.6c
PI229358	95.0 ± 5.0	100.0 ± 0.0	100.0 ± 0.0	98.3 ± 1.2c
SM avg	80.8 ± 3.6a	92.9 ± 2.4b	97.1 ± 1.0b	
		Development days (egg-to-adult)		
Pixie	29.5 ± 0.8	32.3 ± 0.8	30.3 ± 1.7	30.7 ± 0.7
HC83-193	31.1 ± 0.7	31.8 ± 1.2	32.9 ± 0.5	31.9 ± 0.5
HC83-123-9	31.9 ± 1.1	33.5 ± 0.0	—	
PI229358	32.4 ± 1.0	—	—	
SM avg	31.3 ± 0.5			

Averages for the main factors of genotype (in a single column) and soil mixture (SM) (in a single row) followed by the same letter are not significantly different using least significant differences (P = 0.05); mortality data transformed to arcsine $\sqrt{x}$ for analysis. Pixie, susceptible, semidwarf, determinate cultivar, maturity group IV; HC83-193, moderately resistant, semidwarf, determinate germplasm line, maturity group IV; HC83-123-9, highly resistant, semidwarf, determinate germplasm line, maturity group IV; PI229358, highly resistant, plant introduction line, maturity group VII. —, 100% mortality for that portion of the bioassay.

beetle egg masses were collected from a greenhouse colony and placed in petri dishes containing moist filter paper to facilitate hatching and maintain an appropriate humidity. Three petri dishes were used for each pot. Five randomly chosen neonate larvae were placed on an excised leaflet in each petri dish. Leaflets were excised in the early morning (0500–0700 hours). A total of 12 petri dishes was used for each treatment (3 dishes per pot), resulting in a sample size of 60 Mexican bean beetle larvae per treatment. The filter paper was kept moist, and leaflets were replaced at intervals of 3 d or less. All dishes were placed in an environmental control chamber at 23.3°C in complete darkness. Instar development and survival were monitored daily for each bioassay. Following pupation, pupae were removed from the petri dishes, weighed, and placed in plastic cups containing moist filter paper that were covered with clear, labeled lids. At adult emergence, weights, and total days (egg-to-adult) were determined and recorded.

The number of larvae that survived each stadia was determined for each bioassay, and the mean days in each stage was computed using these individuals. For all variables, values from all 3 petri dishes for a given pot were combined and averaged to obtain mean values for each pot before analysis. The values for all surviving individuals corresponding to each pot were used for total days (egg-to-adult), pupal weights, and adult weights. Analysis of variance (ANOVA) was accomplished using the general linear model procedure in SAS (SAS Institute 1988) to accommodate missing data caused by high mortality. A modified statistical model was used that treated the design as a strip plot with growth period as one strip, and genotype and soil moisture as the other to analyze the differences in mortality between soybean growth periods. The arcsine $\sqrt{x}$ transformation was used for analysis of overall percentage of mortality and mortality in each stage. Least significant differences (LSD) (P = 0.05) were used for means separations when overall significance was indicated by an F test. Least squares means were calculated for all variables because of unbalanced data.

Results

Growth Stages V4–R2. ANOVA indicated that the effect of soil moisture on total mortality was significant during the 1st soybean growth period ($F = 29.56$; df = 2, 33; $P < 0.01$), with total mortality decreasing as soil moisture increased (Table 1). Total mortality for the medium and low soil moisture treatments were not significantly different, and both were significantly greater than the high soil moisture treatment. The expression of resistance was greatest on the drought-stressed plants and least on the plants grown with excess water.

The effect of genotype also was highly significant for total mortality ($F = 26.23$; df = 3, 33; $P < 0.01$). Mortality significantly increased for insects reared on the resistant genotypes (Table 1). The 2 highly resistant genotypes (HC83-123-9 and PI229358) were not significantly different, and the susceptible line, Pixie, and the moderately resistant genotype, HC83-193, were significantly different from each other and the other 2 genotypes. Because the soybean is not the best host for the Mexican bean beetle, mortality is often a factor even on susceptible soybean genotypes as compared with preferred *Phaseolus* species (Hammond 1985, Hammond et al. 1995). The genotype × soil moisture interaction was not significant ($F = 1.08$; df = 6, 33; $P > 0.05$).

Total mortality was high for insects on all 4 genotypes in the low soil moisture treatment (Table 1). However, there were differences in the stadia in which Mexican bean beetle larvae died. Mor-

Table 2. Least squares means ± SEM for Mexican bean beetle pupal and adult weights during the V4–R2 soybean growth period

Genotype	Soil moisture level			Genotype avg
	High	Medium	Low	
		Pupal wt, mg		
Pixie	22.29 ± 0.9	18.96 ± 1.2	20.09 ± 1.2	20.29 ± 0.7
HC83-193	21.31 ± 1.2	17.71 ± 0.8	15.26 ± 1.9	20.45 ± 1.1
HC83-123-9	17.63 ± 1.1	14.79 ± 0.0	—	
PI229358	17.18 ± 1.8	—	—	
SM avg	19.60 ± 0.8			
		Adult wt, mg		
Pixie	19.45 ± 0.8	15.94 ± 0.8	17.49 ± 1.1	17.63 ± 0.6
HC83-193	18.90 ± 0.7	15.12 ± 1.2	13.72 ± 3.1	15.92 ± 1.1
HC83-123-9	15.42 ± 1.0	12.25 ± 0.0	—	
PI229358	14.59 ± 0.7	—	—	
SM avg	17.09 ± 0.7			

[a] Pixie, susceptible, semidwarf, determinate cultivar, maturity group IV; HC83-193, moderately resistant, semidwarf, determinate germplasm line, maturity group IV; HC83-123-9, highly resistant, semidwarf, determinate germplasm line, maturity group IV; PI229358, highly resistant, plant introduction line, maturity group VII. —, 100% mortality for that portion of the bioassay.

tality for combined 1st and 2nd instars was 10.0, 58.4, 95.0, and 91.7% for Pixie, HC83-193, HC83-123-9, and PI229358, respectively. Insects died primarily during the 1st and 2nd instars for the highly resistant genotypes, during the later instars for the susceptible genotype, and more equally distributed for the moderately resistant genotype.

Significant differences were not obtained for total days required for egg-to-adult development for the main factors of genotype ($F = 1.25$; df = 3, 15; $P > 0.05$) or soil moisture ($F = 1.21$; df = 2, 15; $P > 0.05$), nor for the genotype × soil moisture interaction ($F = 0.53$; df = 3, 15; $P > 0.05$). Although effects were not significant, trends were observed (Table 1). Least squares means for total days (egg-to-adult) tended to increase from high to low soil moisture treatments. Least squares means for days (egg-to-adult) also increased from susceptible to highly resistant material in the high soil moisture treatment with Pixie, resulting in the fewest days required for development. Because there was 100% mortality for PI229358 in the medium and low soil moisture treatments and also for HC83-123-9 in the low soil moisture treatment, developmental data (days-to-adult) are not available. Days required for development (egg-to-adult) also increased from susceptible to more resistant genotypes.

Pupal and adult weights (Table 2) showed significance for both genotype and soil moisture (for pupal weights: genotype, $F = 4.87$; df = 3, 15; $P < 0.05$, soil moisture, $F = 5.77$; df = 2, 15; $P < 0.05$; for adult weights: genotype, $F = 4.41$; df = 3, 15; $P < 0.05$, soil moisture, $F = 6.11$; df = 2, 15; $P < 0.05$). Pupal and adult weights were greatest for the high soil moisture treatment and declined as soil moisture decreased. Weights were least on susceptible genotypes as compared with highly resistant lines. There was 100% mortality for PI229358 at the medium and low soil moisture treatments and for HC83-123-9 at the low soil moisture treatment. Genotype × soil moisture interactions were not significant for either pupal or adult weights (pupal weights: $F = 0.86$; df = 3, 15; $P > 0.05$; adult weights, $F = 0.69$; df = 3, 15; $P > 0.05$).

Growth Stages R3–R5. ANOVA for total mortality showed the main factors of soil moisture and genotype to be significant ($F = 24.29$; df = 2, 24; $P < 0.01$ and $F = 5.48$; df = 2, 24; $P < 0.05$, respectively). Mortality increased from the high to low soil moisture, and there was greater mortality in the resistant line (HC83-123-9) than in the susceptible lines (Table 3). More importantly, the genotype × soil moisture interaction was significant ($F = 5.43$; df = 4, 24; $P < 0.05$). The importance of this significant interaction is that it indicates a loss in resistance for resistant plants in high soil moisture treatments. Only in the medium soil moisture treatment were differences in resistance detectable. At low soil moisture, mortality was very high with all genotypes, and differences among genotypes were not observed. Least significant differences indicated that total mortality for all the genotypes in the high soil moisture treatment and for Pixie and HC83-193 in the medium soil moisture treatments were not significantly different. Mortality for HC83-123-9 in the medium soil moisture treatment and all the genotypes in the low soil moisture treatment were similar, and significantly higher than the other group.

The trends in development (days egg-to-adult) for the R3–R5 stages were similar to those of the V4–R2 period. Soil moisture level had a significant effect ($F = 35.68$; df = 2, 18; $P < 0.01$) (Table 3). The developmental period (days egg-to-adult) for the high soil moisture treatment was significantly less than the medium and low soil moisture treatments; developmental time (days egg-to-adult) for the medium and low soil moisture treatments were not significantly different. The number of days (egg-to-adult) was less for larvae feeding on plant

Table 3. Least squares means ± SEM for Mexican bean beetle mortality and days egg-to-adult during the R3–R5 soybean growth period

Genotype	Soil moisture level			Genotype avg
	High	Medium	Low	
		Mortality, %		
Pixie	48.4 ± 8.4a	65.0 ± 10.0a	91.7 ± 3.2b	68.3 ± 6.7
HC83-193	58.3 ± 7.4a	55.0 ± 7.4a	88.3 ± 5.7b	67.2 ± 5.8
HC83-123-9	54.9 ± 9.6a	95.0 ± 5.0b	90.0 ± 4.3b	79.9 ± 6.4
SM avg	53.9 ± 4.6	71.7 ± 6.5	90.0 ± 2.4	
		Development days (eggs-to-adult)		
Pixie	28.0 ± 0.8	30.5 ± 0.5	32.6 ± 0.5	30.4 ± 0.7a
HC83-193	27.7 ± 0.2	32.8 ± 1.0	32.9 ± 0.7	31.1 ± 0.9a
HC83-123-9	31.1 ± 0.5	34.5 ± 0.0	33.9 ± 0.4	33.2 ± 0.6b
SM avg	28.9 ± 0.5a	32.6 ± 0.7b	33.1 ± 0.3b	

Means followed by the same letter are not significantly different using least significant differences ($P = 0.05$) for the interaction with mortality and the main factors with egg-to-adult development (genotype averages in columns, soil moisture averages in rows); mortality data transformed to arcsine $\sqrt{x}$ for analysis. Pixie, susceptible, semidwarf, determinate cultivar, maturity group IV; HC83-193, moderately resistant, semidwarf, determinate germplasm line, maturity group IV; HC83-123-9, highly resistant, semidwarf, determinate germplasm line, maturity group IV; PI229358, highly resistant, plant introduction line, maturity group VII.

tissue from the high soil moisture treatment than on the medium and low treatments. Differences in days (egg-to-adult) for genotype were also significant ($F = 9.7$; df = 2, 18; $P < 0.05$) (Table 3). Developmental days (egg-to-adult) for insects feeding on Pixie and HC83-193 were not significantly different, both being less than on HC83-123-9. The genotype × soil moisture interaction was not significant.

Significant differences were not found for pupal weights with soil moisture, genotype, or the genotype × soil moisture interaction. The rank from lowest to highest weight by soil moisture treatment was different for every genotype (Table 4). ANOVA indicated a significant difference in adult weights ($F = 4.33$; df = 2, 18; $P < 0.05$) among genotypes. Adults reared on 'Pixie' and HC83-193 weighed significantly more than that of adults reared on HC83-123-9 (Table 4).

Soybean Growth Stage Effects. When the early (V4–R2) and late (R3–R5) growth bioassays were combined, there was a significant genotype × soil moisture level interaction for percentage of mortality ($F = 4.24$; df = 4, 24; $P < 0.05$) (Table 5). Percentage of mortality was greater on the resistant lines compared with the susceptible Pixie at the high and medium soil moisture treatments, but there was a lack of differentiation at the low soil moisture treatment where Mexican bean beetle mortality was great on all soybean lines.

There also was a significant genotype × soybean growth period interaction for percentage of mortality ($F = 4.06$; df = 2, 24; $P < 0.05$) (Table 6). Mortality was greatest during the V4–R2 stages. However, mortality of insects on Pixie and HC83-193 was significantly different during the V4–R2 stages, but not during the R3–R5 stages. Also, insect mortality on the 2 resistant lines HC83-193 and HC83-123-9, although similar during the V4–R2 stages, were significantly different at the R3–R5 stages. Although the soybean growth stages differed in plant maturity at initiation of the bioassays and the number of days the plants were subjected to the soil moisture treatments, the loss in resis-

Table 4. Least squares means ± SEM for Mexican bean beetle pupal and adult weights during the R3–R5 soybean growth period

Genotype[a]	Moisture level			Genotype avg
	High	Medium	Low	
		Pupal wt, mg		
Pixie	24.13 ± 1.3	23.51 ± 1.0	22.04 ± 2.1	23.23 ± 0.8
HC83-193	24.98 ± 1.4	22.26 ± 2.1	27.27 ± 2.5	24.83 ± 1.2
HC83-123-9	20.51 ± 0.9	19.29 ± 0.0	22.43 ± 1.8	20.74 ± 0.9
SM avg	23.21 ± 0.9	21.69 ± 1.1	23.91 ± 1.3	
		Adult wt, mg		
Pixie	20.32 ± 1.1	20.13 ± 1.1	18.00 ± 2.4	19.48 ± 0.8a
HC83-193	21.92 ± 1.2	18.81 ± 2.0	23.74 ± 2.0	21.49 ± 1.1a
HC83-123-9	16.78 ± 0.9	15.71 ± 0.0	18.25 ± 1.1	16.91 ± 0.7b
SM avg	19.67 ± 0.9	18.22 ± 1.1	19.99 ± 1.3	

Means followed by the same letter in a column are not significantly different using least significant differences ($P = 0.05$). Pixie, susceptible, semidwarf, determinate cultivar, maturity group IV; HC83-193, moderately resistant, semidwarf, determinate germplasm line, maturity group IV; HC83-123-9, highly resistant, semidwarf, determinate germplasm line, maturity group IV; PI229358, highly resistant, plant introduction line, maturity group VII.

Table 5. Least squares means ± SEM for total percentage of Mexican bean beetle mortality for the genotype × soil moisture level interaction for the combined V4–R2 and R3–R5 growth period bioassays

Genotype	Moisture level			Genotype avg
	High	Medium	Low	
Pixie	56.7 ± 6.1a	72.5 ± 5.6b	91.7 ± 1.7c	73.6 ± 4.0
HC83-193	68.3 ± 5.3ab	74.2 ± 8.3b	93.3 ± 3.2c	78.6 ± 3.9
HC83-123-9	70.0 ± 7.6b	96.7 ± 2.5d	95.0 ± 2.7cd	87.2 ± 3.7
SM avg	65.0 ± 3.7	81.1 ± 4.0	93.3 ± 1.5	

Means followed by the same letter are not significantly different using least significant differences (P = 0.05) for the interaction; mortality data transformed to arcsine $\sqrt{x}$. Pixie, susceptible, semidwarf, determinate cultivar, maturity group IV; HC83-193, moderately resistant, semidwarf, determinate germplasm line, maturity group IV; HC83-123-9, highly resistant, semidwarf, determinate germplasm line, maturity group IV; PI229358, highly resistant, plant introduction line, maturity group VII.

tance of HC83-193 can be attributed to the growth stages.

Discussion

Soil moisture affected Mexican bean beetle mortality, days required for development, and pupal and adult weights. Mortality was lower on soybean plants in the high soil moisture treatment, regardless of genotype, than those grown in the medium and low soil moisture treatments during the V4–R2 bioassay. Development days (egg-to-adult) and pupal and adult weights followed the same basic trend with fewer developmental days (egg-to-adult) and higher pupal and adult weights for the high soil moisture treatment than the medium and low soil moisture treatments.

To look at changes in the expression of resistance, the difference between susceptible and resistant genotypes for Mexican bean beetle mortality, developmental (days egg-to-adult), and pupal and adult weights were examined. Loss in the expression of resistance can be defined as having no significant difference between the susceptible and resistant genotypes for a given variable, e.g. soil moisture in our study. Using this definition, a loss in resistance was not observed during the 1st growth period (V4–R2 stages). However, such a reduction in resistance because of soil moisture differences did occur during the R3–R5 stages. Pixie, HC83-193, and HC83-123-9 were not significantly different from each other at the high soil moisture level for mortality.

Table 6. Least squares means ± SEM for total percentage of Mexican bean beetle mortality for the genotype × soybean growth stage interaction for the combined V4–R2 and R3–R5 growth period bioassays

Genotype	Soybean growth stage		Genotype avg
	V4–R2	R3–R5	
Pixie	78.9 ± 4.1ab	68.3 ± 6.7a	73.6 ± 4.0
HC83-193	89.4 ± 3.0c	67.2 ± 5.8a	78.6 ± 3.9
HC83-123-9	94.4 ± 2.6c	79.9 ± 6.4b	87.2 ± 3.7
Bioassay avg	87.6 ± 2.1	71.8 ± 3.7	

Means followed by the same letter are not significantly different using least significant differences (P = 0.05) for the interaction; mortality data transformed to arcsine $\sqrt{x}$. Pixie, susceptible, semidwarf, determinate cultivar, maturity group IV; HC83-193, moderately resistant, semidwarf, determinate germplasm line, maturity group IV; HC83-123-9, highly resistant, semidwarf, determinate germplasm line, maturity group IV; PI229358, highly resistant, plant introduction line, maturity group VII.

This variability in the expression of resistance is important because breeders rely on the comparison of mortality among lines to make selections. These findings indicate the importance of soil moisture relative to the expression of resistance among resistant and susceptible lines. The poor differential in resistance at the low soil moisture treatment was somewhat expected. McQuate and Connor (1990a, b) and Lambert and Heatherly (1991) showed that susceptible genotypes grown under water deficits increased mortality and developmental days of Mexican bean beetle and soybean looper, respectively, as compared with the same genotypes grown with adequate levels of soil moisture.

As soybeans mature, a reduction in the level of resistance was observed, confirming findings in previous studies (McWilliams and Beland 1977, Reynolds and Smith 1985, Nault et al. 1992, Hammond et al. 1995). The significant difference in insect mortality obtained between Pixie and HC83-193 during the V4–R2 growth stages was not present during the R3–R5 stages. Because this reduction in resistance was not observed in HC83-123-9, it seems likely that the reduction in resistance observed in later soybean growth stages may vary by genotype. The insect resistance conferred by PI229358 is known to be controlled by several genes (Rufener et al. 1989). The mechanisms for resistance may differ between these lines. A possible explanation for the differences in the effect of growth stage on the resistance of HC83-193 and HC83-123-9 is that these lines possess a different compliment of resistance genes.

Variability in the expression of resistance under different soil moisture conditions could be a contributing factor that explains why resistance screening from greenhouse-grown plants often produces different results than screening field-grown plants. Greenhouse plants are often overwatered to avoid drying and wilting. Thus, it is likely that greenhouse-grown plants experience consistently higher soil moisture levels than their counterparts in the field, leading to a reduction in the levels of resistance.

These results put forward a new finding that Mexican bean beetle larvae reared on soybean foliage grown under high soil moisture conditions had lower mortality and required fewer days for development. Soil moisture influences the expression of resistance to the Mexican bean beetle as well as the overall suitability of soybean as a host. Future research should explore the genetic, chemical, physical, and nutritional reasons for these findings.

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