PLANT RESISTANCE

Maturity and Environmental Effects on Soybeans Resistant to Mexican Bean Beetle (Coleoptera: Coccinellidae)

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ABSTRACT Entomologists and breeders have met with limited success in the development and release of insect resistant soybean cultivars. Although numerous germplasm lines resistant to defoliating insects have been released, only three cultivars have been made available to growers over the past 20 yr. Researchers have examined potential limitations of insect resistant soybeans, with most of the studies examining the negative impact of plant maturity. This study examined the relative resistance levels of four advanced germplasm lines during the vegetative, flowering, and pod-fill growth stages of soybean development. Resistance was measured by rearing Mexican bean beetle, Epilachna varivestis Mulsant, on excised, field-grown leaves and comparing larval mortality and developmental periods. The study was conducted in two locations for 2 yr. In the 1st yr, mortality was significantly greater, and developmental periods lengthened, on the four resistant soybean lines compared with two susceptible cultivars during the vegetative and flowering stages; however, these differences were slight to nonexistent during the pod-fill stage. Although similar differences were evident the 2nd yr, the levels of resistance were greatly lowered. This reduction in resistance as the plants matured is comparable to that found by other researchers. The loss of resistance in the 2nd yr was unexpected and was attributed to much higher than normal rainfall in July at both locations. We discuss the consequences of lower levels of resistance in maturing plants and under conditions of high rainfall, both for breeding programs and field resistance as required by growers.

KEY WORDS Epilachna varivestis, Mexican bean beetle, soybean
in soybean as the plant matures, we need to determine if this negates its potential value. We need to study resistance throughout the season to determine if levels are sufficient when we require that resistance. We examined four advanced, resistant germplasm lines, obtained from two different breeding programs that have separate pedigrees (with resistance from either 'PI229358' or 'PI1171451'). Our objective was to ascertain the relative resistance between these lines during the season in the vegetative, flowering, and pod-fill growth stages of soybean development. We grew soybean lines in the field at two distinct locations to examine possible effects of environmental variation on the expression of resistance.

Materials and Methods

**Soybean Source.** We planted six soybean lines differing in their resistance to Mexican bean beetle at Wooster, OH, on 22 and 28 May and at West Lafayette, IN, on 23 and 18 May in 1991 and 1992, respectively. Soil types were Wooster silt loam at Wooster and Chalmers silt loam at West Lafayette. Resistant lines 'HC83-123-9' and 'HC83-19-2' were developed in a joint USDA-ARS and Ohio Agricultural Research & Development Center (OARDC), Ohio State University, breeding program at Wooster, OH, and lines 'L86K-73' and 'MBB80-133' were developed in a joint USDA-ARS, Purdue University, University of Illinois and University of Maryland breeding program. 'HC83-123-9' is a selection from the cross 'Pixie' × 'PI 229358' and is of a late maturity group IV with a determinate growth type (Cooper & Hammond 1988). 'HC83-19-2' is an advanced breeding line from the same cross selected for its consistent high levels of resistance and higher yields than 'HC83-123-9' and is of a maturity group IV with a determinate growth type (unpublished data). 'L86K-73' is a selection from the cross 'L73-4673' × 'L76-0038' (Williams) × 'PI1171451' (Elden et al. 1992). These two latter lines are of a maturity group III with an indeterminate growth type. We also planted two lines susceptible to Mexican bean beetle feeding (Ripley, maturity group IV and of a determinate growth type, and Williams, maturity group III and of an indeterminate growth type).

In 1991, we planted soybean seeds from the same initial source. Ohio supplied seeds of 'HC83-123-9', 'HC83-19-2', and Ripley, and Indiana supplied seeds of 'L86K-73', 'MBB80-133', and Williams. Herbicides were applied at recommended rates for weed control. Soybeans, grown for a supply of leaves, were planted in adjacent single rows at least 15 m in length. At harvest maturity in 1991, each location was hand harvested for the six lines to supply seed for 1992.

**Insect Source.** We obtained Mexican bean beetle, *Epilachna varivestis* Mulsant, egg masses from a colony maintained on common greenbean, *Phaseolus vulgaris* L., at the OARDC in Wooster, OH. Egg masses were collected on two consecutive days from the insect cages before the start of each bioassay. We shipped, using overnight services, the egg masses collected on the 1st d to Indiana. Egg masses from the 2nd d were used in Ohio.

**Resistance Bioassay.** We bioassayed soybean lines for insect resistance levels three times during each summer using an intensive evaluation similar to that used by Hammond & Cooper (1989). The first bioassay began when the plants were in the V4-5 stage, the second bioassay began when the soybeans were in the R1-R2 stage, and the last bioassay began when the plants were in the R4-5 stage. Because the bioassays took ≈2 wk and occurred during changing growth stages, we considered the bioassays to be during the vegetative stages, the flowering stages, and the pod-fill stages. Starting dates of each bioassay were: Ohio, 21 June and 9 July for the vegetative stage, 25 July and 1 August for the flowering stage, and 12 August and 31 August for the pod-fill stage in 1991 and 1992, respectively; Indiana, 20 June and 11 July for the vegetative stage, 31 July and 1 August for the flowering stage, and 15 August and 30 August for the pod-fill stage in 1991 and 1992, respectively.

We began a bioassay by collecting 10 fully developed leaflets, chosen randomly, from the upper canopy of each soybean line. Leaflets were returned to the laboratory and placed into individual plastic petri dishes (10-cm diameter) lined with moist filter paper. Ten petri dishes were set up for each soybean line. On hatching, we transferred five neonate Mexican bean beetle larvae into each petri dish (50 larvae per line). Dishes were placed into an environmental chamber at 24°C in complete darkness (see Bufener et al. 1986 for full description of rearing techniques). We monitored instar development and survival daily. Larvae dead or missing on day 1 were considered lost because of handling and were replaced with larvae from the original source. Larvae dead from that point on were considered to have died during the bioassay. Insufficient egg hatch for the first bioassay (vegetative stage) in Indiana in 1991 allowed for the establishment of only eight dishes per line.

We changed leaflets as needed, usually at no more than 3-day intervals. Upon pupation, we placed pupae into small, individual containers containing a moist piece of filter paper. Pupae were checked daily for adult emergence.

**Data Analysis.** We calculated overall percentage of mortality within each dish. Percentage mortality (x) was transformed before analysis by arcsine Vx/100 to control the variability. We calculated egg-to-adult development similarly; data for larvae within a dish for each line were combined. Only data from those insects completing egg-to-adult development were included in the analysis of de-
velopmental data. Because 100% mortality occurred before pupation within some petri dishes during certain trials, the experimental design was not balanced. Therefore, degrees of freedom varied for the analyses for developmental times. Data were analyzed using analysis of variance (ANOVA) or general linear models (GLM) procedures (GLM used with unbalanced data) (SAS Institute 1988). The experiment was analyzed as a $3 \times 6$ factorial (three soybean growth stages by six soybean lines) with 10 samples per line at each growth stage (the 10 dishes per line were considered samples and not replications). Data were analyzed for each state separately during each of the 2 yr.

In 1992, 100% larval mortality occurred on the line IIC83-19-2 within a few days of the onset of the third bioassay (during the pod-fill stage) in Indiana. This mortality was completely unexpected and unexplainable and did not compare with any of the other lines where there was 80–90% survival during the bioassay. Because the mortality was so quick and complete, we believe that the mortality was not caused by resistance in the soybean line, feeling it was related to some unknown factor. Therefore, the data from this line during this bioassay were not included in the analyses (resulting in a reduction in the degrees of freedom).

**Results**

**Mortality.** Mortalities at both locations during 1991 and 1992 are presented in Figs. 1 and 2, respectively. During 1991 (Fig. 1), a significant interaction occurred between the two main factors, growth stage and soybean line (significant differences were also obtained for each main factor) (Ohio: interaction-$F = 7.8$; df = 10, 162; Pr $> F = 0.0001$; growth stage-$F = 116.2$; df = 2, 162; Pr $> F = 0.0001$; soybean line-$F = 54.3$; df = 5, 162; Pr $> F = 0.0001$) (Indiana: interaction-$F = 4.1$; df = 10, 150; Pr $> F = 0.0001$; growth stage-$F = 104.4$; df = 2, 150; Pr $> F = 0.0001$; soybean line-$F = 17.1$; df = 5, 150; Pr $> F = 0.0001$). All four resistant soybean lines grown in Ohio (MBB80-133, L86K-73, HC83-19-2 and HC83-123-9) were highly resistant during the vegetative and flowering stages, with mortality ≥95%. This compared with larval mortality on the susceptible soybean lines between 34 and 66% (we often see these moderate levels of mortality on lines considered susceptible to insect feeding). During the pod-fill stage, we observed less mortality for all lines. However, mortality was much lower for the two Indiana lines, MBB80-133 and L86K-73 (20 and 14%, respectively), compared with the two Ohio lines, IIC83-19-2 and HC83-123-9 (68 and 90%, respectively). We observed similar results in Indiana, with the exception that the reduction of mortality began in the flowering stage and continued into the pod-fill stage. Also, the two Indiana lines lost their resistance by the flowering stage (mortality between 18 and 30% compared with the susceptible with 42%), whereas the Ohio lines maintained either a high level of resistance at the flowering stage (IIC83-123-9 with a mortality of 94%) or at a moderate level (HC83-19-2 with a mortality of 56%). Although there was a difference when resistance diminished among the four resistant lines, there was, nonetheless, greater larval survivorship in the latter soybean growth stages for all soybean lines.

We observed much lower levels of resistance in 1992 (Fig. 2). Significant differences were obtained for the main factors of growth stage and soybean line in Ohio; the interaction was not significant (growth stage-$F = 10.2$; df = 2, 162; Pr $> F = 0.0001$; soybean line-$F = 9.22$; df = 5, 162; Pr $> F = 0.0001$; interaction-$F = 1.2$; df = 10, 162; Pr $> F = 0.413$). In Ohio, a reduction in Mexican bean beetle mortality was observed across soybean lines at each successive soybean growth stage (overall mortality at the vegetative stage = 25.7%, at the flowering stage = 16.7%, and at the pod-fill stage = 9.0%). Significantly greater mortality across all growth stages occurred on the two Ohio lines (overall mortality for IIC83-123-9 = 34.0% and for HC83-19-2 = 27.3%) compared with the remaining lines (overall mortality

![Fig. 1. Percentage of mortality (±SEM) of Mexican bean beetle larvae in Ohio and Indiana during 1991 intensive, resistance bioassays (solid bars represent mortalities during the vegetative stage, narrow cross-bars represent mortalities during the flowering stage, wide cross-bars represent mortalities during the pod-fill stage; soybean lines: 80-133 = MBB80-133, K-73 = L86K-73, 123-9 = HC83-123-9 and 19-2 = HC83-19-2). For each mean, n = 10, except for the vegetative stage in Indiana when n = 8 (see text).](image-url)
<12.0%). Larval mortality was extremely low in Indiana (Fig. 2), and no significant differences were obtained for either main factor or their interaction (Indiana; growth stage—$F = 1.8$; $df = 2, 153$; $Pr > F = 0.16$; soybean line—$F = 0.8$; $df = 5, 153$, $Pr > F = 0.54$; interaction—$F = 1.8$; $df = 10, 153$; $Pr > F = 0.08$).

**Egg-to-Adult Development.** Development of Mexican bean beetles from egg to adult reared on resistant soybean lines in Ohio and Indiana during 1991 and 1992 are presented in Figs. 3 and 4, respectively. During 1991 (Fig. 3), a significant interaction between growth stage and soybean line occurred in both states (both main factors were also significant) (Ohio: interaction—$F = 5.85$; $df = 5, 76$; $Pr > F = 0.0001$; growth stage—$F = 22.02$; $df = 2, 76$; $Pr > F = 0.0001$; soybean line—$F = 25.98$; $df = 5, 76$; $Pr > F = 0.0001$) (Indiana: interaction—$F = 2.34$; $df = 8, 114$; $Pr > F = 0.0232$; growth stage—$F = 374.3$; $df = 2, 114$; $Pr > F = 0.0001$; soybean line—$F = 25.57$; $df = 5, 114$; $Pr > F = 0.0001$). Because larval mortality during the vegetative and flowering stages in Ohio was often 100%, a good comparison of all interactions is difficult in that state. However, egg-to-adult development in Ohio during the pod-fill stages for those lines measured was always less compared with the development during the earlier flowering stage, which indicates a loss of resistance as the plant matured. The reduction in the length of egg-to-adult development as the plant matures is more evident from the Indiana data. With those lines measured, there was faster development when beetles were reared on soybean in the later plant growth stages. This reduction in development periods was observed, not only on the resistant lines, but also on the susceptible lines.

Differences in developmental periods between growth stages and soybean lines were much less in 1992 (Fig. 4), which corresponds to the lower mortalities obtained this year. We obtained a significant difference for the interaction of main factors in Ohio for = 14.24; $df = 10, 158$; $Pr > F = 0.0001$; growth stage—$F = 254.8$; $df = 2, 158$; $Pr > F = 0.0001$; soybean line—$F = 108.0$; $df = 5, 158$; $Pr > F = 0.0001$). In Ohio, developmental times were reduced as the plants matured; the reduction being more evident with the four resistant germplasm lines. Although there were slight reductions with the two susceptible lines, they were not as strong. In Indiana, the interaction between growth stage and line was significant (only the main factor of
The reduction in the relative level of insect resistance as the plants matured compares favorably with recent findings of other researchers. Nault et al. (1992a) had found that bollworm larvae typically preferred older leaves of the resistant line GatIR81-296 over younger leaves throughout the season, indicating that resistance declined with maturity. Nault et al. (1992b) also reported that resistance in GatIR81-296 was reduced in intact reproductive stage plants compared with vegetative stage plants in both choice and no-choice tests. There was greater mortality during the vegetative stages and heavier pupal weights during the reproductive stages. Nault et al. suggested that vegetative stage foliage probably contains a higher level of an inhibitory component in comparison with reproductive stage foliage. Rowan et al. (1993) examined the effect of plant maturity on defoliation levels in the field and found that plant maturity was not associated with defoliation when it occurred in the vegetative or early reproductive stages. However, when insects occurred late, there was a strong correlation between plant maturity and reduced defoliation; either larvae preferred older leaf tissue or the antibiosis expressed in leaves decreased in late growth-stage plants. Rowan et al. (1993) suggested that these observations may help explain the genotype × environment interactions that occur in regional host-plant resistant nurseries, and partially account for the difficulties in developing high-yielding, insect-resistant cultivars.

The differences in resistance between plants of differing maturity have important ramifications for breeding programs. We concur with Nault et al. (1992a) and Rowan et al. (1993) who stated that accurate identification of soybean resistance should be done with lines of similar stage of development when directly comparing soybean lines.

As we develop and release insect-resistant soybean cultivars, we need to educate growers and advisory services on their use and potential limitations caused by plant maturity (Rowan et al. 1993). Because of a loss of resistance as the crop ages, growers need to closely monitor insect pest status later into a season when using resistant varieties. The question arises as to the potential value of these resistant lines. Will soybean cultivars currently being developed maintain sufficient levels of resistance to be of value in the later reproductive growth stages when many insect problems on soybean occur? Will soybean varieties only be of value for early season pests if they are resistant solely in the vegetative and early reproductive stages? Can these lines be of best use against multiple generation pests that begin their population development early in the season? We need to address these questions to determine the future of host-plant resistance as an arthropod management tactic in soybean.

The loss of resistance that we observed in both states from the 1st to the 2nd yr is perhaps even more important. Although we expected the levels of resistance observed in 1991 (based on previous work with this germplasm), we did not expect the near total loss of resistance in 1992. An examination of rainfall pattern and amounts between the two years suggests that the likely reason was the excessive rain that occurred in July of 1992 in both

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**Discussion**

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states. The summer of 1991 was considered to have dry to normal soil conditions (specific data on soil moisture were not taken). Rainfall amounts in 1991 for May, June, July, and August were 8.2, 4.2, 2.2, and 7.8 cm in Ohio (averages of 10.1, 10.1, 10.6, and 9.4 cm), and 12.4, 2.2, 1.3, and 7.9 cm in Indiana (averages of 10.0, 4.3, 6.1, and 8.9 cm), respectively. Compare this with the extremely wet conditions of 1992. Although May and June were relatively dry (Ohio received 5.1 and 5.5 cm, and Indiana received 2.6 and 4.5 cm, respectively), July had much larger amounts of rain in both states. Ohio received 21.1 cm (average for July of 10.6 cm) and Indiana received 30.2 cm (average for July of 6.1 cm). The heavy rainfall began in both states in early July and coincided with the start of the bioassays for that year (9 and 11 July in Ohio and Indiana, respectively). Rainfall in August of 1992 was 10.2 cm in Ohio and 2.2 cm in Indiana.

Direct effects of plant water deficiency and insect resistance in soybean have not been reported in the literature, nor for that matter, with other crops (Smith 1989). McQuate & Connor (1990a) studied the effect of water deficits on Mexican bean beetle on an insect susceptible soybean cultivar and found that larvae preferred to feed on foliage from well-watered plants. McQuate & Connor (1990b) also reported a reduction in larval survival, growth rate, pupal weights and an increase in development time when larvae were reared on foliage from plants subjected to water deficits. Lambert & Heatherly (1991) observed higher insect defoliation in the field when soybean plants were grown in wetter soils or under irrigation. They conducted subsequent laboratory studies and found that soybean looper, Pseudoplusia includens (Walker), larval weights were reduced, and larval developmental periods increased, when reared on soybean grown in soils under reduced soil water potentials. These findings support our results that indicate a loss of resistance and greater susceptibility in 1992 during periods of surplus moisture.

As with plant maturity, we need to consider the impact of water availability in breeding programs (Lambert & Heatherly 1991). We should compare germplasm lines only when they are grown under differing moisture regimes. Changes in the concentrations of feeding deterrents, antibiotic factors, and nutrient levels offer possible explanations. McQuate & Connor (1990a, b) suggest that changes in foliage chemistry caused by water deficits, possibly in the concentration of free amino acids, was the most likely reason for their differences in insect behavior and growth. Further studies are needed to delineate the specific reasons for observed changes in insect resistance that are apparently caused by differing moisture regimes.

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