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Pod Mottle Disease in an Early Season Production System¹

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ABSTRACT The influence of narrow and wide-row soybeans on infestations of bean leaf beetle (BLB), Cerotoma trifurcata (Forster) (Coleoptera: Chrysomelidae) adults, a vector of bean pod mottle virus (BPMV), and associated incidence of bean pod mottle (BPM) disease were investigated in maturity groups IV and V soybeans in Mississippi. Maturity group IV soybeans had greater cumulative BLB numbers and greater incidence of BPM than maturity group V soybeans in 2000, but not in 2001. Row width was not shown to affect beetle numbers in either study year, but a greater incidence of BPM occurred in narrow row soybeans in 2001 and Maturity Group IV soybeans in 2000. There was no significant correlation between numbers of BLB adults and soybean plants infected with BPM virus when data was analyzed within sample dates or by seasonal totals. Greater yields were obtained in maturity group V soybeans than in maturity group IV soybeans in 2000, but not 2001, whereas row width had no significant effect on yield in either 2000 or 2001. The results presented herein suggest that further investigations of soybean row spacing in relation to BLB and BPM disease should consider large experimental plots to minimize beetle dispersal and spread of BPM disease.

KEY WORDS soybeans, planting date, row width, bean leaf beetle, bean pod mottle disease

Row width is one agricultural variable that may be altered by farmers to affect management and economics of soybean production. It has been a common practice in the mid-South in the United States to plant soybeans in wide rows where herbicides may be used effectively to manage weeds (Caviness et al. 1987). Because most farmers produce more than one crop type, soybeans are planted in wide rows so that standard farm equipment may be used for all crops. Due to the advent of more efficient herbicide treatments and Roundup ReadyTM varieties, weed management practices are available to allow narrow-row soybean plantings (Mangold 1980). Soybeans planted in narrow rows achieve canopy closure in a shorter period of time than soybeans planted in wide rows, further limiting weed infestation. Narrow-row plantings of soybeans typically yield more than wide-row plantings in years when sufficient moisture is available for plant development

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(Bowers 1995). The primary reason for greater yields in narrow-row soybeans is the earlier time at which canopy closure is attained. Ninety to 95% sunlight interception during late vegetative and early reproductive stages is a prerequisite for maximum soybean yield (Shibles & Weber 1966). Because yield is a function of canopy light interception, and narrow-row soybeans are more efficient in intercepting available sunlight, greater yields may be expected for narrow-row than wide-row plantings (Ablett et al. 1991).

A relatively recent soybean production recommendation includes early planting of an early season maturity group and is referred to as early season production system (ESPS) (Heatherly 1999). The ESPS involves planting soybean maturity groups III or IV in mid-March to mid-April. Planting early and utilizing an early soybean maturity group allows the farmer to harvest much earlier (ca. 1 mo) than if soybeans were planted in mid- to late May. The critical seed-set development stage for soybeans in the ESPS escapes most harmful environmental stresses and relatively high insect pest infestations.

Narrow-row soybean planting practices have an effect on the behavior of some insect pest species (Hamadain & Pitre 2002). The purpose of this study was to determine effects of narrow-row and wide-row planting practices in the ESPS on bean leaf beetle (BLB), *Cerotoma trifurcata* (Förster), adult populations and associated incidence of bean pod mottle (BPM) disease vectored by the beetles.

Materials and Methods

2000. Soybeans were planted on 28 April 2000 using a John Deere MaxEmerge II planter on the Mississippi Agricultural and Forestry Experiment Station Plant Science Research Farm located in Oktibbeha Co., Mississippi. Planting date for this year was later than recommended for ESPS. Roundup ReadyTM (RR) varieties included a maturity group IV soybean, Pioneer 9492 RR, and a maturity group V soybean, Pioneer 95B95 RR, each planted in wide and narrow rows. Wide-row plots consisted of 12 rows, 96.5-cm wide and 16.5 m long. Narrow-row plots consisted of 24 rows, 48.3-cm wide and 16.5 m long, to achieve plots of the same total area as the wide-row plots. Four treatments were established with four replications in a randomized complete block design. Roundup Ready varieties were used because they represent current soybean production practices in the mid-South (National Agriculture Statistics Service, 2001). DualTM (metolachlor, Ceiba-Geigy Corp., Greensboro, NC) herbicide at a rate of 2.8 L/ha, and Roundup (glyphosate, Monsanto, Chesterfield, MO) herbicide at a rate of 2.1 L/ha were applied at planting and at the V6 stage of plant growth (Fehr & Caviness 1977), respectively. Number of plants/m of row was recorded by counting the number of plants in 3.6 m of row.

Sampling for BLB adults from emergence through the V5 stage of plant growth was conducted by visually sampling individual plants in one row on each sample date. Special attention was directed to cotyledonous leaves because this is a preferred feeding location for the beetles. Visual samples were taken weekly from June 7 to 28 and consisted of three subsamples, each 3.6 m of row (10.4 m² total area) in wide row plots. The sample size in narrow-row plots consisted of two adjacent 3.6 m of row (10.4 m² total area) and contained approximately twice the number of plants as wide-row plots. A random number generator was used to

determine the row to be sampled, excluding rows sampled in the three previous weeks. The number of BLB adults within each subsample was recorded.

After the V5 growth stage, plants were large enough to be sampled weekly with a sweep net (Kogan et al. 1980). Sweep net sampling, using a 38 cm diam. net, was conducted from July 5 to September 15. Each sample consisted of 36 sweeps (13.2 m² total area) taken from a randomly selected row in each wide row plot. Two adjacent rows were sampled in narrow-row plots so that the same total area was sampled in wide-row and narrow-row plots. Sampling on adjacent rows was accomplished by drawing the sweep net across both rows. On July 5, sampling was conducted by both visual and sweep net sampling methods to determine if there was a significant difference in efficiency levels of sampling methods. No significant difference in sampling efficacy was observed. Sample areas were marked to prevent resampling within four weeks. Samples were bagged, transported to the laboratory, and the number of BLB adults within each sample was recorded.

Plants within one row (16.5 m) in each wide row plot with apparent symptoms of BPM were visually identified and recorded weekly throughout the growing season. Characteristic mottling of leaves in the upper canopy (top 2–3 nodes) caused by BPMV (Windham & Ross 1985a) was used to visually identify the disease. As with samples for BLB adults, two adjacent rows were sampled for BPM in narrow-row plots in order to obtain the same total sample area. The number of plants with apparent symptoms of BPM was recorded. Sampling was conducted on a weekly basis beginning on June 7, and care was taken to avoid resampling of the same area within individual plots.

To confirm accuracy of identifying BPM diseased plants in the field, soybean leaves were harvested from the upper two nodes of R2 stage (Fehr & Caviness 1977) plants visually identified as positive or negative for symptoms of the disease. The leaf samples were tested for the presence of BPMV using enzyme linked immuno-sorbent assay (ELISA) (AgDia, Elkhart, IN). Ten symptomatic and ten asymptomatic plants were tested. Because symptoms of soybean mosaic disease may appear similar to those of BPM on soybeans, plants were also tested for soybean mosaic virus (SMV) using ELISA procedures.

Yield measurements were taken by harvesting the middle four rows (66.0 row m) of each wide row plot and an equal area within narrow row plots. Plots were harvested using an MXP four-row plot harvester when the seed was at 12-13% moisture. Due to variance in moisture levels, not all treatments were harvested on the same date. The seed was bagged and taken to the laboratory. Each sample was hand cleaned to remove foreign material and seed weight was measured using a standard scale.

Data were analyzed by Proc GLM using SAS v. 8.2 (SAS Institute 2002). Analysis of variance and correlation procedures were conducted on numbers of bean leaf beetles and BPM symptomatic plants. Fisher's protected LSD was used to determine significant differences between numbers of bean leaf beetles, diseased plants, and yield among treatments.

2001. Soybean plots in 2001 were located in the same field using the same varieties, treatments, and procedures as in the 2000 study. Wide-row plots consisted of 20 rows, each 96.5 cm wide and 16.5 m long, and narrow-row plots consisting of 40 rows, each 48.3 cm wide and 16.5 m long. All plots were planted on April 10 and Dual and Roundup herbicides were applied as in the 2000 study.

Sampling for BLB adults was initiated on May 4 by visual examination of individual plants in one row on each sample date from emergence through the V5 growth stage. Sample sites within each plot were selected and plant samples were taken as described in the 2000 study. Visual samples consisted of 10.4 m^2 of total area within wide row and narrow row plots. Samples were taken to the laboratory and the number of BLB beetle adults was recorded. After the V5 growth stage, samples were taken weekly from June 8 to September 7 using the sweep net sampling method as described in the 2000 study. Sweep net samples consisted of 13.2 m^2 of total area within wide-row and narrow-row plots. On June 8, sampling was conducted by both visual and sweep net sampling methods again to determine if significant difference existed between sampling efficiencies. No significant difference in sampling efficacy was observed.

Sampling for BPM diseased plants within plots throughout the growing season was as described in the 2000 study and was initiated on May 25. Accuracy in field identification of BPM and SM was determined using ELISA procedures. Yield measurements were obtained and data were analyzed using SAS v. 8.2 as described in the 2000 study.

Results and Discussion

There was no significant correlation between numbers of BLB adults and soybean plants infected with BPM virus when data were analyzed within sample dates or by seasonal totals in both 2000 and 2001 (r = 0.0826: 2000 and r = 0.0763: 2001, respectively). Therefore, the data for BLB and BPM diseased soybeans in the two planting systems will be discussed independently.

2000. Soybeans averaged 29 plants per m^2 in wide row plots and 58 plants per m^2 in narrow row plots in 2000.

Adult BLB were first collected on soybeans on June 28, 24 d after emergence of plants in all plots (Fig. 1). At that time, adults in 10.4 m² of foliage numbered 12 ± 1 in narrow-row, maturity group IV soybeans; 15 ± 1 in wide-row, maturity group IV soybeans; 13 ± 1 in narrow-row, maturity group V soybeans; and 12 ± 1 in wide-row, maturity group V soybeans. Initial beetle infestations were not significantly different among planting systems (F = 1.00; df = 3, 12; P = 0.4262). The low number of BLB collected and their late occurrence may be explained by the behavior of BLB as they emerged from overwintering sites adjacent to the field (Schumm et al. 1983). Soybeans did not emerge at the time of peak BLB emergence from overwintering; therefore, emerging adult beetles dispersed from the study area to soybeans planted earlier in the surrounding areas. Bean leaf beetle peak emergence has been reported to occur in mid-May in Illinois (Jeffords et al. 1983).

Number of BLB adults in narrow-row and wide-row plantings remained similar until August 16, at which time significantly greater numbers were observed in wide-row, maturity group IV soybeans than in other plantings (F = 6.68; df = 3, 12; P = 0.0067). Eleven days later, no significant differences were observed in beetle infestations among planting systems (F = 0.18; df = 3, 12; P = 0.9067). Beetle infestations in narrow row, maturity group IV and the maturity group V soybeans had increased to levels similar to that in wide-row, maturity group IV soybeans.

When the seasonal cumulative numbers of BLB adults were compared, significantly greater numbers of beetles were recorded in maturity group IV than in maturity group V soybeans (F = 4.98; df = 1, 14; P = 0.0425) (Table 1).



Fig. 1. Bean leaf beetle (BLB) population levels in narrow-row maturity group IV (NIV), narrow-row maturity group V (NV), wide-row maturity group IV (WIV), and wide-row maturity group V (WV) soybeans in Oktibbeha Co., Mississippi, 2000.

Interaction between maturity group and planting date was not significant (F = 1.00, df = 1, 12, P = 0.3373). Seasonal cumulative numbers of BLB adults recorded from a study conducted adjacent to this study did not show a significant difference in BLB adults between treatments similar to those described in the present study. The lack of observed differences may have been due to the location of the research plots closer to overwintering sites for BLB, resulting in uniform distribution of beetles throughout the study site. In addition, the greater area of early planted soybeans in the study reported herein may have resulted in the attraction of more BLB adults to the study site.

Symptoms of BPM disease on soybeans in treatment plots were first observed June 28 (Fig. 2). Incidence of disease in maturity group IV soybeans was significantly greater than that in maturity group V soybeans (F = 6.68; df = 3, 12; P = 0.0067). Although greater numbers of diseased plants were observed in maturity group IV plantings than in other planting systems throughout the season, significant differences were not observed after August 10. Further research is needed to describe the relationships among maturity group, row width and incidence of BPM disease. The greater incidence of disease in early-maturing group IV soybeans may reflect greater plant susceptibility in early developmental stages to injury by BPM virus (Windham & Ross 1985a).

Table 1. Seasonal total number of bean leaf beetle adults, bean pod
mottle (BPM) diseased plants and yield for narrow-row,
maturity group IV; wide-row, maturity group IV; narrow-row,
maturity group V; and wide-row, maturity group V soybeans in
Oktibbeha Co., Mississippi, 2000.

Main effect	Bean leaf beetles per 13.2 m ² foliage ¹	BPM diseased plants per 16.5 m row ¹	Yield (kg/ha)
Row width			
Narrow	$71\pm10~\mathrm{a^2}$	155 ± 17 a	396 \pm 47 a
Wide	$84~\pm~10~a$	164 ± 17 a	444 ± 47 a
Maturity group			
IV	86 ± 10 a	188 ± 17 a	$324\pm47~\mathrm{b}$
V	$69~\pm~10~\mathrm{b}$	132 ± 17 b	516 ± 47 a

 $^1\!\mathrm{Means}$ in a column within main effect followed by the same letter are not significantly different (P < 0.05).

 2 Least square means (±SE).



Fig. 2. Incidence of bean pod mottle (BPM) diseased plants in narrow-row maturity group IV (NIV), narrow-row maturity group V (NV), wide-row maturity group IV (WIV), and wide-row maturity group V (WV) soybeans in Oktibbeha Co., Mississippi, 2000.

When seasonal cumulative total incidence of BPM diseased soybean plants within plots was analyzed, neither maturity group nor planting date main effects were significant (Table 1). Interaction between maturity group and planting date was also not significant (F = 0.0; df = 1, 12; P = 1.00).

Testing by ELISA for accuracy in disease identification in the field showed a 90% level of accuracy in selecting BPM diseased plants and a 100% level of accuracy in selecting non-infected plants; no plants were determined to be infected by SMV.

There was no significant effect of row-width on yield (F = 6.0; df = 1, 14; P = 0.4519) (Table 1). However, significantly greater yield was obtained from maturity group V than maturity group IV soybeans (F = 48.39; df = 1, 14; P < 0.0001). Interaction between maturity group and planting date was not significant (F = 0.17; df = 1, 12; P = 0.6894). The greater yields for maturity group V soybeans may be due to the relatively late planting in 2000. Dry conditions during the critical growth stages of maturity group IV soybeans did not provide optimum conditions for plant development. According to rainfall measurements recorded at the Mississippi Agriculture and Forestry Experiment Station (MAFES), the total growing season (May–September) rainfall for 2000 was 21.5 cm; this was 24.1 cm below the 10 y seasonal average of 45.6 cm for the study site (MAFES, unpublished). Rainfall was particularly low during the late vegetative and early reproductive growth stages of soybeans in August and September in this study.

2001. Soybeans averaged 29 plants per m^2 in wide row plots and 58 plants per m^2 in narrow row plots in 2001.

In 2001, BLB adults were first observed on May 4, 25 d after plant emergence (Fig. 3). Greater numbers of beetles on soybeans in 2001 (Table 2) than in 2000 (Table 1) can be attributed to greater numbers of overwintering beetles colonizing the earlier planted soybeans in 2001 as compared with the later planting in 2000. Number of BLB adults in maturity group IV soybeans on the initial sampling date was significantly greater than in maturity group V soybean plots (F = 3.47; df = 3, 12; P = 0.0509) (Fig. 3). This difference may reflect an initial BLB preference for maturity group IV soybeans over maturity group V soybeans. However, this difference did not persist after the first sampling date. As in the 2000 study, in the same field, the initial population levels in treatment plots declined after the first week of sampling. This decline may have been due to natural mortality of the overwintering population of adults.

Significantly greater numbers of BLB adults were recorded in wide row, maturity group IV and V soybeans than in narrow row, maturity group IV soybeans on July 11 (F = 2.19; df = 3, 12; P = 0.0036) after which the beetle population declined sharply. This decline in beetle numbers may be related to natural adult mortality in the second generation. The population levels of BLB adults remained low after this time.

The cumulative seasonal total of BLB adults revealed that there was no significant difference in numbers of beetles in narrow row planted soybeans and wide row planted soybeans (Table 2). Also, maturity group did not significantly affect numbers of adult BLB in 2001 (F = 0.39; df = 1, 12; P = 0.5431). Interaction between maturity group and planting date was not significant (F = 0.03; df = 1, 12; P = 0.8686). Seasonal cumulative numbers of BLB adults recorded from a study conducted adjacent to this study showed significantly greater numbers of BLB adults in Maturity Group IV soybeans than in Maturity



Fig. 3. Bean leaf beetle (BLB) population levels in narrow-row maturity group IV (NIV), narrow-row maturity group V (NV), wide-row maturity group IV (WIV), and wide-row maturity group V (WV) soybeans in Oktibbeha Co., Mississippi, 2001.

Table 2. Seasonal total number of bean leaf beetle adults, bean pod
mottle (BPM) diseases plants, and yield for narrow-row,
maturity group IV; wide-row, maturity group IV; narrow-row,
maturity group V; and wide-row maturity group V soybeans in
Oktibbeha Co., Mississippi, 2001.

Main effect	Bean leaf beetles per 13.2 m ² foliage ¹	BPM diseased plants per 16.5 m row ¹	Yield (kg/ha)
Row width			
Narrow	$116\pm10\mathrm{a}^2$	178 ± 22 a	557 ± 53 a
Wide	132 ± 10 a	82 ± 22 b	568 ± 53 a
Maturity group			
IV	$126~\pm~10~\mathrm{a}$	136 ± 22 a	581 ± 53 a
V	123 ± 10 a	124 ± 22 a	544 ± 53 a

¹Means in a column within main effect followed by the same letter are not significantly different (P < 0.05).

 $^{2}\text{Least}$ square means (±SE).



Fig. 4. Incidence of bean pod mottle (BPM) diseased plants in narrow-row maturity group IV (NIV), narrow-row maturity group V (NV), wide-row maturity group IV (WIV), and wide-row maturity group V (WV) soybeans in Oktibbeha Co., Mississippi 2001.

Group V soybeans. The greater number of beetles in Maturity Group V soybeans in contrast to Maturity Group IV soybeans in the adjacent study may have been due to the plants being more attractive to the beetles as they emerge from overwintering. Greater attractiveness of Maturity Group V soybeans may have been due to continuing vegetative growth. This preference may have been weaker as beetles dispersed throughout the study area.

Symptoms of BPM disease on young soybeans were first observed June 1 (Fig. 4). No significant differences in incidence of diseased plants were recorded at this time. In the following week, a significantly greater number of diseased plants was observed in narrow-row, maturity group V soybeans than in narrow-row, maturity group IV soybeans (F = 2.92; df = 3, 12; P = 0.0776).

A greater number of BPM diseased plants were observed in narrow-row soybean plantings of maturity groups IV and V on June 28, July 5, and August 2 (F = 6.68; df = 3, 12; P = 0.0067: F = 1.54; df = 3, 12; P = 0.0123; F = 7.32; df = 3, 12; P = 0.0048, respectively)than in wide-row plantings (Fig. 4). The greater incidence of BPM disease in narrow-row soybeans may be accounted for by the higher concentration of plants within these plots compared with wide-row plots of equal planted area. The close association of these plants could allow for greater movement of viruliferous beetles from plant to plant and row to row.

Observations of BPM disease incidence throughout the sample period were characterized by high variance in observed numbers of diseased plants within sampling dates and fluctuations in observed numbers of diseased plants from sampling date to sampling date. This variance in disease data was apparently due to a clumped distribution of diseased plants in the study area. This clumped distribution occurred as beetles acquired the virus from feeding on infected soybean plants and then moving to adjacent plants to feed, spreading the virus (Windham & Ross 1985a).

The seasonal cumulative total incidence of BPM diseased plants within plots revealed that the number of diseased plants in narrow-row soybeans was significantly greater than the number of diseased plants observed in wide-row soybeans (F = 16.9; df = 3, 12; P = 0.0001) (Table 2). Interaction between maturity group and planting date was not significant (F = 0.13, df = 1, 12, P = 0.7294).

Testing by ELISA for accuracy in disease identification in the field as in the 2000 study indicated a 100% level of accuracy in identifying BPM infected plants and a 90% level of accuracy in identifying non-infected plants; no plants were determined to be infected by SMV.

Neither soybean maturity group nor row width main effects influenced yield in the 2001 study (Table 2). Interaction between maturity group and planting date was not significant (F = 3.35; df = 1, 12; P = 0.092). The greater yields in 2001 compared with 2000 may be attributed to the greater amount of rainfall at the study site in 2001 (155 cm, which is 28 cm greater than the ten year average of 127 cm and 55 cm greater than in the previous season). In 2000, lowest levels of rainfall occurred during July and August when maturity group IV soybeans were entering the reproductive stages of plant development. Row width was not shown to significantly affect soybean yield in this study. It is reasonable to believe that plant growth stage, condition of plants when infested with BLB adults, and plant infection with BPM are factors that could influence soybean yield (Windham & Ross 1985b, Hunt et al. 1995). A limitation in this row spacing study appeared to be the size of soybean treatment plots, because of the ability of bean leaf beetles to disperse within crop production areas. Larger experimental areas would minimize vector beetle movement and spread of BPM virus from plot to plot. This would be particularly significant during the vegetative growth stages of soybean varieties of different maturity groups, when plants are most susceptible to infection by BPM virus. Small plots do not limit to any significant degree the movement of vector beetles from plot to plot throughout the developmental stages of soybean plants of different maturity groups. Dispersal would be most apparent when early maturing soybeans become less attractive than later maturing soybeans growing nearby and in more attractive vegetative stages. Further investigations of soybean row spacing in relation to BLB and BPM disease should consider larger experimental plots than those included in this study.

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