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Date June 1986

THE INHERITANCE OF RESISTANCE TO STEM CANKER IN SOYBEANS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Brian A. Wendel

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ABSTRACT

The fungus, Diaporthe phaseolorum (Cke. and Ell.) Sacc. var. caulivora Athow and Caldwell is the causal agent of the stem canker disease in soybeans [Glycine max (L.) Merr.]. Soybean yield losses up to 80% or more in areas of the southeastern United States during the early 1980s prompted the urgency of determining the inheritance of resistance in order to facilitate development of new varieties with resistance to the pathogen. The objectives of this study were to: (1) compare the relative virulence of two stem canker isolates from Mississippi and two from Tennessee, (2) determine the inheritance of resistance to stem canker among 'Bay,' 'Tracy-M,' 'Bragg' and 'J77-339' soybean varieties, and (3) determine if the gene(s) conditioning resistance is the same in the varieties Bay and Tracy-M.

All possible crosses, including reciprocals were made among the resistant parents, Bay and Tracy-M and the susceptible parents, Bragg and J77-339. During 1984 and 1985, the selfs, F_1, F_2, BC1 and BC2 plants of each cross were tested for their reaction to inoculation with the stem canker pathogen under greenhouse conditions. The Tennessee isolate, labeled FP3, was determined the most virulent from the greenhouse tests conducted with the four isolates. Thus, the FP3 isolate was the only isolate used for testing the selfs, F_1, F_2, BC1 and BC2 populations from each cross combination.

Resistance to stem canker in the four soybean varieties is likely conditioned in the following ways: (1) Bay has a single dominant gene ($R_1R_1r_2r_2$), (2) Tracy-M has two dominant, genes ($R_1R_1R_2R_2$), (3) Bragg

is segregating for the R_2 gene ($r_1r_1R_2$--) and, (4) J77-339 is homozygous recessive for both genes ($r_1r_1r_2r_2$). The single dominant gene present in Bay appears to be the same as one of the dominant genes in Tracy-M. The high level of resistance in Bay and the low level of resistance in Bragg indicates that the R_1 gene confers more resistance than the R_2 gene.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

The fungus, Diaporthe phaseolorum (Cke. and Ell.) Sacc. var. caulivora Athow and Caldwell is the casual agent of the stem canker disease in soybeans [Glycine max (L.) Merr.]. Stem canker has been identified throughout the entire North-American soybean production areas (2). The disease is of economical importance as it is capable under certain circumstances of destroying entire crops of soybeans (2, 16). During the decades of 1950 and 1960, stem canker was reported in northern production areas such as Ontario (13), Minnesota (10) and Iowa (5). It became a major economic problem due to the widespread planting of two extremely susceptible cultivars 'Blackhawk' and 'Hawkeye' (13). The problem was overcome by planting resistant cultivars and following a crop rotation plan. The disease has now shifted into the southeastern soybean production areas during the decades of 1970 and 1980. The following southeastern states have experienced yield reduction in soybeans due to stem canker: Alabama (2), Tennessee (16), Mississippi (19), South Carolina (25), and Georgia (29).

History of the Disease

It is difficult to ascertain the initial recorded occurrence of stem canker on soybeans in the United States. From 1923 until 1949, stem canker and pod and stem blight were classified together as a single fungal disease complex called pod and stem blight of soybeans (18). In deference to the early years when the two diseases were

jointly classified as pod and stem blight it would be beneficial to trace the manner through which the two diseases were separated from each other.

During 1920, Wolf and Lehman (37) observed a new disease of soybeans growing in North Carolina and reported it as Phoma blight. Two years later, Lehman (26) altered the causal name to Phomopsis sojae and called the disease pod and stem blight. In 1923, Lehman (27) attributed the pod and stem blight disease of soybeans to a species of Diaporthe which changed the causal agent to Diaporthe sojae. In 1933 Wehmeyer (35) assimilated the published data on the genus Diaporthe. Wehmeyer's work focused on the actual morphological features of the Diaporthe fungi instead of pathological effects on host plants. As a result of the work, Diaporthe phaseolorum was designated as the type species for those fungi with elongated ostiolar necks. Diaporthe sojae of soybeans was reduced to the varietal status of D. phaseolorum var. sojae and Diaporthe batatatis of sweet potato was reduced to D. phaseolorum var. batatatis (35).

Studies conducted by Luttrell in 1947 (28) compared the morphology, growth in culture, pathogenicity and host range of D. phaseolorum and D. phaseolorum var. sojae. He concluded that positive separation between the two was dependent upon pathogenic differences as well as appearance in culture and that varietal status should be retained for D. phaseolorum var. sojae. Later work by Welch and Gilman (36) in 1948 enabled them to distinguish between two varieties of the Diaporthe genus on the basis of the following criteria: thallism, morphological

features, and pathogenicity. The two varieties included D. phaseolorum var. sojae and var. batatatis. It was determined that D. phaseolorum var. sojae was heterothallic, producing single perithecium in addition to conidia and was a weak pathogen. On the other hand, D. phaseolorum var. batatatis was homothallic, produced caespitose perithecia without conidia and was identified as a strong pathogen. Welch and Gilman's efforts (36) enabled pod and stem blight to be classified as D. phaseolorum var. sojae and the future "stem canker" to be identified as D. phaseolorum var. batatatis. Crall in 1950 (5) proposed that "stem canker" be adapted as a descriptive name for the soybean disease caused by D. phaseolorum var. batatatis to separate it from pod and stem blight induced by D. phaseolorum var. sojae.

Athow and Caldwell (1) in 1954 reinforced the findings of Welch and Gilman that two distinct morphological and pathogenic varieties of D. phaseolorum were found in soybeans. They also concluded that each variety was the causal agent for a specific soybean disease. D. phaseolorum var. sojae was retained for pod and stem blight. A new classification was introduced for stem canker changing D. phaseolorum var. batatatis to D. phaseolorum var. caulivora. The new name of D. phaseolorum (Cke. and Ell.) Sacc. var. caulivora Athow and Caldwell is retained at present. The reason for changing the variety name of D. phaseolorum var. batatatis to D. phaseolorum var. caulivora was that Athow and Caldwell discovered that the stem canker fungus was not identical to D. phaseolorum var. batatatis. This was the result of finding some morphological features such as perithecia and asci size which were different between the two varieties.

Up to this point, there had been no evidence reported that the stem canker pathogen had an imperfect asexual stage. Hildebrand (14) in 1954, reported the occurrence of conidia present in ectostromatic bodies. Frosheiser (10) in 1957, also cited the finding of beta spores as the predominant asexual spores formed. Later, Hildebrand (15) concluded the presence or absence of an imperfect stage had little to do with the diagnosis of stem canker. Kmetz et al. (23) in 1974 also related that D. phaseolorum var. caulivora produced caespitose perithecia with ascospores as well as pycnidia. It was also capable of inducing stem canker as it girdled plants and killed seedlings.

Symptoms and Development of the Disease

It is commonly agreed that ascospores and conidia from overwintered perithecia or pycnidia on soybean debris serve as the primary source for the spread of the inoculum (7, 9, 10, 13). The inoculum is disseminated by splashing rain (32). In previous decades, numerous studies have shown that infected seed is a secondary source of inoculum (10, 15, 23, 24, 30). However, more recent reports indicate that the seed is a likely source of inoculum for previously uninfested areas (2, 10). Chambers (4) reported that planting infested seed in stem canker field residue greatly increased the incidence of stem canker and reduced yields by 2.28 Mg ha^{-1}. Frosheiser (10) and Roy and Miller (31) have suggested that other legumes and cotton may serve as alternate hosts and additional sources of inoculum to infect soybeans.

The germination of ascospores and conidia was reported by Lehman (27) to be similar. First, the spores become saturated and begin to

grow. Secondly a germ tube begins to develop. The third step consists of the guttulae disappearing from the conidia as the oil droplets diminish in size and vacuoles develop in the germinating cell of the ascospore. The fourth step includes the germ tubes increasing in length and penetrating into the host plant. According to Lehman (27), after successful penetration into the plant, the mycelia invade the narrow walled section of the cortex prior to advancing into the stelar tissues as evidenced by darkening of the trachea and pith. The mycelia grow into the lumina of cells in the stem and penetrate the thick walls by means of cell-wall pits. The spread is both inter- and intracellular but no aerial growth occurs on host tissues under ordinary field conditions (27, 35).

The fungus enters the seed so that the seed are internally infected. Kmetz et al. (24) reported that the pathogen produced molded and fissured soybean seed, reducing germination rates and that severity of stem symptoms did not reflect the percentage of seed infected with D. phaseolorum var. caulivora. They also found that the amount of the pathogen isolated from mature seed is miniscule when compared to the amounts of Phomopsis and D. phaseolorum var. sojae obtained. Peterson and Strelecki (30) reported that D. phaseolorum var. caulivora was transmitted at an 8% rate via the seed versus a 48% rate of transmission for D. phaseolorum var. sojae by seed, which was supported later by Kmetz et al. findings.

Plants at all stages of growth are susceptible to infection. There is a tendency for field grown plants to be more susceptible from

midstage of development to maturity than at earlier stages (1, 7, 13, 15, 18, 25); however, seedlings may become infected. According to Hildebrand (15), there are three types of injury to seedlings during the postemergence phase of development. In one case, the cotyledons are attacked and from the cotyledons the fungus advances into the remaining stem tissue. Sinclair (32) supported Hildebrand's findings and related that the presence of small reddish brown lesions on one or both cotyledons is the first sign of stem canker infection. In the second type, lesions appear on the hypocotyl at points where the fungus has attacked. The third type involves the gradual rotting of the root and basal portions of the stem. The fungus was not isolated from healthy plant tissue, thus it was postulated that seedlings were not systemically infected. Frosheiser (10) further supported the idea of nonsystemic infection since the pathogen infected and killed some seedlings, but in others progress was halted and symptoms of the disease were not expressed in the developing plants.

Athow and Caldwell (1) described the disease symptoms on infected plants beyond the seedling stage. They reported that the first noticeable sign of stem canker is a reddish brown discoloration of a leaf scar or of a petiole pulvinus at a basal node. From the initial site of infection, a considerable lesion develops. The canker lesion can develop to a size of several centimeters and can completely girdle the stem.

According to Hildebrand (13), once the fungus penetrates the plant, the mycelia pervade and kill surrounding cells, including the

xylem, which stops the upward flow of water from the roots to parts of the plant above the girdled stem. As the plant becomes girdled, dead, necrotic leaves remain on the plant and stems become dry and brittle (1, 15, 16, 18, 25). With maturation, the lesion changes from reddish brown to shades of brown that range from light to almost chocolate (1, 15, 25, 21). Perithecia have been found on diseased stems near the base of the plant (1, 15, 18).

The manner by which D. phaseolorum var. caulivora infects the soybean is at present still not fully understood. As reported by Sinclair (32) in the Compendium of Soybean Diseases, "the fungus grows from the leaf blade through the petiole to the stem." This contradicts hypotheses of previous researchers. For example, Lehman (27) in 1923 proposed that the pathogen entered through the open stomates in the plant or by wounded tissue. Furthermore, Hildebrand in 1952 (13) postulated that entrance into the plant was accomplished through leaf scars on the basal region of the stem after leaves abcised or through injuries at the junction of the stems and branches. However, Crall in 1956 (7) supported the postulate proposed by Sinclair (32).

According to Athow and Caldwell (1), the amount of yield loss in soybeans from stem canker depends upon plant maturity at the time of infection. The amount of genetic resistance also plays an important role. They concluded that natural infection is not usually observed before the plants are 70 days old. If the pods are just beginning to fill when the pathogen invades the plant, there is a greater loss than if the plants have already filled the pods. Backman et al. (2), Crall

(7), Frosheiser (10), Hilty (16), and Weaver et al. (34) also support Athow and Caldwell's observations. Frosheiser (10) reported that losses reached .69 Mg ha^{-1} when 70 day old Blackhawk soybeans were inoculated with the pathogen.

With the diminished problem of stem canker in the northern soybean production areas, very little was reported about the disease until it became a problem in the southeastern soybean production areas in the 1970s. There have been various levels of the disease present each year since the first recorded outbreak in Mississippi in 1975 (19). Stem canker was reported in Alabama in 1977 where it destroyed several thousand acres of soybeans in Montgomery County (2). During the summer of 1981, stem canker was responsible for the entire loss of yields of some fields in Humphreys County and in Tennessee counties along the Mississippi border extending west to the Mississippi River (16). During the 1982 growing season, soybeans in scattered areas of South Carolina were killed by stem canker (25). Yield losses as high as 80% were reported for the 'Bragg,' 'Hutton' and 'Coker 237' cultivars. In 1983 the disease was responsible for severe crop losses in 19 Alabama counties and for moderate crop losses in 17 additional Alabama counties (34). The cultivar Bragg was removed from the 1984 Georgia variety recommendation list because it had been extremely susceptible to stem canker during the 1983 growing season (29).

With the appearance of the disease in the southeast, there was much testing undertaken to determine which soybean cultivars were resistant or susceptible to the disease. Inoculations of seedlings

grown in the greenhouse and the field were used to obtain the desired information. Keeling (19) in 1982 made use of the toothpick procedure to test eight soybean varieties for their reaction to inoculation with the stem canker pathogen. He concluded that 'Tracy-M,' 'CNS' and 'Peking' were resistant and that Bragg and 'J77-339' were susceptible. During 1983, Krausz and Fortnum (25) determined that 'Coker 338' was susceptible to stem canker. Further testing by Keeling in 1985 (21) with field grown plants, identified an additional 16 nearly-immune and 5 susceptible cultivars.

In addition to artificial inoculations in the greenhouse and the field, tests have been conducted on field sites where the residue of diseased soybean plants from the previous year served as the source of natural inoculum. During 1980 and 1981, Backman et al. (2) conducted field evaluation of several cultivars in three maturity groups for degree of resistance or susceptibility to stem canker. They identified four resistant Group VI cultivars ('McNair 600,' 'Coker 156,' 'Davis' and 'RA680'), three resistant Group VII cultivars ('Braxton,' 'Coker 317' and 'Wright'), and two resistant Group VIII cultivars ('Coker 448' and 'Dowling'). They also determined three susceptible cultivars within Group VII ('RA701,' Coker 237 and 'Brooks'), and three in Group VIII ('RA800,' Hutton and Coker 338). During 1982 and 1983, Weaver et al. (34) tested 41 adapted cultivars in maturity groups V, VI, VII and VIII for degree of resistance or susceptibility to stem canker. From the test they concluded that the two cultivars Tracy-M and Braxton were highly resistant to stem canker. Several cultivars of

the Ring Around Brand like 'RA604,' 'RA702' and 'RA801' were found highly susceptible. 'Jeff,' Coker 237 and Hutton were also found to be highly susceptible. Research conducted by Chambers (4) in 1985 revealed that 'Tracy,' 'Bay,' 'York' and 'Mitchell' were resistant. 'Centennial,' 'Essex' and 'Forrest' were tolerant and J77-339 was susceptible.

Keeling (20) has presented evidence that there are at least six physiological races of stem canker. Three of the six races have been demonstrated to infect Tracy-M and the other three races are non-pathogenic on Tracy-M. J77-339 is susceptible to all six races. Keeling identified the cultivar 'Kingwa' to be resistant to all but the sixth physiological race.

Inheritance of Resistance

As the incidence of stem canker is rising in the southeastern agricultural regions it is important to determine the inheritance of resistance to facilitate development of stem canker resistant soybean varieties.

After the study reported herein was begun, Kilen et al. (22) reported information about the inheritance of reaction to stem canker in soybean. The varieties crossed included Tracy-M, a resistant cultivar and J77-339, a susceptible breeding line. The investigators determined that stem canker resistance in Tracy-M was conditioned by two major dominant genes. The segregation patterns of resistant: susceptible for the F_1 and F_2 generations were 1:0 and 15:1, respectively.

The objectives of this particular study were to: (1) compare the relative pathogenicity of two stem canker isolates from Mississippi and two from Tennessee, (2) determine the inheritance of resistance to stem canker among four soybean varieties, and (3) determine if the gene(s) conditioning resistance is(are) the same in Bay and Tracy-M.

CHAPTER II

MATERIALS AND METHODS

Based on evidence reported by others (4, 19, 34), two resistant and two susceptible varieties were chosen as parents for this study. The two resistant varieties were Bay and Tracy-M and the two susceptible varieties were Bragg and J77-339. The parents had the following pedigrees: Bay = York x 'R62-550' (3); Tracy-M = 'D60-9647' x 'D60-618' (11); Bragg = 'Jackson' x 'D49-2491' (17); and J77-339 = 'J74-57' x 'D74-7631' (12). Bay is in maturity group V whereas, Tracy-M and J77-339 are in group VI and Bragg is in maturity group VII. All possible crosses, included reciprocals, were made among the four parents in the greenhouse and in the field during the spring and summer of 1984. The F_1, F_2, BC1 and BC2 generation plants from each cross combination were produced in the greenhouse during the winter and spring of 1985. Plants from each generation as well as parents were grown in 20 cm diameter plastic pots containing approximately 3300 gm of 'Promix BX.' Plants were fertilized four times at two week intervals using 7.60 g l^{-1} of soluble 20-20-20 fertilizer. Foliar applications of 0.94 g l^{-1} of 'Vendex' (Hexakis 2-methyl-2-phenylpropyl-distannoxane), 5.62 g l^{-1} of 'Kelthane-35' (1,1-Bis(Chlorophenyl)-2, 2, 2,-trichloroethanol) and 4.69 g l^{-1} of 'O-Mite' (Propargite 2-p-tert-butylphenoxy cyclohexyl-2-propynl sulfite) were applied weekly on an alternating basis in order to control spider mites [Tetranychus urticae Koch.] and insects.

Four isolates of Diaporthe phaseolorum var. caulivora were evaluated for use in this study. Two isolates (82-3 and 82-5) were obtained from B. L. Keeling, Plant Pathologist, USDA, Stoneville, Mississippi, and two (EA21 and FP3) were obtained from J. W. Hilty, Plant Pathologist, Department of Entomology and Plant Pathology, The University of Tennessee, Knoxville. Stock cultures of each of the isolates were maintained on potato dextrose agar (PDA) with a pH of 5.6 ± .02. The stock cultures were stored at 4C. Cultures to be used for inoculation were obtained by transferring mycelium to petri dishes containing PDA. The transfers were placed into plastic bags and incubated at 21C for 7 days under 5 h da^{-1} of fluorescent lighting (33). The toothpick method of inoculation described by Crall (6) and used by others (10, 15, 19) to inoculate soybean plants was also used in this study. Dry sterilized 0.5 cm sections of flat wooden toothpicks were autoclaved in potato dextrose broth (19) at 120C for 15 minutes in order to impregnate the toothpick sections with broth. After cooling, toothpicks were placed into the petri dishes containing one-week old cultures of the fungus. The cultures containing the toothpick sections were incubated for 14 days at 21C prior to inoculation of plants.

Seedlings were inoculated with the mycelium-covered toothpick sections 14 days after planting. Following inoculation, seedlings were placed in a moisture chamber for four days in which the relative humidity and temperature were maintained at approximately 100% and 24C, respectively. Dry, sterilized toothpick sections were inserted in

plants as controls. After four days in the moisture chamber, plants were removed and placed on benches in the greenhouse. The plants were grown under 14^{+}h photoperiod until maturity. During periods of time when the natural photoperiod was less than 14 h, 'Grow-Lux' fluorescent bulbs were used to extend it to 14 h.

Six F_1 seedlings of ten of the crosses, and three F_1 seedlings of the remaining two crosses were inoculated with isolate FP3, two weeks after planting at the node below the plant apex. The remaining F_1 seedlings of each cross (numbering 3 to 50) served as the progenitors of the F_2, BC1 and BC2 seed. The reaction of inoculated F_1 seedlings was evaluated 20 and 40 days after inoculation as being either resistant or susceptible to the stem canker pathogen on the basis of the general health of the plant and lesion size. The ratings were based on a scale of 1 to 5 as follows:

Score

1 = Lesion length of 1-10 mm.
2 = Lesion length of 11-20 mm.
3 = Lesion length of 21-50 mm.
4 = Lesion length of greater than 51 mm and/or the plant exhibited stunted growth and yellow or necrotic leaves.
5 = Plant killed by pathogen.

Plants receiving ratings of 1, 2, or 3 were considered resistant and those receiving ratings of 4 or 5 were considered susceptible.

At maturity, all F_2, BC1 or BC2 seeds were harvested from the individual F_1 plants. For each inoculation test, F_2 plants from each cross combination were grown three per pot and were replicated seven

times (total of 21 plants per test) for 11 of the 12 crosses, and six times for the twelfth cross because of the smaller quantity of seed. Each of the parent varieties were grown four plants per pot and replicated four times (total of 16 plants) per test. The inoculation tests of F_2 plants and parents were conducted 12 times for a combined total of 252 plants for 10 of the 12 crosses; 231 and 198 for the other 2 crosses; and 192 plants per parent variety except for Tracy-M with only 176 plants. After inoculation and four days in the moisture chamber, the pots of each test were arranged in a randomized complete block design on greenhouse benches.

All of the F_2 and parent seedlings were inoculated 14 days after planting with the stem canker fungus at the first node below the plant apex. Six of the inoculated F_2 seedlings per cross and four seedlings per parent variety were simultaneously implanted with a sterilized toothpick section, one node below the node in which the mycelium-covered toothpick was inserted.

The plants were rated either resistant or susceptible at 20 and 40 days after inoculation and at growth stage R7.5 (8). This stage is the point after one normal pod on the main stem has attained its normal pod color (8). The 20 and 40 day ratings were dependent upon two characteristics. The first was the general health of the plant as described for the seedling tests. The other characteristic was length of the lesion produced. The description of ratings from 1 to 5 is as follows:

Score

1 = Lesion length 1-5 mm greater than control.
2 = Lesion length 6-20 mm greater than control.

3 = Lesion length 21-44 mm greater than control.
4 = Lesion length 45 mm greater than control.
5 = Plant killed by stem canker.

Plants were scored again at the growth stage R7.5 for resistance versus susceptibility on the basis of three criteria:

1. extent of the lesion spread--if the lesion had spread extensively along the plant's stem and had killed the plant or greatly deteriorated the plant's health it was classified susceptible.
2. retention of dead, dry, necrotic leaves--if it was apparent that the reason for the retention of leaves was due to stem canker, then the plant was categorized susceptible.
3. podfill--if the plant retained shrivelled pods without seeds or more than 50% of the pods produced contained degenerate seeds, the plant was considered susceptible.

The three criteria were evaluated and weighed together in order to determine the reaction of the plant to the disease. If the plant's premature death could not be attributed to stem canker then the plant was not included in the final resistant:susceptible ratios.

The presence of a larger number of resistant plants than expected for the J77-339 variety caused the necessity of retesting the resistant classified J77-339 plants to distinguish between the truly resistant plants and those which might have been "escapes." The variety Bragg and resistant plants from susceptible x susceptible progeny crosses were also retested.

With the exception of growing four plants per pot, the culture of the F_3 and parent plants was similar to the previous generations. Another alteration included the removal of the terminal bud at the V2 stage of growth (8). This step would encourage rapid axillary branch development. If two branches developed, both were artificially inoculated with the fungus. Two to ten seedlings of each parent and F_3 progeny of each cross were tested for their reaction to the pathogen in each of two inoculation tests. Inoculation of the seedlings occurred three weeks after planting. Inoculation was delayed a week to allow sufficient time for the axillary branches to develop. Two of the inoculated seedlings of each parent and F_3 plants were also inserted with a sterile toothpick to act as a control. The plants were visually evaluated for their reaction to the stem canker pathogen at 40 days after inoculation. The scoring of plant reaction to stem canker was similar to the scoring of the F_2 plants regarding the plant's general health as previously described. A plant was classified resistant if the plant's reaction to stem canker produced a tan colored lesion of up to 10 mm greater than the control. A plant was labeled susceptible if the plant's reaction to the stem canker pathogen produced a dark brown or black lesion 10^+ mm greater than the control. Also, a plant received a susceptible rating if stem canker killed the plant. The lesion size ratings were changed because the small tan reactions to inoculation were atypical of the lesions present in susceptible plants at the R7.5 stage of growth. However, dark black lesions, regardless of the size, typically were present in a susceptible plant rating and

often, plant death when ratings were given to the F_2 and parent populations rated at the R7.5 growth stage.

CHAPTER III

RESULTS AND DISCUSSION

Choice of Most Virulent Isolate

Two greenhouse seedling inoculation tests of parent varieties were conducted to determine the relative virulence of the individual isolates. The criteria used for the determination of the degree of virulence for each isolate included the following:

1. the number of seedlings classified diseased per given isolate,
2. the percentage of seedlings classified diseased on a variety-isolate basis, and
3. the mean lesion length on a variety-isolate basis.

Table 1 shows that isolates FP3 and EA21 caused higher numbers of diseased seedlings being classified susceptible than either isolates 82-5 or 82-3 as evidenced by 30 and 28 diseased seedlings for FP3 and EA21 versus the 19 and 8 diseased seedlings for 82-5 and 82-3, respectively. In addition, isolates FP3 and EA21 caused higher percentages of diseased seedlings in the susceptible cultivars than either isolate 82-5 or 82-3 (Table 2). Within the Bragg variety, isolate FP3 caused 33.3 and 45.5% more diseased seedlings than 82-5 or 82-3, respectively. Within the J77-339 variety isolate EA21 caused 29.2 and 50.0% more diseased seedlings than either isolate 82-5 and 82-3, respectively. Table 3 contains the results of the two tests for mean isolate lesion length. EA21 and FP3 caused larger lesions than either 82-5 or 82-3. Isolates EA21 and FP3 caused lesions of 15.25 and 18.08 mm versus the

Table 1. Relative virulence of four Diaporthe phaseolorum var. caulivora isolates as evidenced by the ratio of healthy to diseased plants for two resistant and two susceptible varieties.

	Varieties				
	Resistant		Susceptible		
Isolate	Bay	Tracy-M	Bragg	J77-339	Total
EA21	22:2	22:2	17:7	7:17	68:28
FP3	23:1	23:1	10:14	10:14	66:30
82-5	21:3	24:0	18:6	14:10	77:19
82-3	24:0	24:0	21:3	19:5	88:8
Total	90:6	93:3	66:30	50:46	----

Table 2. Percent† diseased plants of two resistant and two susceptible soybean varieties following inoculation with four isolates of D. phaseolorum var. caulivora.

	Varieties			
	Resistant		Susceptible	
Isolate	Bay	Tracy-M	Bragg	J77-339
	%			
EA21	8.3	8.3	29.2	70.8
FP3	4.1	4.1	58.3	58.3
82-5	12.5	0.0	25.0	41.6
82-3	0.0	0.0	12.5	20.8

†Based on 24 plants total (six tests, four plants per test).

Table 3. Average[†] lesion length produced by four isolates of D. phaseolorum var. caulivora on two resistant and two susceptible soybean varieties.

	Varieties			
	Resistant		Susceptible	
Isolate	Bay	Tracy-M	Bragg	J77-339
	mm			
EA21	4.33[a]	3.92[a]	15.25[a]	32.83[a]
FP3	4.25[a]	6.58[a]	18.08[a]	19.75[ab]
82-5	5.59[a]	4.75[a]	6.91[a]	9.08[ab]
82-3	4.25[a]	3.25[a]	6.08[a]	4.42[b]

[†]Means of two replications with six plants each.

[††]Means followed by the same letter are not significantly different ($P \leq 0.05$) based on t test.

6.91 and 6.08 mm lesions caused by isolates 82-5 and 82-3, respectively on Bragg. The same trend is also applicable to the J77-339 variety. Within the J77-339 variety, the mean lesion length caused by isolate EA21 was larger than those within the Bay and Tracy-M varieties. Within the J77-339 variety, there was a significant difference in lesion lengths between isolate EA21 and isolate 82-3.

On the basis of the three criteria evaluated to determine the best isolate to use for testing of later materials (F_2, BC1, etc.), it was determined that isolates FP3 and EA21 were of equal virulence and both were more virulent than isolates 82-5 and 82-3. Because of the larger lesions caused and a higher percentage of diseased plants particularly on susceptible varieties, FP3 was chosen as the isolate to be used throughout the remainder of this study.

Optimum Postinoculation Time for Rating Plants for Reaction to Stem Canker

Accurate classification of soybean plants as being either resistant or susceptible to the stem canker pathogen can only be achieved by determining the best postinoculation time at which ratings should be given. The selfed, F_1, F_2, BC1 and BC2 plants tested in this study were rated for their reaction to stem canker at 20 and 40 days and at the R7.5 stage of growth.

The scoring of 10 of the 12 F_2 progeny crosses as well as the Bragg and J77-339 parents, for their reaction to the stem canker pathogen, showed a progressive increase in the ratio of susceptible versus resistant plants as the inoculation-to-rating interval increased

(Table 4). However, this pattern was applicable only to the resistant x susceptible, and susceptible x susceptible progeny plants. The resistant x resistant crosses progeny and Bay and Tracy-M parents, reflected no change in the ratio of resistant: susceptible plants in comparison. Presented in Table 5 is the actual increase in percentage of susceptible plants over the time periods during which the plants were evaluated for their reaction to stem canker.

Within each of the time frames there is a consistent increase in the percentages of susceptible plants within the resistant x susceptible, and susceptible x susceptible progeny. This trend also applied to the susceptible parents. From the rating at 20 days versus the rating at growth stage R7.5, the Bay x susceptible progeny reflected increases of 22.4 and 21.5%, respectively. Progeny of the Tracy-M x susceptible crosses gave susceptible plant increases of 17.0 and 13.7%, respectively. Susceptible plant increases of 23.9 and 10.8% were observed in the progeny from the Bragg x resistant crosses. Progeny of the J77-339 x resistant crosses yielded increases of 20.5 and 23.4%, respectively. The susceptible x susceptible crosses reflected increases of 71.5 and 77.8% within the susceptible category (Table 5). The resistant x resistant crosses did not show any appreciable increase in percentage of susceptible plants.

The lack of uniformity in the segregation ratios of a given cross over intervals of time demonstrates the problems of rating susceptible x resistant progeny crosses at 20 or 40 days after inoculation. Ratings given at both times were not good indicators of the segregation

Table 4. Changes in classification of parent and F_2 plants as resistant versus susceptible with increased intervals of time postinoculation with isolate FP3 14 days after planting.

Parent or Cross	20 Days	40 Days	R7.5 Growth Stage
		Parents	
Bay	182:0	180:2[†]	162:3[††]
Tracy-M	167:0	162:0	143:2
Bragg	170:20	106:81	16:170
J77-339	92:95	31:156	3:184
		F_2 Generation	
Resistant x Resistant			
Bay x Tracy-M	228:3	228:3	188:3
Tracy-M x Bay	249:0	248:1	226:1
Resistant x Susceptible			
Bay x Bragg	247:3	226:20	181:56
Bay x J77-339	234:13	197:50	174:64
Tracy-M x Bragg	247:2	236:13	196:35
Tracy-M x J77-339	243:6	219:30	182:35
Bragg x Bay	245:4	219:30	175:60
Bragg x Tracy-M	246:21	240:7	214:27
J77-339 x Bay	226:21	194:53	167:69
J77-339 x Tracy-M	230:17	199:46	166:72
Susceptible x Susceptible			
J77-339 x Bragg	192:58	110:138	14:234
Bragg x J77-339	156:31	86:101	10:177

[†]Totals of resistant to susceptible are the combined results of 11(-) or 12 repeated tests.

[††]Totals are not the same because of a loss of some plants to factors other than stem canker.

Table 5. The percentage increase of susceptible plants of the parent and F_2 plants within time intervals postinoculation with isolate FP3 14 days after planting.

Parent or Cross	Increase in susceptible plants		
	20-40 Days	20 Days-R7.5 growth stage	40 Days-R7.5 growth stage
	%		
	Parents		
Bay	1.1	1.2	0.1
Tracy-M	0.0	1.4	0.0
Bragg	32.8	80.9	48.1
J77-339	32.6	47.6	15.0
	F_2 Generation		
Resistant x Resistant			
Bay x Tracy-M	0.0	1.2	0.0
Tracy-M x Bay	0.0	0.0	0.0
Resistant x Susceptible			
Bay x Bragg	6.9	22.4	15.5
Bay x J77-339	14.9	21.5	6.6
Tracy-M x Bragg	4.4	17.0	12.6
Tracy-M x J77-339	9.6	13.7	4.1
Bragg x Bay	10.4	23.9	13.5
Bragg x Tracy-M	2.4	10.8	8.4
J77-339 x Bay	12.9	20.5	7.6
J77-339 x Tracy-M	11.9	23.4	11.5
Susceptible x Susceptible			
J77-339 x Bragg	32.4	71.5	39.1
Bragg x J77-339	37.4	77.8	40.4

ratio of resistant:susceptible plants. There has been one previous report on the reaction of segregating soybean progeny to inoculation with the stem canker pathogen (22). The plants in that study were evaluated at 40 days after inoculation. If the evaluation had occurred at the growth stage of R7.5, a different ratio might have been observed.

All of the reports have dealt with the reaction of pure-line soybean varieties or breeding lines to stem canker and not segregating populations. For those varieties and breeding lines it was feasible to evaluate seedling tests of highly resistant or highly susceptible varieties for reaction to stem canker at 10 days after inoculation. Forty days after inoculation has also been found to be sufficient time to rate soybean varieties (31). However, Weaver et al. (34) pointed out that there are problems in the classification of varieties for reaction at these time intervals. For one thing, they noted that the evaluation of toothpick-inoculated, greenhouse-raised plants of the Peking variety seemed to react differently to stem canker than toothpick-inoculated field grown plants. They postulated that some inate morphological defense mechanism within the variety is capable of overcoming the effects of the toothpick inoculation method. Keeling (21) also reported that a moderately susceptible variety such as Bragg, or a cross containing Bragg germplasm, which only gradually demonstrates expression of its reaction to stem canker, could conceivably be given a rating at 20 or 40 days after inoculation which would not reflect the actual reaction of Bragg to the stem canker pathogen.

The 10, 20 or 40 day postinoculation ratings should only be used for the most resistant varieties and resistant x resistant crosses. The ratings of reaction to stem canker taken at the growth stage R7.5 are best suited for the resistant x susceptible, susceptible x susceptible crosses and susceptible varieties. The evaluation of these groups at such a time will avoid errors in the final analysis of a plant's disease reaction to stem canker. One problem associated with the evaluation of plants after the R7.5 growth stage is that a small percentage of the plants per given cross cannot be included in the final count due to the inability to ascertain if stem canker was the primary cause of plant death. The best time to evaluate the plant for its reaction to stem canker is probably at the R7.5 growth stage (8). In this study disease ratings made at growth stage R7.5 gave the best fit for the genetic models tested.

Assignment of Genotypes to Parent Varieties

Disease resistance in crop plants is often conditioned by one or two major dominant genes. Kilen et al. (22) reported that the inheritance of resistance to stem canker in soybeans within the cross, Tracy-M x J77-339, was conditioned by two major dominant genes from the Tracy-M variety. J77-339 showed no resistance to stem canker. The information was determined by evaluation of plants at 40 days postinoculation. The F_1 and F_2 expected ratios were 1:0 and 15:1 of resistant:susceptible plants respectively.

The study reported herein included Tracy-M and J77-339 as well as two additional varieties, Bay and Bragg. The number of plants of each parent variety which received scores of 1-5 are shown in Table 6. As mentioned earlier, plants receiving scores of 1, 2, or 3 were classified as resistant and those receiving a score of 4 or 5 were classified susceptible. Bay and Tracy-M parents had higher numbers of plants with a resistance rating of 1 than ratings of 2 or 3. However, Bay had slightly more plants rating 3 and 4 than Tracy-M. Based on the reaction of Bay plants to stem canker infection and of progeny from crosses involving Bay, its resistance is proposed to be conditioned by a single dominant gene ($R_1R_1r_2r_2$) which is the same as one of the two genes conditioning resistance in Tracy-M ($R_1R_1R_2R_2$), Table 7. Furthermore, it is proposed that the R_1 gene confers more resistance to stem canker than does the R_2 gene (Table 8). The expression of resistance in Bay and Tracy-M is very similar, thus the one homozygous dominant gene present in Bay, $R_1R_1r_2r_2$, would confer more resistance than a variety containing the homozygous $r_1r_1R_2R_2$ gene. However, the presence of both the dominant R_1R_1 and partially dominant R_2R_2 genes in Tracy-M would likely confer a slightly higher level of resistance than Bay has with the single gene.

The J77-339 and Bragg parents show fewer number of individuals with resistant ratings of 1, 2, and 3 and a greater tendency towards susceptible ratings of 4 and 5. J77-339 is extremely susceptible to stem canker as evidenced by only 33 out of 187 plants receiving ratings of 1, 2 or 3 at 40 days postinoculation (Table 6). The final ratings

Table 6. Number of plants of each of the four parent soybean varieties receiving scores of 1-5 at 40 days postinoculation with isolate FP3.

Parent Variety	Score:	Parental Plant Reaction to Inoculation with Stem Canker				
		Resistant†			Susceptible††	
		1	2	3	4	5
Bay		141	21	7	3	0
Tracy-M		150	18	1	0	0
Bragg		38	49	19	58	23
J77-339		9	13	11	18	136

†1 = 5 mm greater than control lesion size, 2 = 6-20 mm greater than control lesion size, 3 = 21-45 mm greater than control lesion size.

††4 = 46+ mm greater than control lesion size and decadent plant health, 5 = plant killed by pathogen.

Table 7. Proposed genotype and classification of four soybean varieties based on resistant:susceptible plant reaction to isolate FP3 40 days postinoculation.

Parent	Genotype	Classification	Resistant†	Susceptible††
Bay	$R_1R_1r_2r_2$	Resistant	169	3
Tracy-M	$R_1R_1R_2R_2$	Resistant	169	0
Bragg	$r_1r_1R_2$--	Susceptible	106	81
J77-339	$r_1r_1r_2r_2$	Susceptible	33	154

†Number of resistant plants are taken from total of 1-3 resistant ratings from Table 6.

††Number of susceptible plants are taken from total of 4-5 susceptible ratings from Table 6.

Table 8. Possible genotypes obtainable from crossing the four parents, classification of each genotype as resistant or susceptible to the stem canker pathogen, and the order of magnitude of the genes conditioning resistance to stem canker.

Resistant Genotypes	Susceptible Genotypes	Magnitude of Genes
$R_1R_1R_2R_2$	$r_1r_1R_2r_2$	$R_1 > R_2$
$R_1R_1R_2r_2$	$r_1r_1r_2r_2$	
$R_1R_1r_2r_2$		
$R_1r_1R_2R_2$		
$R_1r_1R_2r_2$		
$R_1r_1r_2r_2$		
$r_1r_1R_2R_2$		

at maturity including the reevaluation of the resistant plants yielded 3 resistant:184 susceptible plants (Table 4, p. 24). These numbers are indicative of a 0:1 ratio. A 0:1 ratio of resistant to susceptible plants clearly indicates that J77-339 has no genes present for resistance to stem canker. Thus a homozygous recessive genotype of $r_1r_1r_2r_2$ is likely to reflect J77-339's genotype (Table 7).

Bragg is classified as moderately susceptible to stem canker (21). Thus, it has a slightly higher level of resistance to stem canker than J77-339 as evidenced by 73 more 1-3 resistant ratings than in J77-339 (Table 6). This indicates that Bragg probably has a dominant allele of one of the genes which differentiates it from the J77-339 genotype of $r_1r_1r_2r_2$. If the R_2 gene conditions less resistance to stem canker than the R_1 gene, one would expect fewer resistant plants to result from the selfing of an $r_1r_1R_2$-- genotype versus an $R_1r_1r_2r_2$ genotype. Therefore, Bragg is assigned an $r_1r_1R_2$-- genotype (Table 7) because it is less susceptible compared to J77-339.

If approximately equal numbers of resistant plants occurred within each rating level of resistance (1, 2 and 3), for the varieties Bay, Tracy-M and Bragg, then Bragg would be likely to have a genotype other than the proposed $r_1r_1R_2$--. Table 6 shows that 82.0% of the Bay plants, 89.0% of the Tracy-M and only 20.0% of the Bragg plants have a resistance rating of 1, respectively. It also shows that 12.0% of the Bay plants, 11.0% of the Tracy-M and 26.0% of the Bragg plants received the resistance rating of 2, respectively. It is obvious that the Bay and Tracy-M resistant genotypes, are different from Bragg's genotype,

otherwise there would be more Bragg plants in the resistance rating classes of 1 and 2. These data and the final ratings taken at the R7.5 stage of growth of 162:3 for Bay, 143:2 for Tracy-M and 16:170 for Bragg (Table 4, p. 24) indicate that a heterozygous genotype for Bragg is possible.

Each of the proposed genotypes $R_1R_1r_2r_2$ (Bay), $R_1R_1R_2R_2$ (Tracy-M), $r_1r_1R_2$-- (Bragg) and $r_1r_1r_2r_2$ (J77-339) are also supported on the basis of how well they fit the expected selfed, F_1, F_2, BC1 and BC2 progeny ratios for the cross combinations made among them.

Resistant x Resistant Crosses

Assuming one major dominant gene and one partial dominant gene confer resistance to stem canker in Tracy-M and one major dominant gene in Bay, progeny from crosses between them should segregate 1 resistant: 0 susceptible in the F_1 and F_2 generations. The parent and F_1 and F_2 data of Bay and Tracy-M and their crosses from this study are presented in Table 9. In all cases the data are a good fit to the expected ratios ($P \geq 0.05$). There were no apparent differences between the reciprocal crosses in level of resistance or susceptibility, indicating that the cytoplasmic effects in these two resistant varieties were not different in relation to reaction to stem canker.

Resistant x Susceptible Crosses

Crosses involving the Bay variety and either of the two susceptible varieties, Bragg or J77-339, support the premise that Bay has one major dominant gene for resistance and namely the genotype $R_1R_1r_2r_2$

Table 9. Chi-square tests for reaction of the parents, F_1 and F_2 progeny from the resistant x resistant cross, Bay x Tracy-M and reciprocal to inoculation with D. phaseolorum var. caulivora.

Parent/Cross	Proposed Genotypes	Generation	Expected Ratios	Observed numbers† (R:S)	χ^2	$P > \chi^2$
Bay	$R_1R_1r_2r_2$	P_1	1:0	162:3	0.02	.80-.90
Tracy-M	$R_1R_1R_2R_2$	P_2	1:0	143:2	0.03	.80-.90
Bay x Tracy-M	$R_1R_1r_2r_2 \times R_1R_1R_2R_2$	F_1	1:0	3:0	0.00	.99
		F_2	1:0	188:3	3.05	.05-.10
Tracy-M x Bay	$R_1R_1R_2R_2 \times R_1R_1r_2r_2$	F_1	1:0	6:0	0.00	.99
		F_2	1:0	226:1	0.00	.99

†Based on ratings for resistance versus susceptibility at the R7.5 stage of growth.

(Tables 10 and 11). The Bay x Bragg and the reciprocal Bragg x Bay cross fit the 1:0 expected ratio in the F_1 (Table 10).

The F_2 subpopulations of this cross were expected to segregate equally (1:1) into ratios of either 13:3 or 3:1. Within the seven F_2 subpopulations comprising the Bay x Bragg cross, four fit a 13:3 ratio, while the other three fit the 3:1 ratio as expected (Table 10). In the reciprocal cross (Bragg x Bay), two of the seven F_2 subpopulations fit the 13:3 ratio and the other five best fit the 3:1 ratio which supports the 1:1 expected ratio (Table 10). Of the 14 F_2 subpopulations comprising the Bay x Bragg and Bragg x Bay crosses, six best fit the 13:3 ratio whereas the other eight best fit the 3:1 ratio. Furthermore, the 6:8 observed numbers fit the expected 1:1 ratio.

Table 11 includes the data for Bay x J77-339 and the reciprocal J77-339 x Bay cross. Based on the proposed genotype for each parent, the F_1 generation plants from a cross between them should all be resistant (1:0) and the F_2 should segregate 3:1. The 10 F_1 plants which were tested for reaction to stem canker received resistant ratings which fit the expected ratio of 1:0 (Table 11). The F_2 progeny from the cross and the reciprocal also fit the expected ratio of 3 resistant:1 susceptible. The BC1 and BC2 progeny support the 1:0 expected BC ratio; however, only one plant was obtained in each case.

There was excellent agreement among the three Bay crosses and their reciprocals in support of the one major dominant gene ($R_1R_1r_2r_2$) in Bay. In addition, there were no apparent differences between the reciprocal crosses in level of resistance or susceptibility, indicating

Table 10. Chi-square tests for reaction of the parents, F_1 and F_2 progeny from the resistant x susceptible cross, Bay x Bragg and reciprocal to inoculation with D. phaseolorum var. caulivora.

Parent/Cross	Proposed Genotypes	Generation	Expected Ratios	Observed numbers† (R:S)	χ^2	$P > \chi^2$
Bay	$R_1R_1r_2r_2$	P_1	1:0	162:3	0.02	.80-.90
Bragg	$r_1r_1R_2$—	P_2	1:3	16:170	277.17**	<.001
Bay x Bragg	$R_1R_1r_2r_2$ x $r_1r_1R_2$—	F_1	1:0	6:0	0.00	.99
		F_2	50% 13:0	98:24(4)††	0.06	.80-.90
		F_2	50% 3:1	83:32(3)††	0.49	.30-.50
		F_2 subpopulation	1 3:1	54:21	0.36	.50-.70
		"	2 13:3	17:4	0.00	.99
		"	3 13:3	16:4	0.02	.80-.90
		"	4 3:1	13:6	2.04	.10-.20
		"	5 13:3	47:13	0.33	.50-.70
		"	6 13:3	18:3	0.27	.50-.70
		"	7 3:1	16:5	0.01	.90-.99
Bragg x Bay	$r_1r_1R_2$— x $R_1R_1r_2r_2$	F_1	1:0	6:0	0.00	.99
		F_2	50% 13:3	52:11(2)††	0.06	.80-.90
		F_2	50% 3:1	123:49(5)††	1.12	.20-.30
		F_2 subpopulation	1 3:1	46:16	0.01	.80-.90
		"	2 3:1	26:11	0.44	.50-.70
		"	3 3:1	9:7	3.00	.05-.10
		"	6 13:3	35:7	0.12	.70-.80
		"	7 3:1	14:6	0.27	.50-.70
		"	8 3:1	28:9	0.00	.99
		"	11 13:3	17:4	0.00	.99

†Based on ratings for resistance versus susceptibility at the R7.5 stage of growth.

††The number in parentheses is the number of F_2 subpopulations which fit the 13:3 or 3:1 ratio.

**Significant deviation from the expected ratio at ($P \leq 0.01$).

Table 11. Chi-square tests for reaction of the parents, F_1, F_2, BC1 and BC2 progeny from the resistant x susceptible cross, Bay x J77-339 and reciprocal to inoculation with D. phaseolorum var. caulivora.

Parent/Cross	Proposed Genotypes	Generation	Expected Ratios	Observed numbers† (R:S)	χ^2	$P > \chi^2$
Bay	$R_1R_1r_2r_2$	P_1	1:0	162:3	0.02	.80-.90
J77-339	$r_1r_1r_2r_2$	P_2	0:1	3:184	3.05	.10-.20
Bay x J77-339	$R_1R_1r_2r_2$ x $r_1r_1r_2r_2$	F_1	1:0	4:0	0.00	.99
		F_2	3:1	174:64	0.45	.50-.70
		BC1	1:0	1:0	0.00	.99
J77-339 x Bay	$r_1r_1r_2r_2$ x $R_1R_1r_2r_2$	F_1	1:0	6:0	0.00	.99
		F_2	3:1	167:69	2.25	.10-.20
		BC2	1:0	1:0	0.00	.99

†Based on ratings for resistance versus susceptibility at the R7.5 stage of growth.

that the cytoplasmic effects in the two susceptible varieties when crossed to Bay were not different in relation to reaction to stem canker.

Crosses involving the Tracy-M variety and the susceptible variety, Bragg, support the assumption that Tracy-M has two major genes for resistance (one dominant and the other partially dominant) and likely the $R_1R_1R_2R_2$ genotype (Table 12). Table 12 includes the selfs, F_1, F_2, BC1 and BC2 progeny data for the Tracy-M x Bragg and the reciprocal Bragg x Tracy-M cross. Based on the assumed genotype for each parent the F_1 generation plants from a cross between them should all be resistant (1:0) and the F_2 generation should segregate equally (1:1) into ratios of either 13:3 or 1:0. All 12 of the F_1 plants tested for their reaction to stem canker were classified resistant which fit the 1:0 expected F_1 ratio (Table 12). Six of the eleven F_2 subpopulations best fit a 13:3 ratio, whereas the other five subpopulations best fit the 1:0 ratio as expected. However, the five subpopulations which on an individual basis gave a best fit of 1:0 did not fit a 1:0 ratio when they were combined (Table 12) due to the presence of a few more susceptible plants than expected. Only six F_2 subpopulations comprise the Bragg x Tracy-M reciprocal cross (Table 12). Four F_2 subpopulations of the cross best fit the 13:3 ratio, whereas the other two F_2 subpopulations best fit the 1:0 ratio as would be expected for a 1:1 F_2 ratio of 13:3 or 1:0. Although the individual F_2 subpopulations best fit a ratio of either 13:3 or 1:0, when the totals are combined for each ratio they deviate significantly from the expected ratios ($P \leq 0.05$ and

Table 12. Chi-square tests for reaction of the selfs, F_1, F_2, BC1 and BC2 progeny from the resistant x susceptible cross, Tracy-M x Bragg and reciprocal to inoculation with D. phaseolorum var. caulivora.

Self/Cross	Proposed Genotypes	Generation	Expected Ratios	Observed numbers† (R:S)	χ^2	$P > \chi^2$
Tracy-M	$R_1R_1R_2R_2$	P_1	1:0	143:2	0.03	.80-.90
Bragg	$r_1r_1R_2$—	P_2	1:3	16:170	277.17**	<.001
Tracy-M x Bragg	$R_1R_1R_2R_2$ x $r_1r_1R_2$—	F_1	1:0	6:0	0.00	.99
		F_2	50% 13:0	103:30(6)††	1.26	.20-.30
		F_2	50% 1:0	93:5 (5)††	18.04**	<.001
		F_2 subpopulation 1	1:0	18:0	0.00	.99
		" 2	13:3	17:4	0.00	.99
		" 3	1:0	20:1	0.08	.70-.80
		" 5	13:3	16:5	0.35	.50-.70
		" 7	1:0	20:1	0.08	.70-.80
		" 8	13:3	13:5	0.96	.30-.50
		" 9	1:0	16:1	0.00	.99
		" 11	13:3	11:5	1.64	.20
		" 12	13:3	33:7	0.04	.80-.90
		" 13	13:3	13:4	0.25	.50-.70
		" 17	1:0	19:2	0.19	.50-.70
		BC1	1:0	2:2	1.00	.30-.50
		BC2	7:2	3:2	0.91	.30-.50
Bragg x Tracy-M	$r_1r_1R_2$— x $R_1R_1R_2R_2$	F_1	1:0	6:0	0.00	.99
		F_2	50% 13:3	158:22(4)††	5.03*	.02-.05
		F_2	50% 1:0	56:5 (2)††	15.41**	<.001
		F_2 subpopulation 1	1:0	37:3	3.22	.05-.10
		" 2	1:0	19:2	0.19	.50-.70
		" 3	13:3	38:4	2.35	.10-.20
		" 4	13:3	31:7	0.00	.99
		" 6	13:3	34:5	0.90	.30-.50
		" 8	13:3	55:6	3.18	.05-.10
		BC1	7:2	0:1	3.54	.05-.10
		BC2	1:0	2:0	0.00	.99

†Based on ratings for resistance versus susceptibility at the R7.5 stage of growth.

††The number in parentheses is the number of F_2 subpopulations which fit the 13:3 or 1:0 ratio.

*,**Significant deviation from the expected ratio at ($P \leq 0.05$) and ($P \leq 0.01$).

≤0.01, respectively). Of the 17 F_2 subpopulations common to the Tracy-M x Bragg and Bragg x Tracy-M reciprocal crosses, they divided equally into ratios of 13:3 and 1:0 as expected for the F_2 segregation ratios (Table 12). The BC1 and BC2 progeny of Tracy-M x Bragg cross fit the expected BC ratios of 1:0 and 7:2, respectively. In addition the BC1 and BC2 progeny of the reciprocal cross fit expected ratios of 7:2 and 1:0, respectively. The Bragg x Tracy-M BC1 and BC2 had three plants total which would not be as credible as the nine combined plants for the Tracy-M x Bragg BC1's and BC2's. There were no apparent differences between the reciprocal crosses in level of resistance or susceptibility, indicating that the cytoplasmic effects in resistant Tracy-M and susceptible Bragg were not different in relation to stem canker.

Crosses involving the Tracy-M variety and the susceptible variety, J77-339, also support the premise that resistance to stem canker in Tracy-M is governed by one dominant and one partially dominant gene (Table 13). Progeny obtained from crossing parents with such assumed genotypes should yield an F_1 generation expected ratio of 1:0 and a 13:3 expected ratio for the F_2 generation. Table 13 confirms that the six F_1's tested for their reaction to stem canker were resistant and did fit the expected 1:0 ratio. The 13:3 F_2 expected ratio was also a good fit for the cross. The reciprocal cross of J77-339 x Tracy-M also best fit the F_1 1:0 expected ratio. However, the observed ratios for the combined F_2 populations deviated significantly ($P \leq 0.001$) from the 13:3 expected ratio. In addition, of the 11 individual F_2 subpopulations comprising the J77-339 x Tracy-M cross, seven best fit the 13:3

Table 13. Chi-square tests for reaction of the parents, F_1, F_2 and BC1 progeny from the resistant x susceptible cross, Tracy-M x J77-339 and reciprocal to inoculation with D. phaseolorum var. caulivora.

Parent/Cross	Proposed Genotypes	Generation	Expected Ratios	Observed numbers† (R:S)	χ^2	$P > \chi^2$
Tracy-M	$R_1R_1R_2R_2$	P_1	1:0	143:2	0.03	.80-.90
J77-339	$r_1r_1r_2r_2$	P_2	0:1	3:184	3.05	.10-.20
Tracy-M x J77-339	$R_1R_1R_2R_2$ x $r_1r_1r_2r_2$	F_1	1:0	6:0	0.00	.99
		F_2	13:3	182:35	0.97	.30-.50
J77-339 x Tracy-M	$r_1r_1r_2r_2$ x $R_1R_1R_2R_2$	F_1	1:0	6:0	0.00	.99
		F_2	13:3	166:72(11)††	20.67**	<.001
		F_2 subpopulation 2	13:3	15:5	0.51	.30-.50
		" 3	13:3	18:2	1.10	.20-.30
		" 4	13:3	28:8	0.28	.50-.70
		" 5	13:3	15:5	0.51	.30-.50
		" 6	13:3	14:7	2.93	.05-.10
		" 10	13:3	11:9	9.05**	.001-.01
		" 11	13:3	15:6	1.33	.20-.30
		" 12	13:3	8:10	15.95**	<.001
		" 13	13:3	12:9	7.99**	.001-.01
		" 16	13:3	12:8	5.93*	.02-.05
		" 22	13:3	18:3	0.27	.50-.70
		BC1	1:1	0.2	2.00	.10-.20

†Based on ratings for resistance versus susceptibility at the R7.5 stage of growth.

††The number in parentheses is the number of F_2 subpopulations which fit the 13:3 ratio.

*,**Significant deviation from the expected ratio at ($P \leq 0.05$) and ($P \leq 0.01$).

ratio, whereas the other four did not. Three subpopulations 10, 12, and 13 deviated significantly from the expected 13:3 ratio ($P \leq 0.01$) and subpopulation 16 deviated significantly from the expected ratios ($P \leq 0.05$) (Table 13). There was an apparent difference between the Tracy-M x J77-339 and the reciprocal cross of J77-339 x Tracy-M in the levels of resistance and susceptibility. The reciprocal cross had lower numbers of resistant plants and higher numbers of susceptible plants versus the Tracy-M x J77-339 cross (Table 13). This most likely indicates that the cytoplasmic effects in these two varieties are different in relation to reaction to stem canker. This is especially true of the assumed homozygous recessive $r_1r_1r_2r_2$ genotype of J77-339. The BC1 progeny of the J77-339 x Tracy-M cross did fit the expected 1:1 BC1 ratio; however, only two plants were present in the BC1 generation.

With the exception of the reciprocal cross of J77-339 x Tracy-M, there was good agreement among the three Tracy-M crosses and their reciprocal crosses in support of a proposed two major gene $R_1R_1R_2R_2$ genotype in Tracy-M. Except for the related case, the individual subpopulations of the Tracy-M x susceptible crosses are a good fit to expected ratios ($P \geq 0.05$).

Crosses involving the Bragg variety and either of the two resistant varieties, Bay or Tracy-M support the assumption that Bragg has the $r_1r_1R_2$-- susceptible genotype. Crosses involving the J77-339 variety and either of the two resistant varieties support the assumption that J77-339 has a $r_1r_1r_2r_2$ susceptible genotype. Furthermore, the J77-339 x Tracy-M cross demonstrated possible cytoplasmic effects

which increased the number of susceptible plants in comparison to the number of susceptible plants found in the Tracy-M x J77-339 cross.

Susceptible x Susceptible Crosses

The assumed genotype of the susceptible Bragg variety, $r_1r_1R_2$-- has been supported well in the Bragg x resistant variety crosses. The selfs of Bragg in the tests resulted in a 16:170 ratio at growth stage R7.5. However, Bragg is moderately susceptible to stem canker and slowly differentiates its reaction to the disease. At 40 days postinoculation the best fit was a 1:1 ratio. Sixty days postinoculation has been reported as the best time to evaluate Bragg soybeans for their reaction to stem canker. There was no 60 day postinoculation rating. The lack of best fit for a genetic model of 1:3 for the Bragg variety was the lack of sensitivity to be able to distinguish those plants which possessed some resistance to stem canker; possibly the $r_1r_1R_2R_2$ genotype. It is possible that several moderately resistant plants could have been classified susceptible because of the extent of lesion development on the plants. The gene assumed to be conditioning resistance in the selfed Bragg genotype $r_1r_1R_2$-- is of less magnitude than the R_1 gene so a lower level of resistance if likely found in the resistant Bragg genotype.

The selfs, F_1, F_2, BC1 and BC2 progeny data of Bragg and J77-339 from this study are shown in Table 14. Based on the proposed genotype for each parent, the F_1 generation plants from a cross between them should all be susceptible (0:1) and the F_2's should segregate 1:1 into ratios of either 1:3 or 0:1. The nine F_1 plants tested for their

Table 14. Chi-square tests for reaction of the selfs, F_1, F_2, BC1 and BC2 progeny from the susceptible x susceptible cross, Bragg x J77-339 and reciprocal to inoculation with D. phaseolorum var. caulivora.

Self/Cross	Proposed Genotypes	Generation		Expected Ratios	Observed numbers† (R:S)	χ^2	P > χ^2
Bragg	$r_1r_1R_2$--	P_1		1:3	16:170	277.17**	<.001
J77-339	$r_1r_1r_2r_2$	P_2		0:1	3:184	3.05	.02-.05
Bragg x J77-339	$r_1r_1R_2$-- x $r_1r_1r_2r_2$	F_1		1:0	0:3	0.00	.99
		F_2	50%	1:3	7:58(1)††	7.01**	<.01
		F_2	50%	0:1	3:120(2)††	3.07	.02-.05
		F_2 subpopulation	1	1:3	7:58	7.01**	<.01
		"	2	0:1	3:102	3.08	.02-.05
		"	3	1:0	0:18	0.00	.99
		BC1		0:1	0:3	0.0	.99
J77-339 x Bragg	$r_1r_1r_2r_2$ x $r_1r_1R_2$--	F_1		0:1	0:6	0.00	.99
		F_2	50%	1:3	7:35(1)††	1.55	.20-.30
		F_2	50%	0:1	7:199(10)††	35.24**	<.001
		F_2 subpopulation	2	0:1	0:20	0.00	.99
		"	8	0:1	1:20	0.00	.99
		"	9	1:3	7:35	1.55	.20-.30
		"	10	0:1	1:20	0.00	.99
		"	11	0:1	1:20	0.00	.99
		"	14	0:1	0:20	0.00	.99
		"	18	0:1	1:20	0.00	.99
		"	22	0:1	1:20	0.00	.99
		"	31	0:1	0:20	0.00	.99
		"	33	0:1	2:19	0.19	.50-.70
		BC1		0:1	0:7	0.00	.99
		BC2		0:1	0:5	0.00	.99

†Based on ratings for resistance versus susceptibility at the R7.5 stage of growth.

††The number in parentheses is the number of F_2 subpopulations which fit the 1:3 or 0:1 ratio.

**Significant deviation from the expected ratio at ($P \leq 0.01$).

reaction to stem canker were classified susceptible which fit the expected 0:1 ratio. Two of the three F_2 subpopulations best fit the 0:1 expected ratio and the third subpopulation came closer to fitting the 1:3 ratio than the 0:1 ratio. However, it deviated significantly ($P \leq 0.01$) from the 1:3 ratio. The F_2 progeny of the reciprocal (J77-339 x Bragg) cross did not segregate 1:1 into ratios of 0:1 or 1:3. Of the 11 F_2 subpopulations composing the cross only one best fit the 1:3 ratio, whereas the other 10 best fit the 0:1 ratio individually. The reason that the 10 F_2 subpopulations do not best fit the 0:1 ratio collectively is because of a few more resistant plants than were expected in the F_2 subpopulations. The BC1 and BC2 progeny of both the susceptible x susceptible crosses support the 0:1 expected BC ratios. With the testing of 15 BC1 and BC2 progeny these numbers will give more credence to results from the testing of these BC's than in other BC1 or BC2 progeny with fewer numbers tested. There were no apparent differences between the reciprocal crosses in level of resistance or susceptibility, indicating that the cytoplasmic effects in these two susceptible varieties were not different in relation to reaction to stem canker.

Within the susceptible x susceptible crosses, the selfs, F_1, BC1 and BC2 data support the expected Bragg and J77-339 assumed genotypes of $r_1r_1R_2$-- and $r_1r_1r_2r_2$, respectively. In the Bragg x J77-339 cross, the equal 1:1 segregation of F_2 subpopulations into 1:3 or 0:1 ratios was more nearly realized than in the reciprocal J77-339 x Bragg F_2 subpopulations. The F_2 subpopulations are the only segment of the data

that do not support the assumed models, yet they all produced at least one 1:3 ratio and several 0:1 ratios. However, when crossing a homozygous susceptible parent (J77-339) to a segregating susceptible parent (Bragg), it is likely that some of the actually resistant F_2 plants may have been misclassified as susceptible due to a low level of resistant genes in the resulting progeny. There is an additional possibility that when crosses were made more susceptible Bragg plants ($r_1r_1r_2r_2$ or $r_1r_1R_2r_2$) than resistant plants ($r_1r_1R_2R_2$) were used. If plants with the homozygous recessive genotype ($r_1r_1r_2r_2$) were used in crossing, all progeny would be expected to be susceptible. On the other hand, if a resistant Bragg plant ($r_1r_1R_2R_2$) was crossed with the homozygous susceptible J77-339, then the F_2 progeny would be expected to segregate 1 resistant ($r_1r_1R_2R_2$):3 susceptible ($r_1r_1R_2r_2$, $r_1r_1r_2r_2$). The most difficult genotypes to classify would be the $r_1r_1R_2r_2$. Also, if Bragg is segregating for the R_2 gene any individual plant could have a $r_1r_1R_2R_2$, $r_1r_1R_2r_2$, or $r_1r_1r_2r_2$ genotype. The proportion depends on the frequencies of the R_2 and r_2 alleles in the Bragg variety, which were assumed to be equal. The level of dominance is also important and the combination of unknown level of dominance and unknown gene frequency in addition to difficulties in classifying each plant into either of two categories contribute potential bias in the evaluation of the Bragg genotypes.

CHAPTER IV

SUMMARY AND CONCLUSIONS

The results of the greenhouse seedling inoculation tests indicated that the four isolates tested in this study were all virulent on the four soybean varieties tested. Isolates FP3 and EA21 were of nearly equal virulence, and both were more virulent than isolates 82-5 and 82-3. Therefore, FP3 was used as the source of inoculum when the progeny generations were tested for their reaction to the stem canker pathogen since it produced slightly larger lesions than EA21.

The optimum postinoculation time for rating plants for reaction to stem canker was found to be dependent upon the actual genotype of the parents used within a given cross. Resistant parents and resistant x resistant progeny crosses could be classified accurately at either 20 or 40 days postinoculation or at the R7.5 growth stage. On the other hand, resistant x susceptible and susceptible x susceptible progeny crosses as well as susceptible parents were classified more accurately for their reaction to the stem canker pathogen at the growth stage R7.5.

The assignment of proposed genotypes for each of the varieties was based upon the segregation ratios of selfed, F_1, F_2, reciprocal F_1 and F_2 progeny and limited numbers of BC1 and BC2 generations. The Bay genotype, $R_1R_1r_2r_2$, was supported by each of the above criteria as a best fit in all possible crosses. The Tracy-M genotype, $R_1R_1R_2R_2$, was supported by all possible progeny crosses except for what appeared to

be a cytoplasmic difference in the reciprocal cross of J77-339 x Tracy-M. The Bragg genotype, $r_1r_1R_2$-- was supported in all of the resistant x susceptible crosses. There were slightly more F_2 subpopulations which segregated 0:1 than expected in the susceptible x susceptible progeny cross, yet there is a good chance that the subjectivity used to distinguish between a resistant or susceptible plant from a susceptible x susceptible cross could result in some misclassifications. Also some of the Bragg plants used in the original crosses could have been susceptible ($r_1r_1r_2r_2$) and one would expect slightly more 0:1 F_2 ratios if susceptible Bragg plants were crossed to susceptible J77-339 plants ($r_1r_1r_2r_2$). The J77-339 genotype, $r_1r_1r_2r_2$, was best supported in all the crosses to have no resistance to stem canker.

The results of this study indicate that the inheritance of resistance to stem canker in the soybean variety Tracy-M is conditioned by two major genes, namely R_1 and R_2. The data from all of the Tracy-M crosses, except for the reciprocal J77-339 x Tracy-M cross F_2 subpopulations, best fit a genetic model of two major genes governing resistance to stem canker. On the other hand, resistance in Bay seems to be conditioned by only one gene, R_1, but it is the same as one of the genes in Tracy-M. The data from all the progeny of the Bay crosses also support the one completely dominant gene model conditioning resistance to stem canker. The resistance reaction of Bay to stem canker is very similar to that of Tracy-M. This indicates that the single R_1 gene in Bay conditions nearly the same amount of resistance

governed by both the R_1 and R_2 genes in Tracy-M. If this is the case, then the R_1 gene confers more resistance than the R_2 gene. Furthermore, it appears that the R_2 allele is only partially dominant to the r_2 allele.

The results of this study indicate that Bragg is not as susceptible to stem canker as J77-339 but it does not have the level of resistance of Bay or Tracy-M. It does appear to have a low level of resistance; therefore, it appears that Bragg is segregating for resistance ($r_1r_1R_2$--) and the resistance comes from the R_2 gene not contained in Bay. J77-339 is homozygous recessive for both genes, $r_1r_1r_2r_2$.

In addition to being homozygous recessive for stem canker resistance, it appears that factors within the J77-339 cytoplasm also contribute to increased susceptibility to the disease when J77-339 is used as the female parent. The J77-339 x Bay cross produced 2.35% more susceptible progeny than the reciprocal Bay x J77-339 cross. The same trend was magnified in the J77-339 x Tracy-M cross as there were 14.13% more susceptible progeny than in the reciprocal Tracy-M x J77-339 cross.

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