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## A Method for Determining Stem Canker Resistance in Soybean<sup>1</sup>

By JOHN M. DUNLEAVY<sup>2</sup>

Stem canker disease of soybean is caused by the fungus, *Diaporthe phaseolorum* var. *caulivora*, and at present there are no soybean varieties known to be highly resistant to this disease. Stem canker takes its name from the resemblance of the discolored area of an infected stem to a canker. As the infected area on a stem enlarges, the stem is girdled and the portion of the plant above the girdled area is killed. Stem canker seriously affects soybeans in the north-central region of the United States and has been reported to cause heavy losses (Athow and Caldwell, 1954; and Dunleavy, 1954, 1955). A description of the disease and the casual organism has been published by Welch and Gilman (1948) and Athow and Caldwell (1954). Differences in varietal susceptibility have been reported by Hildebrand (1953a) and Beeson and Probst (1955).

The incidence of stem canker varies considerably from year to year. In addition, incidence of the disease may be very high in one portion of a field and extremely low in another portion. Because of the great variation experienced when results from different years are compared, as well as variation within a single field in a given year, selecting resistant soybean varieties on the basis of field reaction is difficult. In order to develop stem canker resistant varieties of agronomically acceptable soybeans, highly resistant lines must be found. The investigations reported here were undertaken in the hope of providing an improved, dependable method of determining stem canker resistance.

In a search for stem canker resistant plants in 1953, toothpick tips were inserted in the base of soybean stems of the varieties to be tested (Crall 1952). Stems were inoculated a few inches above the soil surface and the stem wound sealed with petrolatum. Results were disappointing because a high percentage of plants was killed in most cases, leaving a very narrow margin on which to differentiate resistance and susceptibility. Crall (1956) reported natural infection of stems occurred largely through both blade and petiolar portions of leaves. Because variation in the formation of a petiole abscission layer among different varieties of soybeans might

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play an important part in resistance or susceptibility of varieties to stem canker, a petiole inoculation study was conducted.

#### MATERIALS AND METHODS

Inoculum consisted of fungus mycelium grown on toothpick tips prepared as described by Crall (1952) and Hildebrand (1953b). Plants were prepared for inoculation in 2 ways. In the first method, petioles were cut 1 inch from the pulvinus, and the toothpick tips were forced into the distal end of the petiole stub. In the second method, stem tops were severed from the main stem 1 inch above the third node from the top and the toothpick tip inserted in the stem tip. In each case the cut surface of the plant was covered with a thin layer of petrolatum to prevent desiccation of the wounded plant tissue and inoculum.

All plants used for inoculation studies had bloomed prior to inoculation and in most cases pod development had begun at the upper-most node. All petiole stub inoculations were made on field grown plants, whereas, the stem tip inoculations were made on greenhouse grown plants.

#### RESULTS

Petioles of plants representing 12 soybean varieties were inoculated with *D. phaseolorum* var. *caulivora*. One petiole per plant and 10 plants per variety were inoculated. Percentage of infected petioles remaining on stems after 11 days was recorded (Table 1). There was considerable variation between varieties in the retention of infected petioles. Stems of some varieties dropped almost all inoculated petioles, whereas, other lines retained almost all infected petioles. In general, when petiole retention was high, percentage stem infection was high, and when petiole retention was low, percentage stem infection was low. The varieties Cypress and Earlyana, however, were exceptions.

**Table 1**

Percentage of petioles remaining on stems and percentage of infected stems occurring in 12 varieties of soybean 11 days after inoculation with *D. phaseolorum* var. *caulivora*.

Variety	Percentage of petioles remaining	Percentage of stems infected
Hawkeye	70	90
Chief	70	60
Cypress	80	20
Earlyana	0	60
Patoka	30	50
Wabash	0	0
A5-067	0	10
A7-1953	40	50
A7-6520	60	60
C-683	30	40
C-739	80	100
H-3665	10	30

This method of testing for stem canker resistance was utilized in a number of other stem canker studies because it offered a wider base for differentiating degree of resistance among varieties. Use of the method, however, showed it had several serious disadvantages. High winds that occurred 4-8 days after inoculation frequently removed petioles from stems that otherwise might have remained attached and resulted in stem infection. In cases where the percentage of petioles remaining was high and the percentage of stem infection low, it could not be ascertained if the low infection was the result of disease resistance of the plant or some irregularity of the inoculum or the procedure of its application. In addition, little evidence was found to support the belief that infection occurred primarily in leaf blades or petioles.

A new approach to the problem was begun by considering the fact that in stem canker disease the fungus grows through the host tissues until the stem has been girdled and the upper portion of the plant killed. This girdling action of the fungus might logically be expected to be slower in a stem canker resistant variety of soybean than in a susceptible variety. Since the capacity of the fungus to girdle the stem of a given soybean variety is largely determined by the rate of growth of the fungus in that particular tissue, a good measure of stem canker resistance might be the rate of fungus growth through stem tissue. Preliminary tests indicated that the rate of growth of the stem canker fungus is nearly constant, for a given variety, in the portion of the stem above the woody tissue near the base. It thus appeared that the upper portion of the stem was the most logical area of the plant to test for stem canker resistance. Consequently, an experiment was designed to determine if it was possible to obtain quantitative measurements of stem canker resistance.

Since it is well established that *D. phaseolorum* var. *sojae* is less pathogenic than *D. phaseolorum* var. *caulivora* (Welch and Gilman, 1948, and Athow and Caldwell, 1954), the former fungus was selected for testing along with the stem canker fungus because, being less pathogenic, one would expect its rate of growth in soybean stems to be slower than that of the stem canker fungus. Three soybean varieties were selected for the test: Hawkeye, a susceptible variety (Beeson and Probst, 1955); A6K-1040, a line that was resistant in previous tests; and Harosoy, a resistant variety (Beeson and Probst, 1955, and Johnson et al., 1955).

Ten soybean plants of each of the above varieties were inoculated in stem tips with *D. phaseolorum* var. *caulivora*. Ten additional plants of each variety were inoculated in the same way with the pod and stem blight fungus, *D. phaseolorum* var. *sojae*. Growth of the fungi in stems was recorded periodically (Figure 1). From the beginning of the experiment the growth rate of the stem canker

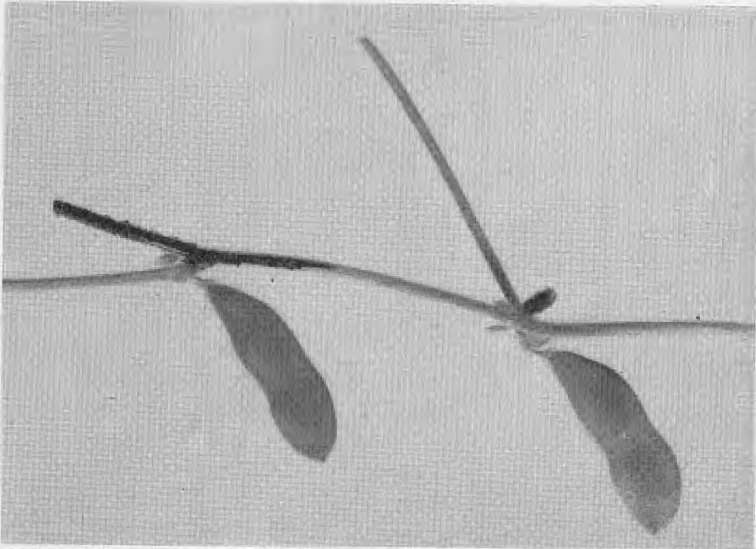


Figure 1. A soybean stem tip that has been inoculated with *D. phaseolorum* var. *caulivora*. Notice the dark discoloration produced by the fungus as it progressed down the stem.

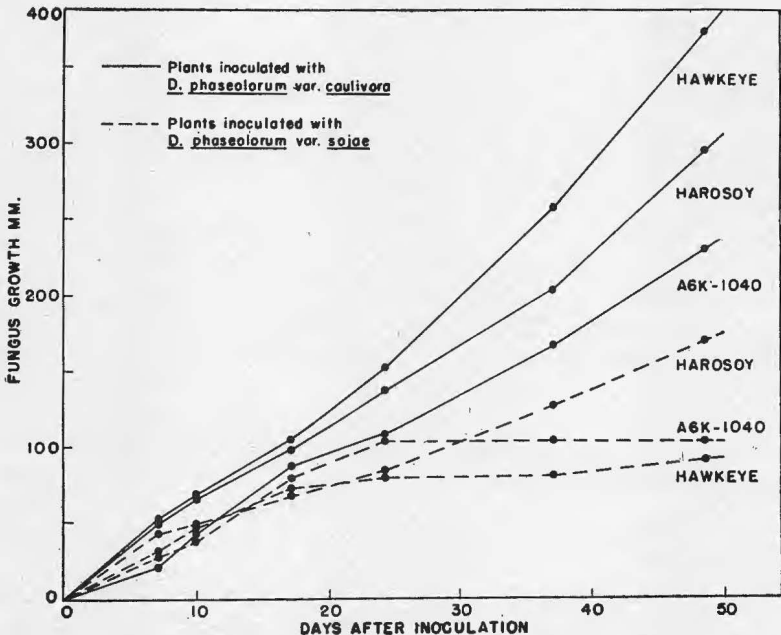


Figure 2. Rate of growth of *D. phaseolorum* var. *caulivora* and *D. phaseolorum* var. *sojae* in stems of 3 varieties of soybean.

fungus was much reduced in the stems of line A6K-1040 (Figure 2). Growth was most rapid in the stems of the variety, Hawkeye, and growth in the stems of Harosoy was intermediate. This trend in growth rate was maintained until the end of the experiment.

Considering the plants inoculated with the pod and stem blight fungus growth rates in line A6K-1040 and Hawkeye were similar. The rate of growth in Harosoy was initially greater than in Hawkeye, but 21 days after inoculation was approximately the same. After this, however, the average rate of growth increased sharply over A6K-1040 and Hawkeye. This is explained by the fact that 100 percent of the A6K-1040 plants inoculated with the pod and stem blight fungus confined growth of the fungus above the first node adjacent to the inoculated tip of the stem. In the variety, Hawkeye, the fungus was confined above the first node in 90 percent of the plants but in Harosoy it was confined in only 40 percent of the plants (Table 2). Thus the fungus continued to grow past the first node of 60 percent of the Harosoy plants and considerably increased the average length of stem penetrated for this variety.

**Table 2**  
Percentage of stems in which *D. phaseolorum* var. *caulivora* and *D. phaseolorum* var. *sojae* did not pass beyond the first node adjacent to the inoculated stem tip.

Variety	<i>D. phaseolorum</i> var.	<i>D. phaseolorum</i> var.
	<i>caulivora</i>	<i>sojae</i>
	%	%
A6K-1040	10	100
Hawkeye	0	90
Harosoy	0	40

Observations during the experiment showed that the rate of growth of the stem canker fungus in stems is retarded at the nodes, but as soon as the fungus is able to penetrate this barrier the initial rate of growth is resumed. This means that care should be taken in making comparisons of growth rate between varieties which differ greatly in internode length. Varieties with a greater number of nodes per unit length of stem will consequently have a slower rate of growth of the fungus than varieties with fewer nodes per unit length of stem.

Results of the stem tip inoculation experiment clearly demonstrated that stem canker resistance of varieties, previously tested and of known resistance, was indicated by rate of growth of the fungus in soybean stems. The data also indicated that this method of inoculation might be of use in determining differences in pathogenicity of previously untested strains of either the stem canker or pod and stem blight fungus, if such differences exist. The method could be used to measure differences in pathogenicity of 2 or more strains of fungus in a single variety by comparing rates of growth.

This method of determining stem canker resistance has many advantages. It gives a quantitative measurement of varietal resistance that can readily be used in selecting resistance suitable for use in breeding programs. It will indicate physiological resistance, a desirable type of resistance, especially where breeding procedures are concerned. This disease can reliably be produced in years when environmental conditions are unsuitable for natural stem canker infection to occur. The method is quicker than that previously used where toothpick tips were inserted in the bases of stems, because holes in the woody stem bases had to be made prior to insertion of the inoculum. The method is easier because workers can stand instead of kneeling as was formerly necessary. An adequate check on the method itself is provided in that a plant is not assumed to be resistant if the inoculum fails to infect the plant. Inoculation failures are thus easily detectable and can be eliminated from consideration of results of tests. If all inoculations failed in a given line being tested, one would suspect an immune reaction, and additional tests would be necessary in such a case.

There are several disadvantages of the method. Morphologic resistance cannot be detected. Care must be taken in comparing fungus rates of growth between varieties that vary widely in internode length. Plants must be individually inoculated. This makes the inoculation procedure both time consuming and expensive if large numbers of plants are to be tested. It is believed, however, that the advantages of the method far outweigh the disadvantages and that for the immediate future it offers pathologists and agronomists interested in locating stem canker resistance an improved technique for detecting resistance.

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