# Methods of Inoculating Sugar Beets with Aphanomyces Cochlioides Drechs<sup>1</sup>

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In the program of breeding sugar beets resistant to Aphanomyces cochlioides Drechs., several hundred lines and varieties are tested each year in fields naturally infested with the fungus. In these tests a number of the lines, especially those which are not derived from resistant selections, are found to be very susceptible compared with resistant lines. A quick method of testing the resistance of lines under controlled conditions in the greenhouse would, therefore, be very useful in the breeding program. Since disease exposure in field tests is not always uniform, a method of obtaining artificial localized epidemics in the field would also be very desirable. With these objectives in view, studies were made to develop methods of inoculating sugar beets with A. cochlioides in the greenhouse and in the field.

# Greenhouse Tests

### **General Methods**

In preliminary studies, practically all plants died, and it was not possible to differentiate between resistant and susceptible lines when the inoculum was introduced into the soil before the seedballs were planted. Since it had been reported earlier by Bockstahler and Henderson (2)<sup>3</sup> that the greenhouse reaction of three sugar beet trains corresponded closely to the field determinations when the seedlings were transplanted to soil inoculated with A. cochloides, further studies were made in which inoculum was applied to the soil after the seedlings had emerged. In these tests, plants of resistant and susceptible varieties were grown in flats of autoclaved soil or sand and were inoculated by burying lengths of string on which the fungus had been cultured, between the rows of plants and by pouring a suspension of zoospores along the rows. In both cases, the resistant and susceptible varieties could be differentiated according to differences in number of plants which survived. The zoospore method was chosen for additional tests because it is quicker and easier.

Zoospore inoculum is readily obtained in the laboratory in the following manner, which is an adaptation of a method used by others and reported earlier in a preliminary paper (5) :

The fungus is grown for about one week in 250-ml. flasks, each containing a sterile decoction of 5 maize kernels in 50 ml. of tap water. The broth is then decanted from the flasks, and the flasks with the mycelial mats are half filled with sterile tap water. Within 12 to 36 hours the submerged mycelial mats produce large quantities of zoospores, often more than 100,000 per ml., as determined with a haemocytometer. If the spore suspensions are

<sup>&</sup>lt;sup>1</sup> CooDerative investigations of the Field Croos Research Branch, Agricultural Research Service, Inited Status, Construction of the Field Cross Research Agricultural Experiment Station, Pater Status, Construction Station, Statistical Experiment Structural Assistant Pathologist, Field GooDS Research Branch, Agricultural Research Service, United Stages Department of Agricultura Creater to Branch, Agricultural Research Service, Numbers in parentheses refer to literature cited.

decanted and additional water is added to the flasks, additional crops of spores may be obtained. Temperature is an important factor affecting the production of zoospores in the laboratory. Counts of zoospore suspensions from flasks containing submerged mycelial mats kept for 24 hours at  $15^{\circ}$ ,  $20^{\circ}$ ,  $27.5^{\circ}$  and  $30^{\circ}$  C. indicate that the optimum temperature for zoospore production in the laboratory is near  $20^{\circ}$  and  $25^{\circ}$  (Table 1). Mc-Kecn (4) previously reported a similar effect of temperature on the time required for the fungus to produce zoospores after immersion of infected sugar beet seedlings in water.

Table I.-Influence of Temperature on Number of Zoospores Produced by Mycelial Mats of Aphanomyces Cochlicides Submerged in Water for 24 Hours.

		Temperature °C.				
	15	20	25	27^5	30	
No. of zoospores per c.c.	23,000	64,000	64,000	28,000	26,000	

Tests were made to determine the zoospore concentration which would result in the best differentiation between a resistant variety, S. P. 48B3-00, and a susceptible one, S. P. 19-00. The plants were grown in 4 inch pots of autoclaved soil at 75° and 85° F. Four suspensions, containing approximately 740, 1,850, 3,700 and 7,400 zoospores per ml. were prepared by diluting a heavy suspension of zoospores obtained from submerged cultures. Two weeks after the plants had emerged, 50 ml. of inoculum were poured into each pot. Counts of surviving plants were made 31 days after inoculation and are presented in Table 2. Under the conditions of this experiment, the lowest concentration. 37000 zoospores per 4 inch pot, was inadequate for differentiating between the resistant and susceptible varieties, as evidenced by the large number of healthy plants of both varieties. It was concluded that a concentration of 100,000 to 200,000 zoospores per pot is needed for differentiating between resistant and susceptible varieties.

	Average Number of Plants of Each Variety Alive 30 Days After Inoculation <sup>1</sup>			
Number of Zoospores	75° F.		85°' F.	
per 4-in. Pot	S. P. 48B3-00	S. P. 1-9-00	S. P. 48B3-00	S. P. 1-9-00
370,000	6.0	2.3	7.0	0.3
185,000	13.7	2.3	12.0	1.0
92,500	16.0	4.7	13.0	3.7
37,000	22.0	20.0	12.7	9.3
0	21.7	22.6	22.0	24.0

Table 2.-Effect of Number of Zoospores of Aphanomyces Cochlioides on Development of Blackroot in Resistant, 48B3-00, and Susceptible, S. P. 1-9-00, Varieties of Sugar Beets Grown at Two Temperatures in the Greenhouse.

<sup>1</sup> Average of 3 replicates.

#### **Comparison of Greenhouse Reaction with Field Reaction**

In a series of two greenhouse tests, 45 lines and varieties which had been included in field tests at East Lansing, Michigan, and Waseca, Minnesota, in 1951 were inoculated with *A. cochlioides*. The plants were grown in 4 inch

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pots of autoclaved soil at  $75^{\circ}$  F. and were inoculated by pouring 50 ml. of a suspension containing approximately 120,000 zoospores into each pot. In the first test, there were 3 pots of each line and in the second there were 5. Except for the resistant and susceptible check varieties, Acc. 1177 and Acc. 1178, respectively, the lines in each test were different.

Counts of surviving plants were made about one month after inoculation. Surviving plants were classified as lightly infected if less than onefourth of the hypocotyl was discolored and as severely infected if more than one-fourth of the hypocotyl was discolored and reduced to a black thread. Before analyses of variance were made, percentage data were converted to degrees according to Bliss' (13) method from tables in Hayes and Immer (3).

To compare the greenhouse reaction with the field reaction, each line was assigned a numerical rating. In the first test, as shown in Table 3, this rating is the percentage of plants surviving, since this was the way in which the lines differed most. In the second test, the lines differed more on the basis of the percentage of plants which were lightly infected, hence this was the basis of the comparative rating. The field reaction of each line is represented by a numerical rating ranging from 0 (very susceptible) to

Table 3.—Comparison of Reactions of 45 Sugar Beet Lines Inoculated with Aphanomyces Cochlioides in the Greenhouse.

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	No. of Plants <sup>1</sup>		Pet. of Plants:		
Line	Inoculated	Field Rating	Surviving	Lightly Infected	
50A3-0	90	5.75	5T5	17~3	
EL 1007	84	5.75	39.9	16.1	
50A4-00	90	5.0	56.4	26.6	
48B3-00	90	5.0	42.3	13.6	
48B3-00	90	5.0	42.3	13.6	
50A2-00	90	4.5	42.5	22.3	
Ace. 1174	90	4.5	40.9	18.4	
Ace. 1170	90	4.0	45.2	22.3	
Ace. 1179	90	4.0	28.0	8.5	
49111-01	90	3.75	36.9	6.0	
Ace. 1173	90	3.5	35.7	18.4	
S. L. 944	90	3.5	27.0	8.5	
50104-0	88	3.5	31.5	10.5	
S. L. 1-300	90	3.25	28.2	16.1	
S. L. 9090114	80	2.5	19.4	0	
Ace. 1171	90	2.5	37.7	8.5	
49B10-00	90	2.5	35.9	15.3	
486-0	90	2.5	31.4	10.5	
S. L. 944H1	90	1.25	13.1	0.0	
S. P. 1-9-00	68	1.0	22.2	0.0	
SL0531-2-4	90	0.5	30.6	10.5	
S. L. 92H1	90	0.5	18.3	0.0	
Ace. 1177	90	5.0	45.0	20.4	
Ace. 1178	70	1.0	20.2	0.0	

Test 1

Difference required for

Greenhouse Rating<sup>2</sup>

Table 3 (Continued)

			Greenhouse Rating		
	No. of Plants <sup>1</sup>		Pet. of Plants:		
Line	Inoculated	Field Rating	Survivirtg	Lightly Infected	
50B92-13	72	7.0	78.0	58.2	
50B92-25	100	7.0	71.6	52.7	
50B4-24	100	6.75	71.6	40.3	
50C3-17	100	6.75	90.0	49.5	
50B92-26	97	6.75	68.0	48.5	
50B92-24	96	6.75	69.2	38.5	
50B1-1	100	6.5	64.9	34.5	
50B94-33	100	6.5	66.4	46.6	
50A7-00	93	5.0	69.0	61.2	
50B4-15	100	5.0	67.2	31.6	
50B3-0	100	5.0	68.9	57.0	
50C3-31	100	5.0	80.0	60.5	
50B92-37	47	5.0	81.9	41.9	
50B4x33	100	5.0	80.0	50.4	
50B3x06	100	3.25	63.4	42.1	
50B12-3	100	3.0	60.7	34.3	
50B10-3	100	2.75	67.2	41.2	
50B57x2	100	2.5	60.7	20.6	
50C3-10	100	2.5	57.4	28.4	
50B88x2	100	2.5	81.9	44.4	
50B69x2	72	2.25	60.9	17.6	
483-0	44	0.0	41.4	4.5	
Acc. 1177				45.5	
Acc. 1178					

Difference required for significance, odds 19:1

 $^1$  Before inoculation plants were thinned where necessary in order to equalize stands as much as possible.  $^2$  Expressed as  $\sin^2 \odot$ .

7 (very resistant). A rating of 5 means that a line was as resistant as the resistant check variety, Ace. 1177, which was the standard for comparison. Stand, root size and shape, and severity of root rot were criteria used in determining field ratings.

The results of the two tests are presented in Fable 3 and are summarized in Table 4. In each of the tests, there were differences betwen varieties *in* their reaction to the disease. In most cases, the reaction in the greenhouse was similar to that in the field. Because of the heterozygosity of many of the lines, it is believed that precision in evaluating the lines would have been gained by using a larger number of replications.

# **Field Tests**

Three methods of inoculation were compared in preliminary experiments in the field. Zoospore suspensions were applied to rows of emerging seedlings with a sprinkling can; artificially infested soil was applied with the seed at planting by means of a fertilizer distributor mounted on a Planet Jr. planter; and autoclaved sugar beet seed balls, coated with vermiculite and nutrient broth and artificially infested with the fungus, were applied in the row with the seed with the fertilizer distributor. Infection occurred only when the latter method was used. Stands in inoculated plots were lower than in noninoculated plots. Also, when sugar beet seedlings were grown in the greenhouse in pots containing soil taken from inoculated field plots one month alter the inoculum was applied, most of the seedlings became infected with *A. cochlioides* and damped-off. Most oi the seedlings grown in soil taken from adjoining noninoculated plots remained alive.

Table 4.—Comparison of Black Root Resistance Rati Assigned on the Basis of Field Tests and Greenhouse Tests.

> .Number of JLines in Each Field Rating Clas With a Greenhouse Rating:

Field Rating	Number of	Less thai
Classes	of Lines	Ace. 117

6-6.9 5-5.9 4-4.9 3-3.9 2-2.9 1-1.9 0-0.9

As these tests were limited in scope they are merely indicative of the possibility of inoculating seedlings by this method. More extensive tests are required before an evaluation can be made of the suitability of this method for obtaining a localized epidemic in the field.

#### Summary

1. Methods for growing inoculum and inoculating voting sugar beet plants with Aphanomyces cochlioides are described.

2. In greenhouse tests, sugar beet lines and varieties differed in their reaction to A. *cochlioides*. In general, the relative degree of resistance of each line or variety tested in the greenhouse was similar to that observed in the field.

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