Field Inoculation of Sugar Beets with Aphanomyces Cochlioides Drechs[1]

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Introduction

In field tests to evaluate the resistance of sugar beet strains to black root caused by the water mold, *Aphanomyces cochlioides* Drechs., a severe disease exposure is desirable. In tests made on land successively cropped to sugar beets, extreme local differences in degree of disease exposure are often evident. One apparent requirement for uniform disease exposure is uniform distribution of the pathogen in the plots. The present study was made, therefore, to develop methods of introducing artificial inoculum of *A. cochlioides* to field plots in order to insure a uniform disease exposure.

No reports were found in the literature concerning methods of inducing extreme field exposures to *A. cochlioides*. Jones and Drechsler (1)[3] obtained limited infection of peas with the root-rot fungus, *Aphanomyces euteiches* Drechs., when the following kinds of inoculum were applied in the field: greenhouse soil previously inoculated with cultures of the fungus, zoospores, and cultures rich in oospores. Kendrick (2) obtained infection of radishes when he spread soil infested with the root-rot fungus, *Aphanomyces raphani* Kendr., on a disease-free plot area before planting.

Materials and Methods

Preliminary studies were made to determine substrates suitable for growing field inoculum. Since *A. cochlioides* requires a relative humidity of at least 98 percent in its immediate environment for growth (3), it follows that the substrate should be capable of retaining sufficient moisture for growth of the fungus. The number of plots to be inoculated in field tests is sometimes too great to make hand application of inoculum feasible; hence, it is desirable that the substrate be of size and consistency that permit quick and uniform application with a mechanical device, such as a planter or fertilizer distributor.

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[3] Numbers in parentheses refer to literature cited.

The following substrates came nearest to fulfilling these requirements and were tested separately in the field after preliminary greenhouse tests:

1. Steamed soil, infested with cultures of the fungus.

2. Oat-grain medium, prepared by adding approximately 250 ml. each of oat grains and water to one-quart fruit jars and sterilizing for 2 hours on 2 successive days.

3. Sorghum-grain medium, prepared according to the method outlined by Whitehead (4).

In each test, the inoculum was applied in the drill row with a V-belt planter. The seed of all sugar beet varieties included in the tests was treated with a fungicide to reduce the incidence of pre-emergence damping-off caused by fungi other than *A. cochlioides.*

Experimental Results and Discussion

In a field test at St. Paul, Minnesota, in 1954, artificially infested soil was applied in the drill row at the rate of approximately one-fourth pint per 10 feet of row. Immediately after application of inoculum, a sugar beet variety susceptible to black root was planted with a Planet Jr. hand planter.

Reduced stands and occurrence of black root in inoculated plots indicate that infection resulted from the inoculation treatment (Table 1). The disease exposure was not so great as was desired, however, as it was less than that which occurred naturally in a nearby field planted with the same variety but where successive crops of sugar beets had been grown.

Table 1.—Artificial Inoculation of Field Plots of Sugar Beets with *Aphanomyces cochlioides:* Infested Soil Applied by Planter before Seeding.[1]

Treatment	Plants per Plot		Plants with Black Root at Thinning	Yield of Roots
	Before Thinning	At Harvest		
	Number	Number	Percent	Pounds
Artificially infested soil	45	19	25	31.4
Control (no treatment)	87	31	0	48.4
L.S.D. (P = 0.05)	25	6		9.8

[1] Data expressed as means of five 4-row plots, each 10 feet long.

In a field test at Waseca, Minn., in 1955, oat-grain inoculum was applied in the drill row in soil naturally infested with *A. cochlioides.* Seed of a black-root-resistant variety and of a sus-

ceptible variety were planted with the oat-grain inoculum. Controls consisting of 1) noninfested oat grains and 2) no treatment were included. The oat grains were placed on top of the seed on the V-belt and applied at the rate of 75 ml. per 20 feet of row. As the oat-grain inoculum tended to be wet and sticky, immediately before application it was mixed with dry sawdust which absorbed much of the excess moisture and was then removed with a screen.

Reduced stands indicate that incidence of black root increased upon application of the oat-grain inoculum at the time of planting (Table 2).

Table 2.—Artificial Inoculation of Field Plots of Sugar Beets with *Aphanomyces cochlioides:* Oat-Grain Inoculum Applied by Planter with Seed.[1]

Treatment	Inches Containing Plants in 240-Inch Row before Thinning		Plants per Plot at Harvest	
	US 400	S.P. 486-0	US 400	S.P. 486-0
	Number	Number	Number	Number
Oat-grain inoculum	79	59	13.6	9.8
Control (noninfested oat grains)	106	103	17.0	12.8
Control (no treatment)	113	109	14.6	13.6
L.S.D. (P = 0.05) for comparing:				
Varieties	16		2.0	
Treatments	22		2.8	

[1] Data expressed as means of 5 single-row plots, each 20 feet long.

In tests at the Plant Industry Station, Beltsville, Md., inoculation of sugar beet seedlings in cold frames was obtained upon mechanical application of 150 ml. of sorghum-grain inoculum per 20 feet of row. As in the 2 previously described tests, prevalence and severity of black root in infested plots was greater than in noninfested plots. The average stand of inoculated plots was reduced 58 percent, 73 percent of the surviving plants showing black-root symptoms in contrast to 23 percent of the surviving plants in noninoculated plots. In this test as in the others, seedlings with black-root symptoms when placed in Syracuse dishes containing a shallow layer of water, usually yielded *Aphanomyces cochlioides.*

Since each of the different inoculation media—infested soil, oat grains, and sorghum grains—was tested separately at different

times, in different places, and under different environmental conditions, no direct comparison can be made of their relative efficacy in field inoculation. The relatively lower degree of black root obtained with the infested soil inoculum may be attributed in part to the dry weather that prevailed during the early period of the test. In greenhouse experiments by McKeen (3), abundant black root occurred in sugar-beet seedlings grown in moist naturally infested soil (43% water content) but none occurred in dry soil (20% water content). Hence, it follows that adequate soil moisture is a requisite for successful field inoculation with *A. cochlioides.*

To insure uniform disease exposure, uniform distribution of the fungus in the inoculum substrate is essential. In the case of grain inoculum this can be ascertained visually; in the case of the soil inoculum it cannot. On the other hand, the infested soil inoculum is relatively easier to prepare and to maintain, especially in larger quantities.

The sorghum-grain inoculum, since it is less sticky than the oat-grain inoculum and has smaller kernels, is easier to apply through the planter.

Summary

Methods for field inoculation of sugar beet seedlings with *Aphanomyces cochlioides* are described. In field tests it was demonstrated that black-root incidence can be enhanced by mechanical application of artificial inoculum in the drill row at planting. This method is proposed as a means of increasing the uniformity of disease exposure in field tests.

Literature Cited

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