In Vitro Inoculation of Sugar Beet Seedlings with Aphanomyces Cochlioides Drechs.[1]

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Introduction

Pathogenesis in *A. cochlioides* Drechs. is dependent to a great extent upon high soil moisture. Free water is necessary not only for zoosporangium and zoospore formation, but also for movement of the zoospores through the soil and infection of the host plant (1, 2)[2]. If moisture should become limiting during this period of motility and infection, the effective exposure time of the sugar beet to infection and the relative number of infections sustained by the seedlings would be reduced. Furthermore, the number of infections sustained by the seedlings might show some relationship to the severity of black root symptom expression. To investigate these proposed relationships, *in vitro* inoculations were performed using standardized concentrations of zoospores and definite times of exposure of the sugar beet seedlings to the zoospore inoculum.

Materials and Methods

The culture of *A. cochlioides* used in this investigation was obtained from diseased seedlings growing in artificially infested field soil. For zoospore production, cultures of *A. cochlioides* were grown for five days in 125 ml. flasks containing 50 ml. of a liquid nutrient medium. This medium consisted of Difco-Bacto-Peptone, 1.0 g., Difco-Bacto-Yeast Extract, 1.0 g., potassium mono-basic phosphate, 0.5 g., magnesium sulfate, 0.5 g., and dextrose, 2.0 g., dissolved in 1000 ml. of distilled water. At the end of the growth period, the nutrient medium was decanted, the culture was washed with sterile distilled water and then resuspended in 50 ml. of sterile distilled water and placed at 20° C. for 24 hours. Zoospore concentrations were determined using a Levy-Hausser counting chamber. Sugar beet seedlings, used in these tests, were grown in steam-sterilized soil from G.W.359 segmented seed, treated with N. I. Ceresan. All isolations from inoculated sugar beet seedlings were made on corn meal agar medium.

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[2] Numbers in parentheses refer to literature cited.

Experimental Results

Zoospore concentrations, able to cause uniform infection within a 24-hour period, were determined in the following manner. Zoospore suspensions containing 20, 200, 2,000 20,000 and 200,000 zoospores/ml. were made up in sterile distilled water and used for inoculum. Four-day-old sugar beet seedlings, washed free of soil, were laid in culture dishes containing 20 ml. of zoospore suspension. Four dishes of twenty seedlings each were prepared for each inoculum concentration and maintained at 20° C. for 24 hours. Seedlings in sterile distilled water were used as checks. Following this exposure to inoculum, the seedlings were removed from the zoospore suspensions and washed vigorously in three changes of distilled water. A one-centimeter section of hypocotyl was removed from each seedling and transferred to a corn meal agar plate. Mycelium of *A. cochlioides*, growing from the infected tissue, was visible in 24 to 48 hours. All hypocotyl sections from seedlings exposed to concentrations of 20,000 zoospores/ml. or greater were infected. At the lesser zoospore concentrations of 2,000, 200, 20 and 0, infection decreased to 80, 10, 2.5 and 0 percent respectively (Table 1).

Table 1.—Infection of Sugar Beet Seedlings Exposed for 24 Hours to Various Concentrations of Zoospores of *A. cochlioides*.

Number of Zoospores/ml.	Hypocotyl Infection	
	Number	Percent
0	0	0.0
20	2	2.5
200	8	10.0
2,000	64	80.0
20,000	80	100.0
200,000	80	100.0

Four-day-old sugar beet seedlings were next exposed to suspensions containing 20,000 zoospores/ml. for 2, 4, 8, 16 and 24-hour periods. Four culture dishes, containing 20 seedlings per dish were prepared for each time period. Inoculation and isolation procedures were identical to those in the previous experiment. Seedlings exposed for 2, 4, 8, and 16 hours were placed in dishes of distilled water after washing and plated at the same time as the 24-hour treatment. This provided the same period of time for development of all infections. In all treatments, 80 percent or more of the seedlings exposed to the inoculum were infected (Table 2).

Table 2.—Infection of Sugar Beet Seedlings Exposed to a Standard Zoospore Inoculum of *A. cochlioides* for Various Periods of Time.

Hours of Exposure to Inoculum	Hypocotyl Infection	
	Number	Percent
0	0	0.0
2	64	80.0
4	66	82.5
8	78	97.5
16	80	100.0
24	80	100.0

The mycelial growth produced from infected hypocotyl tissue was progressively more dense as time of exposure increased. This indicated that a greater number of infections had taken place with a corresponding increase in time.

To determine if increase in time of exposure to inoculum would cause an increase in severity of disease symptoms, inoculated four-day-old sugar beet seedlings were transplanted to bench plots of steam-sterilized soil in the greenhouse. Times of exposure to inoculum, inoculation and isolation procedures were the same as above. Disease readings were made eight days after transplanting, after which the seedlings were removed from the soil and the entire hypocotyl excised and plated on corn meal agar.

Seedlings exposed to the zoospore inoculum for 16 and 24 hours showed, in addition to the usual hypocotyl blackening, wilting and collapse of the hypocotyl tissue. Wilting was also evident in the 8-hour treatment, but most of the seedlings showed only a severe hypocotyl discoloration. In seedlings exposed for 2- and 4-hour periods, hypocotyl blackening was not as severe as in the 8-hour treatment and the majority of the seedlings displayed no above-ground symptoms of disease. However, isolations from these symptomless plants showed Aphanomyces to be present in most cases in a slightly discolored area, 1 to 3 mm. long, at the base of the hypocotyl. To distinguish between these plants and plants showing definite above-ground symptoms, two infection categories were set up. Apparent infection values were based on the percentage of seedlings showing above-ground symptoms. Total infection values were based on the percentage of seedlings from which *A. cochlioides* was isolated (Table 3).

Discussion

This investigation has shown that differences in time of exposure of sugar beet seedlings to a standard zoospore inocumum

Table 3.—Infection and Symptom Expression in Sugar Beet Seedlings Exposed to a Standard Zoospore Inoculum of *A. cochlioides* for Various Periods of Time.

Hours of Exposure to Inoculum	Apparent Infection		Total Infection	
	Number	Percent	Number	Percent
0	0	0.0	0	0.0
2	16	20.0	78	97.5
4	30	37.5	78	97.5
8	62	77.5	80	100.0
16	78	97.5	80	100.0
24	80	100.0	80	100.0

of *A. cochlioides* result in different intensities of disease expression. These differences in disease expression are believed to be a function of the number of infections sustained by the seedlings. Although it was not possible to determine the actual number of infections, it appears that a certain minimum number are required to induce typical black root symptoms. On the other hand, there is apparently a level above which the infections are so numerous as to cause wilting and rapid death of the seedling. Such symptoms suggest penetration of the normally resistant vascular tissue or production of toxic products resulting from the large number of infections.

Inoculation of sugar beet seedlings *in vitro* allows close control of inoculum concentration and environmental factors affecting the infection process. Such control is not possible when soil is used as the infection medium. This technique could be useful in investigations of nutritional and biological factors affecting infection and disease development. Also by providing uniformly infected seedlings, a more accurate screening of lines of sugar beets for resistance to *A. cochlioides* would be possible.

Literature Cited

(1) DRECHSLER, CHARLES. 1929. The beet water mold and several related root parasites. Jour. Agr. Res. 38: 309-361.

(2) MCKEEN, W. E. 1949. A study of sugar beet root rot in southern Ontario. Can. Jour. Res. C. 27: 284-311.