Evaluation of Sugarbeet Breeding Lines in Greenhouse Tests for Resistance to *Aphanomyces cochlioides*[*]

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INTRODUCTION

Blackroot disease, caused chiefly by the beet water mold, *Aphanomyces cochlioides* Drechs., has been responsible for serious losses to sugarbeet crops in the Great Lakes area of the United States. Although losses have diminished considerably since the introduction of black root-resistant cultivars, the disease still remains a threat to sugarbeet production. Non-resistant cultivars still show high incidence of black root in some field tests. Futhermore, cultivars designated as resistant are not immune to black root and may suffer loss under conditions highly favorable to the disease - as when soil is warm and wet. Maintaining and increasing Aphanomyces resistance thus remain major objectives in breeding programs to develop improved sugarbeet cultivars for the Great Lakes Area.

Differences in degree of resistance among sugarbeet breeding lines are readily determined in controlled inoculations with pure cultures of *A. cochlioides* in the greenhouse. Results of screening breeding lines for resistance in tests with zoospore inoculum of *A. cochlioides* have been reported (1,3). Subsequently, we have developed methods for production and employment of oospore inoculum (4) in our annual greenhouse screening of

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several hundred breeding lines. Most of the breeding lines are derived from selections for agronomic characters and for resistance to other diseases. Selection for these characters often results in decreased resistance to *Aphanomyces* hence an important objective of the screening tests is to identify and subsequently eliminate the more susceptible lines.

In this report, we summarize results obtained in a series of greenhouse tests conducted in 1977-1982 that show the range of Aphanomyces resistance among our breeding lines derived from plants selected in the greenhouse for Aphanomyces resistance and from other sources.

METHODS AND MATERIALS

Inoculum - The culture of *A. cochlioides* employed was maintained on 0.5-1.0% oatmeal agar slants. Oospores for inoculum were obtained in general accordance with a previously-described methodology (4), by incubating the fungus in darkness in flasks of 0.5-1.0% oatmeal homogenate broth, adjusted to near pH 6.5. To maximize oospore yields, the following incubation procedures were also followed: The volume of broth equaled 1/5 the capacity of the flask; temperature limits ranged from 15 to 20°C; and flask closures were restricted to plastic caps or foil wraps. After a 35-45 day incubation period, mycelial mats were comminuted in a blender for 5 min. After oospore density was determined, the resultant suspensions were mixed, at 104 spores/cc of carrier. The carrier in 1977-1979 tests was vermiculite, and in 1980-1982 tests was arcillite - a granular calcined, montmorillinite clay proprietary product distributed commercially as a soil conditioner. After the inoculum had been air-dried; it was stored in plastic-lined paper bags at 4°C.

Screening tests - Tests were conducted in a greenhouse at 25°C, with a range of approximately 18-40°C. At planting, seedballs and 10 cc of inoculum were placed on the surface of 500 cc of steam-sterilized 1:2:1 (v:v:v) planting mix of vermiculite:peat:arcillite in each plastic pot of 10.2 cm diameter, then covered with an additional

100 cc of planting mix.

Pots of entries were arranged 11 per 35.6 x 45.7 cm tray on a greenhouse bench. Each tray also included one pot of commerical cultivar, US H20, to serve as a standard for comparison. US H20 is moderately resistant to *A. cochlioides* and produces satisfactory crops under disease exposures that damage non-resistant cultivars.

Entries in each experiment were replicated 4-6 times in randomized blocks. Blocks were usually planted on separate dates at 1-2 week intervals. In most experiments, more than one batch of inoculum was used, but all pots in a block were inoculated with the same batch.

Stand counts were made approximately 10 days after planting when emergence was completed and when initial symptoms of black root were apppearing. Disease incidence and severity subsequently increased until approximately 30 days after emergence when some plants showed signs of symptom remission. Entries were then rated numerically according to severity of symptoms from 0 (no apparent symptoms) to 5 (dead) and a disease index (DI) was computed for each pot. DIs were subsequently converted to percent of the DI of US H20 in the same tray. After DIs were computed, entries were also rated, according to general level of Aphanomyces resistance, as superior; intermediate; or inferior - depending on whether the entry mean DI was, respectively, significantly greater, the same, or significantly less than that of US H20 (P=0.05).

In pots of entries that appeared to be more resistant than the nearest US H20 check, the healthiest-appearing plants were selected as sources of Aphanomyces resistance (2). Seed produced on the selections were included in subsequent greenhouse screening tests.

The entries in the tests included mutigerm and monogerm types. The breeding history of most included sources of Aphanomyces resistance derived from selections in black root nurseries in the 1940's and 1950's as well as other sources, such as selections for resistance to *Cercospora beticola* leaf spot disease, resistance to *Rhizoctonia*

solani crown rot disease and for improved agronomic qualities.

RESULTS AND DISCUSSION

Variability - The mean DI of US H20 check variety, on which entry DI ratings were based, varied from 2.3 to 3.2 in the 11 tests (Table 1). In 10 of the tests there were significant differences among blocks in mean DI level. These differences are attributed to differences in infectivity among some of the 27 batches of inoculum employed and/or variability in environmental conditions among blocks, including temperature, humidity and soil moisture. Within blocks, variability among pots of US H20, as indicated by the coefficient of variability (standard deviation/general mean), ranged from 5.5 to 25.1 pct. These values fall within the normal range of coefficient of variability that we have consistently obtained in other sugarbeet pathology experiments in the greenhouse.

Differences among entries - The mean DIs of entries ranged from 55 to 161 pct of US H20 check (mean = 88.4 pct). In each test there were significant differences in DI among the entries (P=0.05) according to the LSD test. Almost a quarter of the entries (25.5 pct) were rated superior to US H20, 74 pct were rated intermediate and only 0.5 pct were rated inferior.

Table 2 shows a higher proportion of superior entries from among lines selected for Aphanomyces resistance in greenhouse tests than among entries from lines derived from other sources. The table also indicates a rise in the general level of Aphanomyces resistance among monogerm lines since the report of similiar tests conducted in 1957-1961 (3). At that time the mean DI of 1023 monogerm lines was 98.7 pct of check cultivars US 400 and US 401, which are somewhat less resistant than US H20, whereas in the present series of tests, the mean DI of 1574 monogerm lines was 87.0 pct of US H20.

There is no indication that the mean DI of all entries progressively decreased during the 1977-82 test period, inasmuch as concurrent selection for agronomic characters

Table 1. Summary of 1977-82 greenhouse screening tests of East Lansing sugarbeet breeding lines for resistance to *Aphanomyces cochlioides*.

Year and test no.	No. entries	DI[a] US H20[b] Mean	±S.D.	Entries in pct of US H20 Mean	(range)	Pct entries in each class[c] Superior	Intermediate	Inferior
1977-1	371	3.1	± 0.7	86.0	(55-129)	28.0	71.2	0.8
1978-1	281	3.0	± 0.6	91.6	(59-141)	11.4	87.5	1.1
1979-1	283	2.6	± 0.5	89.9	(58-122)	16.6	83.4	0
1979-2	73	3.1	± 0.4	83.3	(65-110)	20.5	79.5	0
1980-1	316	3.1	± 0.3	88.4	(64-110)	39.2	60.8	0
1980-2	69	3.2	± 0.4	91.0	(72-111)	10.1	89.9	0
1981-1	388	2.7	± 0.4	86.7	(65-120)	36.3	63.2	0.5
1981-2	103	2.6	± 0.4	86.2	(62-161)	35.9	62.1	1.9
1981-3	72	2.9	± 0.4	88.3	(68-130)	4.2	94.4	1.4
1982-1	206	2.3	± 0.2	92.1	(67-122)	21.4	77.7	0.9
1982-2	146	2.9	± 0.4	87.9	(64-112)	23.3	76.7	0
Total	2308	2.9	-	88.4	(55-161)	25.5	74.0	0.5

[a]Disese index (DI) = 0(no symptoms)-5(dead).
[b]Commercial cultivar included as standard for comparison.
[c]Evaluation classes included: Superior = DI in pct of US H20 significantly less than that of US H20 which = 100 (P=0.05); Intermediate = DI not significantly different from that of US H20; Inferior = DI Significantly greatly than that of USH20.

Table 2. Distribution of East Lansing sugarbeet breeding lines into disease resistance classes in 1977-1982 greenhouse screening tests of resistance to *Aphanomyces cochlioides*.

Breeding lines			Pct. entries in each class[a]			Mean DI[b]
Seed tube	Parental source	No.	Superior	Intermediate	Inferior	
Multigerm	Aphanomyces selections	176	48.3	51.7	0	81.7 A
Multigerm	Other[c]	558	12.2	86.4	1.4	94.4 C
Monogerm	Aphanomyces selections	162	54.9	45.1	0	82.0 A
Monogerm	Other[c]	1412	24.5	75.1	0.4	87.6 B

[a]Superior = DI in pct. of US H20 significantly less than that of US H20 (P=0.05); Intermediate = DI not significantly different from that of US H20; Inferior = DI significantly more that that of US H20.

[b]DI based on index from 90 (no symptoms) to 5 (dead) and expressed in pct. of US H20, which = 100. Values followed by same capitol letter indicate distributions that do not differ significantly (P=0.01) according to Chi-square test of independence.

[c]Other sources include selections for resistance to other diseases and for agronomic qualities.

and for resistance to other diseases tended to lower the level of Aphanomyces resistance. On the other hand, annual selection for Aphanomyces resistance and elimination from the breeding program of lines with DI's in the upper quartile helped maintain the DI mean at 86 pct of US H20, noted at the beginning of the test period. Moreover, the mean percentage of entries rated superior increased from 19.6 for the 1977-79 period to 30.0 for the 1980-82 period.

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