Reaction of Sugar Beet Strains to *Aphanomyces Cochlioides'*

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Several species of fungi including *Aphanomyces cochlioides* Drechs, are known to be the causal agents for the seedling diseases of sugar beets that growers commonly call "black root." General measures for control have been summarized by Coons and Kotila (2)[3]. The general root disease complex that starts in the seedling stage of the sugar beet plant and may persist throughout the life of the plant has been differentiated into two phases, first an acute phase in which the plants are killed as they grow out of the seed ball or within a week or two after emergence from the soil, and second a chronic phase affecting plants not damped off in which lateral rootlets and the terminal portion of the tap root are chiefly affected. *A. cochlioides*, although capable of causing damping off, is chiefly responsible for the chronic phase of black root—Kotila and Coons (5) ; Coons, Kotila, and Bockstahler (3) .

Observations as made in Michigan, Ohio. Wisconsin, and Minnesota in 1941-1945 by these investigators had indicated that the sugar beet variety, U. S. 216, showed definite resistance to the chronic phase of black root and that a hybrid, S. P. 1-9-00, was highly susceptible. Supporting observations were made by Torrie, Downie, and Culbertson (6) on the same material. On the basis of these findings various inbred lines and their hybrids, as available from the leaf spot resistance project of the Division, were supplied for tests in 1944 at. Holgate, Ohio (Kotila and Bockstahler). and at the South East Experiment Station of the Minnesota Agricultural Experiment Station, Waseca, Minn. (Culbertson). In these tests U. S. 216 and S. P. 1-9-00 were included as, respectively, resistant and susceptible checks. The extremely wet conditions in 1944 seriously influenced the stands in

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[3]Italic numbers in parentheses refer to literature cited.

the field plantings at Holgate, Ohio, and it seemed clear that other pathogens as well as *A. cochlioides* were concerned in the death of the seedlings. Differentiation among the varieties for resistance to *A. cochlioides* was not clear-cut. Similarly, at Waseca, Minn., very rainy weather following the planting period brought about an almost complete loss of stands. However, from such plants as survived under the severe conditions, selections were made by Culbertson[4] and brought to seed under conditions permitting open pollination.

In 1945, plans were developed by the Division of Sugar Plant Investigations for repetition of the test of inbred lines and their hybrids at Waseca, Minn., on the field where the previous planting had failed. One important, change was introduced in the test, namely, all seed was treated with a fungicide (Arasan) prior to planting since it had been concluded from the 1944 tests that the acute phase of the seedling diseases should be excluded so far as possible in order to limit the exposure to *A. cochlioides.*

Two series of investigations on the reaction of sugar beets to black root as caused by *A. cochlioides* were assigned in 1945 to the writers: (1) Field tests to determine the extent to which resistance already exists in sugar beet varieties and strains, and (2) greenhouse experiments to develop a methodology by which controlled exposures may be used in testing for resistance and susceptibility.

Materials and Methods

Field Experiments.- Tests were made at Waseca, Minn., of 216 open-pollinated seed progenies of mother beets that were selected by J. O. Culbertson in 1944 because they survived in heavily infested soil. These were compared with U. S. 216 and S. P. 1-9-00 in duplicate randomized 16-foot progeny rows in a field in which sugar beets had been eliminated completely by black root in 1944. As check varieties, U. S. 216, which had been reported to be resistant, and S. P. 1-9-00, which had appeared to be very susceptible, were planted in paired rows at 10-row intervals.

All seeds were treated with Arasan to protect against seed-borne pathogens and to reduce pro-emergence damping off. Data were taken on the percentage of post-emergence survival, relative vigor of top growth, and vigor of roots.

The mean survival percentage for each progeny was calculated after obtaining data on the initial and final stand of a measured, non-thinned section of each row. The mean percentage of survival for the two check varieties was determined in a similar manner, using data from the 62 paired check rows. The percentage data were trans-

[4]Dr. J. O. Culbertson transferred on February 15, 1945, to Division of Cereal Crops and Diseases. Breeding materials as obtained in his investigations were made available by him for this work.

formed to degrees by means of tables prepared by Bliss (1) and reprinted by Hayes and Immer (4).

Progeny rows were grouped into three classes on the basis of the average vigor of top growth in comparison with U. S. 216 in the two nearest check rows. The class designations were as follows: Class 1, growth exceeding that of U. S. 216; class 2, growth equalling; and class 3, growth inferior. These numerical ratings of the two rows of each progeny were averaged to obtain a mean top growth vigor index for each progeny.

A similar system of classification was used in comparing the vigor of the roots of each progeny row with that in the two nearest check rows of U. S. 216. These ratings were made only on the 59 progenies that exceeded U. S. 216 in vigor of top growth and in percentage of post-emergence survival.

Twelve inbred lines, 10 hybrids. 1 synthetic variety, and 1 commercial variety were compared at Waseca in an adjoining plot. These were tested also in soil that was only slightly infested with *A. cochlioides* at University Farm. St. Paul. These lests were made in eight randomized blocks in 33-foot single-row plots at Waseca and in 20-foot single-row plots at University Farm. Data were recorded on the yield of roots at Waseca and University Farm and on vigor of tops at W[T]aseca only. In summarizing the root yield data in these trials at Waseca and University Farm, the inbred lines were classified as resistant or susceptible on the basis of their performance in these and previous unpublished studies.

In addition to the tests of progenies from selected roots and the tests of inbreds and their hybrids just described, 11 sugar beet varieties were grown in 4-row plots, 132 feet in length, on heavily infested soil in order that selections could be made from the most resistant sorts. U. S. 216 and S. P. 1-9-00 were planted at intervals throughout the field as check varieties. A mass-selected variety designated as 1942 Minnesota Synthetic No. 1 was included. This variety was developed by J. O. Culbertson in cooperation with the Minnesota Agricultural Experiment Station. It is the result of repeated mass selection of roots for high root yield and high sucrose percentage. The selections w^ere made at Waseca, Minn., from varieties grown in leaf-spot-resistance evaluation tests and from plantings of genetic material. The plots were located on soils infested with *A. cochlioides.* Freedom from leaf spot and root rot was considered in making the selections.

Greenhouse Experiments.—-Five strains of sugar beets that had been tested under extremely severe natural infestations in the field at Waseca were grown in the greenhouse in both naturally infested and artificially infested soils. The objectives of this experiment were

two-fold: (1) To develop methods of testing for resistance under controlled conditions and (2) to learn whether the reaction of strains under field conditions was similar to their reaction under greenhouse conditions in soils in which the only pathogen present was *A. cochlioides.*

Soil from the field trials at Waseca and a standard greenhouse mixture were used in these experiments. The five strains were grown in untreated naturally infested Waseca soil, in steamed Waseca soil, in steamed greenhouse soil, in steamed Waseca soil to which an inoculum consisting of a pure culture of *A. cochlioides* had been added, and in steamed greenhouse soil plus inoculum. The inoculum was grown in potato dextrose broth and after maceration was added uniformly to the soil.

Pertaining to their resistance to *A. cochlioides,* the five strains may be characterized as follows:

S. P. 1-9-00	Very susceptible to *A. cochlioides* under field conditions.
U. S. 216	Moderately resistant under field conditions.
Improved U.S. 215 x 216	U.S.D.A. variety resistant tio leaf spot. It has about the same degree of resistance to *A. cochlioides* as U. S. 216, one of its parents.
Commercial No. 3	A locally adapted variety that showed resistance in 1945 field trials at Waseca.
Progeny No. 59	An open-pollinated progeny of a selected mother beet. The progeny was superior to U. S. 216 in percentage survival and vigor of roots in 1945 trials at Waseca under severe natural infestation with *A. cochlioides.*

Seeds were treated with Arasan and germinated in clean quartz sand. With the exception of Progeny No. 59 thirty healthy seedlings of each strain were transplanted in each plot of each soil treatment. Because of scarcity of seed some of the plots of Progeny No. 59 were limited to 15 seedlings. A split-plot type of experimental design was used. The soil treatments were the main plots and were replicated five times with the strains arranged at random in each soil treatment. Each main plot consisted of two greenhouse flats placed end to end. Data were collected on the average percentage survival of the seedlings of each strain and on the vigor of the seedlings in each soil treatment.

The percentage data were transformed according to Bliss's method before applying the analysis of variance. Each of the five soil treatments were analyzed as separate randomized block experiments.

Each seedling was given a vigor rating based on a series of 10 plants used as standards, ranging from dead to exceptionally vigorous. The percentage of the seedlings of each strain which fell in each vigor class in each soil treatment was determined.

Experimental Results

Field Experiments.—The mean post-emergence survival percentages for U. S. 216 and S.P. 1-9-00, as determined from the 62 paired check rows, were 21.8 and 8.7, respectively. According to the analysis of the transformed percentage data, these two varieties differ significantly in their rates of survival. Of the 216 progenies, 152 had a higher survival rate than U.S. 216. Three of them had lower survival percentages than S. P. 1-9-00.

When the progenies were compared with U. S. 216 in regard to vigor of top growth the following results were obtained:

Classification	Number of progenies
Superior to U. S. 216	70
Equal to U. S. 216	61
inferior to U. S. 216	85
Total	216

Of the 70 that were superior in top growth, 11 were inferior in percentage of post-emergence survival and were not used in further studies. The roots of the 59 progenies with superior top growth and percentage of survival were lifted and compared with roots from the adjacent U. S. 216 check rows. On the basis of their average vigor in both replicates, the following results were obtained:

Classification	Number of progenies
Superior to U. S. 216	50
Equal to U. S. 216	6
Inferior to U. S. 216	3
Total	59

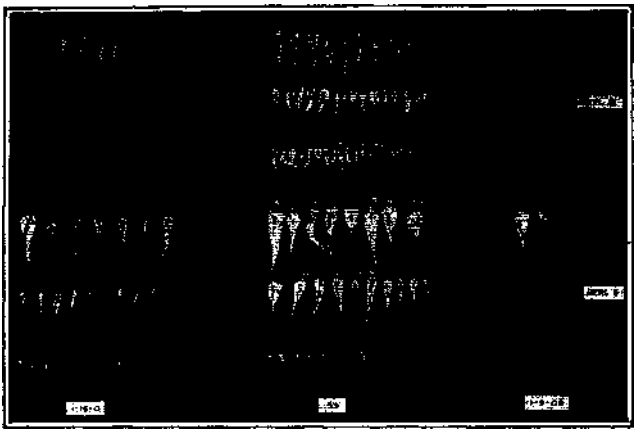

Figure 1.—Roots of three strains of sugar beets grown in non-thinned rows in soil with a heavy natural infestation of Aphanomyces cochlioides. Left to right: U.S. 216, Progeny No.59, and S.P. 1-9-00.

There were striking differences in the vigor of the roots of the various progenies. Figure 1 shows the roots from Progeny No. 59, which was one of the superior progenies, in comparison with roots from adjacent rows of the two check varieties.

In the test of 24 inbred lines, hybrids, synthetics, and commercial varieties at Waseca in heavily infested soil some strains were almost completely eliminated, while others maintained good stands and produced quite vigorous roots despite the extremely adverse conditions. A fairly close relationship between the vigor of top growth and the yield of roots was also observed in this test. This relationship is of interest because it permitted the discarding of a high percentage of the seed progenies without resorting to lifting and examining roots. The data on the vigor of the top growth of the strains in comparison with U. S. 216 may be summarized as follows:

Classification	Number of strains
Superior to U. S. 216	2
Equal to U. S. 216	10
Inferior to U. S. 216	11
Total	23

One of the two strains that had mean vigor ratings which were significantly superior to that of U. S. 216 was unrelated to U. S. 216. S.P. 1-9-00 and U. S. 200 were among those with significantly poorer mean vigor ratings.

On the basis of their performance in 1945 at Waseca in heavily infested soil, at University Farm in lightly infested soil, or in previous studies, there were among these 24 strains 8 susceptible inbreds, 4 resistant inbreds, 4 crosses between susceptible inbreds, 5 crosses of susceptible x resistant inbreds, and 1 cross between resistant inbreds in addition to the commercial variety and the mass-selected synthetic variety designated as Minnesota Synthetic No. 1. The root yields of the 24 strains, summarized according to this classification, are presented in table 1.

Table 1.- Summary of root yield data from tests of 24 sugar beet strains on heavily infested soils at Waseca, Mimi., and on lightly infested soils at University Farm, St. Paul, Minn., in 1945.

Type of material	Number of strains in each group	Root yield in tons per acre			
		Waseca		University Farm	
		Range	Mean	Range	Mean
Susceptible inbreds	8	0.4-2.3	1.4	6.7- 9.7	8.5
Resistant inbreds	4	3.0-5.0	4.1	8.1-12.1	10.5
Susceptible x susceptible inbreds	4	1.2-3.9	2.2	9.6-10.5	10.1
Susceptible x resistant inbreds	5	2.0-5.9	3.9	10.9-12.9	12.0
Resistant x resistant inbreds	1	---- - ----	5.5	---- - ----	12.3
Mass selected synthetic variety	1	---- - ----	4.8	---- - ----	13.1
Commercial locally adapted variety	1	---- - ----	4.2	---- - ----	12.1

Analysis of variance of the data from the tests at Waseca and University Farm showed that in both experiments varietal differences exceeding that required for statistical significance at the 1 percent level of significance were present. It is apparent that the yield of the resistant inbreds is significantly greater than that of the susceptible inbreds and that certain of the hybrids were considerably superior to others.

Figure 2 shows a four-row plot of Minnesota Synthetic No. 1 in comparison with a commercial variety and S.P. 1-9-00. Tests of this mass-selected variety and of seed progenies of selections from it indicate that it contains a relatively high percentage of individuals with resistance equal to or higher than that of U. S. 216. Two synthetic varieties that included among other components selections from leaf-spot-resistant strains obtained from Italy had many plants in the four-row plots that appeared to be more resistant than U. S. 216. Hybrids between U. S. 216 and miscellaneous inbreds exhibited a degree of resistance equal to that of IT. S. 216. S.P. 1-9-00 was very susceptible in this experimental planting.

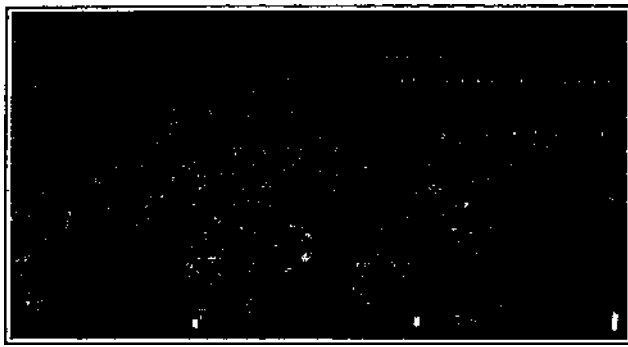

Figure 2.—Comparative effect of black root on stand and top growth of three varieties of sugar beets. Left to right: Commercial No. 1, Minnesota Synthetic No. 1, and S.P. 1-9-00.

Greenhouse Experiments.—The average percentage survival of the five strains in each of the five soil treatments as contrasted with the average percentage survival of three of the strains in the field at Waseca are tabulated in table 2.

The tests in the naturally and artificially infested Waseca soils and the artificially infested greenhouse soil showed statistically significant differences in the percentage of survival of the strains although the two steam-sterilized soils gave no significant differences.

S.P. 1-9-00 was considerably inferior to the other four strains in both of the artificially infested soils and was significantly lower than

Table 2.—Average percentage survival of seedlings of five sugar beet strains grown in the greenhouse and of three strains grown in the field.

Strain No.	Percentage survival of seedlings					
	Test in field at Waseca, Minn.	Test conducted in greenhouse				
		Waseca soil			Greenhouse soil	
		Untreated	Steamed	Steamed plus inoculum	Steamed	Steamed plus inoculum
S. P. No. 1-9-00	9	71	83	11	83	39
U. S. 216	22	75	81	36	75	61
Imp. 215 x 216	....	72	83	28	80	66
Commercial	....	70	90	18	89	53
Progeny No. 59	52	89	87	20	72	67

Progeny No. 59 in the naturally infested soil in the field and in the greenhouse. It is of some interest that the commercial variety was. in general, somewhat inferior to Progeny No. 59. U.S. 216, and Improved U.S. 215 x 216. This was most noticeable in the two soils that were steam-sterilised before adding inoculum.

Seedlings of Progeny No. 59 had the highest average vigor index in the naturallv and artificially infested Waseca soils and had the highest percentage of seedlings with above-average vigor in all three soils in which *A. cochlioides* was present. Seedlings of U.S. 216 and Improved U. S. 215 x 216 in the infested soils consistently had a higher average vigor index than seedlings of S.P. 1-9-00, which was of equal or higher vigor in the steam-sterilized soils and of lowest vigor in the infested soils.

Summary

Aphanomyces cochlioides, one of the pathogens responsible for black root of sugar beets, causes serious yield reductions and abandonment of large acreages of sugar beets in the North Central States.

Field and greenhouse experiments made in 1945 in Minnesota show that the sugar beet varieties U. S. 216 and S.P. 1-9-00 differ significantly in their resistance to *A. cochlioides.*

A number of inbred lines of sugar beets and crosses between them were studied in replicaled field experiments in heavily infested soils and in lightly infested soils. In general when one or both of the parent inbreds were resistant the hybrids significantly outyielded hybrids between two susceptible inbreds.

In preliminary greenhouse experiments the reaction to *A. cochlioides* of three sugar beet strains corresponded closely to the field determinations for these strains.

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