Toothpick Method of Inoculating Sugar Beets for Determining Pathogenicity of Rhizoctonia solani[1]

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Uniform infection in the field of underground parts of plants by soil-borne organisms is frequently mitigated by many factors including an apparent heterogeneous infestation of the soil by the pathogens. Thus it is difficult to predict the presence or absence of an organism such as *Rhizoctonia solani* Kuhn (*Pellicularia filamentosa* (Pat.) Rogers), an economically important crown rot causal organism of sugar beets in the irrigated fields of western Nebraska and other areas.

Attempts to provide a natural uniform soil infestation by sugar beet monoculture at the Scotts Bluff Experiment Station, Mitchell, Nebraska, did not result in an "epidemic" by the soil-borne organism but rather a variability in amount of infection of the plants in this plot from season to season (1)[3]. Greenhouse experiments to determine the crown rot potentialities of isolates of *R. solani* are affected by the comparatively slow growth of the sugar beet plant and the difficulty in obtaining crown rot symptoms by soil infestation prior to planting as a means for inoculation. Our work in 1953 and that of others (3) has shown that the induction of the seedling disease phase is not a criterion to determine crown-rot-inducing potentialities of *R. solani* isolates. This paper includes attempts to correct the difficulties in testing the sugar beet reaction to the fungus isolates and to ensure a positive inoculation of each individual root by employment of a toothpick inoculation technique.

Experimental Procedures

A modification of a toothpick method of inoculation first employed by Young (4) for ear and stalk rots of corn and by Gaskill (2) for storage-rot of sugar beets was used in these experiments. The fungus cultures were grown on two percent potato dextrose agar and quill-type, wooden toothpicks were added to the Petri dishes containing the medium. The entire preparation was accomplished about one month prior to inoculation to ensure adequate contamination of the toothpicks.

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At time of inoculation the sugar beets were three to four months of age. Sugar beets showing any indication of a disease were removed prior to inoculation. Each isolate was tested in duplicate with six plants per replicate and on two dates of inoculation. The toothpicks were inserted diagonally downward into the exposed basal portion of the crown of the sugar beet to a depth of approximately one and one-half inches. In the 1955 and 1956 field trials and 1953 greenhouse tests the toothpicks were forcibly inserted into the crown with the aid of pliers. In 1957 the contaminated tips of the toothpicks were inserted into holes punctured with a 6-penny nail the diameter of which was slightly less than the widest diameter of the toothpick. The insertions were made on the north side of the beets to decrease possible desiccation by the sun's rays. One toothpick per sugar beet was used and was left intact until the final disease reading.

Comparative tests were also conducted in 1957 by toothpick inoculation of the top center of the crowns, bases of leaf petioles, and by hypodermic injections of the crown with water suspensions of the fungus. Each hypodermic injection consisted of one-half milliliter of suspension made by comminuting in a Waring blender the fungus growth from two Petri dishes in one liter of sterilized distilled water. Tests performed on a slide showed that mycelial fragments of the fungus readily passed through the hypodermic needle.

The effects of four isolates on sugar beet seedlings were accomplished by incorporating the fungi in autoclaved soil. Preparatory to this the fungus growth on two percent potato dextrose agar was comminuted in a Waring blender; growth on two Petri dishes sufficed for four replications of each of four isolates. Ten segmented sugar beet seeds were sown in each pot.

The field tests were conducted at the Scotts Bluff Experiment Station, Mitchell, Nebraska. The sugar beet variety, GW304R, was used in the greenhouse tests and the field tests in 1955 and 1956. Varieties GW526 and GW359R were used in the 1953 greenhouse and 1957 field tests as test plants, respectively.

Twenty-four cultures of *R. solani* were tested for their parasitism for sugar beets. These cultures were selected from many isolates originally obtained from diseased sugar beet crowns and roots, potato stems, bean roots, pigweed roots, and one culture from plant residues in soil cropped to sugar beets the previous season.

Experimental Results

In the 1953 preliminary experiments in the greenhouse, two cultures were employed: Culture 1 and Culture 15. The former caused a high percentage of seedling kill while the latter appeared innocuous. None of the 24 sugar beet crowns of the very susceptible variety, GW526, inoculated with either of the two cultures became diseased. A six month period after inoculation of three-month old beets provided ample time for disease development and final disease readings. In this test it was assumed that conditions in the greenhouse did not favor disease development. In reality, as was shown later, the lack of pathogenicity of the isolates was involved. In fact, in the 1957 greenhouse test, Culture 1 again proved nonpathogenic to crowns of older beets, whereas cultures that induced crown or root rot in the field tests behaved similarly in greenhouse tests (Tables 1 and 2).

Table 1.—Percentage of Sugar Beets Infected by *Rhizoctonia solani* Isolates from Western Nebraska Using the Toothpick Method of Inoculation in Field Tests at the Scotts Bluff Experiment Station, Mitchell, Nebraska, 1955 to 1957.

Culture No.	Source of Culture	Plants	
		Number Inoculated	Percent Infected
0	control	288	4
1	potato stem	96	4
2	sugar beet crown	96	98
3	sugar beet crown	96	88
4	sugar beet lateral root	96	21
5	sugar beet root tip[a]	48	63
6	sugar beet root tip	48	31
7	sugar beet crown	24	75
8	sugar beet root tip	24	100
9	sugar beet crown	24	100
10	sugar beet crown	24	8
11	sugar beet crown	24	25
12	sugar beet crown	24	41
13	sugar beet crown	24	0
14	sugar beet crown	24	100
15	sugar beet crown	24	0
16	sugar beet crown	24	8
17	sugar beet lateral root	24	8
18	sugar beet crown	24	58
19	sugar beet crown	24	100
20	sugar beet root tip	24	0
21	sugar beet lateral root	24	100
22	pigweed root	24	38
23	field bean root	24	0
24	soil plant residue	24	17

[a] Refers to tip of tap root for cultures 5, 6, 8, 20.

Table 2.—Percentage of Sugar Beets Infected by *Rhizoctonia solani* Isolates Using the Toothpick Inoculation Method in Greenhouse Tests in 1957.

Culure No.[a]	Source of Culture	Plants	
		Number Inoculated	Percent Infected
0	control	10	0
1	potato stem	10	0
2	sugar beet crown	10	100
6	sugar beet root tip	10	40
7	sugar beet crown	10	100
8	sugar beet root tip	10	100
15	sugar beet crown	10	0

[a] Culture numbers correspond to those in Table 1.

Figure 1.—Sugar beet roots infected by *Rhizoctonia solani* following the toothpick method of inoculation. New toothpicks were inserted to obtain better contrast.

Although plants in many instances were beyond recovery two or three weeks subsequent to inoculation in the field tests in 1955-1957, the final disease readings awaited harvest time. Not all isolates of *R. solani* from diseased sugar beets proved pathogenic for sugar beets by the toothpick method of inoculation (Table 1). Of twenty such isolates (Cultures 2 to 21, inclusive) only nine (Cultures 2, 3, 5, 7, 8, 9, 14, 19, 21) appear definitely pathogenic (Figure 1). Four isolates (Cultures 4, 6, 11, 12) are of intermediate parasitism but possess possible virulence as in-

dicated by infection of 21, 31, 25, and 41 percent of the plants, respectively. Culture 18 may be included in the list of intermediate pathogenicity, but because of its ability to induce rotting of about 50 percent of the inoculated roots, it is possible that inadequate contamination of the toothpicks or some other undetermined variable factor may be instrumental in allaying infection. Upon repeated testing of these four cultures, a more precise cataloging of their parasitic capabilities can be made. The remainder of the sugar beet isolates (Cultures 10, 13, 15, 16, 17, 20) are ascribed to the nonpathogenic category.

A high incidence of crown rot was produced by Culture 2. Over a three-year testing period this culture induced rotting in 98 percent of the beets tested. Culture 2 can be depended upon to cause the disease by the inoculation method described. It may be considered a standard and reliable strain in determining plant reaction. In contrast, a potato isolate (Culture 1) proved consistently innocuous and thus could be considered a good comparative strain. Both of these cultures upon reisolation gave the same results as the original culture that was introduced into the plant. Culture 2, isolated in 1953, and Culture 1, isolated in 1950 retained their pathogenicity or nonpathogenicity for sugar beets for at least four and seven years, respectively. Seven additional isolates used in two to four experiments gave consistent results, making a total of nine cultures that were reliable in successive tests and displaying similar degrees of virulence.

Inoculation of the top center of the crowns and of petiole bases with Culture 2 resulted in crown rot infections in both instances. Inoculation of the crown by the use of a hypodermic needle with the virulent culture did not induce any appreciable decay indicating that desiccation of the wounded area may have been involved in lack or prevention of fungus development.

Inasmuch as *R. solani* may be pathogenic to any part of the sugar beet under natural conditions, root isolates of this organism from different areas were tested for parasitism for the crown area. Isolates from rotted crown of sugar beets did not consistently differ in capabilities to induce crown rot from those obtained from the tip of the tap roots or from lateral roots (Table 1). For example, Cultures 5, 8, and 21 were as virulent in general as isolates from diseased crowns. In other words isolates from lateral or tap roots were not necessarily restricted in their parasitism to these areas but were capable of infecting the crown as well. The cultures isolated from the three areas of the root may belong to any one of three categories: pathogenic, nonpathogenic, or of intermediate parasitism.

Table 3.—Percentage of Sugar Beet Seedlings Infected by *Rhizoctonia solani* Cultures in Greenhouse Tests in 1957.

Culture No.[a]	Percent Compared to Control		
	Pre-emergence	Post-emergence	Total
0 (control)	0	0	0
2	43	34	77
13	99	0	99
17	44	1	45
24	22	8	30

[a] Culture numbers correspond to those in Tables 1 and 2.

Studies to determine the comparative effects of cultures on seedlings and older plants indicated that cultures capable of causing seedling infection or damping-off do not necessarily induce crown rot (Table 1 and 3). Culture 13 can be destructive to seedlings but innocuous when inoculated into the crown. Culture 2 is pathogenic to seedlings and to older sugar beets. Cultures 24 and 17 are relegated to the intermediate parasitic class.

Cross inoculation tests of *R. solani* isolates from other crops grown in western Nebraska included those from potato (Culture 1), field bean (Culture 23), and pigweed (Culture 22). They did not induce crown rot, although the pigweed isolate may be pathogenic. Another isolate (Culture 24) made directly from plant residue in the soil cropped to sugar beets the year prior to isolation also did not exhibit parasitism. The number of isolates from crops other than sugar beets were too few in number to arrive at any definite conclusions with respect to their pathogenicity for sugar beets. In a preliminary manner cross inoculations of sugar beet cultures into other crops have been started and will be continued using the toothpick inoculation procedure. Culture 2 inoculated into field corn stalks under field conditions induced some decay. The testing of isolates from other crops may indicate the possible relationship of crop rotation effects and incidence of crown rot of sugar beets.

Discussion and Summary

The procedure of employing toothpicks contaminated with mycelia and sclerotia of *Rhizoctonia solani* appears promising from several standpoints in view of experimentation over a three-year period. A fairly constant amount of inoculum is brought into intimate contact with the host tissue and possible contami-

nation by secondary organisms is greatly reduced. Dessication of host and fungus is diminished because of the shape of the inserted toothpick. There is a uniformity of wounding associated with the inoculation procedure which may not be true in some other methods. By using this technique, the time of inoculation is known and the progress of the disease can be closely scrutinized. The possibilities of disease escapes are reduced. In event certain plants in a variety indicate differences in their reaction, individual roots can be reinoculated in the field or in storage to confirm the first reading. Thus, disease escapes can be found and eliminated.

Field testing was resorted to because greenhouse tests did not prove adequate owing to the poor growth of the sugar beet and slow development of symptoms. In field tests killing of plants occurred in a few weeks, whereas in the greenhouse evidence of disease did not appear for months.

Pathogenicity of the fungus isolates can be determined by obtaining crown or root infection of older roots of sugar beets. Field tests, which were mainly employed in this work, have shown that pathogenic isolates can be relied upon to retain their pathogenic qualities and produce severe rotting of the crown, root, or complete destruction of the plant. This was particularly exemplified by cultures that have been maintained for four to seven years on artificial media. Similarly, nonpathogenic cultures of *R. solani* retained this innocuous characteristic for as long a period. A group of isolates intermediate in their pathogenicity produced infection in about one-fourth to one-third of the plants inoculated. Thus reliable cultures with different parasitic capabilities have been found to retain their characteristics in successive tests.

The toothpick inoculation method appears promising in determining the reaction of varieties and species of *Beta* and of other crops that may be used in cross inoculation tests. Work of this nature is in progress in the university's greenhouse at the present time. By the toothpick method the fungus is introduced into the plant in an unnatural manner and the resulting tests may not be a true measure of the resistance for it may be too drastic a test. It may be necessary to correlate other techniques to determine the final sugar beet reaction. Regardless of the inoculation method employed it will be artificial in nature either because of the degree of wounding, number of strains used, or the amount of inoculum applied.

The toothpick method may be employed to test weed isolates onto beets and other crops and vice versa. In one field test, a

culture from sugar beets caused some necrosis of corn stalks. Information on the host range of cultures that induce crown rot of sugar beets may suggest the best crop rotations and explain the value of weed control in controlling the root diseases.

Determination of varietal reaction to *R. solani* based on the seedling stage does not truly depict the reaction of more mature beets to the serious crown rot phase that occurs most frequently late in the season. It also complicates the attempts at control of this phase through breeding by the use of seedling reaction as a criterion to judge crown rot of mature beets.

Although the test procedure possesses possibilities, the existence of strains differing in pathogenicity confounds the problem. The use of one or two isolates in determining the reaction of a variety to *R. solani* are fraught with uncertainties. Strains existant in western Nebraska differ in their virulence for sugar beets. A single isolate such as Culture 2 may or may not be relied upon to be a standard in determination of the host reaction. Although Culture 2 appeared pathogenic to three sugar beet varieties, which differ somewhat in their degree of susceptibility on the basis of field tests (1), the experiences with other disease-producing organisms and hosts should serve as a warning against such a simple solution.

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