Correlation of Cultural Characters and Source of Isolates with Pathogenicity of Rhizoctonia solani from Sugar Beet

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ABSTRACT

A crown and two foliar isolates of *Rhizoctonia solani* from sugar beet exhibited significantly greater growth rate in culture than did six root isolates. Root isolates were assignable to anastomosis group 2, whereas the crown and foliar isolates were associated with group 4.

In pathogenicity studies, all isolates incited significant damping-off; crown and foliar isolates caused significantly more foliar blight than did root isolates, with the crown isolate being intermediate; and root isolates caused more severe root rot than did the crown or foliar isolates. Disease reactions were significantly more severe in a susceptible cultivar (GW 674-56C) as compared with a resistant selection (FC 701/2) from GW 674-56C in damping-off and root rot tests. Root isolates X lines interactions were nonsignificant, which indicated that resistance of FC 701/2 is effective against several diverse isolates of the pathogen.

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Sugar beet (*Beta vulgaris* L.) can be affected by damping-off, root rot, crown rot, or foliar blight incited by *Rhizoctonia solani* Kuehn (*Thanatephorus cucumeris* [Frank] Donk). Physiological, morphological, and pathological variation among isolates implicated in these disease syndromes is well documented (1, 5, 6). However, the practical importance of existing races of the fungus in sugar beet could not be assessed previously because sugar beet lines with varying degrees of resistance to highly pathogenic races were not known.

Substantial gains in resistance of sugar beet to Rhizoctonia root rot have been made (2, 3); however, most selections were based on the responses of lines to one isolate of the pathogen. The present research was conducted to further explore physiological specialization among isolates of *R. solani* from sugar beet, and to study the interaction of diverse isolates with sugar beet lines having various degrees of resistance.

MATERIALS AND METHODS.—Isolates of *R. solani* used in this study are designated as inciting root rot (RR), foliar blight (FR), or crown rot (CR). Seven isolates, RR-1, -2, -3, -4, -9, CR-5, and FR-6, were obtained from diseased beets grown in widely scattered areas of eastern Colorado, and isolates FR-7 and RR-8 were from diseased beets grown near Willcox, Ariz. Isolates were representative of *R. solani* as typified by species criteria outlined by Parmeter et al. (7). Isolate RR-9 has been used for initiating epidemics of root rot in breeding nurseries at Fort Collins, Colo. for several years. Cultures were maintained in test tubes on potato-dextrose agar (PDA) or on whole barley grain. Sugar beet cultivar GW 674-56C, and FC 701/2, a Rhizoctonia-resistant selection from GW 674-56C, were used in all pathogenicity tests.

Cultural characteristics.—Rate of growth at 24 C was calculated from the average radial growth which occurred between 2 and 3 days after plating on PDA containing 1 g yeast extract/liter (PDA-YE). Colonies were initiated with 4-mm-diam discs from margins of 1-week-old cultures also grown on PDA-YE. A completely randomized design with 13 replications was used.

Cultural characteristics were recorded 14 days after plating on PDA-YE and oatmeal agar (OA). The latter medium provided more precise differentiation among all isolates, whereas differentiation between root isolates and crown-foliar isolates was more distinct on PDA-YE.

Anastomosis groupings were determined by the method of Parmeter et al. (7). Each sugar beet isolate was opposed with representative cultures of the four known anastomosis groups (supplied by R. T. Sherwood, USDA, ARS, North Carolina State University, Raleigh). Each isolate pair was tested on cellophane (2 cm^2) resting on 2% water agar in 9-cm petri dishes (one pair per dish). Mycelial transfers from the margins of 1-week-old cultures on PDA-YE were plated 3 cm apart in each dish. The dishes were incubated at 24 to 26 C until advancing hyphae slightly overlapped. Hyphal and cytoplasmic fusions were determined by microscopic examination. Three trials were conducted.

Pathogenicity.—1) *Damping-off.*—The nine isolates were tested on two sugar beet cultivars arranged as a randomized block design with six replications. The isolates were grown in 1-liter flasks containing a sand-oatmeal medium (200 g white silica sand, 20 g

ground oatmeal, 50 ml water) for 3 weeks at 24 C. Before inoculation, each culture was triturated and mixed with a spatula to assure uniformity of the inoculum. Plastic pots (3 inch sq) were filled with steam-sterilized soil to within 2.5 cm of the rim. Ten surface-disinfested seedballs of sugar beet were distributed over the soil surface of each pot and covered with about 4 mm of inoculum. Another 1 cm of sterilized soil was added to the surface of each pot. The soil in the pots was irrigated immediately, and thereafter as needed. Noninoculated controls of each sugar beet line were included in each block. Seedballs in these pots were covered with sterile sand-oatmeal medium. Greenhouse temperatures ranged from 25 to 30 C during the experiment. Seedling survival was recorded 21 days after planting, and percentage damping-off was calculated.

2) *Foliar inoculations.*—Each isolate was tested on two sugar beet cultivars arranged as a randomized block design with five replications. Inoculum was prepared by comminuting the mycelial mat from one 1-week-old potato-dextrose-broth culture in 100 ml sterile distilled water in a blender for 30 sec. Two-month-old plants of each sugar beet line grown in individual 4-inch clay pots of sterilized soil were inoculated. Inoculum was atomized onto the foliage at 10 psi; then the plants were placed in a humidity chamber at 100% relative humidity and 27 to 32 C for 48 hr before being placed on the greenhouse bench. Greenhouse temperatures ranged from 25 to 30 C. Supplemental fluorescent light was used at night. Noninoculated plants of each line served as controls. Disease ratings of 0 to 3, in increasing quantity of foliar lesions, were made 72 hr after inoculation when abundant leaf lesions were evident on plants inoculated with the foliar isolates.

3) *Root rot.*—Inoculum for root (via soil) and crown inoculations was grown in sand-oatmeal medium and prepared as described for the damping-off study. Four-month-old sugar beet plants of each line grown in individual 6-inch clay pots of sterilized soil were inoculated. Approximately 5 cc of inoculum was applied either to the crown of the plant or buried 2 cm deep in the soil and 2.5 cm from the tap root. The crowns of all plants were sprayed with a fine mist of water 3 times each day for 5 days to prevent excessive drying of exposed inoculum. Noninoculated controls were included. A randomized block design with three replications was used to evaluate the pathogenicity of each isolate for each method of inoculation. Each isolate was tested on two lines of sugar beet in both methods of inoculation. Roots were removed from pots 60 days after inoculation, washed, and rated 0 to 5 in ascending order of root rot severity. Ratings were based on the relative amount of rotted root tissue, with 0 = healthy and 5 = 100% rot.

RESULTS.—*Cultural characteristics.*—Growth rates of some isolates were significantly different (Table 1). The foliar cultures grew faster than all other isolates, whereas the crown culture was intermediate in growth between the foliar and root isolates. Root isolates RR-1, -2, -3, and -4 grew faster than did RR-9. Cultural characteristics differed appreciably among the isolates on PDA-YE and OA after 14 days' growth (Fig. 1-A, B). Root isolates were distinguishable from the crown and foliar isolates on PDA-YE, whereas each isolate exhibited unique characteristics on OA. All root isolates were in anastomosis group 2, whereas the crown isolate and foliar isolates were in group 4 (7).

Pathogenicity.—1) *Damping-off.*—All isolates induced significant damping-off as compared with control seedlings. The difference between lines was

TABLE 1. Growth rate in culture and pathogenicity on sugar beet of nine isolates of *Rhizoctonia solani* isolated from sugar beet

Isolate	Source	Growth[a, b] in cm/day	Damping-off[a, c] %	Disease severity on	
				Foliage[a, d]	Roots[a, e]
RR-1	Root	0.81 D	76.3 C	0.1 C	3.0 A
RR-2	Root	0.81 D	78.3 ABC	0.0 C	1.9 B
RR-3	Root	0.91 C	81.3 AB	0.0 C	2.7 AB
RR-4	Root	0.94 C	79.4 ABC	0.0 C	2.1 B
CR-5	Crown	1.19 B	89.1 A	0.9 B	0.3 C
FR-6	Foliage	1.48 A	79.4 ABC	2.8 A	0.3 C
FR-7	Foliage	1.52 A	87.5 AB	2.7 A	0.4 C
RR-8	Root	0.78 DE	82.4 AB	0.0 C	2.9 A
RR-9	Root	0.76 E	65.6 C	0.1 C	2.3 AB

[a] Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

[b] Growth rate on potato-dextrose agar, containing 1 g yeast extract/liter, calculated 48 to 72 hr after plating; means of 13 replications.

[c] Data based on percentage survival of control seedlings growing in sterilized soil; means of six replications X two sugar beet lines.

[d] Disease ratings of 0 to 3 in increasing number of leaf lesions; means of five replications X two sugar beet lines.

[e] Disease ratings of 0 to 5 with 0 = healthy and 5 = 100% root tissue rotted; means of six replications X two sugar beet lines.

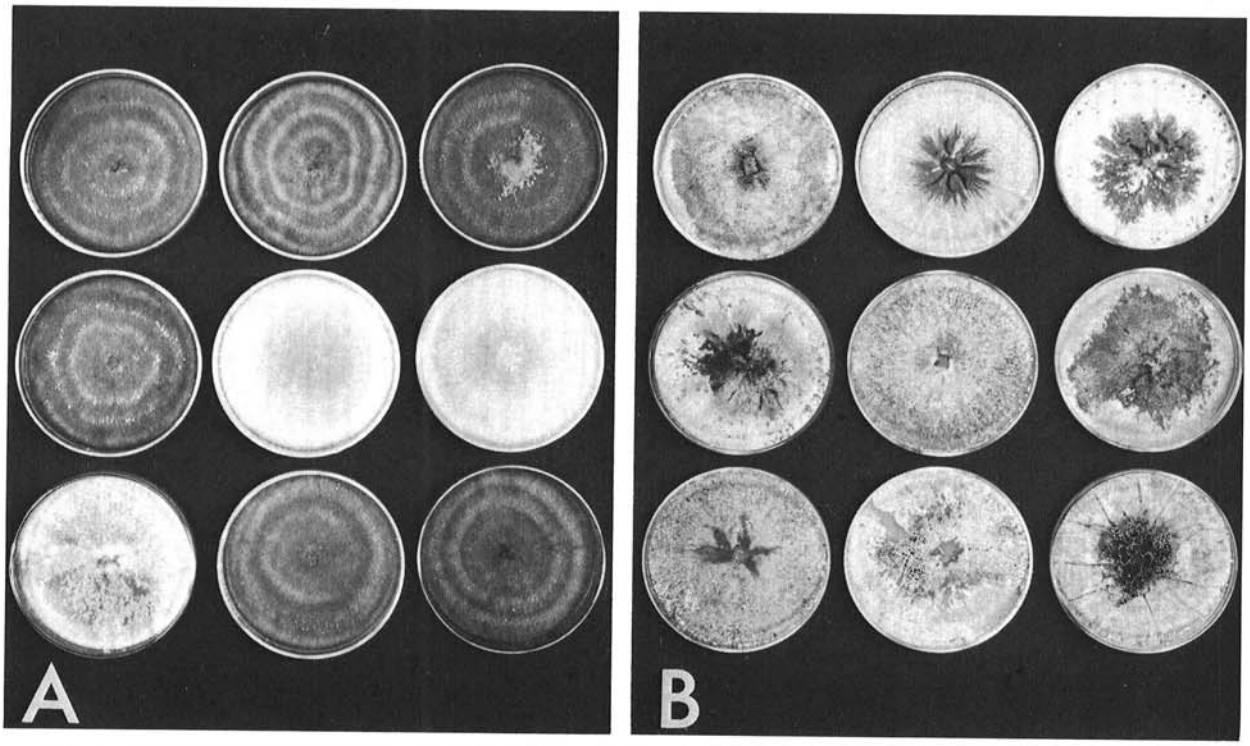

Fig. 1. Cultural appearance of nine 14-day-old isolates of *Rhizoctonia solani* grown at 24 C on **A)** potato dextrose-yeast extract agar; and **B)** oatmeal agar. Left to right, top, RR-1, -2, -3; middle, RR-4, CR-5, FR-6; bottom, FR-7, RR-8, -9. RR = root rot isolate; CR = crown rot isolate; FR = foliar blight isolate.

highly significant, with more damping-off occurring in GW 674-56C (mean = 89%) than in FC 701/2 (mean = 70%). Isolates RR-1 and RR-9 induced significantly less damping-off than RR-3, CR-5, FR-7, and RR-8 (Table 1). Isolate CR-5 caused more damping-off than the other isolates. The isolates X lines interaction was not significant.

2) *Foliar blight.*—Only isolates CR-5, FR-6, and FR-7 incited foliar blight (Table 1). Isolates FR-6 and FR-7 were not significantly different, but both incited significantly more blight than CR-5. The difference between sugar beet lines was not significant.

3) *Root rot.*—Separate analyses of variance performed on data from both inoculation experiments indicated highly significant differences among isolates and between lines. The isolates X lines interactions for both tests were significant. Bartlett's test for homogeneity of error variances of the two analyses showed that the variances were homogeneous. Therefore, a combined analysis was performed which disregarded the method of inoculation. Again, differences among isolates and between lines were highly significant. The isolates X lines interaction also was significant. However, due to the frequency of zeros and, consequently, the lack of variability in the results from inoculations with the crown and foliar isolates, data from these isolates were excluded from the analysis. When only the data from the root isolates were analyzed, the interaction was not significant.

Data in Table 1 clearly show that the root isolates induced significantly more root rot than did the crown or foliar isolates. Also, isolates RR-1 and -8 incited significantly more rot than did RR-2 and -4. Root rot was more severe in line GW 674-56C (mean = 3) than in FC 701/2 (mean = 1) regardless of root isolate.

DISCUSSION.—Root rot is the most widespread and serious Rhizoctonia-incited disease of sugar beet in the USA, and is of primary concern in our breeding program. Differences among root isolates in this study were minor, and consisted primarily of variation in virulence. The nonsignificant isolates X lines interactions in the damping-off and root rot experiments, when the crown and foliar isolates were excluded, indicated that the relative behavior of the root isolates was similar in the susceptible and resistant lines, but no definite conclusions can be drawn. However, in inoculated cultivar tests conducted by cooperators in Ohio (4), Michigan (G. J. Hogaboam & C. L. Schneider, *unpublished data*), and North Dakota (W. M. Bugbee, *unpublished data*), the most Rhizoctonia-resistant lines in the tests were those developed at Fort Collins, Colo. Resistance of sugar beet lines selected at Fort Collins apparently is stable under a wide range of environmental conditions in the presence of other highly pathogenic root isolates of *R. solani*. The significant isolate X lines interaction in the root rot experiments, when all isolates were included in the analysis, may indicate that resistance of FC 701/2 to Rhizoctonia root rot

may not be expressed against foliar blight. Further study is needed to determine the reaction of resistant and susceptible cultivars to foliar isolates. The significant differences between lines in the damping-off and root rot tests indicate that such tests may prove useful for evaluating breeding material for Rhizoctonia resistance in the greenhouse.

Most isolates in anastomosis group 4 studied by Parmeter et al. (7) were obtained from aerial plant parts. Isolates within this group are characterized by their rapid growth and light colored colonies (8). My crown-foliar isolates, and all other isolates from sugar beet seedlings or foliage not reported herein (E. G. Ruppel, *unpublished data*), exhibited the cultural characteristics of group 4. Anastomosis tests established the relationship of these isolates with group 4. Because of the constant association between cultural appearance or anastomosis behavior and pathological activity, it has been possible to distinguish sugar beet foliar isolates from root isolates by either cultural characteristics or anastomosis tests.

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