Artificial Exposure of Sugar Beets to Rhizoctonia Solani[1]

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Introduction

Root rot caused by *Rhizoctonia solani* Kuehn, also known as *Pellicularia filamentosa* (Pat.) Rogers, is a serious disease of sugar beets for which satisfactory control measures are not known. Attempts to breed Rhizoctonia resistant varieties have been impeded by the lack of satisfactory techniques for creating artificial exposure to attack. Such techniques must provide for a high degree of uniformity in the intensity of exposure to the pathogen and also for maintenance of exposure intensities at levels suitable for root selection and progeny testing purposes, respectively. Several studies of inoculation methods have been reported [Houston (8)[3], LeClerg (10 and 11), Kreitlow and Sherwin (9), Erwin (5), and Schuster et al. (14)], but none has been shown by experience to be entirely satisfactory for such purposes. The relation of soil amendments to Rhizoctonia attack of sugar beets has been studied by several authors [Blair (2), Boosalis (3), Dunleavy (4), Holst and Cormany (7), and Sanford (12 and 13)].

Materials and Methods

Twenty-five *R. solani* isolates from Colorado crop plants and soil were tested for pathogenicity to sugar beets at 3 stages of seedling development. Three, replicated, greenhouse pot experiments were involved in this study, each experiment including the full set of 25 isolates. Steamed and non-steamed soils were used in separate experiments. Certain isolates did not attack the plants, and the pathogenic action of others ranged from mild to very severe. Isolates classed as pathogenic, on the basis of these results, were used selectively in all field and greenhouse inoculation experiments initiated during 1958. In earlier inoculation experiments in the field, the inoculum used consisted of a composite of 18 Rhizoctonia isolates of undetermined pathogenicity.

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[3] Numbers in parentheses refer to literature cited.

For field experiments, inoculum was prepared by growing Rhizoctonia isolates for 2 to 3 weeks, at approximately 25° C, in flasks containing moist, sterilized, whole grain. The cultures then were dried in a mechanically ventilated oven at approximately 40° C. Except where otherwise indicated, the dried material was ground in a Wiley mill, passing through a 2-mm wire mesh screen. Except where the use of sorghum grain is indicated, barley grain was used as the substrate for the preparation of all inoculum for field use. For check purposes in certain experiments, the fungus in ground grain inoculum was killed by means of propylene oxide, alone or in conjuction with autoclaving. In some cases autoclaved substrates (ground grain) also were used for check purposes.

Sugar beet strains or varieties used in these investigations were as follows:

A. SP 52108-0: A USDA variety resistant to Cercospora leaf spot (*C. beticola* Sacc.) and to black root of the type caused by *Aphanomyces cochlioides* Drechs.
B. SP 581815-022: B-590♀ × miscellaneous material selected at Fort Collins for Rhizoctonia resistance. The ♀, furnished by The Great Western Sugar Company, had resulted from selection for Rhizoctonia resistance.
C. SP 471001-0: A vigorous inbred, susceptible to Cercospora leaf spot and to Aphanomyces-type black rot.
D. Kleinwanzleben-E: A commercial European variety.
E. SP 5832-0: A monogerm USDA variety, resistant to leaf spot and black root.
F. GW 359: A leaf spot resistant commercial variety widely grown in northern Colorado in recent years. Seed of this variety was used in all experiments reported in this article where the variety is not specifically indicated in the presentation of results.

On the basis of 1957 results at Fort Collins (unpublished), strains C and D were considered quite susceptible to *R. solani* and A and B were thought to have at least some degree of resistance or tolerance to that pathogen. In all experiments involving the application of inoculum in the seed furrow with the seed at time of planting, the seed was treated before planting with an ample quantity of 70 percent maneb dust, the excess removed by vigorous screening. Maneb seed treatment also was used in all other experiments, sometimes in combination with Dieldrin for root maggot control.

All field experiments were conducted on the Hospital Farm near Fort Collins, Colorado. Soil type in the area used for the

experiments is classed as Fort Collins fine sandy loam or Fort Collins loam, light-textured phase. The sprinkler method of irrigation was used exclusively.

Two principal inoculum application methods were tested under field conditions. They involved the placement of ground grain inoculum, respectively: 1) with the seed at planting, and 2) in contact with the sugar beet tap root after thinning. In the first case, a measured amount of sugar beet seed was placed on the seed belt of a drill, and a measured quantity of inoculum was spread uniformly over the seed[4]. The mixture then was drilled the full length of 1 plot row. With the second method, the soil was removed from around the tap root to a depth of 1 to 1¼ inches, a measured quantity of inoculum was placed in contact with the tap root, and the soil was pulled back into its original position (Figure 3). A third method of inoculation, used in a preliminary field trial, involved the application of ground grain inoculum in the center of the foliar rosette, after thinning, allowing it to fall on the surface of the soil at will.

Figure 3.—Post-thinning method of applying Rhizoctonia inoculum to sugar beet plants. The completed job is shown for plant at left where the inoculum is covered with soil.

A preliminary field experiment, dealing with the effects of soil amendments on Rhizoctonia attack, was conducted in 1958. Each of 2 amendments, yellow corn meal and dried beet pulp[5], was applied at 2 rates: 0.2 and 0.4 pound per 17 feet of row. The

[4] This method was suggested by N. R. Gerhold and K. E. Mueller, while serving as Associate Plant Pathologist and Junior Plant Pathologist, respectively, Colorado Agricultural Experiment Station.

[5] LPC dried pulp made by The Great Western Sugar Company, containing the equivalent of 14 percent crude protein.

amendment was distributed in a band approximately 6 inches wide, immediately before planting, and worked into the soil to a depth of about 3 inches. The beet row was planted approximately 1 inch to the left of the center line of the 6-inch amendment band, using a belt-type planter and a uniform seeding rate. On July 29, 3 weeks after planting, living or killed ground-barley (composite Rhizoctonia) inoculum was applied to the right of the center line of the amendment band, approximately 2 inches from the beet row and 1½ inches deep. There were 2 blocks of plots, each plot consisting of 1 row, 17 feet long. The 0.2-pound soil amendment rate was used in block 1; the 0.4-pound rate in black 2. Appropriate soil-amendment and inoculum checks were included in each block.

Two experiments were designed to study the relationship of plant ages and fungus isolates to disease reaction of 4 sugar beet strains growing in an artificial medium in the greenhouse. Sugar beet strains A, B, C, and D were used. All seed was sized between 10/64 and 15/64 inch and planted in a 4.5-inch diameter circle in 6-inch pots. Three Rhizoctonia isolates (B-6, B-12, and S-10), rated respectively as strong, moderate, and weak in pathogenicity, plus check constituted 4 so-called "isolate" treatments. The inoculum consisted of 1 cm mycelial agar disks, and on December 30, 1958, one disk was placed ½ inch deep in the center of each pot to be inoculated. A steamed mixture of 50 percent Canadian peat moss and 50 percent fine, washed, river sand, by volume, as proposed by Baker and coworkers (1), was used as a growth medium. Nutrients supplied in the watering system solution, in parts per million, were as follows: N = 129.4, Na = 8.0, K_2O = 132.0, Mg = 12.0, SO_4 = 48.0, B_2O_3 = 0.5, and P_2O_5 = 35.0. Calcium was incorporated in the sand-peat moss mixture at the rate of 5.7 grams of powdered calcium carbonate per gallon. Greenhouse air temperatures, as recorded by thermograph, were approximately as follows: 6:30 PM to 8:00 AM, 16° C; 9:30 AM to 4:30 PM, 27° C; over-all, 21° C. Soil temperatures, in the pots, averaged approximately 14° and 26° C at 8:00 AM and 4:00 PM, respectively.

In the first of these 2 greenhouse experiments, planting was done on November 13 and December 6, and populations were thinned to 10 plants per pot prior to inoculation. All possible combinations of 4 sugar beet strains, 2 age classes, and 4 isolate treatments resulted in a total of 32 entry numbers for the experiment. A randomized complete block design was used with 3 replications. In the second greenhouse experiment, planting was done immediately before inoculation was performed. Seeding

rates were adjusted according to germination in an attempt to provide 30 potential seedlings per pot. All possible combinations of 4 sugar beet strains and 4 isolate treatments resulted in 16 entry numbers. The pots were arranged in a randomized complete block design with 5 replications.

Additional details regarding materials and methods are given in the report of results for individual experiments.

Experimental Results and Discussion

Inoculum applied with the seed

In a preliminary field experiment with 3 replications, in 1957, ground barley grain (Rhizoctonia) inoculum was applied with the seed at approximately 0.4, 0.8, 1.6, and 3.2 ml per foot of row. Stands for those treatments after thinning, expressed as percentage of the non-inoculated check, were 7, 6, 2, and 0, respectively. In a comparison of ground- and whole-barley inoculum, applied at the rate of 0.4 gram per foot of row, pre-thinning stands were 1 and 28 percent of check, respectively. The greater percentage of killing obtained for the ground inoculum was attributed to more uniform distribution of infective Rhizoctonia units. Where dry, ground, autoclaved barley grain was applied at the rate of 0.4 gram per foot of row, pre-thinning stand was 7 percent of check.

Table 1.—Effects of type and rate of application of Rhizoctonia inoculum, with the seed, on sugar beet stand under field conditions.

Treat. number	Inoculum			Isolates and living plants per plot		
	Substrate	Kind	Amount per ft.	B-7[a]	S-21[a]	Average
			Ml	No.	No.	No.
Comparison of rates:						
1	barley	living Rhizoc.	0.03	30.3	20.7	25.5
2	do	do	0.12	3.0	3.0	3.0
3	do	do	0.29	2.0	3.7	2.8
6	sorghum	do	0.03	22.7	48.3	35.5
7	do	do	0.12	4.0	20.7	12.3
8	do	do	0.29	1.0	8.3	4.7
LSD (5-percent point)				11.1	11.1	
F-value for interaction, isolates × treatments = 5.67**						
Comparison of "checks":						
4	barley	killed Rhizoc.[b]	0.12	96.3	124.0	110.2
5	do	ster. substrate	0.12	33.7	47.0	40.3
9	sorghum	killed Rhizoc.[b]	0.12	96.0	95.3	95.7
10	do	ster. substrate	0.12	50.0	63.7	56.8
11	none	.....	.....	124.7	121.0	122.8
LSD (5-percent point)				25.0	25.0	17.7
F-value for intraction, isolates × treatments = 1.14						

[a] Three-plot averages; counts made in 14 feet of row per plot, 9/19/58, 53 days after planting.
[b] Killing performed by means of propylene oxide.
** F-value exceeds the 1-percent point.

In 1958, ground grain inoculum was applied with the seed, in a field experiment, at 0.03, 0.12, and 0.29 ml per foot of row. Killed Rhizoctonia inoculum and sterilized substrates were applied at the rate of 0.12 ml per foot. A split-plot design was used with main plots (Rhizoctonia isolates) composed of sub-plots of inoculum types and rates. There were 3 randomized complete blocks. The results from this experiment are presented in Table 1 and in Figures 1 and 2. As shown in Table 1, the 2 heavier rates of application of living inoculum resulted in extremely severe reductions in stand for each type of substrate. Stand reductions for the 0.03-ml rate were significantly less severe and probably much nearer the point that would be most suitable for progeny testing purposes. Where barley-substrate inoculum was used at the 0.03-ml rate, the average number of surviving plants per plot (25.5) was 21 percent of that shown for the non-treated check, treatment no. 11. The corresponding percent-of-check value for the 0.03-ml rate of sorghum-substrate inoculum was 29. It is recognized that such a degree of exposure to the pathogen probably would permit the survival of many "escapes." Con-

Figure 1.—Comparison of 3 rates of application of living Rhizoctonia inoculum, with sugar beet seed, at time of planting. Plots with large labels, left to right, received 0.03 ml, 0.12 ml, and 0.29 ml, respectively, per foot of row.

Figure 2.—Effects of killed Rhizoctonia inoculum and sterilized barley substrate, applied with the seed, in comparison with the non-treated check. Labeled plots at left and right received killed inoculum and sterilized substrate, respectively, at the rate of 0.12 ml per foot of row.

sequently, if such a method of inoculation were to be used for individual plant selection purposes, a much more consistent or uniform exposure would be required. For such purposes, and under the conditions of this experiment, it appears that an inoculum application rate of 0.29 ml per foot of row, or slightly higher, would be desirable. The relative pathogenicity of the fungus isolate also must be considered, of course. It is conceivable that a much heavier application rate, using an isolate of only medium pathogenicity, would be more suitable for identification and preservation of individual plants with a modicum though desirable degree of seedling resistance to Rhizoctonia, if such plants exist.

Certain comparisons among "check" treatments are of interest (Table 1). The average stand for each of the treatments involving the application of killed-Rhizoctonia inoculum (treatments 4 and 9) was below that obtained for the basic check treatment (no. 11), and in one case the difference was quite significant. The average stand for each of the sterilized-substrate treatments (nos. 5 and 10), in turn, was far below the average for the corresponding killed-inoculum treatment, both differences being highly significant.

The role of one type of sterilized substrate in promoting damping-off among young seedlings was studied in a preliminary laboratory experiment. Field soil was used without steaming or other fungicidal treatment. Seed was planted in small glass-walled containers, with and without dry, ground, autoclaved barley grain, and the containers then were held at a temperature of 28° C. At intervals of 72 and 96 hours after planting, seedlings were removed from the soil and plated on nutrient agar. The fungi obtained were identified as to genera. The highlight of this experiment was the behavior noted for *Pythium* sp. Seedlings from 5 of 32 seed balls of the check treatment produced *Pythium* sp. on nutrient agar. In contrast, seedlings from 18 of 32 seed balls, planted in containers receiving the sterilized substrate, produced *Pythium* sp. Species of *Pythium* are known to be quite pathogenic to young sugar beet seedlings, and one species has been reported as the chief cause of pre- and post-emergence damping-off in a series of seed treatment experiments conducted in the vicinity of Fort Collins a number of years ago (6). Admittedly the seedling populations involved in this experiment were not large, and soil conditions in the laboratory differed from field conditions. However, the results seem to justify the tentative conclusion that the severe stand losses observed, where sterilized barley-type substrate had been applied with the seed at time of planting, may be attributed largely to stimulation of Pythium activity by the presence of this readily available food material.

Inoculum applied after thinning

Two dates of post-thinning sub-surface inoculation were compared in a preliminary field experiment, with 3 replications, in 1957. Thinning was performed on July 29 at about the usual stage of plant development. Ground barley grain inoculum, composed of a mixture of Rhizoctonia isolates, was applied at the rate of 0.6 gram per plant. Dates of inoculation compared were August 2 and August 15. Living plants in the inoculated plots were counted at time of inoculation and again at harvest, October 21. At harvest, each living plant was classified for disease reaction, as illustrated in Figure 4, and the total weight of roots of living plants was determined for each plot. In the non-inoculated check plots, no loss in stand occurred between thinning and harvest, the average disease rating at harvest was 1.2 (essentially healthy), and the total weight of roots per plot was 36.6 pounds. For plots inoculated on August 2 (4 days after thinning), a 32-percent loss of stand occurred between time of inoculation and harvest, and the average disease rating and total

weight of roots per plot were 3.2 and 19.6 pounds, respectively. Corresponding averages for plots inoculated 17 days after thinning were 2 percent, 2.2, and 36.1. It seems clear that delaying the inoculation 13 days resulted in a decrease in severity of attack. However, not all of this difference can be attributed to plant age effects.

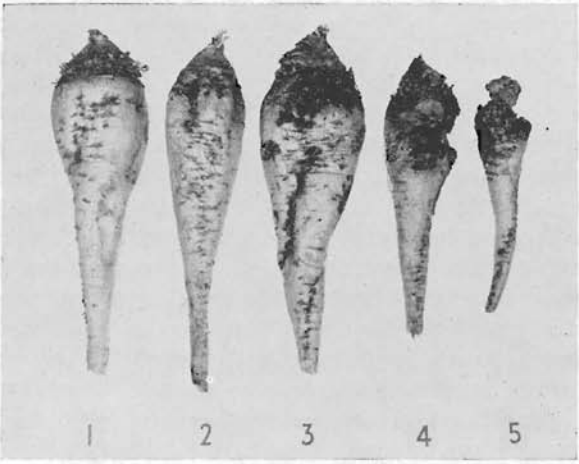

Figure 4.—Rhizoctonia disease ratings.

The relation of plant age to Rhizoctonia attack in the field was studied further in 1958, using the post-thinning sub-surface method of inoculum application, 4 dates of planting, and a single inoculation date. A split-plot design with 4 randomized complete blocks was used. Plant ages constituted the main plots. Each main plot was composed of 3 sub-plots which received living inoculum, killed inoculum, and no inoculum, respectively. Ground barley inoculum, composed of a mixture of 13 pathogenic isolates, was used at the rate of 2 ml per plant. At the time of inoculation (August 1), soil adjacent to all plants in the check plots was manipulated in exactly the same manner as in the inoculated plots. Stand counts made on August 5 represented numbers of plants living at time of inoculation. The lower initial stand counts in age class IV (Table 2) were the result of removal of curly top infected individuals just prior to inoculation and are considered unimportant insofar as the outcome of this experiment is concerned. As shown in Table 2 and Figure 5, there was a strong relationship between plant age and severity of Rhizoctonia attack, where living inoculum was applied, the older plants tending to die later and exhibiting a higher percentage survival at harvest. Effects of killed Rhizoctonia inoculum were

Table 2.—The relation of sugar beet age to severity of Rhizoctonia attack under field conditions[a].

Inoculum	Plant age symbol[b]	No. of days, thinning to inoculation[c]	No. of plants per plot 8/5	Surviving plants		Disease rating 9/27[e]
				Percent[d]		
				8/19	9/27	
Living Rhizoc.	I	0	27.0	5.9	0.0	
	II	11	29.3	8.6	0.8	5.0
	III	22	25.3	83.0	2.1	4.5
	IV	44	18.5	98.6	26.9	4.5
Killed Rhizoc.[f]	I	0	29.5	100.0	97.8	1.2
	II	11	32.3	100.0	100.0	1.1
	III	22	26.8	100.0	100.0	1.3
	IV	44	16.3	100.0	97.7	1.2
Check	I	0	30.3	100.0	100.0	1.1
	II	11	31.0	99.2	99.2	1.1
	III	22	23.5	100.0	100.0	1.1
	IV	44	19.0	100.0	100.0	1.0

[a] Data presented as 4-plot averages; each plot, 2 rows × 12 feet (Counted area, 2 rows × 11 feet).
[b] Planting and thinning dates were as follows: I = 7/3, 8/1; II = 6/27, 7/21; III = 6/13, 7/10; IV = 5/22, 6/18; respectively.
[c] Inoculation performed 8/1/58.
[d] Percentages based on initial stand (8/5).
[e] Basis of ratings: 1 = essentially healthy; 5 = very severe disease.
[f] Killing performed by means of propylene oxide plus autoclaving

negligible. These results indicate that, in a Rhizoctonia resistance breeding program, the intensity of exposure to the pathogen may be regulated to some extent by suitable timing of inoculation with respect to plant age.

Rates of post-thinning inoculum application, sub-surface, were studied in a replicated experiment in the field, in 1958, using 1-, 2-, and 4-ml amounts of ground-barley inoculum per plant. The 13-isolate composite, mentioned in the preceding paragraph, was employed. Killed inoculum and sterilized substrate were applied at the rate of 2 ml per plant. Sugar beet strains C and E were used. Disease effects were negligible in all plots receiving killed inoculum, sterilized substrate, or no inoculum or substrate of any kind. Of 514 plants inoculated with living Rhizoctonia 4 days after thinning, only 2 were alive 45 days later, and both were severely diseased. One of those plants had received 1 ml of inoculum and the other had received 2 ml. These results do not offer any encouragement for control of intensity of exposure to Rhizoctonia, through regulation of amount of inoculum, where the post-thinning sub-surface method is employed. However, further study, involving lower inoculum dosages, is needed before a definite conclusion can be reached in this regard.

A preliminary field trial, with inoculum applied in the petiole-crown region, was conducted in 1958, using 1-, 2-, and

Figure 5. — Influence of plant age on Rhizoctonia attack. Plots 1 and 4 (2-row plots marked at far end with tall white stakes) were planted on June 27 and 13, respectively, and both had received living Rhizoctonia inoculum on August 1, 1958, 12 days before picture was taken.

Table 3.—The influence of soil amendments on Rhizoctonia attack of sugar beets in the field.

Inoculum	Soil amendment and living plants per plot[a]								
	No amendment			Beet pulp			Corn meal		
	Initial stand[b]	Final stand[c]		Initial stand[b]	Final stand[c]		Initial stand[b]	Final stand[c]	
		Surv.	Dis.		Surv.	Dis.		Surv.	Dis.
	No.	Pct.	Pct.	No.	Pct.	Pct.	No.	Pct.	Pct.
Living Rhizoc.	143.5	85.7	40.6	56.5	98.2	5.5	69.5	97.1	17.9
Killed Rhizoc.[d]	133.0	92.9	0.0	88.5	98.9	0.0	62.0	100.0	0.0
None	117.5	97.0	1.4	75.5	100.0	0.0	48.5	100.0	0.0
Average	131.3			73.5			60.0		

[a] Basic data presented as 2-plot averages, each plot 1 row × 17 feet; counts, 14 feet of row per plot.
[b] Initial stand counts 8/2/58; inoculum applied 7/29, 21 days after planting.
[c] Final stand counts 9/17; percentage survival based on initial number of plants; percentage diseased based on number surviving.
[d] Killing performed by means of propylene oxide plus autoclaving.

4-ml rates. Of a total of 128 plants of sugar beet strain C, inoculated with composite living Rhizoctonia 4 days after thinning, all were dead 45 days later. There was no loss due to Rhizoctonia in the non-inoculated check plots. Thus, the use of inoculum in this manner appears rather promising as an inexpensive and effective means of exposing large populations of sugar beets to Rhizoctonia under field conditions. Whether the intensity of exposure to the pathogen can be controlled satisfactorily, and whether exposure in this manner is desirable in other respects, must be determined by further experimentation.

Soil amendment trial

The results of a preliminary soil-amendment experiment, conducted under field conditions, are shown in Table 3. Since results for the 0.2- and 0.4-pound rates were not contradictory, the 2 rates were combined in the preparation of Table 3. In studying these data, it should be noted that they are based on 2 replications, only. However, certain treatment effects or trends are of such magnitude that they appear to be quite meaningful.

It was observed in the field that damping-off was severe in those plots receiving either of the soil amendments and negligible in plots receiving no soil amendment. Since the initial stand counts, shown in Table 3, were made before killing due to the application of Rhizoctonia inoculum had begun, the effects of soil amendments on damping-off, independent of the effects of inoculation, may be observed in the initial stand columns. Average initial stand for the 6 plots given no soil amendment was 131.3 plants per plot, and the comparable averages for the beet pulp and corn meal amendment treatments were 73.5 and 60.0, respectively. Thus, the early losses in stand, indicated by these results and attributed to the respective amendments, were 44 and 54 percent. It is assumed that those losses were primarily due to stimulation, by the amendments, of the activity of pathogenic organisms occurring naturally in the soil.

Stand losses occurring during the 46-day period, between the dates when the initial and final counts were made, provide a basis for appraising effects of inoculation with Rhizoctonia and counter effects of soil amendments. As shown in Table 3, survival percentages ranged from 92.9 to 100.0 for the 2 types of checks (killed Rhizoctonia inoculum and no inoculum), with no obvious effects attributable to soil amendments or to the use of killed inoculum. Where living Rhizoctonia inoculum was used, survival percentages for no-amendment and for beet pulp and corn meal amendments, respectively, were 85.7, 98.2, and 97.1.

Table 4.—The influence of sugar beet strain and fungus isolate on Rhizoctonia attack under controlled conditions; inoculum applied after thinning.

Isolate	Sugar beet strain	Number of living plants per pot[a]										
		Total										Healthy
		1/26	2/2	2/9	2/16	2/23	3/2	3/9	3/16	3/23	3/31	3/31
B-6	A	10.0	7.0	5.8	3.2	1.2	0.0	0.0	0.0	0.0	0.0	0.0
	B	10.0	7.7	7.5	3.3	0.5	0.0	0.0	0.0	0.0	0.0	0.0
	C	10.0	6.2	4.8	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	D	10.0	8.7	7.3	3.8	1.7	0.2	0.0	0.0	0.0	0.0	0.0
B-12	A	10.0	10.0	10.0	9.8	9.7	9.3	8.8	8.3	7.8	5.2	1.0
	B	10.0	10.0	10.0	10.0	10.0	10.0	9.5	9.2	9.2	5.7	0.8
	C	10.0	10.0	10.0	10.0	10.0	9.8	9.8	9.0	7.8	6.0	0.5
	D	10.0	9.8	9.8	9.8	9.7	9.7	9.2	9.0	6.8	4.7	0.8
S-10	A	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
	B	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
	C	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
	D	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Check	A	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
	B	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
	C	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
	D	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0

[a] Data presented as 6-pot averages (6-inch pots). Three replications were planted on November 13, and the other 3 on December 6, 1958. Inoculation was performed on December 30.

Table 5.—The influence of sugar beet strain and fungus isolate on Rhizoctonia attack under controlled conditions; inoculum applied at time of planting[a].

Isolate	Sugar beet Strain	Total no. emerged plants per pot	Living plants as percent of total emergence[b]											
			Total											Healthy
			1/12	1/19	1/26	2/2	2/9	2/16	2/23	3/2	3/9	3/23	4/1	4/1
B-6	A	34.2	97.8	86.5	68.3	45.5	35.5	18.0	9.5	3.2	0.0	0.0	0.0	0.0
	B	30.2	96.2	77.1	57.7	39.7	30.2	19.5	12.4	4.9	1.2	0.0	0.0	0.0
	C	34.2	93.1	80.8	69.4	43.4	31.5	13 7	4.4	2.2	0.0	0.0	0.0	0.0
	D	28.6	97.6	81.2	69.9	55.7	47.1	35 8	15.7	9.1	2.1	0.0	0.0	0.0
B-12	A	32.0	97.8	99.4	100.0	99.2	99.2	99.2	98.6	98.6	98.6	93.9	66.6	11.7
	B	32.4	98.5	97.5	98.9	98.9	98.9	98.9	98.9	98.9	97.4	96.3	73.2	21.5
	C	33.8	92.3	98.2	100.0	100.0	100.0	98.3	96.4	94.2	88.4	84.1	57.0	11.2
	D	30.8	99.4	100.0	100.0	99.4	99.4	98.0	98.0	95.2	93.3	89.3	61.5	11.1
S-10	A	31.2	99.4	100.0	100.0	100.0	100.0	99.4	99.4	99.4	99.4	99.4	98.7	98.7
	B	28.2	97.1	99.4	100.0	99.4	98.6	97.4	97.4	97.4	97.4	97.4	95.5	94.9
	C	39.4	96.4	98.8	100.0	100.0	99.6	99.6	99.6	99.6	99.6	99.6	98.6	95.1
	D	28.6	99.3	98.9	98.9	98.9	98.9	98 9	98.9	98.9	98.9	98.9	98.3	94.7
Ck.	A	31.0	98.7	100.0	100.0	100.0	100.0	100 0	100.0	100.0	100.0	100.0	99.4	99.4
	B	31.8	99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4
	C	33.0	93.4	98.7	98.7	99.2	100.0	100.0	100.0	100.0	**100.0**	100.0	99.0	99.0
	D	30.0	98.1	98.7	98.0	98.6	99.3	99.3	99.3	99.3	99.3	99.3	98.6	98.6

[a] Planting and inoculation performed on December 30, 1958.

[b] Data presented as 5-pot averags (6-inch pots).

Corresponding percentages of diseased plants at harvest were 40.6, 5.5, and 17.9, respectively. It seems clear that the living inoculum caused substantial injury in the no-amendment plots, and it is tentatively concluded that the amendments, where applied, provided some degree of protection. It is assumed that this protective influence was due to stimulation of soil microorganisms detrimental to Rhizoctonia. This apparent protective effect of the amendments was in agreement with results reported by Sanford (13) and Dunleavy (4), but contrary to the observations of Holst and Cormany (7).

Inoculation of plants growing under controlled conditions

The reaction of 4 sugar beet varieties or strains to 3 Rhizoctonia isolates, in a sand-peat moss medium in the greenhouse, is shown in Tables 4 and 5. Since there was very little difference in reaction of the 2 older seedling groups, the results for those groups were combined (Table 4). Results for the youngest seedling group—the group that was inoculated at time of planting—are presented in Table 5. Isolate B-6 killed all seedlings of all sugar beet strains and ages before or during the week of March 9—i.e., within approximately 10 weeks after inoculation. The final killing action occurred within a 3-week period for all strains. Isolate B-12 was much slower in its attack than isolate B-6, and 13 weeks after inoculation it had not killed all the plants of any strain. Isolate S-10 appeared to be almost nonpathogenic under these conditions. It exhibited very little killing and disease in the younger beets, and none in either of the older sets.

Some evidence that sugar beet strain C was more susceptible to Rhizoctonia than the other 3 strains, under the conditions of this experiment, may be found in Tables 4 and 5. That evidence is rather meager and cannot be considered as conclusive. Differences among the other 3 strains appeared to be negligible.

The preparation of a reproducible soil mixture, suitable for Rhizoctonia resistance testing purposes in the greenhouse, represents a substantial problem. It is believed that the sand-peat moss medium used in this study appears rather promising as a solution to this problem.

Summary

A series of greenhouse and field experiments was conducted for the purpose of devising techniques for exposing sugar beets to *Rhizoctonia solani* suitable for disease resistance testing and selection purposes.

Ground and whole barley-grain Rhizoctonia inocula, in dry form, were compared and found to produce relatively even and

sporadic attack of sugar beet seedlings, respectively, when applied with the seed at identical rates. The ground, barley-grain inoculum, prepared from virulent isolates and applied with the seed at time of planting in field plots at the rate of 0.03 ml per foot of row, produced a degree of exposure to the pathogen considered satisfactory for progeny testing purposes.

Where ground, barley-grain inoculum was applied in contact with the tap root and below the soil surface at the rate of 2 ml per plant, from 0 to 11 days after thinning, less than 1 percent of the plants were alive at harvest. Of comparable older plants, inoculated 44 days after thinning, 27 percent survived. Attempts to control intensity of Rhizoctonia exposure by varying the rate of post-thinning application of inoculum were unsuccessful.

Four sugar beet strains, thought to differ somewhat in resistance or tolerance to *R. solani*, were tested under controlled conditions against *R. solani* isolates with past histories of strong, moderate, and weak pathogenicity, respectively. The strains were tested at 3 stages of seedling development. Differences among strains, in apparent resistance, were rather small and inconclusive. However, the relatively consistent pathogenic activity of the Rhizoctonia isolates indicated that the sand-peat moss medium employed in this study may be a useful tool for Rhizoctonia resistance testing and selection work in the greenhouse.

Beet pulp and corn meal, incorporated with field soil immediately prior to planting, caused a severe, early reduction in sugar beet seedling stand where artificial inoculation with Rhizoctonia was not a factor. On the other hand, the presence of either soil amendment apparently interfered substantially with the pathogenic action of Rhizoctonia inoculum applied in a band, approximately 2 inches from the beet row, 3 weeks after planting.

Sterilized barley- and sorghum-grain substrates resulted in severe losses in seedling stand, in the field, when applied with the seed at time of planting. On the basis of results obtained from a laboratory experiment, this occurrence, at least with respect to barley, was attributed to stimulation of the pathogenic activity of *Pythium* sp. occurring naturally in the soil.

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