Seedling Response of Storage-Rot-Resistant Sugar Beets To *Phoma Betae* and *Rhizoctonia Solani*

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ABSTRACT

Five sugar beet germplasm lines resistant to storage rot caused by *Phoma betae* also were resistant to Phoma damping-off in the seedling stage. Seedling resistance to *P. betae* was not significantly affected at 15° or 25°C. Seedlings resistant to *P. betae* were not resistant to AG-2-2 or AG-4 of *Rhizoctonia solani*. Thus, genetic resistance to Phoma damping-off disease, but not Rhizoctonia damping-off, can be selected when mature harvested roots are evaluated for resistance to Phoma storage rot.

Additional Key Words: Seedling disease; seedling disease resistance; black leg

Rot of stored sugar beet (*Beta vulgaris* L.) occurs even if roots are stored properly. Primary causes are the fungal pathogens *Phoma betae* Frank (teleomorph, *Pleospora bjorlingii* Byford), *Botrytis cinerea*, and *Penicillium* spp. *Phoma betae* is the most significant of these pathogens because it affects all life cycle phases of sugar beet and causes seedling disease, crown rot, leaf spot, and storage rot (Edson, 1915; Byford, 1972; Tomkins and Pack, 1932). Also, it is the only recognized seed-borne fungal pathogen of sugar beet (Edson, 1915). This fungus causes storage losses of thousands of tons of sucrose per year (Bugbee and Cole, 1976). Valuable germplasm can be lost when stored mother roots are destroyed by Phoma rot. Breeding lines with resistance to this pathogen have been developed (Bugbee, 1978; Campbell

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and Bugbee, 1985).

Rhizoctonia solani Kuehn (teleomorph, *Thanatephoris cucumeris* [Frank] Donk) causes seedling disease, crown and root rot, and foliar blight, but not storage rot. Germplasm lines resistant to Rhizoctonia root rot have been developed and some have shown resistance in the seedling stage (Campbell and Altman, 1976; Ruppel, 1972; Bugbee, unpublished data). One root rot resistant germplasm line and selections from it also were resistant to storage rot caused by *P. betae* (Bugbee, 1979a). Therefore, *R. solani* was included in this study to determine whether storage-rot-resistant lines also might be resistant to *R. solani* in the seedling stage.

Low levels of resistance to Phoma storage rot (Bugbee, 1973) and to Phoma damping-off exist among cultivars (Osinka, 1985). Whether resistance to both phases of the disease resides within the same cultivar has not been reported.

The objectives of this research were: 1) to determine whether storage rot and damping-off resistance to *P. betae* occurred in the same germplasm line; 2) to determine whether temperature affected a genotypic response to Phoma damping-off; and 3) to detect seedling resistance to *Rhizoctonia solani* in germplasm lines resistant to Phoma storage rot.

MATERIALS AND METHODS

Roots used for storage rot evaluations were grown in a Fargo clay soil type at the North Dakota Agricultural Experiment Station, Fargo. Harvested roots were washed, stored at 4-6°C and 95% relative humidity in perforated plastic bags, and evaluated for resistance to *P. betae* within 90 days. The inoculum for storage rot evaluations was prepared by adding 1 ml of a conidial suspension of a 2- to 3-wk-old culture of *P. betae* to 20 ml molten (50°C) potato-dextrose agar amended with 30 μg/ml of streptomycin sulfate, which was then poured to 10-cm square, sterile, polystyrene disposable Petri dishes. The cultures were incubated until the agar surface was covered with mycelium (usually 4 days); then 1-cm cubes were prepared (Bugbee, 1979b) from field-grown roots and placed (cut surface down) on the cultures and incubated for 14 days at 22°C. Forty- nine cubes were placed in each dish in a sequential order so that each cube could be identified with its numbered, parent root. After incubation, the cubes were cut in half and assigned a storage rot rating based on the distance rot had progressed through the cube: 0 = no rot; 1 = not over 2 mm; 2 = 2-4 mm; 3 = 4-6 mm; 4 = 6-8 mm; and 5 = completely rotted.

Seedling Response to P. betae. Two germplasm lines that were developed for storage rot resistance to *P. betae* (Bugbee, 1978), F1001 (Reg. No. GP-15) and F1002 (Reg. No. GP-16), were compared to the susceptible cultivar American Crystal 2B and the susceptible germplasm line PBP-4 for seedling resistance. The germplasm line F1001 was selected from an introduction from

the Soviet Union and F1002 was selected from FC 701/4, a line developed for resistance to *R. solani* by USDA-ARS at Fort Collins, Colorado.

The inoculum for seedling evaluation was prepared by growing P. betae on green bean agar (GBA) for 10-15 days under light at 23°C. The GBA was prepared by heating 150 g of snap bean (*Phaseolus vulgaris* L.) pods for 30 min at 60°C in 500 ml of distilled water (DW). The bean extract was brought to 1 L by the addition of DW, 20 g of agar was added, and the mixture was autoclaved for 20 min at 121°C. Conidia were collected by flooding cultures with sterile DW and then lightly scraping the agar surface with a spatula to dislodge the conidia. The conidial concen- tration was adjusted to 5 x 10^5/ml with the aid of a cell counter. Seed were inoculated by submerging them in a conidial suspension for 30 min and then incubating the moist seed for 24 h at room temperature and 100% relative humidity before planting. Control seeds were submerged in DW.

To evaluate seedling reaction to *P. betae*, inoculated seeds of each germplasm line were planted in flats (34 x 49 cm with a capacity of 10 L) containing pasteurized sand and grown in a growth chamber at 22°C and 14-h light period with a mix of fluorescent and incandescent light (18,300 lux). Treatments (25 seeds of each line) were arranged in a randomized complete block design and replicated four times. Stand counts were taken when the second leaf emerged and were expressed as percent emergence of uninoculated controls. An analysis of variance was performed on arcsine transformed percentages.

Effect of Temperature on Seedling Response to P. betae. Three germplasm lines, F1004 (Reg. No. GP-94), F1005 (Reg. No. GP-95), and F1006 (Reg. No. GP-96), with improved storage rot resistance were compared with three other germplasm liness for seedling resistance to *P. betae*. The germplasm lines F1004 and F1005 were selected from introductions from the Soviet Union, and F1006 was selected from a population of resistant individuals from the USDA-ARS *Beta* collection (Campbell and Bugbee, 1985). Three other germplasm lines that were used in this experiment were not tested for storage rot resistance. They were 85N0016 which had been developed for high sucrose content, 85N0030 developed for low storage respiration rate, and M-1 developed for resistance to the sugar beet root maggot (*Tetanops myopaeformis*). Ten seeds per germplasm line were planted in 15-cm clay pots containing commercial, pasteurized Sunshine Mix No. 1 (Fisons Western Corp., Vancouver, B.C., Canada) and grown at 15° or 25° in four replicates as in the previously described seedling experiement. After stand counts were taken, surviving seedlings were harvested and the leaves and stems were removed and discarded. Hypocotyls and roots were surface-disinfested in 0.1% sodium hypochlorite for 30 s, rinsed twice in sterile DW, plated on an agar medium selective for *P. betae* (Bugbee, 1974), and incubated at 23°C under constant fluorescent light for 10-12 days.

The number of infected seedlings were counted and the identity of *P. betae* was confirmed by the presence of characteristic holdfasts that formed at the bottom surface of the culture dish (Mangan, 1971; Bugbee, 1974). Data were modified by substracting counts of the uninoculated treatments from counts of inoculated treatments. The three resistant lines, as a group, and the susceptible lines, as a group, were compared by linear contrast.

Response of Seedlings to R. solani. Resistance in germplasm lines F1004, F1005, F1006, M-1, 85N0016, and 85N0030 was tested against local field isolates of *R. solani* from anastomosis groups 4 and 2-2. The test was conducted at 20° or 25°C because of an earlier report of seedling resistance being expressed at 26°C (Campbell and Altman, 1976). Barley was soaked overnight in 1% potato-dextrose broth, drained, and 250 ml were placed in 1 L Erlenmeyer flasks. The barley was sterilized by autoclaving twice for 1 h at a one-day interval. Each flask was inoculated with one-fourth of a young *Rhizoctonia* Petri dish culture that had grown to about 9 cm in diameter. The fungus was grown on the sterile barley at 25°C for 2 wk, air-dried, and ground in a hammer mill to pass through a screen with 3-mm diameter openings. Ground barley inoculum containing 500 or 2500 propagules of *R. solani* was uniformly mixed into the potting soil that was used to cover the seed. The inoculum level was determined from counts of colonies that grew from known weights of ground barley on the selective medium of Ko and Hora (1971). Stand counts were taken when the second leaf emerged.

RESULTS

Seedling Response to P. betae. The level of storage rot resistance among the four germplasm lines in the first experiment is shown in Table 1 (Bugbee, 1978). The storage rot ratings were taken from previous work (Bugbee, 1978) where the ratings of 2.4 for both F1001 and F1002 were significantly lower than the rating of 4.1 for hybrid 2B ($LSD_{.05} = 0.09$). The rating for germplasm line PBP-4 was from a separate evaluation and was not included in the analysis of variance. F1001 expressed resistance to *P. betae* damping-off as shown by a stand count only 3% less than the uninoculated control. The seedling response of F1002 to *P. betae* also was favorable but not statistically different from susceptible PBP-4 or 2B. Figure 1 shows representative seedling damage caused by *P. betae* to the storage-rot-resistant germplasm F1001 and the susceptible cultivar 2B.

Effect of Temperature on Seedling Response to P. betae. More seedling disease occurred at 25° than at 15°C, but ranking of the genotypes with respect to resistance to seedling disease remained the same and the temperature X germplasm line interaction was not significant, so data for the two temperatures were combined (Figure 2). Natural infection of beet seed with *P. betae* is common (Byford, 1972; Edson, 1915). Osinka (1985) reported that differential resistance among 25 inoculated cultivars was obscured by

Table 1. Seedling response of storage-rot-resistant (F1001, F1002) and susceptible (PBP-4, 2B) cultivars to *Phoma betae*.

Cultivar	Storage rot rating[†]	Stand as percent of control[‡]
F1001	2.4	97
F1002	2.4	71
PBP-4	3.5	54
2B	4.1	35
LSD, 0.05	–	36

[†]Storage rot rating indicates distance rot progressed through a 1-cm block of tissue after inoculation and incubation at 22°C for 2 wk: 0 = 0 mm; 1 = not over 2 mm; 2 = 2-4 mm; 3 = 4-6 mm; 4 = 6-8 mm; 5 = entire block. The rating of 2.4 for F1001 and F1002 was significantly lower than 4.1 for hybrid 2B ($LSD_{.05}$ = 0.09) according to earlier work (Bugbee, 1978). The data for PBP-4 was not included in the earlier analysis.

[‡]Mean percentages of four replications. Statistical comparisons were made on arcsine transformed percentages.

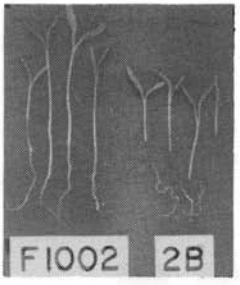

Figure 1. Seedling damage caused by *Phoma betae* to a germplasm line (F1002) selected for resistance to Phoma storage rot compared to damage in the storage-rot-susceptible cultivar 2B. F1002 was selected from FC 701/4, a germplasm line developed at Fort Collins, CO, for resistance to *Rhizoctonia solani*.

the amount of initial seed infection. Osinka showed that meaningful results occurred when uninoculated data were substracted from inoculated data. This adjustment revealed the actual infection rate caused by the inoculation treatment. The effect of natural infection of seed was removed in our test by substracting counts of uninoculated controls from the inoculated treatments. The linear contrast analysis showed a significantly lower stand reduction for the group of storage-rot- resistant germplasm lines than for the susceptible group ($P = 0.001$) (Figure 2). Less than 10% of surviving seedlings became infected in storage-rot-resistant germplasm lines whereas 34-52% became infected in M-1, 85N0016, and 85N0030.

Response of Seedlings to R. solani. Experiments with *R. solani* as the inoculum showed that none of the germplasms possessed resistance to this pathogen in the seedling stage at either of the temperatures tested.

DISCUSSION

Based on the *R. solani* experiment, it does not appear that

storage rot resistance can be used as an indicator of seedling resistance to this fungus.

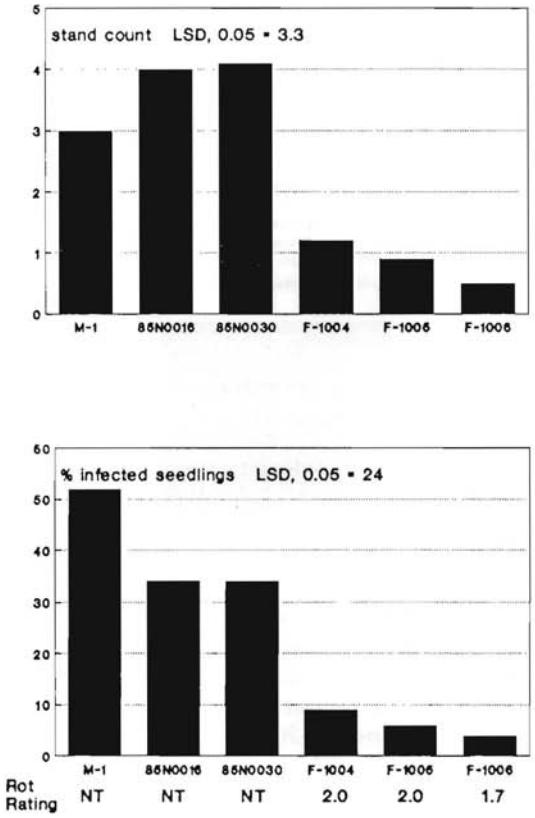

Figure 2. Stand count and percent infected seedlings expressed as the difference between *Phoma*-inoculated and the uninoculated controls. The germplasms M-1, 85N0016 and 85N0030 were not tested (NT)[1] for their reaction to Phoma storage rot.

Seedlings infected with *P. betae* can recover to produce systemically infected, apparently healthy roots (Edson, 1915). Harvested and stored roots, however, will begin to decay after being placed in storage. Our research shows that fewer plants would be infected if cultivars with resistance to storage rot were planted. This also would mean that less inoculum would enter storage piles.

Germplasm lines F1001, F1002, F1004, F1005, and F1006 that

[1]NT = not tested for reaction to storage rot pathogens. Storage rot rating indicates distance rot progressed through a 1-cm block of tissue after inoculation and incubation at 22°C for 2 wk: 0 = 0 mm; 1 = not over 2 mm; 2 = 2-4 mm; 3 = 4-6 mm; 4 = 6-8 mm; 5 = entire block.

were developed for resistance to Phoma storage rot also possessed resistance to *P. betae* damping-off and resulted in reduced stand loss and fewer infected seedlings. The results of our research indicate that genetic resistance to seedling infection by *P. betae* can be developed and should be considered as a complement to fungicidal seed treatments for seedling disease control, especially in those regions of the world where Phoma seedling disease is important.

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