Screening Peas for Resistance to Stem Rot Caused by *Rhizoctonia solani*

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ABSTRACT

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Three inoculation techniques for screening peas (*Pisum sativum*) for resistance to stem rot caused by *Rhizoctonia solani* AG4 were compared. The corn kernel technique gave a consistent separation between resistant and susceptible genotypes in growth chamber and field studies. Among 68 entries, three cultivars, four breeding lines, and one PI line had partial resistance. However, resistance (expressed as a stem reaction) was not uniform within each taxon and varied with environmental conditions.

Rhizoctonia solani Kuehn causes a seedling tip blight, seed rot, and stem rot of peas (*Pisum sativum* L.). A severe outbreak of these diseases occurred in Wisconsin in 1963. The causal organism was identified as *Thanatephorus praticolus*, which is now considered to be anastomosis group (AG) 4 of *R. solani*, whose sexual state is *Thanatephorus cucumeris* (Frank) Donk. The problem is serious when temperatures above 18–20 C persist and soil moisture is high (3). In a previous study, stem canker caused by *R. solani* was found in the later maturing cultivars (2).

Recent work indicates that *T. cucumeris*, or *R. solani*, is a complex species composed of at least four anastomosis groups that can be separated by the ability of field isolates to anastomose (5). Because AG4 isolates caused a severe seed and stem rot of peas, a highly pathogenic isolate of this group was used in our study.

Before 1979, efforts to select pea lines resistant to both seed and stem rot caused by *Rhizoctonia solani* AG4 resulted in the selection of resistant lines with lavender flowers and dark seed coats (7).

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Previous studies with AG4 isolates of *R. solani* indicated that the ability to cause seed rot and stem rot of flax was controlled by two different genetic systems in the pathogen (1). Disease resistance to seed rot and stem rot caused by AG4 isolates of *R. solani* in alfalfa (*Medicago sativa*) has also been found to be controlled by different genetic factors (6). In addition, seed treatment with the fungicide captan provided good protection against seed rot but had no significant effect on stem rot.

For these reasons, we began in 1979 to look for stem rot resistance in field and growth chamber tests. Our principle objectives were to find a reliable technique for screening for resistance to stem rot caused by *R. solani* and to identify promising pea genotypes with moderate to high levels of stem rot resistance.

MATERIALS AND METHODS

The efficacy of two inoculation techniques (corn kernel and cornmeal-sand) for differentiating the relative resistance of 68 pea cultivars and lines was compared in a randomized complete block test with three replicates in sandy loam soil at Becker, Minnesota. Twenty seeds of each cultivar were planted in one-row plots 180 cm long. Rows were spaced 90 cm apart.

In the corn kernel technique, a colonized corn kernel was placed just below the soil surface and in contact with the stem at the two- to three-leaf stage. The corn kernel inoculum was prepared by soaking mature dry dent corn kernels in water in jars for 12 hr, draining the water, and autoclaving the kernels at 121

Table 1. Reaction[a] of 68 pea genotypes to inoculation in the field with *Rhizoctonia solani* AG4 by two inoculation techniques

Genotype	Inoculation technique: Cornmeal-sand	Inoculation technique: Corn kernel
AA-15	3.7	4.0
AA-15-A	3.5	4.5
Alaska M 163	4.5	4.8
Alaska R 28-57	3.7	4.1
Alaska Sweet (346)	4.0	4.6
Alsweet (Morrison)	4.2	4.2
Alsweet E.S.5	3.3*[b]	3.8
Alsweet 4683	3.7	4.6
Alsweet (Stubbs)	3.0*	3.7
Asgrow (3852)	3.7	4.6
Asgrow (4683)	3.7	4.1
Canjoy (1351)	2.5*	3.8
Canner (488)	3.8	4.0
Canner (69315)	3.5	3.8
9901 Canner	4.0	4.8
Challenge	3.8	4.8
Code #1	3.7	4.1
Dark Skin Perfection	2.5*	2.7*
Dark Skin Perfection (5147)	1.8*	2.9*
Dawn	3.8	4.5
Delwiche Commando	3.8	4.5
Early Freezer (63)	3.8	4.5
Early Frosty (812)	3.0*	4.6
Early Perfection (3040)	3.0*	4.2
Early Perfection (8221)	3.8	3.6
Early Perfection (8617)	3.7	4.5
Early Perfection (9021)	3.5	4.5
Early Sweet (7025)	3.8	4.6
Freezer (76110)	2.7*	3.5*

(*continued on next page*)

Table 1. (*continued from preceding page*)

Genotype	Inoculation technique: Cornmeal-sand	Inoculation technique: Corn kernel
Freezer (9888)	4.2	4.5
Freezer (9889)	3.0*	4.0
Green Star	3.0	3.9
Little Marvel	4.7	4.5
M 410	3.7	4.1
Medalist (303)	3.2*	4.5
75 MF 1029	2.8*	3.5
75 MF 1029-F_2	3.0*	4.7
75 MG 75-89 Bk	2.2*	2.1*
75 MG 90-102 Bk	1.8*	2.3*
75 MG 171-194 Bk	2.8*	4.7
Minn. 108	3.2	4.5
Minn. 494 All	3.8	4.4
Neptone	3.8	4.4
Novella	3.8	4.5
Pacemaker	3.7	4.2
Parlay (Fr. 72244)	3.5	4.1
Perfected Freezer	3.3*	4.1
P.I. 257593	3.2*	3.3*
Small Sieve Freezer #1	3.7	4.2
Sprite	3.5	4.5
Target (552)	4.2	4.3
Tempter (68273)	4.0	4.3
Trojan	3.0*	3.9
Venus	3.3*	4.3
Wando	2.3*	3.2*
WSU 23	2.3*	3.7
WSU 28	3.7	4.1
74 SN3	3.1*	3.3*
338	4.1	4.5
339	3.8	4.1
414	3.8	4.5
425	2.7*	4.1
434	4.0	4.5
502	2.2*	3.6
507	3.8	4.3
508	3.7	3.9
4683	3.7	4.3
8615	3.3*	4.1
Average	3.34	4.04

[a] Reaction is based on a disease index: 1 = no symptoms, 2 = one or a few pinpoint dark spots, 3 = necrotic lesions less than 0.5 cm long with 25–50% of stem girdled, 4 = necrotic lesions more than 0.5 cm long with 50–75% of stem girdled, 5 = severe necrotic lesions with plants dead and 100% of stem girdled.

[b] *Indicates significant differences from the control, Little Marvel, within each column (least significant difference, 5% level, square root transformation of data).

C for 30 min and again after 24 hr. A disk from a colony of *T. cucumeris* on potato-dextrose agar (PDA) was placed in each jar. The jars were kept at 24 C for 2 wk and shaken every 3–4 days.

In the cornmeal-sand technique, 1 tbs of inoculum was applied to each seed. The inoculum was prepared by growing the pathogen for 10 days on a medium containing cornmeal, sand, and perlite (1:10:10). Seeds previously treated with captan for seed rot protection were placed in the furrow and covered with up to 1.5 cm of field sand before the inoculum was applied. The seedbed furrows were covered with another layer of sand (2.5 cm) directly after the inoculum was applied.

All seeds were treated with captan in the field to avoid preemergence seed rot. Because environmental conditions such as soil temperature and moisture have a great effect on disease severity (3), the sandy loam soil in the field was irrigated frequently to encourage infection. When most plants had reached the processing maturity stage, the plants were carefully pulled from the soil, and the roots and stems were washed in running water.

Stem rot was rated with the following disease severity index: 1 = no symptoms, 2 = one or a few pinpoint dark spots, 3 = necrotic lesions less than 0.5 cm long with 25–50% of the surface area of the stem girdled, 4 = necrotic lesions more than 0.5 cm long with 50–75% of the stem girdled, and 5 = plants dead with 100% of the stem girdled. Because small-scale index numbers were used to test a large number of pea cultivars, the data were transformed to $\sqrt{x} + 0.5$ for statistical analysis by the least significant difference method. Duncan's multiple range test was used in the statistical analysis of the growth chamber experiments.

Three cultivars and one breeding line that were moderately resistant in the field experiment were retested in a growth chamber to compare the efficacy of the corn kernel technique with a PDA disk technique in differentiating host resistance (4). The cultivar Little Marvel was used as a susceptible control.

In the PDA disk method, a 4-mm agar disk from the colony of an AG4 isolate of *R. solani* was placed next to the plant stem just below ground level and was covered with soil. In all growth chamber tests, seeds were planted in wooden flats containing sterilized sand. After 8 days at 24 C, seedlings were transplanted to 5.5 ×5.5 cm Jiffy Strips containing autoclaved sand and were inoculated by either the corn kernel or the agar disk technique. The seedlings were supplied with nutrient solution.

Effects of soil moisture and soil temperature on disease severity were studied in the growth chamber tests. In a water tank method, the Jiffy Strips were held for 4 days in metal flats containing water to a depth of 2 cm to keep the soil saturated. After 5 days, the water was drained and the soil was allowed to dry. Preliminary studies indicated that uninoculated plants of Little Marvel may survive saturated soil conditions for up to 4 days. Soil temperature was maintained at 27 C by placing the metal flats in a controlled-temperature water bath.

Surface irrigation was used in a second growth chamber where the soil temperature fluctuated between 24 and 29 C, depending on the air temperature and time of irrigation. Surface irrigation was applied frequently, and the metal flats were replaced by wooden ones. The photoperiod was 16 hr, and a light intensity of 7,500 lux was maintained in both growth chambers.

In all growth chamber tests, plants were carefully removed from the soil and the stem disease reaction was scored 1 wk after inoculation. In each test, six plants of each genotype were inoculated in each of three replicates.

RESULTS AND DISCUSSION

Statistical analysis of the field data indicated that cultivars with a disease index up to 3.3 for the cornmeal-sand method and up to 3.5 for the corn kernel

Table 2. Reaction[w] of five pea cultivars to inoculation with *Rhizoctonia solani* by two inoculation techniques under two sets of environmental conditions

Cultivar	Corn kernel technique: Water tank[x,y] Exp. 1	Corn kernel technique: Water tank[x,y] Exp. 2	Corn kernel technique: Surface irrigation[y,z] Exp. 1	Corn kernel technique: Surface irrigation[y,z] Exp. 2	x	Agar disk technique: Water tank[x,y] Exp. 1	Agar disk technique: Water tank[x,y] Exp. 2	Agar disk technique: Surface irrigation[y,z] Exp. 1	Agar disk technique: Surface irrigation[y,z] Exp. 2	x	X
Little Marvel	5.0 a	4.8 a	4.8 a	4.9 a	4.9	2.2 ab	2.7 a	1.8 a	2.7 a	2.4	3.7
Dark Skin Perfection	3.2 bc	3.1 c	3.5 b	4.8 a	3.7	1.8 b	2.4 a	1.6 a	2.4 ab	2.1	2.9
Freezer 76110	2.4 c	3.1 c	2.5 b	4.5 b	3.1	2.0 b	2.1 a	1.7 a	1.6 b	1.9	2.5
USDA SN_3	3.4 b	3.7 b	3.1 bc	4.4 b	3.7	2.2 ab	2.6 a	1.8 a	1.7 b	2.1	2.9
Wando	3.3 b	3.5 bc	3.1 bc	4.5 b	3.6	1.9 b	2.0 a	1.9 a	1.2 b	1.8	2.7

[w] Reaction is based on a disease index: 1 = no symptoms, 2 = one or a few pinpoint dark spots, 3 = necrotic lesions less than 0.5 cm long with 25–50% of stem girdled, 4 = necrotic lesions more than 0.5 cm long with 50–75% of stem girdled, 5 = severe necrotic lesions with plants dead and 100% of stem girdled.

[x] Soil was saturated by holding the Jiffy Strips for 4 days in metal flats containing water to a depth of 2 cm. Soil temperature was maintained at 27 C.

[y] The two columns show the results of separate tests at two different times. Each value is a mean of three replicates (six plants per replicate). Mean separation within each column by Duncan's multiple range test at the 5% level of probability.

[z] Surface irrigation applied frequently. Soil temperature fluctuated between 24 and 29 C.

technique were significantly different ($P \leq 0.05$) from the susceptible check, Little Marvel (Table 1). Both inoculation procedures induced stem rot in pea cultivars and lines tested in the field, but the disease was less severe with the cornmeal-sand technique, which did not differentiate between resistant and susceptible cultivars as well as the corn kernel method. Some cultivars and lines that showed resistance based on the cornmeal-sand technique were susceptible when inoculated with infested corn kernels; that is, the first method may not have been severe enough and may have allowed some plants to escape infection. The corn kernel technique was not laborious, and it provided a way to apply the inoculum directly to the stem of all plants at the same time. It separated susceptible and resistant genotypes and gave consistent resistance ratings under both chamber and field conditions.

The corn kernel inoculation technique also separated resistant cultivars from susceptible cultivars in the growth chamber study where the temperature was controlled (Table 2, columns 1 and 2). On the other hand, when the same technique was used in the second growth chamber, where the temperature fluctuated and surface irrigation was applied frequently, separation of susceptible and resistant genotypes was not consistent. Moreover, the breakdown of resistance in Dark Skin Perfection was noted in one test (Table 2, column 4).

The agar disk method did not separate resistant from susceptible lines within 1 wk (Table 2). However, further tests have indicated that resistant and susceptible genotypes may be distinguished if the test is run for 2 wk or longer.

The three cultivars (Dark Skin Perfection, Freezer 76110, and Wando) and one breeding line (SN3) that showed moderate resistance in the field maintained their resistance when compared with the susceptible cultivar Little Marvel in growth chamber tests. A high level of resistance (disease index of 1) was not found among the 68 genotypes tested (Tables 1 and 2). The moderate resistance found in a few commercial cultivars and breeding lines was not uniform and may break down when environmental conditions favor the pathogen.

The corn kernel inoculation technique, combined with control of moisture and temperature, may provide a good screening procedure to select for moderate resistance to pea stem rot. Selection should be based on plant survival in relation to a susceptible control genotype. To minimize the effect of a pathogen-host-environment interaction, single plant selection for resistance with minimum "escapes" may be started after all plants of the susceptible cultivar collapse and die—in our tests, about 1 wk after inoculation in the water tank method under controlled conditions.

Research on the nature and the heritability of the moderate resistance found in some cultivars and lines in this study is needed. Breeding for moderate resistance to stem rot may be an important step in breeding for resistance to the root rot complex of peas.

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