Seed Electrolyte Loss and Resistance to Fusarium Root Rot of Peas

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ABSTRACT

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Resistance in peas to Fusarium root rot, caused by *Fusarium solani* f. sp. *pisi*, is evidenced by lower disease indices and higher fresh weights of plant tops and roots of resistant lines than of susceptible pea lines. Seed of three *Fusarium*-resistant USDA pea breeding lines (RR-1178, 75-786, and 84-1780) and the susceptible cultivar, Dark Skin Perfection, were stressed by methanol or high humidity and temperature. The relationship between emergence, disease index, fresh weights of plant tops and roots, and flow of electric current through leachates from individual seeds, a measure of electrolyte loss, was calculated by regression analysis on individual seeds and seedlings. Physiological aging (6 days at 35 ± 1 C and 100% relative humidity) slightly increased loss of seed electrolytes during imbibition but did not significantly decrease the level of resistance inherent in all three test lines. However, the methanol stress test significantly increased loss of seed electrolytes with corresponding decrease in resistance to Fusarium root rot. In addition, *F. solani* f. sp. *pisi* sporulated more profusely in leachates from methanol-treated seed of resistant lines than in leachates from the control or physiologically aged seed.

Fusarium root rot of peas (*Pisum sativum* L.), caused by *Fusarium solani* (Mart.) Sacc. f. sp. *pisi* (*F. s.* f. sp. *pisi*) (Jones) Snyd. & Hans., is a serious disease problem of peas in the Pacific Northwest (6). This pathogen primarily infects the cotyledons, cotyledonary attachment area, epicotyl, and hypocotyl (2). Infection occurs early in the growth cycle of the plant, and resistance to *Fusarium* is thought to be genetically linked with resistance to Pythium root rot (11).

In repeated laboratory tests, early radicle growth, rapidity of emergence, and/or resistance to seed and seedling disease caused primarily by *Pythium ultimum* Trow was negatively correlated with electroconductivity readings of the seed steep water (1,7–10,12,14). In general, seeds that give a high conductivity reading germinate and grow slowly (12,14) and solutes are readily leached into the surrounding spermosphere during imbibition (10,12).

Seed imbibition and germination, with a corresponding release of nutrients into the surrounding soil, were shown to trigger germination of chlamydospores of *Fusarium* with subsequent infection of pea seedlings (6). Genetically inherited resistance to Fusarium root rot of peas has been described, and several breeding lines resistant to this disease have been released (2,4,5). Accelerated aging (at 30–40 C, 100% relative humidity [RH]) was reported to significantly increase electrolyte leakage during seed imbibition with a corresponding decrease in seedling vigor (12,14,15). Furthermore, Musgrave et al (13) reported that immersing seed in a 20% methanol solution (v/v) for 2 hr simulated accelerated aging with less time consumed. Krarup and Ross (7), however, reported that round-seeded peas with a low glucose content were resistant to physiologic aging and methanol stress injury.

The recent development of the automatic seed analyzer (16) allows measurement of the flow of electric current through leachates from individual seeds and use of these same seeds in further studies. Because *F. s.* f. sp. *pisi* is similar to *Pythium* in attacking the pea plant early in its life cycle, I investigated whether an increase in the amount of electrolyte lost from imbibing seed of a *Fusarium*-resistant line would reduce the resistant response of that line.

MATERIALS AND METHODS

All seed used in this study was field grown during the 1984 season and was stored at 7 ± 2 C and 45% RH. The seed used did not pass through a screen 18 × 16 mm and had no visible cracks. A 500-g lot of each of three resistant pea lines (75-786, wrinkled seed; 84-1780, wrinkled seed; and RR-1178, smooth seed) and of the susceptible cultivar Dark Skin Perfection (DSP) were surface-disinfected (2) and air-dried, then physiologically aged in the dark at 35 ± 0.5 C and 100% RH for 6 days. Control samples of each lot were also disinfected and left on a laboratory bench. An additional 500-g lot of each line was also subjected to a methanol (MeOH) stress test by immersing in a 20% methanol solution (v/v) for 2 hr. All seed lots, after the designated treatments, were air-dried on a laboratory bench for 48 hr and stored in paper bags in a refrigerator at 3 C until used.

To determine whether physiologic aging or the methanol stress test affected viability of the test line, a random sample of 100 seeds per lot was selected. The seeds were placed in a seed-soaking tray containing 100 individual compartments with each compartment containing about 4.5 ml of glass-distilled water. Each tray was incubated at 22 ± 1 C for 22–24 hr (1). After incubation, the leachate in each compartment containing one seed was read for electric current with the ASAC-1000 automatic seed analyzer (Neogen Food Tech. Corp., Lansing, MI) with a preset voltage of 0.25 to each of the electrodes and all readings displayed as microamperes.

After measuring electric current, the imbibition water was decanted and each seed was planted in flats containing a sandy silt loam artificially infested with *F. s.* f. sp. *pisi* (F51) at an inoculum concentration of about 20,000 colony-forming units per gram (cfu/g) of air-dry soil (2). Seeds were planted in numerical sequence to facilitate keeping track of each seed's electric current reading. All planted flats were placed in a controlled-environment chamber with an illumination of 11,840 lux for a 12-hr photoperiod and day and night temperatures of 23.9 and 18.4 ± 1 C, respectively. All flats were watered as necessary (2).

Ten days after emergence, each seedling was carefully removed (in numerical sequence) from each flat and soil was washed from the roots. All seedlings were rated by a disease index of 0–5, where 5 indicated a completely rotted root. Fresh weights of each plant top (above cotyledonary attachment) and of the roots were also taken.

A completely random, split-plot experimental design was used, and all tests were repeated twice and analyzed by linear regression. Statistical analysis was performed comparing only one treatment with another within a given line.

The ability of *F. s.* f. sp. *pisi* to sporulate in seed leachate water from the

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Table 1. Effect of 6-day physiologic aging or methanol stress on electric current measurements on seed leachates (0.25V), emergence, disease severity, and plant growth on three pea lines resistant to *Fusarium solani* f. sp. *pisi* and the susceptible cultivar, Dark Skin (DS) Perfection

Test line	Treatment	Electric current[u] (microamperes) (avg.)	Emergence[v]	Disease index[w]	Fresh weight (g) Top[x]	Fresh weight (g) Root[y]
DS Perfection (wrinkled seed)	Control	86.3 d[z]	72 a	3.5 a	1.2 a	1.1 a
	6-Day aging	146.8 c	38 b	4.4 b	0.6 b	0.5 b
	MeOH	101.4 b	10 b	4.8 c	0.2 c	0.1 c
RR-1178 (smooth seed)	Control	39.6 a	92 a	2.6 a	1.6 a	1.5 a
	6-Day aging	67.0 b	92 a	2.9 a	1.6 a	1.6 a
	MeOH	89.6 c	69 b	3.6 b	1.2 b	1.1 b
75-786 (wrinkled seed)	Control	42.7 a	97 a	3.0 ab	1.8 a	1.3 a
	6-Day aging	49.2 a	92 a	2.9 a	1.8 a	1.2 a
	MeOH	56.2 b	83 b	3.2 b	1.6 a	1.1 a
84-1789 (wrinkled seed)	Control	49.5 a	96 a	2.6 a	1.9 a	1.4 a
	6-Day aging	51.1 a	100 a	2.7 a	1.9 a	1.3 a
	MeOH	56.9 b	86 b	3.3 b	1.5 b	1.1 b

[u] Data represent average of 100 individual readings (replicated twice).
[v] Percentage of seedlings emerged in *Fusarium*-infested soil from 100 planted. Test was repeated twice.
[w] Based on scale of 0–5, where 0 = healthy plant and 5 = dead.
[x] Above cotyledonary attachment area.
[y] Below cotyledonary attachment area.
[z] Data in each column followed by different letters differ significantly ($P = 0.05$) according to Duncan's multiple range test. Each test line was analyzed separately.

Table 2. Effect of pea seed leachates on the in vitro sporulation of *Fusarium solani* f. sp. *pisi*

Pea line	Treatment	Spore production[y] (conidia/ml [$\times 10^6$], av.)
DS Perfection	MeOH stress	21.5 a[z]
	6-Day aging	7.2 b
	Control	4.9 c
RR-1178	MeOH stress	3.0 d
	6-Day aging	2.1 de
	Control	1.3 e
75-786	MeOH stress	3.2 d
	6-Day aging	1.7 e
	Control	1.1 ef
84-1780	MeOH stress	1.7 e
	6-Day aging	1.3 e
	Control	0.6 f
Water	···	0.09 g

[y] Hemacytometer counts of microconidia and macroconidia of *F. solani* f. sp. *pisi*. (F51) after a 7-day incubation. Data represent an average for three flasks per treatment.
[z] Data followed by different letters differ significantly (P = 0.05) according to Duncan's multiple range test.

various treatments was determined by a bioassay. Seed from the various treatments were disinfected with an Alconox/95% ETOH/15% H_2O_2 treatment (3) and aseptically placed in sterile, glass-distilled water. Ten disinfected seeds from each treatment were placed in 45 ml of sterile, glass-distilled water in 125-ml Erlenmeyer flasks. The flasks (three per treatment) were incubated at 24 ± 1 C in the dark for 24 hr. Before assaying, the leachate in each flask was tested for bacterial contamination and discarded if contaminated. To remove seed fragments and to ensure sterility, the liquid from each flask per treatment was aseptically passed through a 0.2-μ filter and collected. A 5-mm cornmeal agar plug from the margin of a 7-day-old culture of *F. s.* f. sp. *pisi* (F51) was used to inoculate sterile 50-ml flasks containing 10 ml of a seed leachate. The flasks were incubated 7 days at 22 ± 1 C with a 12-hr light period in a rotary shaker-incubator. At harvest, the incubation medium was first strained through a double layer of cheesecloth to remove mycelial fragments. Numbers of microconidia and macroconidia per milliliter of culture medium were determined by hemacytometer counts. Spore counts in each of three flasks were determined separately.

RESULTS

The three *Fusarium*-resistant breeding lines (RR-1178, 75-786, and 84-1780) were definitely more affected by the methanol immersion treatment than by the 6-day accelerated aging test at 35 C (Table 1). It was interesting to note that even the smooth-seeded line (RR-1178) was adversely affected by the methanol immersion. However, all three resistant lines (RR-1178, 75-786, and 84-1780) were still more resistant to *Fusarium* than the untreated susceptible DSP when subjected to methanol stress, as indicated by lower electric current readings, higher emergence rates, and lower disease severity ratings.

The effect of seed leachates on sporulation of *F. s.* f. sp. *pisi* was studied in vitro. The pathogen sporulated more profusely in seed leachates from DSP than in leachates from more resistant lines (Table 2). In addition, the MeOH stress treatment resulted in greater sporulation of *F. s.* f. sp. *pisi* in leachates from all three *Fusarium*-resistant lines than the control or 6-day aging treatment.

Table 3 illustrates the correlation coefficients comparing individual electric current readings of individual seeds with disease index, top weight, and root weight of resultant seedlings of the same test line when grown in *F. s.* f. sp. *pisi*-infested soil. There was a significant positive correlation only within the methanol stress test for each resistant line, which indicates that methanol stress increased electric current readings and disease indices of individual seeds of the resistant lines. Electric current readings were negatively correlated with top and root weights. Resistant line 75-786 was the least affected by the methanol stress test as evidenced by the lack of a significant decrease in fresh weights of tops and roots (Table 1). The susceptible control (DSP) was severely affected by both the accelerated aging and methanol stress test, but the correlation was not significant.

DISCUSSION

In this study, an increase of electrolytes in the seed steep water was significantly correlated with a decrease in resistance of three breeding lines to Fusarium root rot. These results are similar to those from previous studies that demonstrated an association between the amount of electrolyte leached from pea seeds during imbibition and severity of Pythium seed decay (9,10). It is interesting to note that genetic resistance to both *F. s.* f. sp. *pisi* and *Pythium* spp. is thought to be governed by the same genetic factors (11). Both *Pythium* and *F. s.* f. sp. *pisi* are capable of attacking the pea plant early in its growth stage, and both pathogens are influenced by the quantity and quality of the seed and seedling exudate at the infection site (3). An increase in electrolytes most likely indicates an increase in nutrients being exuded during imbibition, which improves the nutritional status of the pathogen at the infection site (Table 2) (3,6). Deceased plant vigor, as indicated by an increase in solute leakage, has also been associated with increased Fusarium root rot severity (6).

Fusarium root rot of peas begins as a black decay in the cotyledonary attachment area. This area is close to the cotyledons, which are the source of solute leakage. There is a direct relationship between the number of chlamydospores stimulated to germinate near pea seeds and the number of infection thalli of *F. s.*

f. sp. *pisi* on nearby epicotyl, hypocotyl, and root tissues (3,6). Thus, leachates from a germinating pea seed exert a significant influence not only on seed infection but also on infection of root and stem tissue contiguous to the seed. Apparently this increase in solute leakage with a corresponding decrease in seed vigor is responsible for a decline in *Fusarium* resistance. These results emphasize the importance of vigorous pea seed and seedlings in any attempt to control Fusarium root rot. This study also indicates the importance of good seed storage conditions and helps to explain why breeding lines or plant introduction accessions found resistant to Fusarium root rot may not be resistant after prolonged storage under less than ideal conditions.

It was interesting to note that all three resistant lines were not affected by the accelerated aging compared with DSP (susceptible), which was severely affected by this treatment. In contrast, the methanol stress test was more severe than 6-day physiologic aging. Methanol stress increased the amount of electrolyte in the steep water and resulted in higher disease indices of the resistant lines in infested soil.

Musgrave et al (13) reported that the effect of methanol stress on pea seed was very similar to accelerated aging and demonstrated a massive leakage of solutes from soybean seed. This leakage occurred during the 2-hr methanol treatment and persisted after treatment. With resistant pea breeding lines, the methanol stress treatment was severe enough to demonstrate a relationship between solute leakage and a breakdown in *Fusarium* resistance, whereas the 6-day accelerated aging process was not. Further studies are needed to determine if reduced seed vigor is responsible for a decline in *Fusarium* resistance or if the decline due to enhanced *Fusarium* nutrition (and virulence) at the infection site or both.

Table 3. Correlation coefficients comparing electric current readings of individual seeds with disease index, top weight, and root weight of the resultant seedlings for four test lines with three treatments when grown in *Fusarium*-infested soil

Test line	Treatment	Disease index	Fresh weight (g)	
			Top	Root
DS Perfection	Control	0.59	−0.62	−0.53
	6-Day aging	−0.05	−0.11	−0.08
	MeOH	−0.14	0.25	0.19
RR-1178	Control	0.30	−0.10	−0.30
	6-Day aging	0.26	−0.31	−0.19
	MeOH	0.59[a]	−0.68	−0.72
75-786	Control	0.17	−0.19	−0.11
	6-Day aging	0.58	−0.59	−0.45
	MeOH	0.70	−0.64	−0.59
84-1780	Control	0.24	−0.40	−0.25
	6-Day aging	0.32	−0.44	−0.08
	MeOH	0.78	−0.73	−0.68

[a] Values exceeding $|r| \geq 0.677$ are significant at $P = 0.05$.

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