The Influence of Seedling Exudates on the Resistance of Peas to Fusarium and Pythium Root Rot

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ABSTRACT

Peas with pigmented seeds, both resistant and susceptible to root rot, produced like amounts of phenols and reducing sugars in exudates from germinating seeds and seedlings. However, only exudates from resistant lines (P.I. Nos. 140165 and 257593) inhibited sporulation of *Fusarium solani* f. sp. *pisi*, growth of *Pythium ultimum* in vitro, and conidial germination of *F. solani* in soil. Lesions, caused by *F. solani*, on epicotyls of 4- and 6-day-old resistant plants were fewer and coalesced less rapidly than on epicotyls of susceptible plants. Factors, other than phenols and reducing sugars in seed and seedling exudates may also play a role in the resistance of peas to *F. solani* and *P. ultimum*.

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Root rots play a major role in limiting production of processing and seed peas (*Pisum sativum* L.) in the Pacific Northwest (6, 10, 19, 20). Results from previous studies (10, 20) have shown that *Fusarium solani* (Mart.) f. sp. *pisi* (F. R. Jones) Snyd. & Hans. and *Pythium ultimum* Trow are the two major pathogens of pea roots in the Pacific Northwest. Sources of resistance to these pathogens have been described (8, 9, 12, 13, 14, 15, 16), and most lines reported to be resistant have dark-colored seeds and purple or lavender flowers.

Phenolic compounds in the exudates from germinating seeds and seedlings, are reported to be responsible for resistance in peas to *Ascochyta pisi* Lib. (1) and in beans (*Phaseolus vulgaris* L.) to *F. solani* (Mart.) Appel & Wr. f. sp. *phaseoli* (Burk.) Snyd. & Hans. (21). These phenolic compounds were found to be more abundant in bean and pea selections with colored seeds and/or flowers than in selections with less pigment. Pea cultivars with wrinkled seeds were reported to be more susceptible to pre-emergence attack by *Pythium* because of a higher sugar content in exudates, as compared to smooth-seeded varieties (5).

In previous studies (11, 12) on the resistance of Plant Introduction (P.I.) lines with colored seed, the following observations were made: (i) resistance to *F. solani* f. sp. *pisi* was not altered by an increase in inoculum density; (ii) populations of *Fusarium* increased less in soil surrounding resistant pea roots than in soil surrounding susceptible pea roots; and (iii) both *Fusarium* and *Pythium* sporulated or grew less profusely in exudates from germinating seedlings of resistant peas than in exudates from susceptible ones.

In the present study I wished to determine: (i) whether the inhibition of root pathogens by exudates from seedlings of resistant pea selections was related to their content of phenols or sugars; (ii) whether this inhibition also occurred during seed germination in soil; and (iii) whether resistant lines possess additional mechanisms of resistance to root rot, other than inhibition of pathogens by seedling exudates.

MATERIALS AND METHODS.—*Sources of germplasm.*—In a continuing search for pea germplasm resistant to root rot (11, 12), 115 of 900 lines tested have shown a level of resistance greater than that of 'Dark Skin Perfection' (D.S.P.). Two of these lines (P.I. 140165 and P.I. 257593), with colored seed and well-defined resistance to *F. solani* f. sp. *pisi* and *P. ultimum*, were used in this study. Dark Skin Perfection and three other susceptible lines with colored seed (P.I. 138945, P.I. 165965, and P.I. 253968) were included as controls.

Phenols and sugars in seedling exudates.—Seedling and root exudates were collected according to procedures previously described (12, 18). Analyses of all exudates for total phenols and reducing sugars were made according to the procedures of Johnson and Schaal (7) and Miller (17), respectively. Collections and analyses were repeated three or more times. Sporulation of *F. solani* f. sp. *pisi* and growth of *P. ultimum* in the exudates were also measured (12).

Germination of conidia in soil near pea seeds.—Macroconidia of *F. solani* f. sp. *pisi* were washed from 7-day-old cultures on fresh potato-dextrose agar with sterile glass-distilled water. The suspension was diluted to 3×10^6 spores/ml in a volume of 300 ml. This suspension was then treated with 200 μg/ml of the vital fluorescent stain 'Calcofluor' (22) and left standing on a laboratory bench for 8 h. The labeled conidial suspension was sprayed and thoroughly mixed into 1 kg of uncropped sandy loam soil (10), bringing the soil moisture to 16%. Infested soil was distributed in flat-bottomed petri dishes, 150 g per dish, and six surface-disinfested seeds (12) were placed in each dish. Beads of sterile glass, approximately seed size, were used as controls. The dishes were incubated on a laboratory bench. After a 24- and 48-h incubation at 20-25 C, three seeds were removed from each dish, and soil adhering to each seed surface was washed onto separate microscope slides (2).

The labeled conidia in soil smears were viewed with a Zeiss Model WL microscope equipped with a H30 mercury vapor lamp, a BG excitation filter, and an ultraviolet-absorbing barrier filter. This study was repeated three times.

Numbers of infection sites on susceptible and resistant

TABLE 1. The in vitro effects of phenols and reducing sugars in seedling exudates on the sporulation of *Fusarium solani* f. sp. *pisi* and growth of *Pythium ultimum*

Pea line	Seed coat color	Root rot reaction[a]	Phenols[b] (mg/100 ml)	Reducing sugars[c] (mg/ml)	Sporulation[d] of *F. solani*	Growth[d] of *P. ultimum* (mg)
138945	Mottled	S	0.14	0.21	17.0×10^5 a[e]	4.4 c
140165	Mottled	R	0.14	0.18	0.6×10^5 c	0.0 d
165965	Mottled	S	0.11	0.19	10.2×10^5 b	4.9 c
253968	Mottled	S	0.10	0.18	14.2×10^5 a	6.7 b
257593	Mottled	R	0.20	0.22	0.9×10^5 c	0.0 d
D.S. Perf.	Green	S	---	0.17	16.6×10^5 a	14.2 a
H_2O control	---	---	---	---	0.3×10^5 c	0.0 d

[a] Root rot reaction: S = susceptible as cultivar 'Dark Skin Perfection', R = more resistant than cultivar 'Dark Skin Perfection'.
[b] mg/100 ml using a gallic-acid standard.
[c] Total reducing sugars were determined by use of a dinitrosalicylic-acid reagent and recorded as mg/ml of a glucose equivalent.
[d] Sporulation of *F. solani* was measured by haemocytometer counts of spore suspensions. Growth of *P. ultimum* was recorded as oven-dry wt of mycelium.
[e] Data within the same column followed by the same letter are not significantly different at the 5% level.

seedlings.—Pea seedlings were grown in plastic containers (5.7 × 5.7 cm) in soil prepared as above containing *F. solani* f. sp. *pisi* at an inoculum density of 2.5^5 - 10^5 spores/g. Two surface-disinfested seeds of each test line were planted 2 cm deep in 100 g of soil. The containers were covered with aluminum foil for 48 h to maintain soil moisture during germination. The containers were then randomly positioned on a laboratory bench under fluorescent light with ca. 9,684 lx (900 ft-c) and a 12-h photoperiod. After 4 and 6 days, ten seedlings of each pea selection were removed and stained according to the procedure of Cook and Snyder (3). The number of lesions on the epicotyl of each plant was counted with the aid of a dissecting microscope. Seedlings grown in noninfested soil served as controls. This study was repeated two times, with five replications of two plants.

RESULTS.—*Phenols and reducing sugars.*—Seedling exudates from both susceptible and resistant colored-seeded P.I. lines contained comparable amounts of phenols (Table 1). In contrast, exudates from D.S.P., a green-seeded variety, contained only trace amounts of phenols. Even though exudates from the colored-seeded susceptible lines contained phenols, germination of *F. solani* f. sp. *pisi* was not inhibited, and growth of *P. ultimum* was affected only slightly. Amounts of reducing sugars produced by susceptible lines were comparable to those produced by the resistant P.I. lines 140165 and 257593.

Germination of conidia in soil near germinating seeds.—Germination of *F. solani* f. sp. *pisi* macroconidia after 24 h was less frequent in soil around seeds of the resistant selections 140165 and 257593 than around seeds of the susceptible lines (Table 2). After 48 h, the conversion of conidia to chlamydospores increased around seeds of the resistant lines. However, in soil around susceptible seeds, the conversion to chlamydospores did not increase, but the numbers of conidia with actively growing hyphae did increase.

TABLE 2. Macroconidial germination of *Fusarium solani* f. sp. *pisi* in soil near germinating pea seed, as determined by fluorescence microscopy

Pea line	Root rot reaction[a]	Incubation time (h)	No. macroconidia observed[b]	Macroconidial germination[c] (%)
138945	S	24	511	66.4
		48	336	77.8
140165	R	24	761	46.8
		48	643	38.5
165965	S	24	226	65.7
		48	316	70.6
253968	S	24	283	70.2
		48	233	81.6
257593	R	24	992	48.0
		48	546	40.4
D.S. Perf.	S	24	629	72.6
		48	634	75.6
Glass beads (control)		24	559	28.0
		48	244	13.9

[a]Root rot reaction: S = susceptible as cultivar 'Dark Skin Perfection', R = more resistant than cultivar 'Dark Skin Perfection'.

[b]Average of three experiments with three seeds of each pea selection per incubation time.

[c]Macroconidia converted to chlamydospores were not counted as germinated.

TABLE 3. Infection sites on 4- and 6-day-old pea seedlings (epicotyls) grown in soil artificially infested with *Fusarium solani* f. sp. *pisi*

Pea line	Root rot reaction[a]	Incubation time days	No. of infection sites[b]
P.I. 140165	R	4	3.3
		6	7.6
P.I. 253968	S	4	11.7
		6	38.4[c]
P.I. 257593	R	4	9.4
		6	15.6
D.S. Perf.[a]	S	4	41.3[c]
		6	---[d]

[a]Root rot reaction:S = susceptible as cultivar 'Dark Skin Perfection', R = more resistant than cultivar 'Dark Skin Perfection'.

[b]These data represent averages of three experiments with five replications of 10 plants per test line.

[c]Lesions were beginning to coalesce.

[d]Lesions had coalesced and could not be counted.

Numbers of infection sites on resistant and susceptible seedlings.—Infection sites on 4-day-old epicotyls of resistant seedlings (140165 and 257593) were fewer than on epicotyls of D.S.P. and 253968 (Table 3). After 6 days, the numerous lesions on D.S.P. and 253968 had started to coalesce, but the relatively few lesions on the resistant lines had not.

DISCUSSION.—Clauss (1) and Statler (21) concluded that phenols, present in seed coats and cotyledons of dark-seeded pea and bean selections, were responsible for root rot resistance. The present study demonstrated that total quantities of phenols and reducing sugars in exudates from pea selections are probably not related to Fusarium and Pythium root rot resistance. If specific phenols or reducing sugars in exudates from resistant lines are responsible for the demonstrated inhibition of *F. solani* f. sp. *pisi* and *P. ultimum,* they must be present in larger amounts in the exudates from resistant plants.

I could find no correlation between amounts of phenols and resistance in comparisons of root exudates from 1-wk-old plants or from 3-wk-old root tissue of susceptible and resistant plants (J. M. Kraft, *unpublished*). Statler (21) concluded that phenolic compounds are important in the resistance of bean plants to *F. solani* f. sp. *phaseoli* only in seed germination and early seedling development. Phenols detected in exudates from dark-seeded pea lines were found to originate primarily in the seed coat (J. M. Kraft, *unpublished*). If specific phenols are involved in the inhibition of root

pathogens of peas, then perhaps peas are similar to beans in expressing resistance only during the early stages of growth.

As illustrated in Table 1, there were no large differences in amounts of reducing sugars exuded by susceptible and resistant pea selections. This perhaps reduces the importance of pathogen nutrition at or near the seed surface as a factor in resistance or susceptibility.

The resistance to spread of infection by *F. solani* f. sp. *pisi* in the resistant pea selections 140165 and 257593 is of interest. Whether this resistance is related to differences in pisatin production, as initially described by Cruickshank and Perrin (4) is under investigation. Perhaps the inhibition of conidial germination and growth of *F. solani* in soil around resistant seedlings was sufficient to reduce the inoculum level to a point were numbers of infection sites were reduced (Table 3). However, reduced numbers of infection sites does not fully account for the observed resistance to spread of infection.

Research is in progress to determine whether specific phenolic compounds or other components of seed and seedling exudates are involved in the resistance mechanism. Also to be determined, is whether resistance to spread of infection is independent of the pathogen-inhibitory compounds in seed and seedling exudates. Such information should expedite selecting peas for resistance to Fusarium and Pythium root rot.

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