Resistance in Peas to Fusarium and Pythium Root Rot

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ABSTRACT

Accessions 140165, 183910, 194006, 210587, and 223285 exhibited resistance to *Fusarium solani* f. sp. *pisi*, *Pythium ultimum*, and the combination of these two pathogens at all inoculum levels tested. In 28 days, populations of *F. solani* f. sp. *pisi* increased more in rhizosphere soil from roots of susceptible Dark Skin Perfection than in soil from the roots of resistant lines. Furthermore, *F. solani* f. sp. *pisi* did not sporulate as readily in seedling exudates from the resistant lines as in seedling exudates from Dark Skin Perfection. An increase in population of *P. ultimum* could not be detected in the rhizosphere of either susceptible or resistant lines; however, *P. ultimum* did not grow as readily in seedling exudates from the resistant lines as in exudates from Dark Skin Perfection. Phytopathology 60:1814-1817.

Additional key words: controlled environment, selective media, seed exudate collection.

Root diseases of peas (*Pisum sativum* L.) are a serious production problem in the USA (19, 21, 24) and especially in the Pacific northwest (5, 11, 22). *Fusarium solani* (Mart.) Appel & Wr. f. sp. *pisi* (F. R. Jones) Snyd. & Hans. and *Pythium ultimum* Trow are the principal pathogens involved in this disease complex in the Pacific northwest (11). Major emphasis in evaluating peas for root-rot resistance has been focused on these pathogens.

There are reports on evaluation of pea lines for resistance to *Aphanomyces euteiches* Drechs. (4, 13, 14, 16), *F. solani* f. sp. *pisi* (3, 7, 8, 13, 15, 16), and *Pythium spp.* (2, 17). Although sources of resistance to each pathogen have been found, there are no reports on the evaluation of peas for resistance to the pea root-rot complex of the Pacific northwest.

Our objectives were to determine (i) if resistance to both *F. solani* f. sp. *pisi* and *P. ultimum* can exist in the same pea plant; (ii) if this resistance is affected by changes in inoculum concn or exposure to both pathogens simultaneously; (iii) what effect a resistant or susceptible pea plant has on the population of *P. ultimum* or *F. solani* f. sp. *pisi* in soil; and (iv) the effect of seedling exudates on growth of these fungi in culture. A portion of the work was reported previously (12).

MATERIALS AND METHODS.—Beginning in 1966, we screened Plant Introduction accessions in a composite soil collected from several pea fields in Washington, Oregon, and Idaho known to be infested with *F. solani* f. sp. *pisi* and *P. ultimum*. Plants were grown in this soil on a greenhouse bench. After 28 days, plants were dug (26 plants/3 replications) and compared with a standard commercial variety, Dark Skin Perfection (DSP). Of 600 lines tested, 58 were less diseased (i.e., they had more vigorous root systems and fewer rotted lateral roots) than DSP. These resistant lines were further evaluated for specific resistance to *F. solani* f. sp. *pisi* and *P. ultimum*.

Tests for specific resistances were conducted in controlled environment rooms at 24 C, with a 12-hr photoperiod and 1,100-1,500 ft-c of illumination at plant height. All pea lines tested were planted in an uncropped, nonpasteurized, desert, sandy loam soil (11) artificially infested with either or both pathogens. Control plants were grown in noninfested soil.

Inoculum of *F. solani* f. sp. *pisi* was produced by incubation of the fungus in Kerr's liquid medium (6) in shake culture for 7 days at 24 C. Inoculum of *P. ultimum* was incubated on sterile vermiculite saturated with a complete basal medium (9) for 7 days at 26 C. Soil was infested with each pathogen according to the method previously described (11).

Infested soil was prepared for use by air drying for 14 days to induce both organisms to form resistant structures (1, 6). Propagule numbers were determined by the soil dilution plate technique using Nash & Snyder's PCNB (18) and Tsao & Ocana's $P_{10}PV$ (23) media for *F. solani* f. sp. *pisi* and *P. ultimum*, respectively. The levels of these fungi in rhizosphere soil at harvest were also determined by the dilution plate technique. The soil that adhered to the roots of each test line after digging was shaken into paper bags and stored for 1 week or less at room temp until assayed.

Inoculum levels were adjusted by adding noninfested soil. Unless stated otherwise, population levels of *F. solani* f. sp. *pisi* and *P. ultimum* varied between 6,000-10,000 propagules/g and 700-2,500 propagules/g, respectively. In treatments where peas were grown in soil containing both pathogens, infested soils were mixed proportionally. This resulted in a population level of 1,000-5,000 propagules of *F. solani* f. sp. *pisi* and 500-1,000 propagules of *P. ultimum*/g of air dry soil.

Seed were dusted with *N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide (captan) to prevent seed rot, and planted (4 seeds/pot) in 10-cm plastic pots containing infested or noninfested soil. All seed was planted approximately 3 cm deep and covered with sterile, coarse sand to insure uniform emergence. All

pots were watered with Millipore-filtered (0.45 μ) tap water with soil moisture adequate for uniform disease expression of both pathogens (11). A disease index, developed by Lockwood (13), was used where 0 = healthy and 9 = death. All tests were repeated at least twice with six replications/treatment.

The ability of *P. ultimum* and *F. solani* f. sp. *pisi* to grow or sporulate in seedling exudates was determined by use of a rapid bioassay. Several lots of untreated seed from various resistant P.I. accessions and DSP (10 seeds/lot) were disinfested (10) and aseptically placed in sterile 125-ml Erlenmeyer flasks containing 50 ml of sterile, glass-distilled water. The flasks (10/pea accession) were incubated at 24 C in an incubator-shaker set to make one cycle/sec. The length of the incubation period was adjusted so that 90-100% of the seeds had germinated and had radicles approximately 1 cm long when harvested. Before assaying, the liquid in each flask (exudate plus water) was tested for bacterial contamination, and any flasks found to be contaminated were discarded. To remove seed fragments and to insure sterility, the liquid from each flask was separately passed through a 0.22 μ Millipore filter.

A 5-mm potato-dextrose agar plug from the margin of a 24-hr-old culture of *P. ultimum*, or from a 72-hr-old culture of *F. solani* f. sp. *pisi*, was used to inoculate sterile 125-ml flasks containing 25 ml of a seedling exudate. Both *P. ultimum* and *F. solani* f. sp. *pisi* were incubated 7 days at 24 C in an incubator-shaker. Mycelial mats of *P. ultimum* were washed in distilled water, dried at 65 C for 24 hr, and weighed to the nearest mg. Variation in the wt of mycelial mats among six replicates/treatment was 10% or less. To detect differences in sporulation of *F. solani* f. sp. *pisi*, the incubation medium was first strained through a double layer of cheesecloth to remove mycelial fragments. Counts of micro- and macroconidia per ml of culture medium were determined by use of a hemacytometer. Spore counts in each of six flasks/seedling exudate were determined separately.

RESULTS.—Selected pea accessions when grown in soil artificially infested with *F. solani* f. sp. *pisi* and/or *P. ultimum* were more resistant than DSP to both pathogens and to a combination of them (Table 1).

TABLE 1. Root rot development in pea lines grown in soil artificially infested with *Fusarium solani* f. sp. *pisi*, *Pythuim ultimum*, or both

P.I. accession no. or var.	Disease index[a]		
	F. solani f. sp. *pisi*	*P. ultimum*	Combination
140165	3.1 b[b]	2.4 a	2.5 a
183910	2.3 a	2.6 a	4.4 a
194006	2.3 a	2.9 a	3.3 a
210587	3.9 b	3.6 a	4.2 a
223285	2.6 a	3.1 a	3.2 a
Dark Skin Perfection	6.1 c	6.2 b	7.3 b

[a] Average of three experiments with six replications of four plants/treatment. Disease index is based on a 0-9 rating, where 0 = healthy and 9 = dead.

[b] Duncan's multiple range test used at 5% level. Treatments with same letter are not significantly different.

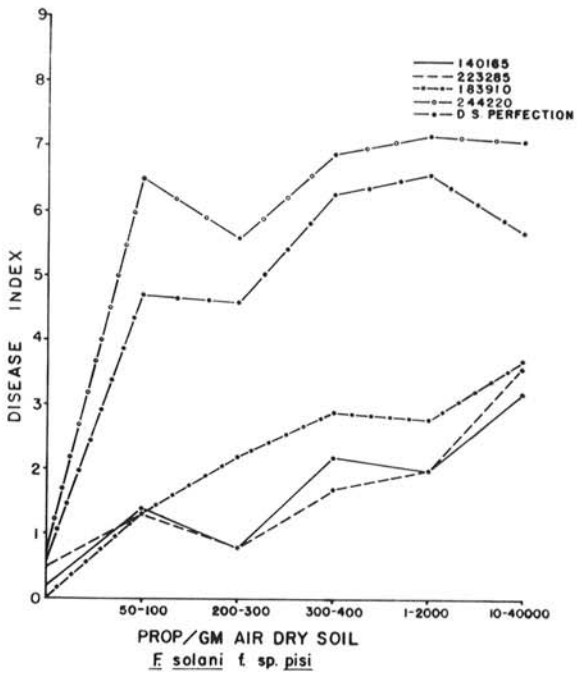

Fig. 1. Effect of inoculum concn of *Fusarium solani* f. sp. *pisi* in soil on severity of root rot in resistant and susceptible pea lines after a 28-day growth period.

Figure 1 illustrates the effect of varying soil inoculum concn of *F. solani* f. sp. *pisi* from a low (50-100 propagules/g) to a high (10,000-40,000 propagules/g of soil) on disease severity. Accessions 140165, 183910, and 223285 were more resistant to *F. solani* f. sp. *pisi* than the susceptible accession 244220 or DSP at all inoculum levels.

Rhizosphere.—To determine the effect of resistant or

TABLE 2. The effect of resistant and susceptible peas on the population of *Fusarium solani* f. sp. *pisi* in the rhizospheres from 28-day-old plants starting with a low and a high soil population

P.I. accession no. of var.	Propagules of *F. solani* f. sp. *pisi*/g of soil[a]		
	Initial	Rhizosphere soil	Disease index
140165	170	870	2.3
	10,500	16,000	4.3
183910	170	1,260	1.4
	10,500	16,600	2.3
210587	170	1,000	3.1
	10,500	15,400	4.6
223285	170	1,095	2.3
	10,500	17,600	3.3
Dark Skin Perfection	170	5,100	5.2
	10,500	29,500	6.6

[a] Average of two experiments with six replications of four plants/treatments.

TABLE 3. The effect of resistant and susceptible pea lines on the build-up of *Fusarium solani* f. sp. *pisi* and *Pythium ultimum* in rhizosphere soil

P.I. accession no. or var.	Propagules of *F. solani* f. sp. *pisi* and *P. ultimum* per gram of soil[a]				Disease index
	Initial count		Rhizosphere count		
	F. solani	*P. ultimum*	*F. solani*	*P. ultimum*	
140165	5,000	2,310	4,300	340	4.6
223285	5,000	2,310	2,950	400	4.1
Dark Skin Perfection	5,000	2,310	23,000	530	7.3

[a] Average of two experiments with six replications.

susceptible peas on the population of *F. solani* f. sp. *pisi* in rhizosphere soil, plants were grown in soil with a low (170 propagules/g of soil) and a high (10,500 propagules/g of soil) population (Table 2). The fungus did not increase in population as profusely, in either the low or high inoculum level rhizosphere soils, around the resistant pea roots as it did around DSP roots.

Two resistant accessions and DSP were grown in soil infested with both *F. solani* f. sp. *pisi* and *P. ultimum*. *Fusarium solani* reproduced profusely only in rhizosphere soil surrounding DSP roots (Table 3). There was no detectable increase in population of *P. ultimum* in soil surrounding either the resistant or DSP roots. This was also true when peas were grown in soil infested only with *P. ultimum*.

Seedling exudates.—The effect of seedling exudates on the growth or sporulation of *P. ultimum* and *F. solani* f. sp. *pisi* was studied in vitro. *Pythium ultimum* grew more in the exudates from DSP than in other seedling exudates or water (Table 4). Similarly, *F. solani* sporulated more profusely when incubated in seedling exudates from DSP than in seedling exudates from the more resistant lines.

DISCUSSION.—These studies indicated that a measurable level of resistance to two important root rot pathogens exists in the same pea accession. The resistance expressed by several test lines to the individual pathogens was not altered by subjecting the root system to both pathogens simultaneously. This is noteworthy because several pea lines not discussed in this paper had resistance to only one pathogen or were susceptible to both.

Resistance to *F. solani* f. sp. *pisi* was expressed over a range of inoculum concn. This is similar to what Lockwood (14) found in evaluating various P.I. accessions for resistance to *A. euteiches*. An inoculum concn for *P. ultimum* was not discussed in this paper for 2 reasons: (i) when we lowered the population below 500 propagules/g, disease severity on DSP was drastically reduced; and (ii) it was difficult to raise the inoculum concn above 2,500 propagules/g due to the technique used in this study.

TABLE 4. The effect of seedling exudates on the growth of *Pythuim ultimum* and sporulation of *Fusarium solani* f. sp. *pisi*[a]

Source of exudate	Incubation time	*P. ultimum*, dry wt.	Spore production *F. solani* f. sp. *pisi* condia/ml
Water control		1.0	9×10^4
P.I. 140165	24 hr	2.9	90×10^4
P.I. 183910	24 hr	2.4	93×10^4
P.I. 210587	48 hr	1.9	19×10^4
P.I. 223285	24 hr	3.2	100×10^4
Dark Skin Perfection	48 hr	10.1	400×10^4

[a] The pH of all exudates and water varied between 4.8 and 5.5 initially, and between 4.6 and 5.8 at the end of each experiment.

Fusarium solani f. sp. *pisi* did not increase as readily in rhizosphere soil around resistant roots as DSP. Nor did this fungus sporulate as profusely in the seedling exudates from the resistant lines as in the exudates from DSP. Although *P. ultimum* did not increase in the rhizosphere, mycelial growth was retarded in seedling exudates from the resistant lines, but was not affected by exudates from DSP. *Pythium ultimum* produces relatively few sporangia and oospores which are often embedded or on the surface of cortical tissues, which may explain the failure of DSP to stimulate an increased population of *P. ultimum* in rhizosphere soil. In contrast, *F. solani* f. sp. *pisi* produces abundant conidia on the surface of the host which can be readily washed off to the surrounding soil.

The seedling assay technique was rapid, and can be easily adapted for working with small seed lots. Seedling exudates can be obtained in 24-48 hr, and the seedlings can be saved by transplanting to soil. Whether the results of a bioassay will always agree with results obtained by growing plants in infested soil will require further experimentation.

Reyes & Mitchell (20) found that *F. solani* f. sp. *pisi* increased in population in rhizospheres of susceptible peas, but not in rhizospheres of nonhosts. The present study illustrates that *F. solani* f. sp. *pisi* did not readily increase in population in rhizosphere soil from resistant pea roots, as compared to rhizosphere soil from susceptible DSP roots. Seed leachates, and perhaps seedling and/or root exudates, may contribute to that resistance. Further work should help to determine what mechanisms are involved in this resistance.

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