Response of Susceptible and Moderately Resistant Pea Genotypes to Interaction Between *Rhizoctonia solani* and Three Other Stem and Root Rot Pathogens

M. A. SHEHATA, Associate Scientist, Department of Horticultural Science and Landscape Architecture, F. L. PFLEGER, Associate Professor, Department of Plant Pathology, and D. W. DAVIS, Professor, Department of Horticultural Science and Landscape Architecture, University of Minnesota, St. Paul 55108

ABSTRACT

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Susceptible and moderately resistant peas (Little Marvel, Dark Skin Perfection, Minnesota 494-A11, and PI 257593) grown in the greenhouse in pasteurized soil beds were inoculated with *Rhizoctonia solani* AG4, *Fusarium solani* f. sp. *pisi*, *Pythium ultimum*, and *Aphanomyces euteiches* separately and in combinations. These combinations were *R. solani* plus *F. solani* f. sp. *pisi*, *R. solani* plus *P. ultimum*, *R. solani* plus *A. euteiches*, and the four-pathogen combination. Stem rot severity varied with the pathogen used in the inoculation process, either alone or in combination, as well as with cultivar. Severe stem and root rot developed when Little Marvel, a susceptible genotype, was inoculated with *F. solani* f. sp. *pisi* alone or in combination with *R. solani* or when all pathogens were combined by sequential inoculation. PI 257593, however, which is moderately resistant to either *R. solani* or *F. solani* f. sp. *pisi*, failed to maintain stem and root rot resistance when inoculated with either *R. solani* plus *F. solani* f. sp. *pisi* or with the four-pathogen combination. A similar relationship was found between *R. solani* and *F. solani* f. sp. *pisi* on Minnesota 494-A11, which is moderately resistant to *F. solani* f. sp. *pisi*, as reflected by root rot and plant mortality.

Stem and root diseases caused by *Rhizoctonia solani* (Kühn), *Fusarium solani* (Mart.) Appel & Wr. f. sp. *pisi* (Jones) Snyd. & Hans., *Pythium ultimum* (Trow), and *Aphanomyces euteiches* (Drechs.) continue to be a major problem on green peas, *Pisum sativum* L., and are often considered the limiting factor in production (2,4,7,8,13,15,18,22). These four pathogens may be present and active in the same soil, causing pea diseases independently or in combination, depending on the soil and environmental conditions that exist throughout the growing season.

Interaction between soilborne plant pathogens has been reported in some crops. All possible combinations of *F. solani* f. sp. *phaseoli*, *P. ultimum*, and *R. solani* were investigated on Red Kidney beans, and no combination was found to incite more severe root rot than that caused by *F. solani* f. sp. *phaseoli* alone (16). A synergistic relationship was found, however, when plants were inoculated with *P. ultimum* plus *F. solani* f. sp. *phaseoli*, whereas an antagonistic relationship was reported with *R. solani* plus *P. ultimum*, and finally, no interaction was found with the combination of *R. solani* plus *F. solani* f. sp. *phaseoli* (17). A synergistic relationship between *R. solani* and *F. solani* was found on potato tubers, and disease severity varied with the sequence of pathogen infection (5).

Limited studies on the interaction of various combinations of several pathogens have been reported in peas. Pea plants inoculated with *Pythium* spp. and *F. solani* f. sp. *pisi* developed more severe root rot symptoms than plants inoculated with either pathogen alone (6). Infection of peas by both *A. euteiches* and *P. ultimum* did not increase the severity of pea root rot over that caused by *A. euteiches* alone (1). The severity of pea root rot caused by either *P. ultimum* or *F. solani* f. sp. *pisi* or the two in combination was found to be affected by soil-water tension and temperature (12).

Although resistance to stem rot caused by *R. solani* AG4 has been investigated (19), the relationship between *R. solani* stem rot and other root rot pathogens is not well understood. The objective of this study was to determine the response of susceptible and moderately resistant pea genotypes to the interaction between *R. solani*, *F. solani* f. sp. *pisi*, *P. ultimum*, and *A. euteiches*.

MATERIALS AND METHODS

Four pea genotypes, Minnesota 494-A11, Dark Skin Perfection, PI 257593, and Little Marvel, were used. Three of these gentoypes have been shown to have moderate resistance to single pathogen inoculations as follows: Minnesota 494-A11 to *A. euteiches*, *F. solani* f. sp. *pisi*, and *P. ultimum* (10); Dark Skin Perfection to *R. solani* AG4 (19); and PI 257593 to *F. solani* f. sp. *pisi* (11), *P. ultimum* (21), and *R. solani* AG4 (19). Little Marvel was included because in our screening tests, we have found it susceptible to all of these pathogens.

The reactions of each of the four pea genotypes to the four pathogens and combinations of the four were studied in greenhouse tests during the winters of 1980–1981 and 1981–1982. Pasteurized soil consisting of loam, peat moss, and sand (1:1:1, v/v) was placed in transite benches 1 m above the gravel floor. Each bench was subdivided into plots separated by plastic sheets. Alleys 15 cm wide between plots were filled with dry sand to reduce contamination among pathogens.

In each year, a completely randomized block design was used in which there were four genotypes and nine pathogen treatments. There were three replicates. Plots consisted of four single rows 105 cm long spaced 12.5 cm apart, with one row for each genotype. Seeds were treated with 1% sodium hypochlorite for 15 min and rinsed several times with water before planting. Twenty-five seeds were planted per row and the stands reduced to 20 seedlings by removing weak and late-germinated plants.

Air temperature in the greenhouse was maintained near 23 C during the day and 19 C at night and a high level of soil

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moisture was provided by frequent irrigation.

One week after planting, seedlings were inoculated with *R. solani*, *F. solani* f. sp. *pisi*, *P. ultimum*, or *A. euteiches* separately or with the three possible two-pathogen combinations containing *R. solani* or with the four-pathogen combination. Inoculum consisted of corn kernels colonized by *R. solani* for 2 wk (19), a microconidial and macroconidial suspension of *F. solani* f. sp. *pisi* at 1×10^6/ml (14), agar disks 4 mm in diameter taken from 4-day-old cultures of *P. ultimum* (9), and a zoospore suspension of *A. euteiches* at 1.5×10^5/ml (3). Plants were inoculated singly or in the appropriate pathogen sequence by placing a single infected corn kernel or an agar disk of *P. ultimum*, by pipetting 1 ml of zoospore suspension of *A. euteiches*, or by pipetting 1 ml of conidia of *F. solani* f. sp. *pisi* into a depression measuring 1 × 0.5 cm made in the soil adjacent to the stem of each plant. The inoculum and hole were then covered gently with the soil medium. The sequence of inoculation was *R. solani*, *F. solani* f. sp. *pisi*, *P. ultimum*, and *A. euteiches*. When control plants of each genotype were at full bloom, all control and inoculated plants of that genotype were gently removed, washed, and rated separately for stem and root rot based on a 1–5 index in which 1 = no symptoms and 5 = severe symptoms with dead plants. Percentage of dead plants and dry weights of plants per plot also were determined. Combined analyses of variance were used in testing the effect of pathogen treatments on each pea genotype. Data on the percentage of dead plants were adjusted by arc sine transformation before statistical analysis.

Table 1. Response of Little Marvel and PI 257593 peas after inoculation with *R. solani* alone and in combination with *F. solani* f. sp. *pisi*, *P. ultimum*, and *A. euteiches*[x]

Pathogen and treatment no.	Little Marvel				PI 257593			
	Stem rot index[y]	Root rot index[y]	Dry wt g/plot	Dead plants (%)	Stem rot index[y]	Root rot index[y]	Dry wt g/plot	Dead plants (%)
R. solani (1)	4.2 b[z]	1.4 e	4.5 bcd	43 bc	3.8 b	1.2 d	16.6 a	18 bc
F. solani (2)	4.9 a	3.7 bc	5.1 abc	67 ab	4.1 b	2.6 b	15.4 ab	28 b
P. ultimum (3)	1.8 c	3.9 b	3.6 de	37 bc	1.4 c	2.1 bc	17.0 a	4 cd
A. euteiches (4)	1.2 d	2.5 d	5.4 ab	17 c	1.1 c	1.6 cd	17.7 a	1 d
1 + 2	5.0 a	4.9 a	3.5 de	80 a	4.6 a	4.9 a	7.8 bc	82 a
1 + 3	4.3 b	3.7 bc	3.9 cde	43 bc	4.0 b	1.7 cd	16.0 ab	30 bc
1 + 4	4.4 ab	3.0 cd	4.5 bcd	35 bc	4.0 b	1.7 cd	16.8 a	30 bc
1 + 2 + 3 + 4	5.0 a	4.9 a	3.0 e	82 a	5.0 a	4.8 a	6.9 c	81 a
Control	1.1 d	1.1 e	6.4 a	0 d	1.2 c	1.2 d	20.4 a	0 d

[x]Values are means of 120 plants in three replicates in two combined experiments (20 plants per replicate).
[y]Based on a 1–5 scale (1 = healthy plants and 5 = severe root rot, plants dead).
[z]Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.

Table 2. Response of Dark Skin Perfection and Minnesota 494-A11 peas after inoculation with *R. solani* alone and in combination with *F. solani* f. sp. *pisi*, *P. ultimum*, and *A. euteiches*[x]

Pathogen and treatment no.	Dark Skin Perfection				Minnesota 494-A11			
	Stem rot index[y]	Root rot index[y]	Dry wt g/plot	Dead plants (%)	Stem rot index[y]	Root rot index[y]	Dry wt g/plot	Dead plants (%)
R. solani (1)	3.3 bc[z]	1.2 d	11.1 a	17 b	4.3 ab	1.2 f	9.3 bc	31 b
F. solani (2)	5.0 a	2.8 b	8.6 ab	69 a	3.8 b	2.4 b	11.0 abc	26 b
P. ultimum (3)	2.1 cd	2.4 bc	8.7 ab	10 bc	1.5 c	2.1 cd	11.6 ab	2 c
A. euteiches (4)	1.6 d	1.9 c	11.5 a	7 bc	1.2 c	1.8 e	13.8 ab	0 c
(1) + (2)	5.0 a	4.9 a	5.5 bc	90 a	4.9 a	4.8 a	6.4 c	73 a
(1) + (3)	3.6 b	2.2 c	8.1 abc	20 b	4.2 b	2.3 bc	9.4 abc	28 b
(1) + (4)	3.2 bc	1.9 c	9.6 a	21 b	4.1 b	1.9 de	9.5 abc	28 b
(1 + 2 + 3 + 4)	5.0 a	4.9 a	4.7 c	84 a	5.0 a	4.9 a	6.4 c	77 a
Control	1.3 d	1.1 d	11.7 a	0 c	1.3 c	1.2 f	14.2 a	0 c

[x]Values are means of 120 plants in three replicates in two combined experiments (20 plants per replicate).
[y]Based on a 1–5 scale (1 = healthy plants and 5 = severe root rot, plants dead).
[z]Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.

RESULTS AND DISCUSSION

The most severe stem rot was found on all four genotypes inoculated with either *R. solani* or *F. solani* f. sp. *pisi* alone, with the combination of these two pathogens, or with the four-pathogen combination (Tables 1 and 2). PI 257593, moderately resistant to *R. solani* and *F. solani* f. sp. *pisi*, tended to have more stem rot when inoculated with the combination of these two fungi than when inoculated with either alone (Table 1).

As expected, *R. solani*, when used as the sole pathogen in an inoculation on the stem, did not cause root rot (19), and pea stems did not develop resistance to this pathogen with age (Shehata et al, *unpublished*). All other pathogens, however, caused root rot when inoculated singly on the stem, with the exception of *A. euteiches* on PI 257593. Interestingly, the combination of the stem rot pathogen, *R. solani*, plus *F. solani* f. sp. *pisi*, a root and stem rot pathogen, caused more root rot on all genotypes than when the latter pathogens were used alone (Tables 1 and 2). As the disease index indicates, the *R. solani* plus *F. solani* f. sp. *pisi* combination produced root rot symptoms as severe as those produced by the four-pathogen combination. Plant mortality and plot dry weight decrease also indicated that this combination was as severe as the four-pathogen combination (Tables 1 and 2).

The *R. solani* plus *F. solani* f. sp. *pisi* combination increased disease severity in PI 257593 over that caused by either pathogen alone even though this genotype has some resistance to those two fungi. This was strongly evident in greater plant mortality and loss of plant dry weight. A similar tendency was seen for Minnesota 494-A11, which has some resistance to *F. solani* f. sp. *pisi* (Tables 1 and 2).

These results may indicate the importance of pathogen interactions on the performance of gentoypes that heretofore have been evaluated via inoculation with a single pathogen. Equally important is that in breeding programs, consideration should be given to developing multiple disease resistance by simultaneous screening with more than one pathogen. Disease reaction caused by a single pathogen or by the combination of more than one pathogen, however, may vary not only with pea genotype and disease period (Shehata et al, *unpublished*) but also with such variables as method of inoculation (Shehata et al, *unpublished*; 19), crop species (5,17), environmental conditions (Shehata et al, *unpublished*; 12,19,20), sequence of infection (5), inoculum density, and virulence of isolates used (3).

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