Predisposition of Bean Roots to Attack by the Pea Pathogen, *Fusarium solani* f. sp. *pisi*, Due to Temporary Oxygen Stress

D. E. Miller, D. W. Burke, and J. M. Kraft

Research soil scientist and research plant pathologists, Western Region, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Irrigated Agriculture Research and Extension Center, Prosser, WA 99350.
Cooperative investigations of the U.S. Department of Agriculture and Washington State University. Scientific Paper 5578 of Washington State University, Pullman 99164.
Accepted for publication 9 June 1980.

ABSTRACT

MILLER, D. E., D. W. BURKE, and J. M. KRAFT. 1980. Predisposition of bean roots to attack by the pea pathogen, *Fusarium solani* f. sp. *pisi*, due to temporary oxygen stress. Phytopathology 70:1221-1224.

Bean plants were grown in cells containing field soil naturally infested with either bean or pea root pathogens and in fumigated soil, with or without reinfestation with the pea pathogen *Fusarium solani* f. sp. *pisi*, or the bean pathogen, *F. solani* f. sp. *phaseoli*. With normal aeration, *F. solani* f. sp. *pisi* had a negligible effect on bean growth, whereas *F. solani* f. sp. *phaseoli* severely injured the plants. After a temporary imposition of low soil oxygen levels, however, the pea pathogen, alone or in combination with other fungi, injured the bean roots and permanently reduced water absorption and plant growth. This injury was less than that caused by *F. solani* f. sp. *phaseoli* under the same conditions.

Miller and Burke (3) report that beans (*Phaseolus vulgaris* L.) are predisposed to severe root rot caused by *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burk.) Snyd. and Hans. when exposed to minimal soil aeration. Short periods of poor soil aeration increased *Fusarium* injury so that the plants did not recover when adequate soil aeration was resumed. In *Fusarium*-free soil, such treatment had little effect on plant growth. The pea pathogen, *F. solani* (Mart.) Sacc. f. sp. *pisi* (Jones) Snyd. and Hans., produces superficial lesions and sporulates on bean hypocotyls and roots (2) but causes no measurable effect on bean growth under normal field conditions (1). Under similar conditions *F. solani* f. sp. *phaseoli* infects beans and sporulates on them, but not on peas (2). However, A. J. M. Smucker (*personal communication*) observed that pea plants grown under poor soil aeration were infected and damaged by *F. solani* f. sp. *phaseoli*. This study was conducted to determine if a brief exposure to low soil oxygen will result in injury to bean plants by *F. solani* f. sp. *pisi*.

MATERIALS AND METHODS

Bulk supplies of surface soil (0–15 cm) and subsoil (60–75 cm) of Warden sandy loam (coarse-silty, mixed mesic Xerollic Camborthids) were collected separately from two adjacent fields. One field, after repeated bean crops, was naturally infested with *F. solani* f. sp. *phaseoli* but not with *F. solani* f. sp. *pisi*, as determined by soil platings on modified Nash's medium (5). The other field, after repeated pea crops, was infested with *F. solani* f. sp. *pisi*, but not with *F. solani* f. sp. *phaseoli*. Both fields were infested with *Pythium ultimum* Trow., *Thielaviopsis basicola* (Berk. and Br.) Ferraris, and *Rhizoctonia solani* Kühn. Supplies of the surface soil and subsoil were fumigated with methyl bromide (2–3 g/kg soil). Conidia from pure cultures of either *F. solani* f. sp. *phaseoli* or *F. solani* f. sp. *pisi* were added to portions of the surface soil while inoculum was not added to other portions. The pathogen inoculum densities (200–1,000 ppg of dry soil) were sufficient to cause typical bean or pea root rot (1), as determined in soil platings on modified Nash's agar (5). The soil was fertilized to adequate levels of N, P, K, and Zn, moistened to about 15% water by weight and packed into cells as described previously (3).

In the first experiment involving the naturally infested soils, surface soil was used in the top 18 cm of each cell. The upper 14 cm was packed to a bulk density of 1.2 g/cm^3 and the 14- to 18-cm depth was packed to 1.55 g/cm^3. Subsoil at 1.2 g/cm^3 was used below 18 cm. In the second experiment, repeated three times, we used fumigated surface soil only, infested or not infested with conidia of the pathogens as described above and packed into the cells in three layers in the same manner as before. In all cases, soil water potential was maintained at −150 mb (3).

Three bean seedlings (cultivar Red Mexican UI-36) barely germinated in vermiculite were transplanted into each cell and covered with 1 cm of autoclaved sand. After emergence they were illuminated with fluorescent lamps (light intensity near the top leaves was about 12,900 lx with a cycle of 16 hr on and 8 hr off) and maintained at 22–24 C for the duration of each test. In each experiment, 36 cells were used with 12 cells each of fumigated soil, soil infested with *F. solani* f. sp. *pisi*, and soil infested with *F. solani* f. sp. *phaseoli*. The cells were arranged in six replications of a split-plot design; half the cells received normal aeration and half were subjected to temporary oxygen stress.

The surface of the sand covering the soil was about 0.5 cm below the tops of the cells. Five days after the seedlings were transplanted, one-half of the cells of each soil were sealed below the foliage around the seedling stems with plastic tape and paraffin, leaving a space within the cells above the sand. The sealed cells were subjected to low soil oxygen levels for 3 days by passing nitrogen gas across the soil surface under the seal. During the nitrogen treatment, soil-gas compositon was monitored and analyzed with a gas chromatograph (3,4). After 3 days, the cells were opened to the atmosphere.

Each cell was equipped with a calibrated water supply flask. Water-use rates were measured from the day the aeration variable was applied until harvest. The plants were harvested 4 wk after planting and green top weights and fresh root weights above, within, and below the compacted layer (14–18 cm) were determined. Roots and hypocotyls were indexed for degree of necrosis by using a 0–5 scale, on which 0 = healthy and 5 = severe necrosis. Sections of the hypocotyl of each plant were surface sterilized with a 10% Clorox solution and plated on modified Nash's agar (5) to verify that lesions on the hypocotyl contained the appropriate organism, *F. solani* f. sp. *pisi* or *F. solani* f. sp. *phaseoli*, easily identified by colony characteristics. A small sample of rhizosphere soil in each cell was removed at harvest and assayed for inoculum density of the appropriate pathogen on Nash's agar.

RESULTS

In the first study, top and root weights of beans were reduced in soil naturally infested with either *F. solani* f. sp. *pisi* or *F. solani* f. sp. *phaseoli* compared to those of similar plants growing in the fumigated soil (Table 1). Yields were lower still in the low soil

oxygen treatment. During and after the period of oxygen stress, plants growing in soil infested with either pathogen showed similar symptoms of dark color, temporary wilting, and reduced growth. Water use by infected plants was reduced compared with that of noninfected plants growing in the fumigated soil (Fig. 1). Examination of the roots at harvest revealed considerable root injury from *Pythium ultimum* and *Thielaviopsis basicola* in addition to *Fusarium* injury. Thus, the effects of the *Fusarium* pathogens alone could not be definitely evaluated.

The effects of pathogens other than the *Fusarium* species was removed by using fumigated soil reinfested with conidia from pure cultures of either *F. solani* f. sp. *pisi* or f. sp. *phaseoli*. The results were similar to those of the first study in which, with temporary low soil oxygen levels, top and root weights were highest in soil not containing the *Fusarium* pathogens, severely reduced in *F. solani* f. sp. *phaseoli*-infested soil, and slightly reduced in *F. solani* f. sp. *pisi*-infested soil (Table 2). However, with normal aeration, yields of tops and roots were essentially the same in noninfested soil as in soil infested with *F. solani* f. sp. *pisi*. Infection with *F. solani* f. sp. *phaseoli* reduced the ability of roots to penetrate the compact soil layer and to ramify in the bottom section. This effect was increased by poor soil aeration. The roots and hypocotyls subjected to temporary oxygen stress in *F. solani* f. sp. *pisi*-infested soil exhibited more necrosis than those in soils not infested with the pathogens, but much less than those exposed to *F. solani* f. sp. *phaseoli* (Table 3).

Hypocotyl sections with lesions were infected by the appropriate organism, ie plants growing in the soil infested with *F. solani* f. sp. *pisi* were infected only by *F. solani* f. sp. *pisi* and plants growing in the soil infested with *F. solani* f. sp. *phaseoli* were infected only by *F. solani* f. sp. *phaseoli*. Lesions were not observed on the plants growing in soils not infested with the pathogens.

Water-use rates decreased when plants were oxygen stressed for 3 days (Fig. 2). This decrease was more pronounced in plants growing in soil infested with *F. solani* f. sp. *phaseoli* and *F. solani* f. sp. *pisi* than in noninfested soil. The decrease in water use corresponded to a decrease in root weight (Table 2) and an increase in disease index (Table 3). Decreased daily water use in plants exposed to oxygen stress remained throughout the remainder of the study. In the soil containing the bean pathogen, *F. solani* f. sp. *phaseoli*, 3 days of oxygen stress permanently damaged the plants and daily water-use rates decreased with time. Many plants

TABLE 1. Fresh top and root weights[a] of 1-mo-old bean plants as affected by natural soil infestations of *Fusarium solani* f. sp. *pisi* or *F. solani* f.sp. *phaseoli* and by temporary oxygen stress

	Fresh tops (grams/cell)		Fresh roots (grams per cell)							
			Top section		Compacted middle section		Bottom section		Total roots	
Soil treatment	Wt	Avg	Wt	Avg	Wt	Avg	Wt	Avg	Wt	Avg
Control (fumigated soil)										
Normal aeration	40.9	36.4	12.4	12.7	2.4	2.0	9.8	8.5	24.6	23.2
Low O_2 for 72 hr	31.9		13.0		1.7		7.2		21.9	
Loss due to O_2 stress (%)	22		...		29		26		11	
Infested field soil[b]										
F. solani f. sp. *pisi*										
Normal aeration	27.8	22.5**[c]	10.0	9.3**	1.7	1.2**	10.9	7.2	22.6	17.8*
Low O_2 for 72 hr	17.2		8.6		0.8		3.4		12.9	
Loss due to O_2 stress (%)	38		14		53		69		43	
F. solani f. sp. *phaseoli*										
Normal aeration	22.1	17.4**	10.6	8.8**	1.3	0.8**	6.6	4.3**	18.4	14.0**
Low O_2 for 72 hr	12.6		7.1		0.4		2.0		9.5	
Loss due to O_2 stress (%)	43		33		69		70		48	

[a] Average weights (grams per cell) from six replications of three plants in each cell.
[b] Infested also with natural populations of *Pythium ultimum, Thielaviopsis basicola,* and *Rhizoctonia solani.*
[c] *, ** *Fusarium*-infested soil means were significantly less than fumigated soil means at 5 and 1% probability respectively.

TABLE 2. Fresh top and root weights[a] of 1-mo-old bean plants grown in fumigated soil infested with *Fusarium solani* f. sp. *pisi* or *F. solani* f. sp. *phaseoli* and exposed to temporary oxygen stress

	Fresh tops (grams/cell)		Fresh roots (grams per cell)							
			Top section		Compacted middle section		Bottom section		Total roots	
Soil treatment	Wt	Avg	Wt	Avg	Wt	Avg	Wt	Avg	Wt	Avg
Control (fumigated soil)										
Normal aeration	33.9	30.6	18.3	18.6	1.1	1.1	6.3	5.1	25.8	24.8
Low O_2 for 72 hr	27.3		18.8		1.1		3.9		23.8	
Loss due to O_2 stress (%)	19.5		...		0		38.1		7.8	
Fumigated soil, reinfested										
F. solani f. sp. *pisi*										
Normal aeration	32.7	27.2*[b]	16.8	15.9*	1.4	1.0	5.8	4.0†	24.0	20.8*
Low O_2 for 72 hr	21.7		15.0		0.6		2.1		17.7	
Loss due to O_2 stress (%)	33.6		10.7		57.1		63.8		26.2	
F. solani f. sp. *phaseoli*										
Normal aeration	19.1	11.7**	9.3	6.2**	0.9	0.6**	4.8	2.7**	14.9	9.5**
Low O_2 for 72 hr	4.3		3.2		0.2		0.6		4.1	
Loss due to O_2 stress (%)	77.5		65.6		77.8		87.5		72.5	

[a] Average weights from three experiments, each involving six replicate cells which contained three bean plants (a total of 18 cells per treatment).
[b] †,*,** *Fusarium*-infested soil means were significantly less than noninfested soil means at $P = 0.10$, 0.05, and 0.01, respectively.

TABLE 3. The effect of soil oxygen stress on necrosis[a] caused in bean hypocotyls and roots by *Fusarium solani* f. sp. *pisi* and *F. solani* f. sp. *phaseoli* in fumigated soil

Soil treatment	Hypocotyl		Roots			
			Top section		Middle section	
	Rating	Avg	Rating	Avg	Rating	Avg
Control (fumigated soil)						
Normal aeration	0.4	0.5	0.5	0.5	0.6	0.7
Low O_2 for 72 hr	0.6		0.5		0.8	
Increased necrosis due to O_2 stress (%)	50		...		33	
Fumigated soil, reinfested						
F. solani f. sp. *pisi*						
Normal aeration	0.6	0.9†[b]	0.6	0.8	0.8	0.7
Low $_2$ for 72 hr	1.2		1.1		0.6	
Increased necrosis due to O_2 stress (%)	100		83		...	
F. Solani f. sp. *phaseoli*						
Normal aeration	3.8	4.3**	2.8	3.8**	3.0	3.6**
Low O_2 for 72 hr	4.8		4.7		4.2	
Increased necrosis due to O_2 stress (%)	26		68		40	

[a] Rating Scale: 0 = no necrosis, 5 = severe necrosis. Botton section is omitted because many inoculated cells had no roots in bottom section.
[b] †,** *Fusarium*-infested soil means were significantly more than noninfested soil means at $P = 0.10$ and 0.01, respectively.

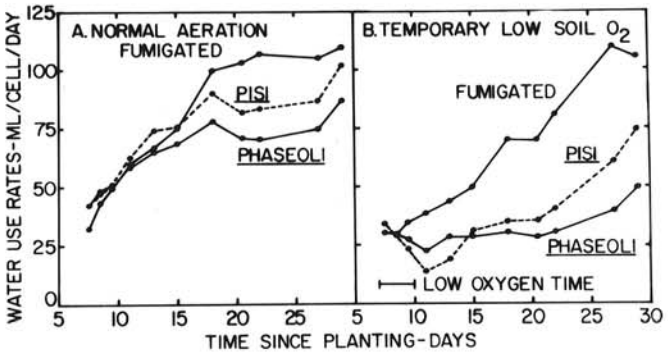

Fig. 1. Water use rates by bean plants as affected by soil infestation with *Fusarium solani* f. sp. *pisi* or *F. solani* f. sp. *phaseoli* and temporary low soil oxygen. Soils were naturally infested with the pathogens by repeated cropping to peas or beans.

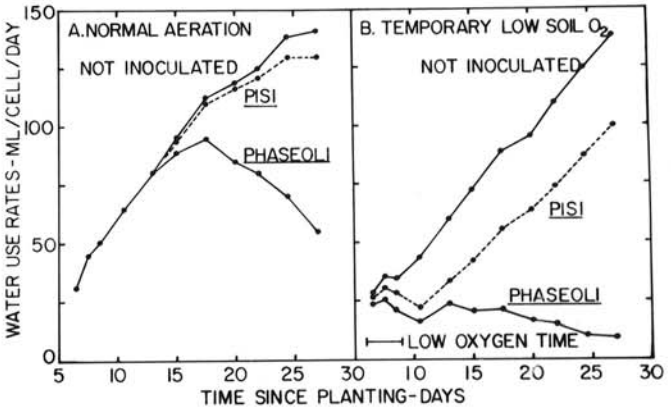

Fig. 2. Water use rates by bean plants as affected by soil infestation with *Fusarium solani* f. sp. *pisi* or *F. solani* f. sp. *phaseoli* and by temporary low soil oxygen. Fumigated soils were equally reinfested with conidial suspensions of the respective pathogens.

eventually died or remained so severely injured that they extracted little soil water. In soil containing the pea pathogen, *F. solani* f. sp. *pisi*, plants recovered somewhat in their ability to extract soil water, and then daily water use rates increased with time.

DISCUSSION

The predisposing effects of brief oxygen stress made possible infection and permanent damage to the bean roots by *F. solani* f. sp. *pisi*, previously not considered pathogenic to beans (1), significantly reducing plant growth (Table 2) and reducing plants' ability to use water (Fig. 2). In soil not containing the pathogens, oxygen stress retarded plant water use for only 2 or 3 days, and the plants fully recovered and showed no permanent effect of temporary oxygen stress as measured by water use.

In plants not stressed for oxygen, the pea pathogen, *F. solani* f. sp. *pisi* alone had little, if any, effect on plant growth or on their ability to absorb water. In comparison, the bean pathogen, *F. solani* f. sp. *phaseoli*, after the first week of plant growth, permanently decreased plant growth and water use, and imposition of oxygen stress greatly accentuated plant damage (Table 2).

The data in Table 1, involving soils infested naturally by several root pathogens, and the data in Table 2, involving only the two *Fusarium* pathogens, respectively, showed similar trends with respect to plant and root growth. Also, the trends in plant water use were similar in naturally infested and artificially infested soils (Figs. 1 and 2). These observations tend to support those previously made in field experiments (1,2), that the respective *Fusarium* pathogens are the most important and essential members of the pathogen complex which affects yields of beans and peas. However, the populations of *Pythium, Thielaviopsis,* and possibly other microorganisms in field soil used in this experiment caused root necrosis and significantly affected plant growth in the confinement of the cells (Table 1). Under field conditions in soils of the type used in this study, the root pathogens *P. ultimum* and *T. basicola,* even though prevalent, caused no significant yield reductions to beans or peas in the absence of the host-specific *F. solani* f. sp. (1). Furthermore, these pathogens have caused no measurable permanent damage to beans temporarily oxygen stressed in the field by excessive irrigation (author's *unpublished observations*). Unless the field is infested also by *F. solani* f. sp. *phaseoli,* the plants revive, as they did in this study in the absence of pathogens (4). Whether or not *F. solani* f. sp. *pisi* is capable of significant damage to bean plants under oxygen stress in the field remains to be determined.

LITERATURE CITED

1. BURKE, D. W., and J. M. KRAFT. 1974. Responses of beans and peas to root pathogens accumulated during monoculture of each crop species. Phytopathology 64:546-549.
2. KRAFT, J. M., and D. W. BURKE. 1974. Behavior of *Fusarium solani*

f. sp. *pisi* and *Fusarium solani* f. sp. *phaseoli* individually and in combination on peas and beans. Plant Dis. Rep. 58:500-504.
3. MILLER, D. E., and D. W. BURKE. 1975. Effect of soil aeration on Fusarium root rot of beans. Phytopathology 65:519-523.
4. MILLER, D. E., and D. W. BURKE. 1977. Effect of temporary excessive wetting on soil aeration and Fusarium root rot of beans. Plant Dis. Rep. 61:175-179.
5. PAPAVIZAS, G. C. 1967. Evaluation of various media and antimicrobial agents for isolation of *Fusarium* from soil. Phytopathology 57:848-852.