The Effect of Plant Age on Severity of Pea Wilt Caused by Fusarium oxysporum f. sp. pisi Race 5

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Scientific Paper No. 4339 and Project No. 1987, College of Agriculture Research Center, Washington State University, Pullman.

Accepted for publication 29 January 1976.

ABSTRACT

NYVALL, R., and W. A. HAGLUND. 1976. The effect of plant age on severity of pea wilt caused by Fusarium oxysporum f. sp. pisi race 5. Phytopathology 66: 1093-1096.

Peas grown in soil infested with *Fusarium oxysporum* f. sp. *pisi* race 5 and transplanted after 3 or 7 days to noninfested soil exhibited mild disease symptoms as measured by plant dry weight. Peas grown 14 or more days in infested soil exhibited severe disease symptoms and usually died within 21 days. Dry weight loss was 57% greater after 14 days in infested soil than after 7 days and 50% greater after 28 days than after 14 days in infested soil. Peas grown 21 or more days in noninfested soil, then transplanted to infested soil or to soil artificially inoculated with chlamydospores exhibited only mild disease symptoms and the fungus was not isolated from the upper portions of the plants. However, peas grown 14 days or less in noninfested soil then transplanted to infested soil developed severe disease symptoms and died. The population of race 5 was highest in the rhizosphere of 14-day-old plants but probably had little effect on disease severity since plants were infested 3 days after sowing. Seven- and 21-day-old plants exposed to infested soil for various time periods and transplanted into noninfested soil exhibited wilt symptoms and these became progressively more severe the longer the plants were exposed to infested soil. Wilt severity is dependent on the age of plants at infection and length of time of root-fungus contact.

Additional key words: Pisum sativum, soil fungi.

Fusarium wilt of peas (*Pisum sativum* L.) caused by *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. f. sp. *pisi* (Lindf.) Snyd. & Hans. race 5 Hag. & Kr. has been a serious disease in northwestern Washington since 1963 (7, 8). There has been no work on the relationship of age of peas to disease severity per se as caused by *F. oxysporum* f. sp. *pisi*. However, Hepple (9), working with peas growing in soil naturally infested with *F. oxysporum* and *F. oxysporum* var. *redolens,* observed that invasion of peas by these fungi occurred through moribund cotyledons. In a later paper (10) she concluded that there was no correlation between the onset of wilting and plant maturity when peas were grown under different day-lengths.

The relationship of age of various host plants to susceptibility and to the incidence of fungi upon and within roots has been discussed by several workers (4, 5, 12, 13, 15, 16). However, none of the authors of those papers discusses the relationship between the age of the host and severity of disease caused by soil-borne fungi.

Research on the interaction of race 5 and peas, specifically the age when peas were most susceptible to infection, was undertaken to find possible means of control. This paper gives attention to (i) the age at which peas are most susceptible to race 5, and (ii) the length of time different aged peas must be exposed to infested soil to display wilt symptoms.

MATERIALS AND METHODS

Peas (cultivar Darkskin Perfection) were inoculated by one of three different methods: (i) planting in naturally infested soil, (ii) placing with a syringe a suspension of chlamydospores around the roots of a plant growing in vermiculite (soil drench tests), and (iii) placing a chlamydospore suspension at several sites on roots with a syringe (soil injection tests).

Natural infested soil tests.—Infested Puget silty clay loam (pH 5.8) from a field in which peas had died from Fusarium wilt, contained about 10,500 propagules of *Fusarium* spp. per gram of soil; of these about 8,500 per gram were race 5. It was not possible to find a noninfested Puget silty clay loam; therefore, noninfested soil was a greenhouse mixture of silt, loam, peat, and sand (2:2:1, v/v), pH 6.0.

Ten seeds were planted in soil in 15 cm diameter plastic pots and thinned to five seedlings per pot after emergence. To determine the relationship of time seedlings must be exposed to infested soil and wilt severity, plants were grown 3, 7, 14, 21, or 28 days in infested soil, removed by washing the soil from the root-soil mass and transplanted to noninfested soil. To determine at what age peas can be infected and exhibit wilt symptoms, peas were started in noninfested soil and then transplanted to infested soil at the five time intervals listed above.

The effect upon disease severity of early seedling infection and exposure time of seedlings to infested soil, in relation to disease severity, was determined by growing seedlings in noninfested soil for 7 or 21 days, transplanting to infested soil for 3, 7, or 14 days and, at the end of each exposure period, transplanting to noninfested soil. The control plants were grown and transplanted to noninfested soil at the same time intervals.

Soil drench tests.—Peas were grown in vermiculite in plastic pots (15 cm in diameter) and fertilized every 7 days with a solution of 20-20-20 fertilizer. Dexon sodium [4-(dimethylamino) phenyl] diazenesulfonate was added to the vermiculite at planting time to control *Pythium* spp. Three, 7, 14, or 21 days after planting, 5 ml of a chlamydospore suspension (5×10^4 chlamydospores/ml) of race 5, produced by the method of Alexander et al. (1), was poured around the base of each plant. Three days after inoculation, the pots were submerged in a 1% solution of NaOCl for 3 minutes, then five successive water rinses for 1 minute each. Roots were examined microscopically 3 days after inoculation, by which time infection had occurred. Race 5 was isolated by this procedure from less than 1% of the vermiculite fragments surrounding pea roots. The controls were: (i) plants not inoculated but placed into NaOCl and then into five water rinses and (ii) plants inoculated but not placed into NaOCl or succeeding water rinses.

Soil injection tests.—Peas were grown in 2-liter polyethylene bags filled with noninfested soil as described earlier (14). At 3, 7, 14, or 21 days after planting, 10^4 chlamydospores of race 5 in a water suspension were injected with a syringe at five equidistant sites on the taproot. The control was sterile distilled water (SDW) placed at the five inoculation sites.

Disease assessment.—Disease severity was measured by: (i) visual wilt symptoms; i.e., gray-green color of the leaves and stems, and downward curling of leaf edges; (ii) dry weight of the aboveground plant portions (dried in a mechanical convection oven at 43 C for 7 days); (iii) plant height (number of nodes per plant either at plant death or at maturity). At death or maturity each plant was assayed for race 5 by placing an internode from 15 cm above the cotyledon on Nash's pentachloronitrobenzene (PCNB) agar. Race 5 was verified according to the criteria of Nyvall and Haglund (14).

Population estimates in the rhizosphere.—Race 5 was isolated from the rhizosphere using a modified procedure of Johnson et al. (11, p. 29-30). Peas were grown for 7, 14, 21, or 28 days in infested soil. The root systems were removed and nonrhizosphere soil loosely adhering to the roots was shaken off and the roots were air-dried at room temperature (22-24 C). The roots then were placed in 90 ml of SDW and allowed to stand for 1 hour, then vigorously shaken for 5 minutes twice at 1 hour intervals to dislodge rhizosphere soil.

Both rhizosphere and nonrhizosphere soil were diluted 1:1,000 and 1 ml of the final dilution was placed on solidified PCNB agar and incubated for 7 days at 24 C ± 2 C in diffuse daylight, then colonies were counted. Water into which roots were placed was evaporated and the weight of rhizosphere soil for each plant was obtained to compute the final soil dilution. The results are the averages of three replicates of 25 plants each.

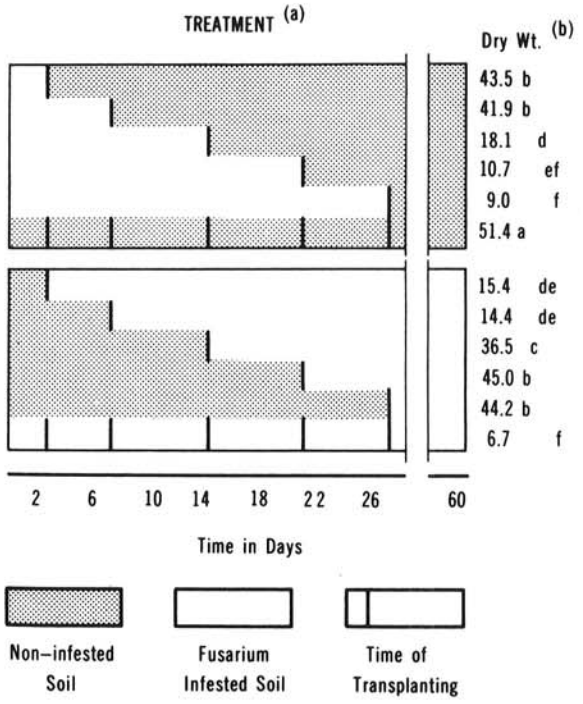

Fig. 1. Fusarium wilt on peas, exposed for 3, 7, 14, 21, or 28 days to soil infested with *Fusarium oxysporum* f. *pisi*. Severity expressed as dry weight of aerial portion of plant. Dry weight is based on average of three replicates of 50 plants each. Numbers followed by different letters differ significantly from each other according to Duncan's multiple range test, $P = 0.05$.

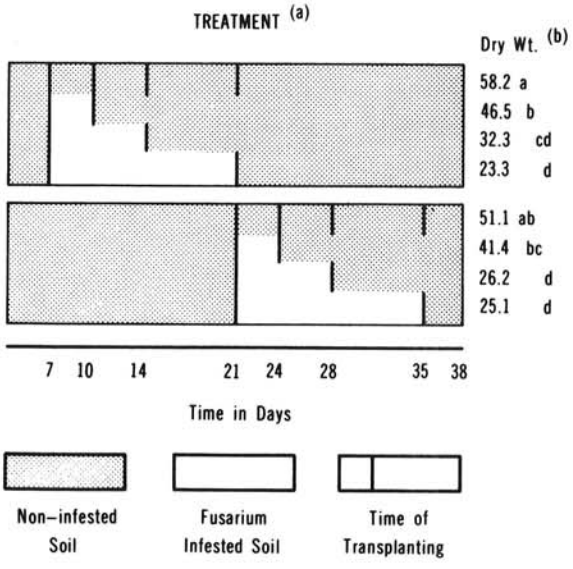

Fig. 2. Fusarium wilt of 7- and 21-day-old pea seedlings exposed for 3, 7, or 14 days in soil infested with *Fusarium oxysporum* f. sp. *pisi*. Disease severity is expressed as reduction in dry weight of aerial portion of plant. Dry weight is based on average of three replicates of 50 plants each. Numbers followed by different letters differ significantly from each other according to Duncan's multiple range test, $P = 0.05$.

RESULTS

Natural soil tests.—Peas exhibited few wilt symptoms when grown in infested soil for 3 or 7 days (Fig. 1). The greatest reduction in dry weight occurred between 7 and 14 days and a 50% loss in root dry weight resulted from exposure to infested soil for 14 to 28 days. One hundred percent of the plants from all sampling dates were infected by race 5; however, the fungus rarely was isolated 15 cm above the cotyledon of peas transplanted after 3 or 7 days of exposure in infested soil. Furthermore, pods containing peas formed only on plants grown in infested soil for 7 days or less.

The age of peas is reported from the time of planting. Peas transplanted from noninfested to infested soil at 21 and 28 days had no symptoms and little loss in dry weight (Fig. 1); however, race 5 was isolated from all roots. Peas transplanted to infested soil at the age of 3 or 7 days exhibited severe wilt symptoms and 14-day-old plants had mild symptoms. Race 5 was isolated less frequently from the tops of peas transplanted at 14 than from those transplanted at 3 and 7 days although all roots were infected. Fifty-one percent of the peas transplanted into infested soil at 14 days bore pods but all seedlings transplanted at 3 or 7 days died prior to pod formation.

Soil drench tests.—Severe wilt symptoms developed and a corresponding reduction in dry weight occurred when 3, 7, and 14-day-old seedlings were exposed to race 5 chlamydospores for 3 days (Table 1). Less than 1% of the plants inoculated when 21 days old wilted and no reduction in dry weight was observed (Table 1). However, when 21-day-old plants were exposed to race 5 for the remainder of the growing period, severe wilt developed together with a loss in dry weight. Race 5 was not isolated from the tops of 21-day-old plants exposed to wilt for 3 days. Race 5 was recovered from 75% of the plants inoculated at 14 days and from 100% of the plants inoculated at 3 and 7 days.

Soil injection tests.—Severity of wilt symptoms decreased with increased age of plants. The average number of nodes per plant at death was 11, 14, 17, and 20 when plants were inoculated at 3, 7, 14, and 21 days, respectively (average of three replicates, 25 plants per replicate). The average number of nodes per plant in the control was 20.

Relationship between plant age and length of time exposed to infested soil to wilt symptoms.—Twenty-one-day-old pea plants were more severely diseased than 7-day-old peas when exposed to infested soil for 3 or 7 days (Fig. 2). However, the onset of visual symptoms in both ages of peas occurred the same time after transplanting (7 to 10 days). Wilt symptoms were similar when peas of both ages were exposed to infested soil for 14 days. The dry weight of the control plants (grown only in noninfested soil) was about twice that of plants exposed to infested soil for 7 or 14 days.

Rhizosphere.—The number of *F. oxysporum* propagules was greatest in the rhizosphere of 14-day-old plants (Fig. 3), but declined in the rhizosphere of older plants. During this period the *F. oxysporum* populations outside the rhizosphere remained between 5,000-6,000 propagules/g of soil. The increase in *F. oxysporum* in the rhizosphere occurred between 7 and 14 days. With the exception of 7-day-old plants, almost all *F. oxysporum* colonies from the rhizospheres were race 5.

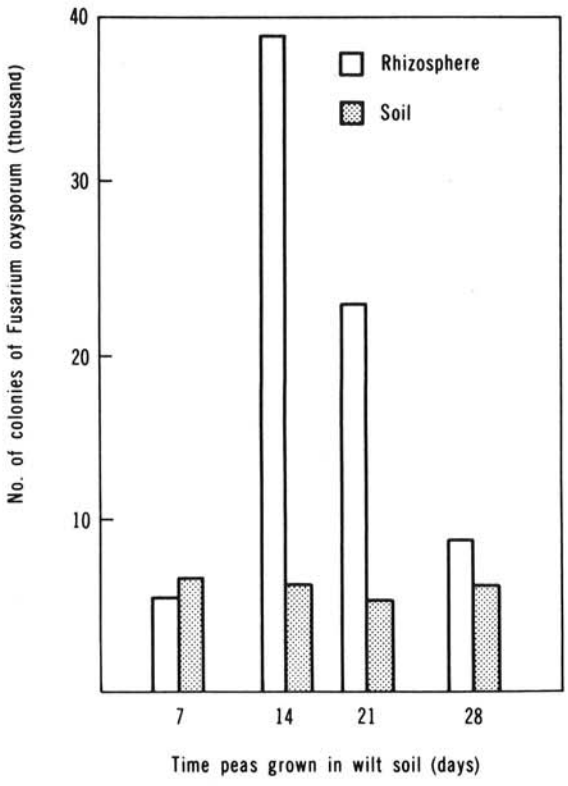

Fig. 3. Populations of *Fusarium oxysporum* in the rhizosphere of pea.

DISCUSSION

The results of this study suggest two things: (i) plants infected when older (21 or more days) would not be so

TABLE 1. Reduction in dry weights of pea plants grown in vermiculite and inoculated with chlamydospores of *Fusarium oxysporum* f. sp. *pisi* race 5 at 3, 7, 14, and 21 days after planting

Age of peas at inoculation (days)	Dry weight (g)[b]		
	Inoculated[a]		Not Inoculated[c]
	A	B[d]	
3	3.5	3.1	19.7
7	2.5	3.0	19.8
14	4.0	3.7	19.2
21	19.2	6.2	19.2

[a]Peas inoculated by placing a chlamydospore suspension around the base of each plant. After 3 days the chlamydospores were killed by submersing the vermiculite containing the plants into a 1% solution of NaOCl for 3 minutes, then into five successive water rinses for 1 minute each.

[b]Average of four replicates, 25 plants per replicate.

[c]Plants not inoculated, but placed into NaOCl and then into five successive water rinses.

[d]Plants inoculated, but not placed into NaOCl or succeeding water rinses.

severely wilted as plants infected while younger, and (ii) wilt severity in younger plants was proportional to the time of root-fungus contact. In all instances, wilt symptoms decreased in proportion to the age of plants at inoculation; i.e., the younger the plant when inoculated, the more severe the symptoms. Race 5 always was isolated from the tops of plants inoculated at 3 and 7 days of age but infrequently from 14-day-old and rarely from 21-day-old plants. This suggests that older plants, when infected, must have a defense mechanism that inhibits disease development. Beckman et al. (3) found that resistance to Fusarium wilt in banana was partly due to physical occlusions in the xylem that prevented distribution of the pathogen in the host; occlusions were less frequent in susceptible bananas (2). Other possibilities are that plants reached maturity before disease developed fully or older plant roots were not in contact with race 5 for as long a time as the roots of young plants. Plants of two ages, 7 and 21 days, were grown in infested soil for different lengths of time. Twenty-one-day-old plants had less severe wilt than 7-day-old plants when both were grown for 3 and 7 days in infested soil. However, when both ages were grown for 14 days in infested soil there was no difference in wilt symptoms.

We conclude there is a relationship between age of plants at infection and wilt severity. However, the length of time roots are in contact with the fungus is the most important factor determining wilt severity. When an older plant becomes infected, wilt symptoms are mild. However, under field conditions there obviously is going to be continuous root-fungus contact from the time peas are sown until maturity. As an aid in disease control, perhaps the plant root could be protected for a period of time with a soil fumigant until the plants are old enough to resist disease development.

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