Ecology and Epidemiology

Effects of Inoculum Density and Placement on Fusarium Root Rot of Peas

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Cooperative investigations of the ARS, USDA, and the Washington State University Agriculture Research Center, Prosser 99350. College of Agriculture Research Center Scientific Paper 7402.
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The authors wish to thank Ginny Coffman and Bill Boge for their technical help.
Accepted for publication 17 June 1986 (submitted for electronic processing).

ABSTRACT

Rush, C. M., and Kraft, J. M. 1986. Effects of inoculum density and placement on Fusarium root rot of peas. Phytopathology 76:1325-1329.

Pea roots became infected when inoculum of *Fusarium solani* f. sp. *pisi* was placed in the lower 10 cm of 30-cm containers, but no disease symptoms appeared. A high inoculum density of 5,000 colony-forming units (cfu) per gram of soil, placed in the lower 10 cm, failed to cause any measurable plant stress, but when inoculum was placed in the upper 10 cm or mixed throughout the containers, dry top and root weight, plant height, and leaf area were all significantly lower than control plants growing in uninfested soil. Plants growing in the high inoculum density soil (5,000 cfu per gram of soil) exhibited severe root rot symptoms earlier than plants growing in soil infested with 100 cfu per gram of soil. There was no significant difference between plant stress measurements whether inoculum was mixed throughout the container or placed in the upper 10 cm. Top weight, root weight, rate of node appearance, leaf area, and plant height were measured to evaluate the effect of Fusarium root rot on plant growth and development. Leaf area was the most sensitive indicator of plant stress. As early as 21 days after emergence, control plants had significantly greater leaf area than plants growing in infested soil. This study suggests that, in the absence of other stress factors, inoculum of *F. s.* f. sp. *pisi* deep in the soil has no detrimental effect on pea growth and development up to the time of flowering, when the upper 20 cm of the root system is free from infection.

Additional key word: *Pisum sativum*.

In the Pacific Northwest, peas (*Pisum sativum* L.) are the primary rotation crop grown with wheat (*Triticum aestivum* L.). Wheat yields have increased dramatically over the last 20 years, whereas those of peas have remained static (16,21). One factor that affects pea productivity is Fusarium root rot caused by *Fusarium solani* (Mart.) Sacc. f. sp. *pisi* (Jones) Snyd. & Hans. Loss due to this disease typically ranges from 10 to 40% annually (20). In southeastern Washington and northeastern Oregon, *F. s.* f. sp. *pisi* is the primary pathogen in a complex that also includes *Pythium ultimum* Trow and *Rhizoctonia solani* Kühn (16,17).

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Peas are infected by *F. s.* f. sp. *pisi* soon after seed germination. Chlamydospore germination and successful infection of the hypocotyl and root system is dependent on host exudates and soil moisture (9,14,15). Although the root system is infected early, disease symptoms on the root usually do not begin to appear until approximately 14 days after plant emergence. The duration of the incubation period between initial root infection and subsequent symptom expression and plant stress is affected by many biotic and abiotic factors. In general, any factor that stresses the pea plant will predispose the plant to root rot and shorten the incubation period (20). This also holds true for root rot of beans (22,23). Cultural practices that result in vigorous plant growth will lengthen the incubation period and reduce the level of stress despite extensive infection of the root system (7,20,23).

Infection of the root system is unavoidable because of the distribution of the pathogen. In field soils that have been previously cropped to peas, propagules of *F. s.* f. sp. *pisi* can be detected as deep as 60 cm (2,16,20). Propagules at this depth presumably are the result of progressive infection of the pea root

system by the pathogen. Because *F. s.* f. sp. *pisi* is a soil inhabitant, it survives primarily as chlamydospores over long periods (24). A 3–5-yr rotation out of peas, however, will eliminate the pathogen from the plow layer, but have little effect on its survival in the subsoil (Kraft, *unpublished*). The effects of *F. s.* f. sp. *pisi* on plant growth and development when the pathogen is restricted to the subsoil have not been previously investigated. Therefore, the primary objective of this study was to determine how placement of inoculum of *F. s.* f. sp. *pisi* at different soil depths affects the development of Fusarium root rot and growth of peas in the absence of other stress factors.

MATERIALS AND METHODS

Soil and inoculum preparation. Potting medium consisted of a nonsterile Warden fine sandy loam mixed with 20% peat (w/w) to prevent compaction. This soil type is conducive to Fusarium root rot, but is not naturally infested with *F. s.* f. sp. *pisi*. Chlamydospore inoculum was prepared as previously described (19). Stock soil containing 350,000 cfu per gram of soil was diluted with the potting mix to achieve final inoculum densities of either 100 or 5,000 cfu per gram of soil. In order to insure a uniform distribution of the pathogen, the appropriate amount of stock soil was added to 3.6 kg of potting medium and mixed in a twin shell blender. This medium was then further diluted and mixed in a cement mixer. Random samples were taken from the prepared soil media and plated onto a selective medium for Fusarium (24) to determine actual inoculum densities. The infested soil media were stored in plastic bags at room temperature for 7 days before use.

Inoculum placement and experimental design. Polyvinylchloride tubes, 10 × 30 cm, were covered on one end with perforated plastic to allow subirrigation. The containers were then filled with combinations of infested and uninfested soil to a bulk density of approximately 1.2 g/cm^3. Infested soil was placed in the upper or lower 10 cm of each container or mixed throughout. Containers with only uninfested soil were included as controls. Water was added as needed during the study to maintain adequate soil moisture levels to eliminate water stress as a variable. Five seeds were planted in each container and after emergence plants were thinned to two per container. There were 10 replicates of each inoculum density-inoculum placement combination. Five of the replicates were harvested 35 days after emergence and the remaining five were harvested 2 wk later, at first flower (49 days after emergence). The experiment was repeated three times.

Disease and plant stress evaluations. At each harvest date, the two plants of cultivar Dark Skin Perfection in each container were assigned a disease index rating between 0–5 as previously described (19). A value of 5 indicated a dead plant and a disease index of 0 was assigned plants with root systems completely devoid of root rot symptoms. Plants were then placed in drying ovens at 80 C and after 48 hr root and top weights were determined. To further quantify the amount of plant stress caused by Fusarium root rot, plant height, leaf area, and rate of node appearance were also measured. Plant height measurements were taken 21, 30, 37, and 47 days after emergence; leaf area was measured 21, 33, and 48 days after emergence; and node counts were taken weekly. Leaf area was determined with a Li-Cor area meter (Li-Cor, Inc., Lincoln, NE) by taking a leaf from the most distal node with fully expanded leaflets common to all plants. The exception to this was the last measurement at 48 days after emergence. At that time, some of the plants growing in the soil infested with 5,000 cfu per gram of soil had died, and the last measured node on many of these plants was not fully developed. A split plot analysis of variance was run on all data and means were compared using Duncan's multiple range test or Fisher's LSD.

RESULTS

Fusarium root rot symptom development as indicated by disease index was affected by inoculum density and inoculum placement (Table 1). Plants exposed to high inoculum density had more root rot at both harvest dates than those exposed to the low inoculum density when the inoculum was mixed throughout the container or placed in the upper 10 cm. There was no significant difference in disease index for plants exposed to inoculum placed in the upper 10 cm or mixed throughout at a given density. However, placement of the inoculum in the lower 10 cm resulted in significantly less disease. The disease index was significantly higher at the second harvest date than at the first in low inoculum density treatments when the inoculum was in the upper 10 cm or mixed throughout. This did not happen with the high inoculum density treatments because extensive root damage had already occurred by the first harvest. Plants growing in containers with inoculum in the lower 10 cm did not develop severe root rot symptoms although the roots were infected by *F. s.* f. sp. *pisi* as determined by plating roots on a medium selective for *Fusarium* (24).

Root and top weights were also affected by inoculum density and placement. When inoculum was restricted to the lower 10 cm, root and top weights were equivalent to the controls (Table 1). Plants grown in the low inoculum density soil regardless of placement also had root and top weights equivalent to the controls at first harvest. However, by the second harvest, root and top weights were significantly less than controls. At both harvest dates, the plants growing in soil with high inoculum in the upper 10 cm or mixed were severely stunted. Essentially no growth occurred between the first and second harvest when plants were exposed to the high inoculum level placed in the upper 10 cm or mixed.

The appearance of stem nodes was the least sensitive measurement of plant stress evaluated (data not shown). Plants exposed to the low inoculum density had the same number of

TABLE 1. Effects of inoculum density and placement of *Fusarium solani* f. sp. *pisi* on disease index, top weight, and root weight of pea

Variable/inoculum density (cfu/g)	1st Harvest[x] Inoculum placement[y]			2nd Harvest Inoculum placement		
	High	Low	Mixed	High	Low	Mixed
Disease index						
Control	0.60 a[z]	0.50 a	0.60 a	0.80 a	0.67 a	1.05 a
100	3.82 b	0.40 a	3.42 b	4.40 b	0.60 a	4.45 b
5,000	4.80 c	0.75 a	4.94 c	4.95 c	0.57 a	5.00 c
Top weight (g)						
Control	2.86 a	2.84 a	2.66 a	5.26 a	5.29 a	5.39 a
100	2.54 a	2.84 a	2.56 a	3.64 b	5.07 a	4.20 b
5,000	1.16 b	2.82 a	1.18 b	1.54 c	5.63 a	1.15 c
Root weight (g)						
Control	0.35 a	0.34 a	0.33 a	1.07 a	1.08 a	1.01 a
100	0.33 a	0.39 a	0.41 a	0.70 b	1.16 a	0.79 a
5,000	0.15 b	0.39 a	0.14 b	0.24 c	0.98 a	0.15 b

[x] Harvests at 35 and 49 days after emergence.

[y] Infested soil placed in the upper (high) or lower (low) 10 cm of each container or mixed throughout. Containers with only uninfested soil were included as controls.

[z] Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

nodes as control plants for the duration of the study. Plants growing in high inoculum density soils had the same number of nodes as the control plants for the majority of the study and differences were not significant.

Internode length or plant height was a better measurement of plant stress than node number, but it was also relatively unsensitive. Plants grown in the high inoculum density soil were significantly shorter than the control plants at all four measurement dates. However only with the last measurement was there a difference between the controls and plants exposed to the low inoculum density (Fig. 1). Placement of inoculum in the lower 10 cm did not affect plant height.

Leaf area was the most sensitive indicator of plant stress (Fig. 2). Twenty-one days after plant emergence, there was a significant difference between the control plants and those grown in the low inoculum density soil placed in the upper 10 cm. Placement of inoculum in the lower 10 cm of the containers had no measurable effect on leaf area. By the final measurement date, 48 days after emergence, large differences in leaf area existed among treatments. The leaf area values for the high inoculum density treatments were especially low because many of these plants had died and the final node measured had an undeveloped leaf.

DISCUSSION

Root symptoms of Fusarium root rot usually appear first at the cotyledonary attachment area. During germination, sugars, amino acids, and other organic compounds that leak from the cotyledons are available as a food source for *F. s.* f. sp. *pisi* (9,14,15). Eventually the entire root system will exhibit root rot symptoms, but the appearance of these symptoms and associated plant stress is highly dependent on the environment in which the plant is growing. Biotic and abiotic factors such as root pruning by *Pythium*, spp., exposure to toxic microbial metabolites, tillage pans, poor aeration, and drought can all perpetuate plant stress, thereby increasing the severity of root rot (1,3,8,10,11). In the past, the primary measure of disease severity has been the disease index. Often disease index is assigned while plants are relatively young and, for this reason, the index is inordinately weighted toward symptoms on the cotyledonary attachment area, hypocotyl, and epicotyl. Our results suggest that root disease symptoms alone are a poor indicator of actual disease severity or plant stress. This is especially true when the symptoms are restricted to the cotyledonary attachment area. Similar observations have been made by workers studying Fusarium root rot of beans (5,6,27). In our study, plants with a disease index of 3.4–3.8 at the first harvest 35 days after emergence had the same top and root weight as control plants with an index of 0.5. The deterioration of the root system on these infected plants was minimal, despite severe disease symptoms at the cotyledon attachment area. Plants exposed to the high inoculum density with disease index values between 4.8–4.9 had severely rotted root systems, in addition to the cotyledon attachment area, and plant growth was significantly reduced. By the second harvest date 49 days after emergence, the root systems of plants exposed to the low inoculum density were also severely rotted and plant growth was retarded. This indicates that one of the most critical factors in determining the amount of plant stress caused by Fusarium root rot is the timing of root deterioration and not symptom expression at the cotyledon attachment area. Although roots are infected by *F. s.* f. sp. *pisi* soon after contact with infective propagules, plant stress resulting from disease is minimal until symptoms begin to appear on the root system. Any factor that stresses the host root system will shorten the incubation period between infection and root deterioration (2,3,11,16,22,23). This emphasizes the importance of providing the plant with the most stress-free environment possible.

The last 20–30 days of the growing season are usually the most critical in determining pea yields in the Pacific Northwest. Temperatures are high, precipitation low, and vegetative biomass accumulation with associated high transpiration rates has peaked. Because peas in this area are nonirrigated, available soil moisture is restricted to subsoil moisture stored from winter and spring precipitation. Therefore the lower root system is all important. Any factor that restricts root growth or function will ultimately

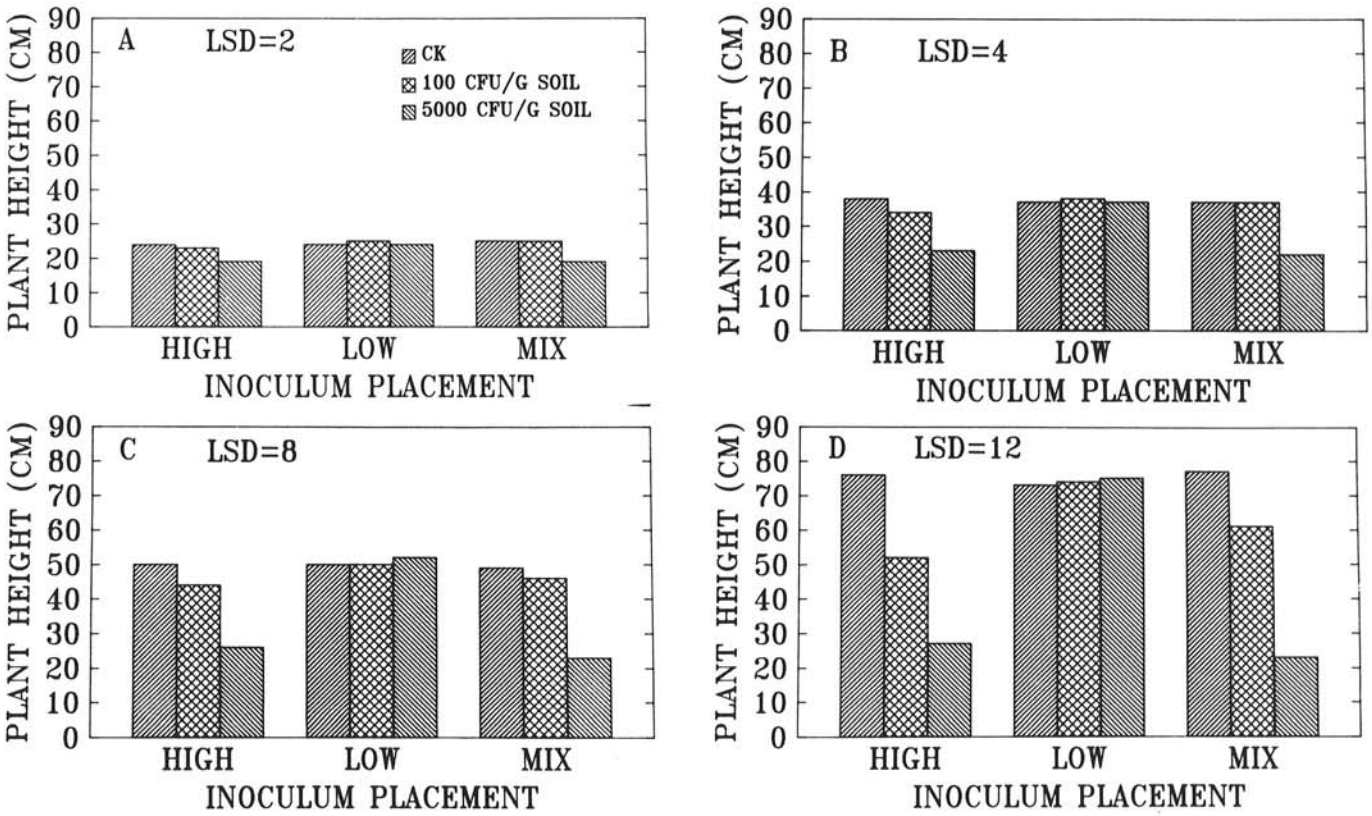

Fig. 1. Effects of inoculum density and placement of *Fusarium solani* f. sp. *pisi* on plant height. Plant height was measured four times during the study: 21, 30, 37, and 47 days after plant emergence. Infested soil was placed in the upper (high), or lower (low) 10 cm of each container, or mixed throughout the entire container (mixed). LSD value in figures was computed at the 5% level of significance.

reduce yield, but many of these can be manipulated (3,18,20). Plow pans can be broken with various tillage procedures and *F. s.* f. sp. *pisi* propagules can be eliminated from the plow layer by 3–5 year rotations (Kraft, *unpublished*). However, the effects of *F. s.* f. sp. *pisi*, surviving in the subsoil, on pea growth and development is unknown. The results of our study indicate that when *F. s.* f. sp. *pisi* is restricted to the lower root system in the absence of other stress factors, plant growth and development is not reduced by root rot up to the time of flowering even though the roots are infected. Factors that affect the incubation period of Fusarium root rot on the lower portion of the root system deserve further investigation. It is unknown for instance whether in the absence of any other stress factor if the pathogen is capable of deteriorating juvenile root tissue. If pathogenesis is dependent on tissue maturation or the beginning of senescence either natural or induced by external stress factors, then the lower root system might be protected long enough to produce a crop if these external factors could be managed. In this study, *F. s.* f. sp. *pisi* was the only stress factor to which plants were exposed. In the field, however, even if *F. s.* f. sp. *pisi* was removed from the plow layer, results could be considerably different because external stresses are always present to varying degrees. Working with Fusarium root rot on beans, Burke and Barker (5,6) found that plants with roots growing from an island of sterile soil into an infested field soil were stunted. They attributed this to Fusarium root rot. Other stress factors that were possibly involved, such as Pythium root rot or a compaction layer in the field soil, were not investigated.

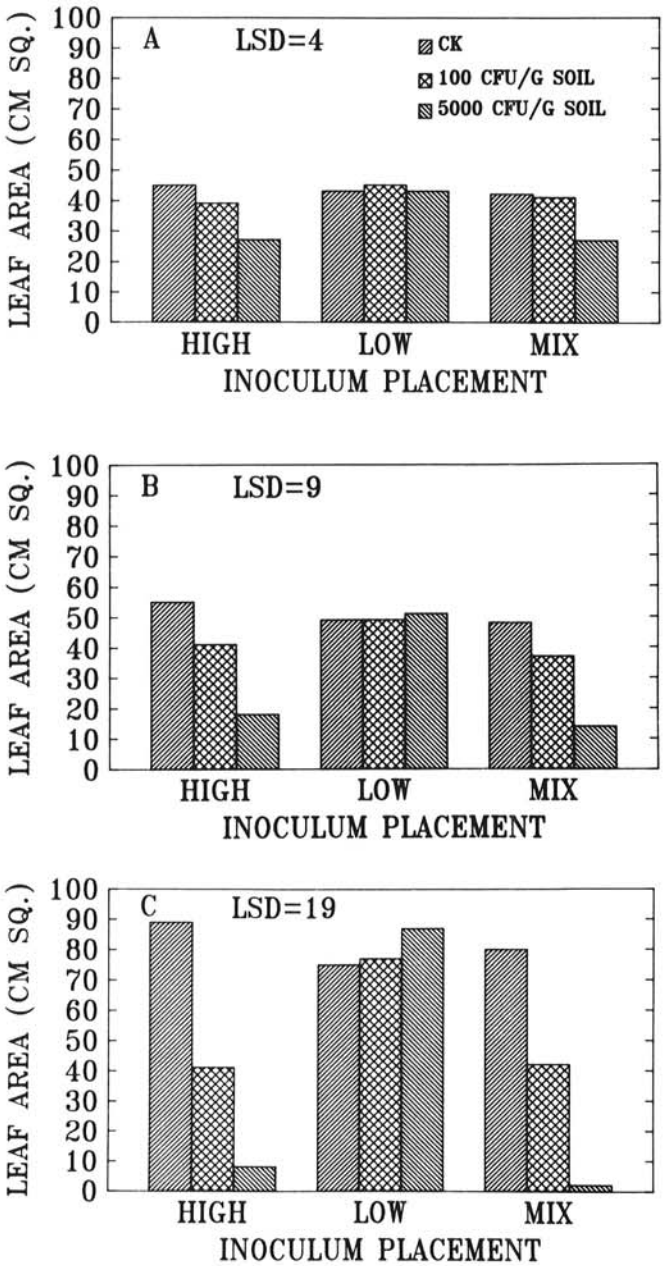

Fig. 2. Effects of inoculum density and placement of *Fusarium solani* f. sp. *pisi* on leaf area. Area of leaflets at a single node were measured three times during the study: 21, 33, and 48 days after plant emergence. Infested soil was placed in the upper (high), or lower (low) 10 cm of each container, or mixed throughout the entire container (mixed). LSD value in figures was computed at the 5% level of significance.

When working with soilborne pathogens, it is often difficult to determine whether an observed plant stress symptom is the result of pathogenic activity, some abiotic factor, or a combination of the two. The separation of these factors depends in part on the ability to measure the plant's initial responses to stress. The most sensitive measurement of disease stress in this study was leaf area. The rate of appearance of leaf nodes has been previously reported to be a sensitive indicator of plant stress (4,12,13,26), but we found node appearance to be the least sensitive of all measurements taken. Only toward the end of the experiment, when infected plants were extremely stressed, did node development differ from control plants. The fact that node development in peas is a relatively stable process, however, could be useful in studies of plant stress. By measuring factors such as available soil moisture, temperature, and disease symptom appearance, then relating them to node development, leaf area measurements at a given node could be taken at the end of the growing season and correlated with the onset of any particular stress. A simple model could then be developed to determine how stress at any given time during the growing season ultimately affects yield.

If pea yields in the Pacific Northwest are to be improved, it is important to determine how the various components of the disease complex interact to cause plant stress. This study suggests that more attention be directed to studies of the lower root system. Pumphrey et al reported that water stress could account for over 60% of the year-to-year variation in pea yield (25). Because most of the available soil moisture during flowering and pod filling is below 20 cm, anything that inhibits root function at this depth will have a direct influence on yield. Identifying the factors that affect the timing of root rot development on the lower root system should be a fruitful area for future research.

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