

**Soil Moisture, Root System Density, and Infection of Roots of Pinto Beans
by *Fusarium solani* f. sp. *phaseoli* Under Dryland Conditions**

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ABSTRACT

Dryden, P., and Van Alfen, N. K. 1984. Soil moisture, root system density, and infection of roots of pinto beans by *Fusarium solani* f. sp. *phaseoli* under dryland conditions. *Phytopathology* 74:132-135.

Fusarium solani f. sp. *phaseoli* is a chronic pathogen of dryland pinto beans (*Phaseolus vulgaris*) in the southwestern United States. Root system density in the soil was studied in relation to pathogen propagule density, root infection, and soil water availability. Root system density varied with the time elapsed after planting and depth in the soil, with the majority of the roots being located between 15 and 45 cm deep. Pathogen-propagule density corresponded to that of the root system, except that few propagules were found below 60 cm. Root infection occurred primarily above 45 cm.

Soil water availability was greatest below 60 cm during the month when the beans were maturing. The spatial relationship in the soil between root system density, infection by the pathogen, and soil water availability indicates that infection of roots occupying the soil volume between the surface and 45 cm deep should have little deleterious effect on a plant's water relations because of the lack of available water above 45 cm. Approximately 50% of the roots were in this region of the soil.

Root rot caused by *Fusarium solani* f. sp. *phaseoli* (Burk.) Snyder & Hans. is a chronic problem in the dryland pinto beans cultivated in the San Juan Basin of the Four-Corners area of Utah, Colorado, Arizona, and New Mexico. Few fields have escaped infestation, and by the end of each season most plants show some degree of root rot. Yield losses of up to 84% have been estimated (8). Hypocotyl lesions are the most obvious symptom of the disease; however, Burke (3) has concluded that disease development on the lateral roots is more important than that on the hypocotyl or tap root regions. He found that subsoiling increased yield (5), probably because roots penetrating the subsoil are healthy and grow abundantly even when the surface soil is infested (4,10). To date, however, the prevalence of rootlet infection has not been measured as a function of the strata of soil in which the roots are growing.

Availability of water appears to be an important factor in plant

tolerance to the disease. In relatively moist soils, roots easily penetrate the plow sole (4,10) and, presumably, grow there undamaged by infection. In fact, irrigation at the Southwestern Colorado Experiment Station has increased yields up to 10-fold (A. Foster, *personal communication*), although factors other than disease were surely involved.

In the San Juan Basin, low rainfall and low humidity make lack of water a major limiting factor affecting bean yields. Root rot may aggravate climate-induced water stress by damaging the lateral root system. If this is the case, future control strategies will need to consider both root system and pathogen distributions in relation to soil moisture. Our objective in this study was to determine the relationship between root system density, pathogen inoculum location, and soil moisture under dryland conditions.

MATERIALS AND METHODS

All soil samples were taken from the Colorado State University San Juan Basin Research Station near Cortez, CO. The soil at this location is a Witt series silty clay loam. Soil samples were taken

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with an 8-cm-diameter soil auger, the perimeter of which was 5 cm from randomly selected bean plants. The soil core was separated into 15-cm depth increments, and 20 cm³ (~3.93 cm) of soil from the bottom of each depth increment was set aside for pathogen propagule counts and water content determination. The remaining soil was used to determine root length. Four replicate cores were collected during the first weeks of July, August, and September.

Water content was determined gravimetrically. Propagules were counted by suspending them in water and quantitatively plating them on selective agar (12). In previous experiments, only one strain of *F. solani* f. sp. *phaseoli* (isolated from either plants or soil) produced symptoms in inoculated plants. This clone had a characteristic colony morphology and produced macroconidia, but not microconidia. Only colonies of this pathogenic strain were counted on dilution plates.

To determine root length per soil depth increment, soil was wet-sieved through a 246- μ m (60-mesh) screen. Roots were stored in 50% ethanol until counted by the line intersect method (13). Instead of separating organic matter from the roots prior to counting, each sample was mixed with a warm agar solution (5 g/L) and allowed to solidify on a tray (made by folding a sheet of cellulose acetate upon which a grid had been traced). The roots settled to the bottom of the tray before the agar solidified, allowing the operator to count live roots through a dissecting microscope. Color was the principal criterion used to distinguish live from dead roots. Two grid sizes (1- or 2-cm) were used, depending on sample density. Each intersect count was kept between 50 and 500. Larger samples were divided into subsamples, counted individually, and the counts were summed.

To determine the efficacy of the method, particularly the ability to separate roots from organic matter, greenhouse-grown roots were washed, weighed, and added to fallow field soil, treated as field samples, and counted. Replicate samples of roots were counted directly without being mixed in soil. The means of four replicates differed only by 4%, which was not statistically significant, $P = 0.01$, according to Student's t -test.

Isolation was used to assess degree of root colonization. In September and October (at harvest), five additional cores were taken to a depth of 60 cm and divided into four 15-cm increments. Roots were separated by sieving, and rootlets having diameters between 0.25–1 mm were cut into 1-cm lengths, surface sterilized with sodium hypochlorite, rinsed in sterile water, and a total of 15 cm of roots was plated onto PCNB agar for each sample. Optimal conditions for surface sterilization were found to be 2 min in 20% Clorox® (Clorox Corp., Oakland, CA) containing 0.02% sodium dodecyl sulfate. After 1 wk of incubation at 25 C, the number of 1-cm pieces from which pathogen colonies developed was counted. Data were subjected to a factorial analysis of variance.

Since soil cores had to be stored for up to 3 days at room

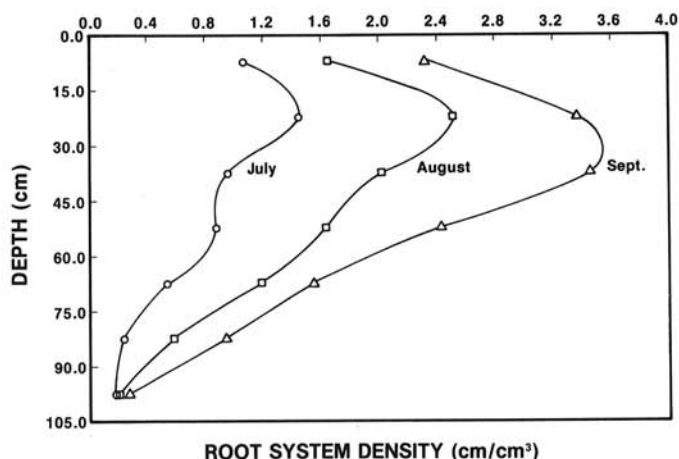


Fig. 1. Root system densities 5 cm from bean plants growing in dryland soil as determined by using the line intersect method (13). The data are means of four replications. Samples were taken during July (○), August (□), and September (△).

temperature before sieving, an experiment was conducted to ascertain whether colonization was occurring during storage. Greenhouse-grown roots were added to field soil (inoculum level, 900 propagules per gram) and incubated in the same manner as field samples. Predominantly *Mortierella* sp. and rarely saprophytes were isolated. Therefore, it was concluded that pathogens isolated from field samples had colonized the roots prior to collection.

The relation between soil water content and water potential was investigated using a composite sample of the various depths. The soil was moistened, then allowed to air-dry. At intervals during the drying cycle, water content was measured gravimetrically and water potential data, obtained by using a thermocouple psychrometer, were recorded. Data from three drying cycles were pooled.

The relative importance of the various soil layers in supplying water to roots were roughly assessed by using a simplification of Nimah and Hank's (11) formula. We have simplified this formula as follows:

$$A(z,t) = -(WP_{\text{root}} - WP_{\text{soil}}) \times RDF \times K(WC).$$

This relation states that root water extraction or water availability (A) at depth z and time t is a function of the differential between root and soil water potentials (WP), the proportion of active roots (RDF) at depth z and the hydraulic conductivity at depth z [$K(WC)$]. We estimated $K(WC)$ from the soil water characteristic curve (Fig. 2) by using the formula of Kunze et al (9) with $n = 10$ moisture-content classes. Availability terms for each depth were normalized by dividing each term by the sum of the terms for that month. The resultant relative availabilities were comparatively insensitive to the exact shape of the moisture characteristic curve. For instance, when other arbitrarily drawn curves (curves b and c, Fig. 2) flanking the experimentally determined one (curve a, Fig. 2) were used in the calculations of $A(z,t)$, very little change in the values was obtained. Likewise, the formula of Nimah and Hanks (11) was relatively insensitive to the differential between root and soil water potential. Thus, we arbitrarily estimated WP_{root} to be -15 bars.

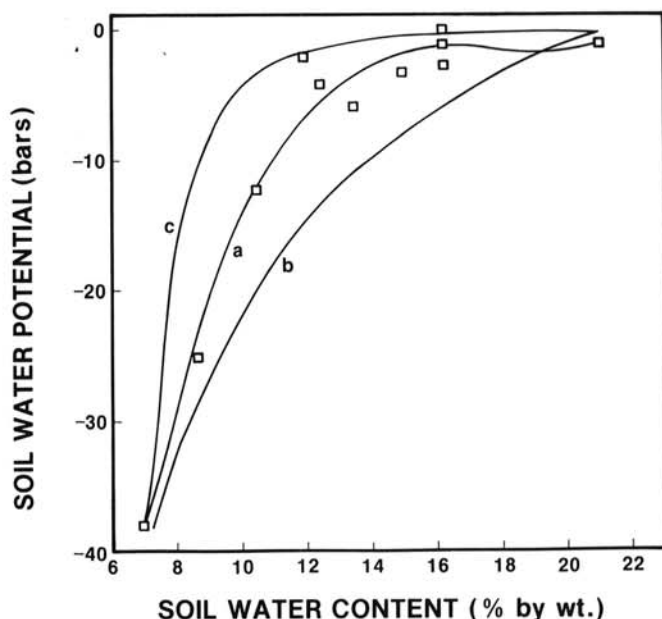


Fig. 2. Relationship between soil water content and soil water potential. Curve "a" represents a third-order polynomial fit of the data. This curve was used to calculate the $K(WC)$ parameter of the relationship listed in the legend to Fig. 3. Because of the error possible from extrapolating data from this curve, we tested the sensitivity of the curve shape to the determination of $K(WC)$ by arbitrarily drawing curves "b" and "c" and then determining the values of $K(WC)$ from these curves.

RESULTS

Soil propagule counts. Inoculum density in the soil did not change with time, but it did differ with soil depth (Table 1). It was significantly lower at depths >60 cm than at depths ≤45 cm.

Root system density. The differences in root growth with both time and depth were significant, but there was no interaction between the two. Root system density increased as expected over the growing season (Fig. 1). It was greatest in the interval between 15 and 30 cm during July and August, but by September it was in the 30- to 45-cm zone. For all 3 mo, the midpoint of cumulative root system density (ie, the depth at which half the roots were above and half below) was in the 30- to 45-cm interval.

Soil water content. Soil water content data are reported in Table 2. The soil dried progressively, mostly above the 60-cm depth. Rainfall during late August partially refilled the upper soil profile so that the water content at the 15-cm level was greater in September than in August. Water potential values were estimated from the soil moisture characteristic curve (Fig. 2). Water availability estimates at different soil depths and sampling times are shown in Fig. 3.

Root isolations. Root lesions were quite prevalent, ranging from 0.27 lesions per centimeter of root at 60 cm deep to 0.83 lesions per centimeter at 15 cm; these values were significantly different, $P = 0.05$. The number of lesions was always greater than the number of pathogen colonies that were isolated from the roots. Pathogen colonies were frequently isolated from roots without symptoms, yet were inconsistently isolated from lesions. Christou and Snyder (6) reported similar initially symptomless infections and poor efficiency of isolation from root lesions of *F. solani* f. sp. *phaseoli*. We felt, however, that since the etiology of the lesions could not be definitely determined without microscopic examination, that a more conservative approach would be to quantitate colony isolation from roots rather than numbers of lesions. Table 3 reports the number of rootlet infections by the pathogen at different soil depths. Two-way analysis of variance revealed no differences between months and no interaction. Root infection dropped off significantly in the 45- to 60-cm zone. We expected it to be quite low below this depth, since inoculum was

almost totally absent below 60 cm.

Fusaria other than the specified pathogenic strain were also isolated. Only the strain considered in this study, however, reproduced hypocotyl symptoms upon inoculation.

Disease incidence. Large populations of propagules of *F. solani* f. sp. *phaseoli* were present in the plots used in this study; these propagules were found to be responsible for hypocotyl lesions on the beans. On a hypocotyl lesion disease rating scale from 1 to 5 (with 5 = dead plants), the average rating in the location of the plots was 1.93. This corresponded to a 20–40% necrosis of the cross-sectional area of the bean hypocotyl. At the end of the growing season, 3% of the surveyed plants had been killed by the disease. However, we have found that hypocotyl lesions are not a reliable indication of disease stress on plants (*unpublished*). The lesions cause stress once the hypocotyl is girdled. Prior to girdling, however, the lesions have no detectable effect on the water status of the infected plant. Of the plants that were selected for root

TABLE 1. Populations of *Fusarium solani* f. sp. *phaseoli* at various soil depths during the cropping season

Depth (cm)	Sampling period			Mean
	July	August	September	
0	588 ^{x,y}	480	1,112	727 a ^z
15	397	600	590	528 a
30	589	893	859	780 a
45	970	724	359	685 a
60	44	227	14	11 b
75	2	21	6	9 b
105	0	1	3	1 b

^xNumber of viable colonies per 20 cm³ of soil.

^yValues are means of four samples.

^zMeans followed by the same letter are not significantly different, $P = 0.05$, according to Fisher's protected least significant difference test.

TABLE 2. Soil water content and calculated water potential (WP) at various soil depths during the cropping season

Soil depth (cm)	June		July		August		September	
	Soil water (%)	WP (-bars)	Soil water (%)	WP (-bars)	Soil water (%)	WP (-bars)	Soil water (%)	WP (-bars)
0	3.7	86.1	4.1	78.8	3.0	99.4	8.5	24.8
15	20.4	1.5	16.7	1.4	8.1	28.1	9.5	18.1
30	20.2	1.5	17.9	1.6	12.0	6.8	11.6	7.9
40	18.3	1.6	19.3	1.7	15.7	1.5	14.7	2.0
60	18.2	1.6	19.6	1.7	17.8	1.6	17.3	1.5
75	18.3	1.7	19.5	1.7	18.0	1.6	17.5	1.5
90	17.6	1.6	19.8	1.7	17.3	1.5	16.8	1.5
105	18.5	1.7	18.5	1.7	17.5	1.5	16.1	1.5

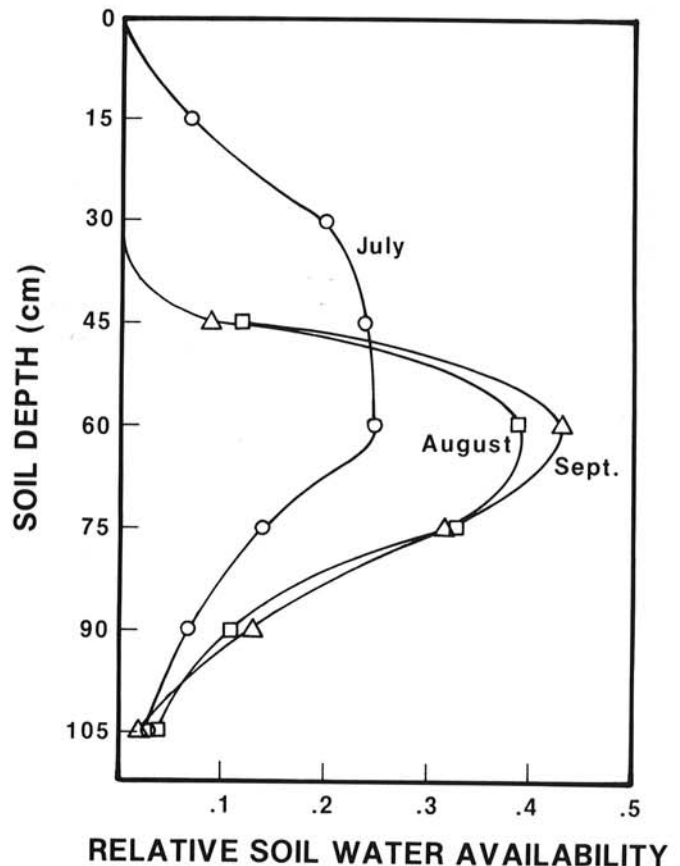


Fig. 3. Relative soil water availability in a soil profile as determined from the relationship $A(z,t) = -(WP_{root} - WP_{soil}) \times RDF \times K(WC)$. See Materials and Methods for description of parameters. The availability curves were calculated from July (○), August (□) and September (△) data. Relative soil water availability is unitless.

sampling, no more than 40% of the cross-sectional area of the hypocotyl was damaged.

DISCUSSION

Disease severity estimates of *F. solani* f. sp. *phaseoli* are hampered by the lack of knowledge concerning the relative importance of the effects of pathogen-caused lesions on plant vigor. Hypocotyl lesions are more easily seen than root lesions and are frequently used to evaluate severity of the disease (8). Unlike Keenan et al (8), we have not been able to use hypocotyl lesions as a reliable indicator of pathogen effects on yield in the San Juan Basin (*unpublished*). Thus, we were interested in assessing the importance and frequency of lateral root infections. Under dryland conditions, soil moisture availability and thus the plant's ability to extract water from the soil are the limiting factors in yield. Yields may be severely reduced if roots are invaded by the pathogen.

Like Christou and Snyder (6), we found that *F. solani* f. sp. *phaseoli* was not consistently isolated from root lesions that developed on roots grown in pathogen-infested soil. The numbers of lesions on roots always exceeded the number of pathogen colonies that were isolated per centimeter of root length. We also found, as did Christou and Snyder (6), that symptomless infections were common. This situation indicated recent invasion by the pathogen (6). The results of histological studies by Christou and Snyder (6) suggested that root infections developed slowly.

We estimated disease severity in roots by isolation of the pathogen rather than by counting lesions. We felt that this was a more conservative assessment of root colonization than was the counting of lesions. Since root lesions only yielded the pathogen at a frequency of 5–25% (6), the etiology of lesions could not be determined without histological studies, which were impractical in these experiments. The results of our study clearly showed the extent of root colonization by the pathogen. Pathogen inoculum was uniformly distributed in the soil to depths of 45 cm, which was where the bulk of the plant roots were found. Colonization of the roots of this depth was also essentially uniform, with about one colony of the pathogen being isolated from the roots each 2 cm of root length. We have not shown that infection of the roots results in physiological stress to the plants. Physiological studies are necessary before the extent of host damage due to a specific amount of root infection can positively be quantified.

In other studies, we found a high correlation between leaf water potential and yield of the plant (P. Dryden and N. K. Van Alfen, *unpublished*). Under dryland conditions, soil water availability becomes a limiting factor in yield. Water uptake by plants depends on a complex interaction among root characteristics and growth, soil water potential, and hydraulic conductivity (7). Each of these factors vary spatially and temporally, making field studies of uptake very difficult, although some have been published (1,2). In our study, we have attempted to correlate root distribution, propagule and root infection frequency, and estimated soil water availability at different depths in a vertical transect. The results of these studies showed that in July, roughly 20% of a plant's roots were below 60 cm with approximately the same percentage of the available water in this stratum. However, as the soil dried, the stratum containing these 20% of the roots then held almost 50% of the available water. This was because a small drop in soil water content reduced hydraulic conductivity much more quickly than it reduced water potential.

Pathogen infection of roots can act to reduce the root density by killing roots and perhaps reduce the functional efficiency of the remaining infected roots. The soil stratum below 60 cm contained significantly fewer pathogen propagules than in the upper strata. As severity of disease increased in the upper strata more reliance was placed on the deeper roots for maintaining the plant's water balance. The stratum from 45–60 cm deep appeared to be where pathogen infection of roots could significantly affect the plant's health. Late in the growing season about 50% of the available water was present in this stratum. The frequency of root infections was

TABLE 3. Proportion of root pieces infected with *Fusarium solani* f. sp. *phaseoli* at various soil depths

Depth interval	Sampling time	
	September	October
0–15	0.52 ^{ab}	0.48 a ²
15–30	0.63 b	0.61 a
30–45	0.55 b	0.64 a
45–60	0.22 a	0.39 a

¹Number of pathogen colonies isolated per centimeter of root. Fifteen 1-cm-long root pieces were plated out from each sample. Five samples were collected at each soil depth each sampling time.

²Root infection at depths followed by the same letter is not significantly different, $P = 0.05$, according to Fisher's protected least significant difference test.

high within this soil depth, and these infections could seriously affect the plant's ability to extract 50% of the water that was potentially available to it. Soil water availability above 45 cm was only a fraction of that in the deeper soil late in the growing season; thus, infection of roots above 45 cm should have little effect on plant water relations.

Assessment of total root infection under dryland conditions alone is not likely to be a more accurate indicator of disease severity than are estimates of hypocotyl lesion damage. Since nearly 50% of the roots were in soil depths containing little available water late in the growing season, infection of 50% of the roots by *F. solani* should have a minimal effect on the plant's ability to extract water. If the remaining 50% of the roots became infected, severe water stress could develop that would result in yield reductions.

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