Radial Extent of the Bean Spermosphere and its Relation to the Behavior of Pythium ultimum

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ABSTRACT

Bean seed exudates diffused through moist soil and stimulated sporangial germination of *Pythium ultimum*. In soil contiguous to the seed, 78% sporangial germination occurred within 3-4 hr after sowing. Extensive mycelial growth in soil and subsequent host penetration and infection occurred within 24 hr. Bean seed exudates, in sufficient quantity to stimulate sporangial germination of *P. ultimum* and chlamydospore germination of *Fusarium solani* f. *phaseoli* and to support vegetative growth, diffused through soil maintained at 28% moisture (pF 1.7) up to 10 mm from the seed surface within 24 hr. A lower soil moisture content (15.7%, pF 2.0) resulted in a decrease in the magnitude of the bean spermosphere. Phytopathology 61:165-168.

The spermosphere has been defined as the zone of influence of the seed on the soil microbial population (20, 23). The development of this zone results primarily from soluble organic materials released by the seed which stimulate an increase in the activity of the saprophytic microflora (15) and under certain conditions may favor pathogenesis by soil-borne plant pathogens (18).

While much is known about the effects of seed and root exudates on the soil microflora (15), little information is available as to the volume of soil around the seed or root into which exudates diffuse. Most studies indicate that the influence of plant exudations, as assessed either by bacterial or fungal plate counts or percentage propagule germination, occurs in close proximity (1-2 mm) to their respective planes or surfaces (6, 8, 14). Distances ranging from 5-20 mm, however, have been reported (2, 12, 19). The significance of such a zone of influence in relation to the disease cycle of specific plant pathogens remains obscure.

This investigation determined the zone of influence of the bean spermosphere under known soil moisture regimes and its relation to the behavior of *Pythium ultimum* Trow.

MATERIALS AND METHODS.—A Chular sandy loam agricultural field soil with the following characteristics was used: pH 7.3, organic matter 1.03%, sand 71.2%, silt 20.8%, and clay 8.0%. The soil was collected from the Salinas Valley in California and stored at 24 C in polyethylene bags.

Pythium ultimum was established in the soil as sporangia (22). The resulting population was ca. 2.0×10^5 propagules/g soil (21). *Fusarium solani* f. *phaseoli* was established in soil as chlamydospores, following the method of Nash et al. (11). The resulting population was ca. 1.5×10^5 propagules/g soil, estimated by the method of Nash & Snyder (10). Infested soils were stored in polyethylene bags.

Forty-two g of pathogen-infested soil were poured into a plastic cylinder placed on the sintered glass plate (medium porosity) of a Haines' (7) apparatus (Fig. 1). The soil occupied a volume of 34 cc and a depth of 3 cm. One bean seed, *Phaseolus vulgaris* L. 'Pinto', was placed in the center of the soil volume, which was then compressed to a bulk density of 1.24. The soil was wetted to saturation, and a known suction applied by adjusting the height of the water miniscus in the side arm. The time required to saturate and reach equilibrium depended on the suction employed, but never exceeded 20 min. The drying boundary of the soil, as determined by the Haines' apparatus, shows the relationship between soil moisture and the corresponding water suction, pF (Fig. 2). Fifty-cm suction (pF 1.7) resulted in a soil moisture of 28.3%, 100-cm suction (pF 2.0) in a soil moisture content of 15.7%. The latter value was taken as field capacity as recommended by Salter (16). The permanent wilting point was determined by a pressure membrane apparatus (13).

Soil samples, prepared as described above, were incubated for periods up to 24 hr. At the end of the incubation period, the soil core was plunged out of the cylinder and separated into halves by breaking at the fracture zone. Seven 2-mm soil sections were removed, starting adjacent to and proceeding radially away from the seed surface (Fig. 1). Four quadrants were sampled per seed. Soil sections were prepared for estimation of percentage germination of *F. solani* f. *phaseoli* chlamydospores and *P. ultimum* sporangia, using a modification of the method of Nash et al. (11). Soil samples were placed in glass vials and diluted 1:3 with sterile distilled water. The sample was then gently agitated and 0.1 ml of the soil suspension immediately removed, placed on a glass slide, smeared, and dried over a bunsen flame. The preparation was stained with 0.1% acid fuchsin in 85% lactic acid and observed microscopically at ×100 and 430. Values recorded represent the mean of three counts, 100 chlamydospores/count for *Fusarium* and three counts of 50 sporangia each for *Pythium*.

No seed germination occurred during the incubation period. Pathogen-infested soil without a bean seed served as a control. The possibility that seed exudation occurred during the brief saturation period necessitated a further control. Soil samples were prepared as de-

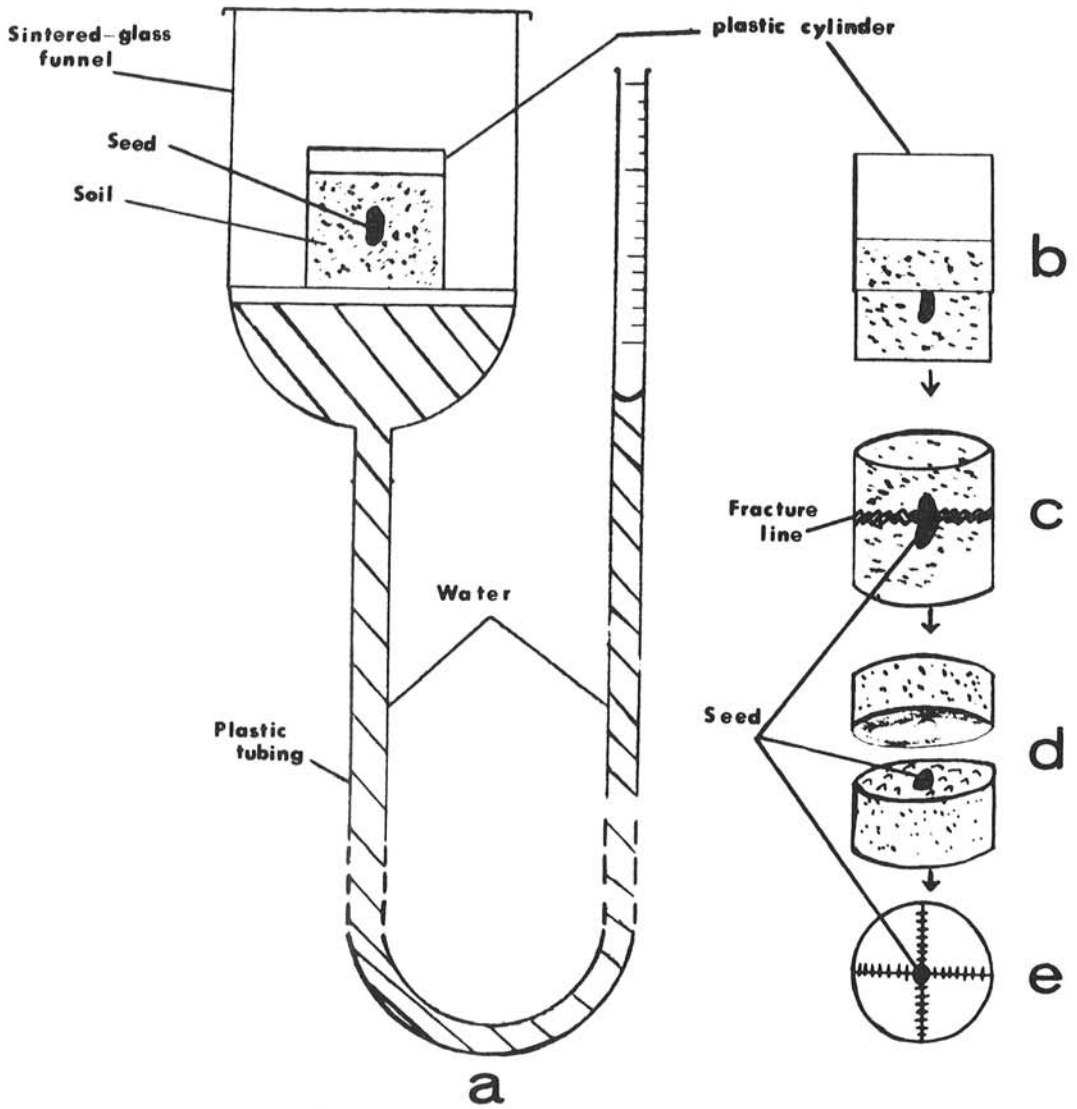

Fig. 1. Diagram illustrating (a) apparatus used for determining soil moisture characteristics and method of measuring the zone of influence of the spermosphere. Cylinder is removed from apparatus; (b) soil core extracted; (c) fractured; and (d) separated into halves. Seven 2-mm soil sections (e) were removed starting adjacent and proceeding radially away from the seed surface.

scribed above with a seed. Immediately upon reaching equilibrium at a known suction, the soil was removed, fractured, and separated into halves. The bean seed was carefully removed and the soil halves were subsequently incubated for 24 hr at 24 C in a moist chamber. Two-mm thick soil sections were taken adjacent to the depression in the soil made by the seed and assessed as described. All experiments were repeated at least 5 times.

RESULTS.—Sporangia of *P. ultimum* began to germinate in soil 0-2 mm from the seed surface within 1.5 hr after sowing seed, and reached a max germination of 78 ± 4% after 3-4 hr incubation at both 50- and 100-cm suction. Extensive mycelial growth, resulting in a "balling" of the soil around the seed, occurred within this zone after 12-hr incubation. Mycelial lysis and production of terminal and intercalary sporangia were observed in soil taken from soils subjected to 50- and 100-cm suction for 24 hr.

Extensive mycelial growth occurred 2-4 mm from the seed surface at both 50- and 100-cm suction after 6-hr incubation. By 12 hr, sporangial germination was observed 8-10 mm from the seed surface at 50-cm suction and 4-6 mm at 100-cm suction. Germ tubes varied in length from 20 μ to greater than 1,500 μ. Numerous germ tubes, due to their extensive length, were dislodged from the parent sporangium during the preparation of soil smears. Germlings in various stages of hyphal lysis and production of terminal and intercalary sporangia were also observed in the same soil

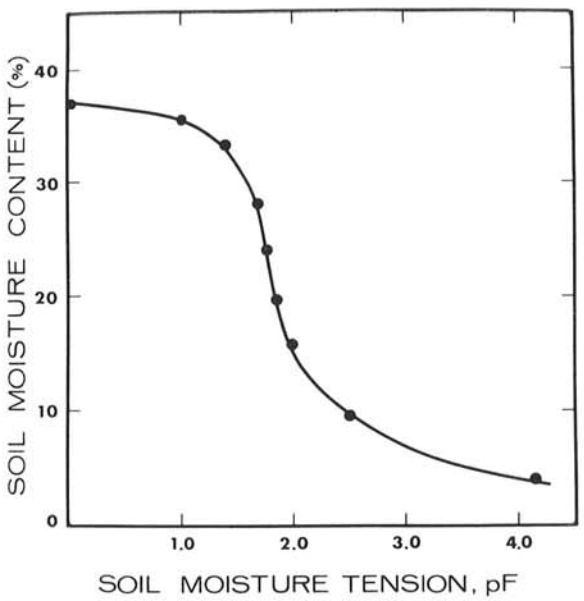

Fig. 2. Moisture characteristics of Salinas Valley sandy loam soil.

preparations. Consequently, quantitative estimation of the percentage germination was not possible. Germination at distances greater than 2 mm from the seed surface occurred erratically throughout the entire incubation period, and thus obviated estimation of the percentage germination at a prescribed distance from the seed surface. No differences were noted in the percentage and rate of sporangial germination in samples from the 0-2 mm range taken from the 4 quadrants. No sporangial germination was observed in the controls.

Infection of bean seed occurred with equal rapidity at both 50 and 100 cm of water suction. Removal of seed coats (prior to radicle emergence) from bean seed 24 hr after sowing in infested soil revealed water-soaked lesions on the cotyledons along the suture and brownish discoloration of the radicle. Free-hand sections from these areas showed abundant nonseptate mycelium ramifying intercellularly. Isolations from these areas consistently yielded *P. ultimum*.

In view of the erratic germination of sporangia at distances greater than 2 mm from the seed surface, further attempts to measure the zone of influence of the spermosphere were made using *Fusarium solani* f. *phaseoli* as a biological assay organism according to the methods of Cook & Snyder (3).

Chlamydospore germination within 0-2 mm of the seed surface was initiated 4-5 hr after sowing seed in infested soil. Germ tube rate of growth was slow, rarely exceeding 100 μ in length after 24 hr. Germ tubes were not dislodged from chlamydospores during the preparation of soil smears.

Chlamydospore germination was recorded 10-12 mm from the seed surface after 24-hr incubation at 50-cm water suction, and 6-8 mm at 100-cm water suction (Table 1). Although the percentage germination varied considerably among replications (reflecting seed variability), similar patterns were obtained. Chlamydospore germination was consistently observed 2-4 mm further at 50-cm suction than at 100-cm suction. Although quantitative data were not taken, lysis of germlings was not observed at distances greater than 4 mm from the seed surface after 24-hr incubation, and germ tubes appeared to be of greater diam than those within the 0-4 mm of the seed surface. No chlamydospore germination was observed in the controls.

Discussion.—High soil-moisture conditions, field capacity (FC), and above, are usually associated with increased incidence of damping-off by *Pythium ultimum*. Such conditions are believed to act directly on the host by decreasing host vigor and increasing seed exudation (1, 9). This, in turn, influences disease incidence by stimulating spore germination and supporting vegetative growth. This hypothesis is supported by several studies which have correlated the quantity of seed exudation with increased incidence of damping-off (4, 9, 17).

Our results indicate that, in addition to direct effects on the host, high soil-moisture conditions provide a suitable environment for the rapid diffusion of seed exudates through soil. Both the rate and percentage of sporangia germination by *P. ultimum* and chlamydospore germination by *F. solani* f. *phaseoli* support this contention. Based on the findings of a previous study (22), estimates of the quantity of exudate released by the germinating seed in soil, as assessed from the percentage sporangia germination, indicate that at least 30 μg of glucose equivalents/g soil were exuded within 3-4 hr after sowing seed.

Pythium ultimum, in order to compete effectively for ephemeral substrates or the colonization of young susceptible host tissues, must rely on rapid spore germination and high hyphal growth rates (5). We showed that within 1.5 hr after sowing seed in soil, sporangia

Table 1. Effect of soil moisture on chlamydospore germination of *Fusarium solani* f. *phaseoli* around germinating bean seeds

Water suction, cm	Distance from seed surface, mm						
	0-2	2-4	4-6	6-8	8-10	10-12	12-14
50	26.0[a]	23.3	23.5	14.0	10.3	2.8	0.0
	31.1	34.2	36.4	14.5	4.5	1.2	0.0
100	48.5	35.2	28.6	6.0	0.0	0.0	0.0
	29.3	31.9	24.6	8.5	1.2	0.0	0.0

[a] Data represent the per cent chlamydospore germination of *Fusarium solani* f. *phaseoli* in each 2-mm soil section from two experiments after 24-hr incubation at 24 C.

of *P. ultimum* were stimulated to germinate and reached a percentage germination of about 78% in 3-4 hr in soil contiguous to the seed. Host penetration and infection occurred within 24 hr. The rapidity with which these processes take place give *P. ultimum* a competitive advantage in the colonization of young susceptible host tissues. High soil-moisture conditions allow seed exudates to diffuse through soil in sufficient quantities to stimulate spore germination and support vegetative growth to a distance of about 10 mm from the seed surface. In view of the rapid rate of germ tube growth through soil [ca. 300 μ/hr (22)], it is conceivable that this organism has the capacity to traverse relatively large expanses of soil and colonize seeds prior to emergence. A lower soil moisture apparently decreases the distance to which exudates diffuse, thus reducing the inoculum potential [sensu Garrett (5)] by (i) decreasing the number of propagules affected; and (ii) decreasing the nutrients available to the propagules for germination and vegetative growth. Although seed colonization in this investigation occurred with equal rapidity at both 50- and 100-cm water suction, this is attributed to the high inoculum density which apparently masked the effect of an increase in the inoculum potential at the higher soil moisture level.

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