

Vertical Distribution in Soil of and Induction of Disease by Strands of *Phymatotrichum omnivorum*

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ABSTRACT

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Soil cores to a depth of 90 cm were taken in cotton fields infested with *Phymatotrichum omnivorum* near Marana, AZ, from June 1979 through April 1980. Total viable strand lengths (VSL) of *P. omnivorum* of 0.1 and 0.2 cm (per 50 g soil) were recovered at depths of 15-30 and 30-60 cm, respectively, in June 1979, whereas no viable strands were recovered at 0-15 or 60-90-cm depths. VSL increased during July and peaked during August and September, when maximum VSL of 0.1, 0.4, 1.0, and 0.6 cm (per 50 g soil) were recorded for depths of 0-15, 15-30, 30-60, and 60-90 cm, respectively. VSL declined after November. In April 1980, VSL at depths of 0-15, 15-30, 30-60, and 60-90 cm were 0.0, 0.2, 0.3, and 0.05 cm, respectively. All infected taproots contained viable strands at depths of

0-15, 15-30, and 30-45 cm during field collections made in July and August 1978 and 1979. Percentages of roots from which viable strands were recovered fell to 35-50% (0-15-cm root depth), 53-77% (15-30-cm root depth) and 76-96% (30-45-cm root depth) in later collections made from November to February (before plow down). *P. omnivorum* was isolated from internal noncortical tissue of all infected cotton roots collected from the field in August. Isolations fell to 40-80% in September and no positive recoveries were made after October. Inoculation of cotton plants with strands in field, laboratory, and greenhouse studies did not result in disease. Further studies indicated that sclerotia germinated only from broken strand fragments attached to the sclerotia.

Phymatotrichum omnivorum (Shear) Dug. is a soilborne plant pathogen that induces serious losses in the southwestern United States and in northern and central Mexico. The fungus produces a root-rot disease (commonly referred to as *Phymatotrichum* or Texas root rot) of over 2,300 species of dicotyledonous plants (3,11,12). Although *P. omnivorum* has been studied since 1888 (9), aspects of the life cycle of the pathogen are still not well understood.

P. omnivorum produces strands and sclerotia. Sclerotia develop exclusively on maturing strands. The importance of strands in the life cycle of the fungus is not known. Information regarding strands primarily concerns their survival on the surface of roots or in rotting root tissue, although two reports concern their potential to be pathogenic. Dana (1) claims to have successfully inoculated a single cotton plant using strands of *P. omnivorum*. Neal and McLean (6) could not induce disease by use of strands for inoculum.

Several individuals investigated the survival of strands on infected cotton roots. Wheeler (16) demonstrated that strands can survive for 12 mo on infected cotton roots in Arizona. McNamara et al (4,5) found viable strands on decayed cotton roots in Texas fields left fallow for 5 yr; however, quantitative data on the survival and pathogenicity of strands is not available.

Strands form on the exterior of roots, but only fungal hyphae, which are formed from strands, penetrate internal tissues. Taubenhaus and Ezekiel (14) isolated *P. omnivorum* from the interior of infected but living roots. Because they could not isolate the fungus from decayed roots, they concluded that *P. omnivorum* could survive in infected cotton root tissue but not in decayed root tissue. Taubenhaus and Killough (15) recovered *P. omnivorum* from recently killed cotton roots but not from those dead longer than 26 days.

Infected living roots carrying strands have successfully been used as inoculum. Strands growing from these roots were capable of inciting disease (7,14,15). However, introduction of plants with decayed roots in an infection court seldom resulted in disease

(7,14,15), presumably due to a lack of strand production from the roots.

Ratliffe (10) suggested that a saprophytic stage of *P. omnivorum* occurred on dead cotton roots in Texas. However, this view was not supported by others (8,14,15).

The present study concerns the distribution and viability of strands in soils and on infected taproots of cotton, the changes in strand populations with time, and the role of strands in the initiation of *Phymatotrichum* root rot.

MATERIALS AND METHODS

Recovery of *P. omnivorum* from soil. The vertical distribution of strands in naturally infested soil was determined by taking soil cores with a hydraulically operated coring device (Bull Soil Corer, A.D. Bull Enterprises, Chickasha, OK 73018) mounted on the back of a pickup truck. The apparatus is capable of taking 5-cm cores to a depth of 1.8 m. The truck was driven directly into the field for core sampling.

Collections were made monthly from June 1979 through April 1980 from two infested cotton fields near Marana, AZ. A collection consisted of three to six cores taken from within a particular root-rot circle in each of the two fields, which consisted of Gila silt loam (GSL) and Pima clay loam.

Immediately after collection, each core was divided into the following segments relative to the soil line: 0-15, 15-30, 30-60, and 60-90 cm. Each segment was placed in a plastic bag. The samples were taken to the laboratory under ice, stored under refrigeration, and processed for strands and sclerotia within 7 days.

Strands and sclerotia were extracted by suspending 50 g of soil in 800 ml of water and then rapidly decanting the suspension onto a 0.149-mm (100-mesh) sieve. The extraction of each 50-g sample was repeated at least three times to assure complete removal of the propagules. Strands and sclerotia were washed from the sieve into petri dishes. They were handpicked under a dissecting microscope and placed on water-agar to which was added streptomycin sulfate (200 mg/L) and penicillin G (200 mg/L) after autoclaving (AB medium). Strands and sclerotia were examined for germination after 3 days.

During the extraction process, fragmentation of strands occurred. Because fragmentation was not consistent with respect to length or number of fragments, strand numbers could not be used

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to quantify strands removed from soil or to compare samples. Strands per sample were quantified by taking the sum of the lengths of viable strands in a sample. Strands were measured using a compound microscope with an ocular micrometer. The total length of the nonviable strand fragments also was determined. For routine analysis, two 50-g samples were taken from each core segment. The total length of strands obtained from the two samples were averaged to obtain a strand-length value per core segment. These values were then averaged with values obtained from other core segments to obtain the viable strand length (VSL) or the total (viable and nonviable) strand length (TSL) per 50 g of soil.

Recovery of *P. omnivorum* from roots. Infected cotton roots were collected at monthly intervals from October 1978 through February 1980 from infested areas in two separate fields near Marana, AZ, and composited for a total of 20–50 roots per collection. The roots were kept moist, transported under ice, and stored under refrigeration. Each root was divided into the following segments with respect to the soil line: 0–15, 15–30, and 30–45 cm. All root segments were examined for strands within 2 days. Strands were handpicked from each segment under a dissecting microscope and placed on AB medium.

Root segments used for strand determinations also were used to determine whether *P. omnivorum* could survive within the root. Each root segment was stripped of cortical tissue, washed under tap water, and blotted dry. Five to six small pieces of tissue were aseptically removed from the vascular and pith tissues of each segment, surface-sterilized in 0.5% sodium hypochlorite for 1–3 min, blotted dry, and placed on AB medium. *P. omnivorum* growing from any of the five to six chips constituted a positive

recovery.

Disease induction from strands and sclerotia. Strands and sclerotia were produced in the laboratory by the following method. Sorghum seeds were washed under tap water and soaked overnight. The seeds were added to 15-cm petri plates and autoclaved 1 hr on two separate days. Sterile seeds were colonized with a known pathogenic culture of *P. omnivorum*. After 1 mo, 2-cm square blocks of infested seeds were cut from the plates and incubated in sterile 15-cm glass petri plates for 2 wk at 30 C. The blocks were then placed into 1-L jars containing 900 g of unsterile, air-dried GSL, which was sieved (20 mesh), adjusted to 20% moisture by weight, and covered with a lid containing holes for aeration. The cultures were incubated at 30 C for 1 mo, after which large numbers of mature strands and sclerotia could be separated by wet-sieving from the soil cultures.

Field plots were set up in cotton fields with no history of Phymatotrichum root rot to determine whether strands could induce disease in mature cotton plants. The treatments consisted of inoculating 3-mo-old flowering plants (Delta Pine 61) with strands removed from naturally infected plants in the field and with naturally infected cotton roots bearing strands. With the aid of a dissecting microscope, strands were removed from infected cortical tissues of roots and stored in tap water in an ice chest 1–2 hr before use. For each inoculation, a trench 30-cm deep was dug to expose the healthy taproot. Twenty to 30 strands in water were placed on or near the exposed surface of each root at depths between 15 and 30 cm. The trench was then carefully filled with moist soil.

Infected roots (15–20 cm in length), used as inoculum, were positioned parallel and adjacent to healthy taproots, one infected

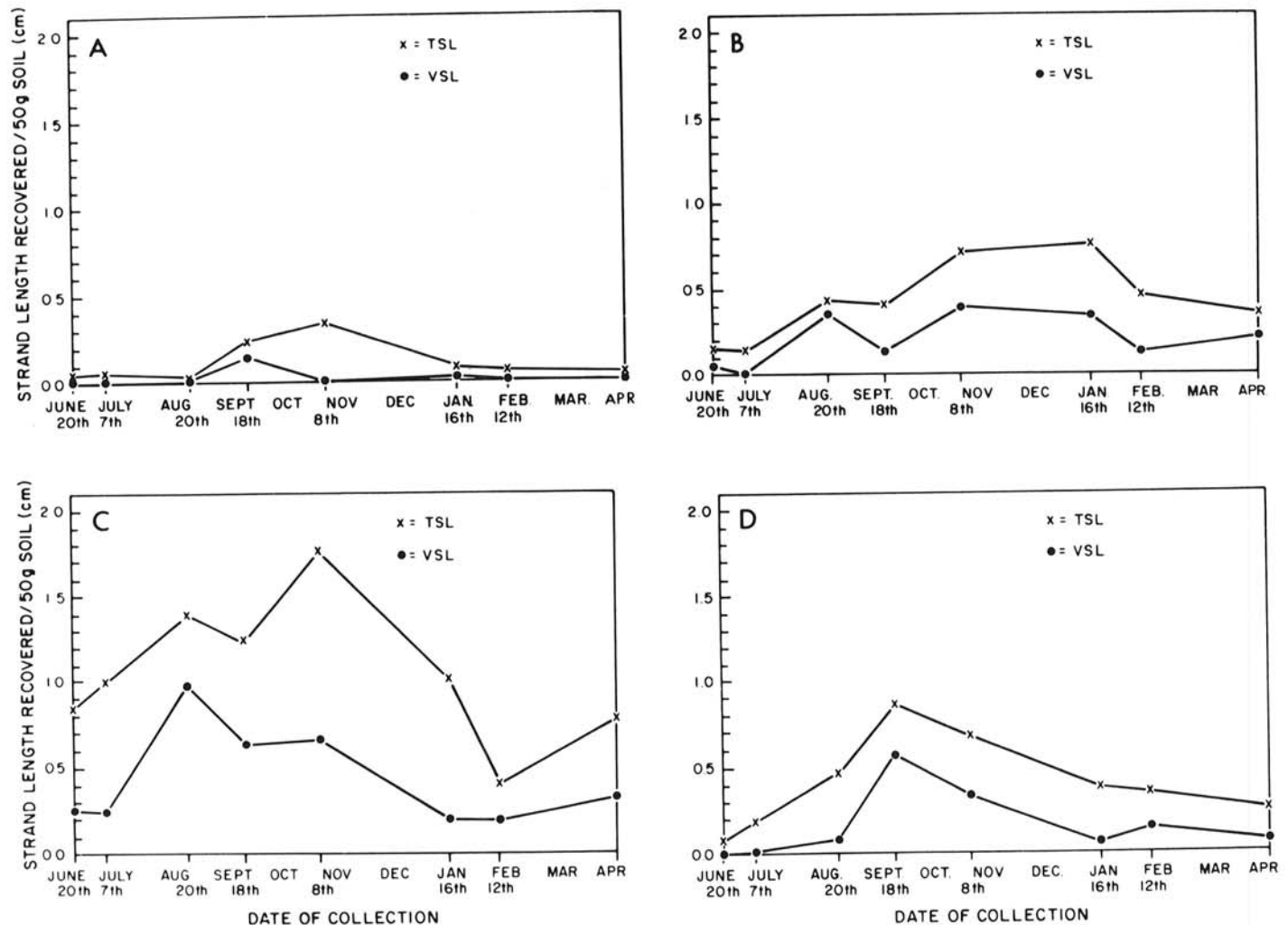


Fig. 1. Total strand length (TSL) and viable strand length (VSL) of *P. omnivorum* recovered from depths of: A, 0–15; B, 15–30; C, 30–60; D, 60–90 cm in infested cotton soils, 1979–1980.

root per plant. The field was irrigated approximately 1 hr after the plants were inoculated. Strands representative of those used as inocula were taken to the laboratory to determine viability. Eight weeks after inoculation all plants were dug and examined for disease.

Cotton plants (DP 61) were grown in unsterile or sterilized GSL in 15-cm pots in growth chambers maintained at 28–30 C. Sclerotia or strands, wet-sieved from soil cultures, were positioned 5-cm deep on or near the taproot of each cotton plant in several experiments with plants ranging in age from 5 to 20 wk. Periodic examinations were made on propagule growth and lesion development.

RESULTS

Recovery of *P. omnivorum* from soil. Strands of *P. omnivorum* were found throughout the year to depths of 90 cm in infested areas in GSL and Pima clay loam at Marana, AZ. Strands were never recovered from adjacent disease-free areas or at depths below root penetration. The appearance of Phymatotrichum root rot in July 1978 and 1979 preceded the increase in VSL at both locations. In June 1979, VSL of 0.1 and 0.2 cm per 50 g of soil at the GSL location were recovered at depths of 15–30 and 30–60 cm, respectively. No strands were recovered at 0–15 or 60–90-cm depths at either location. VSL increased during July and peaked during August or September, when VSL were about 0.1, 0.4, 1.0, and 0.6 cm for depths of 0–15, 15–30, 30–60, and 60–90 cm, respectively. TSL also increased and peaked during these same periods; maximum TSL values of 0.3, 0.7, 1.8, and 0.8 cm were recorded for soil depths of 0–15, 15–30, 30–60, and 60–90 cm, respectively (Fig. 1).

VSL and TSL began declining in November. By the middle of April 1980, VSL of 0.0, 0.2, 0.3, and 0.05 cm were recovered from 0–15, 15–30, 30–60, and 60–90 cm, respectively (Fig. 1). Plots of TSL versus VSL indicated that these two indices fluctuated similarly with time (Fig. 1). Strands obtained from wet sieving field soil ranged in length from 0.1 to 1.5 cm.

Fifteen sclerotia were recovered from 120 soil cores taken to depths of 90 cm from June 1979 through April 1980. Only four of the 15 sclerotia germinated when placed on AB medium. When 100 uninjured, mature sclerotia were sieved from soil cultures and placed on AB medium or moist field soil, germination occurred only from the strand fragments attached to the sclerotia and not from the sclerotial body. However, if the sclerotial rind was ruptured, then germination occurred from the underlying exposed cells.

Recovery of *P. omnivorum* from roots. Strands ranged in length from 0.5 to 5 cm and formed a continuous interconnecting network extending along the surface of infected taproots of mature cotton plants. Although strands were found on all infected taproots and less frequently on smaller, lateral roots, sclerotia were never seen on approximately 500 cotton plants examined over a 3-yr period. To determine the relationship between soil depth and strand viability, infected taproots of cotton were collected from depths to 45 cm from October 1978 through February 1980. All roots at depths of 0–15, 15–30, and 30–45 cm contained viable strands when collections were made on 5 August 1979. By the middle and latter part of August, viability remained at 100% at depths of 15–30 cm and 30–45 cm but decreased at 0–15 cm. Collections made in the same areas in January and February indicated that strand survival was highest at the lowest soil depths. For example, 96% of roots at a depth of 30–45 cm contained viable strands, whereas only 34% levels occurred at depths of 0–15 cm (Table 1).

P. omnivorum was readily isolated from the pith or stele tissue of infected roots that were not completely rotted. As the root deteriorated and plant death occurred, the fungus became more difficult to recover from interior root tissue. Positive recoveries of *P. omnivorum* were 100% in July and August. By the middle of September, however, the recovery fell to 60–70%. Isolation of *Phymatotrichum* from rotted interior root tissue was not possible after 20 October 1979.

Ability of strands and sclerotia to induce infection. In the replicated field plot at Marana no disease occurred after 2 mo in

any of 40 plants where strands were placed on the taproot. The strands germinated but were not able to invade root tissue or induce lesions. The strands used as inoculum, which were all viable when recovered from the soil, were taken to the laboratory and placed on water agar. When infected roots were used as inoculum, 45% of the plants became infected. No disease occurred in uninoculated controls.

No disease or root lesions occurred in any of several experiments over a 2-yr period in which strands were placed on the taproots of cotton of varying ages growing in sterile or unsterile GSL in temperature chambers or in soil maintained at 28–30 C. Successful infections occurred when sclerotia were placed in or near the taproot or when infected sorghum seed was used as inoculum.

When roots of cotton containing strands of *P. omnivorum* were buried in moistened, raw GSL, strands grew from living but not from dead root tissue. In one experiment, sorghum-seed-block-inoculum was placed adjacent to taproots of cotton plants 10 wk old growing in raw GSL in pots in temperature tanks maintained at 28–30 C. The inoculum was removed after varying periods (3, 6, 9, and 12 days) to determine the minimum time needed for infection. After 6 wk, the percentage of dead plants was 60, 85, 100, and 100, respectively.

DISCUSSION

Marana, AZ (760 m elevation, 32° N latitude), was selected as a location for these studies because *Phymatotrichum* root rot induces major losses in the area. At this location, cotton is normally planted in April and harvested in the fall and early winter. *Phymatotrichum* root rot generally appears in cotton from early July until the middle of September. The highest number of viable strands recovered from the soil was in August and September when disease incidence was at maximum levels. Highest numbers of viable strands were also found on infected roots during this same period.

Wheeler (16) and McNamara et al (4,5) established that strands can survive at least 1 yr on dead roots. Results of our study on the recovery of strands from surfaces of infected roots showed that strand viability increased with soil depth. This suggests that lower soil depths are more conducive to strand survival and that strands may remain viable for longer periods of time at lower soil depths. Factors influencing production and/or survival of strands at various depths are unknown.

Survival of *P. omnivorum* within root tissues was not correlated with survival of strands on root surfaces or in soil. *P. omnivorum* was isolated from internal tissues of infected roots in August but not in October. Thus, overwintering within root tissues probably does not occur.

Infested sorghum-seed inoculum removed from the taproots of cotton after 3, 6, 9, and 12 days resulted in 60, 85, 100, and 100% disease, respectively. This suggests that *Phymatotrichum* may require a food base to initiate disease but that once the fungus becomes established in the plant, the food base may no longer be

TABLE 1. Relationship of cotton root depth to *Phymatotrichum omnivorum* strand viability^a

Collection date	Number of host roots in sample	Percent of roots from which viable strands were recovered at soil depths (cm) of		
		0–15	15–30	30–45
1979				
5 Aug.	20	100	100	100
10 Aug.	19	89	100	100
29 Aug.	20	50	100	100
1980				
24 Jan.	22	50	77	85
9 Feb.	30	48	53	76
28 Feb.	29	34	74	96

^a Viability was determined by removing five strands from each root surface and placing them on water agar containing 200 ppm streptomycin sulfate and penicillin G.

needed.

The importance of sclerotia in initiating disease was demonstrated by the fact that they did induce disease, in contrast to strands, which did not. However, sclerotia recovered from field and laboratory cultures have two strands attached, and sclerotial germination occurs from the strand fragments rather than the sclerotial body. This suggests an association between strands and sclerotia.

Lyda (2) has reported sclerotia of *P. omnivorum* to be the infective propagules. The role of the strand needs further investigation. Strands may function as extensions of the sclerotia. A system of strands and sclerotia would occupy a greater soil volume than sclerotia alone.

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