

DISTRIBUTION AND ETIOLOGICAL ROLE OF STRANDS
OF PHYMATOTRICHUM OMNIVORUM (SHEAR) DUG.

by

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A Thesis Submitted to the Faculty of the
DEPARTMENT OF PLANT PATHOLOGY
In Partial Fulfillment of the Requirements
For the Degree of
MASTER OF SCIENCE
In the Graduate College
THE UNIVERSITY OF ARIZONA

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To Lou-Anne, Mom, and Dad

ACKNOWLEDGMENTS

The author expresses sincere gratitude and appreciation to Dr. Richard B. Hine, for his direction, encouragement, and helpful suggestions during the course of this study. The author also expresses deep appreciation to Dr. Stanley M. Alcorn and Dr. Iraj J. Misaghi for their many constructive criticisms and helpful suggestions throughout this study and during the preparation of this thesis.

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ABSTRACT

Viable strand lengths (VSL) of Phymatotrichum omnivorum (per 50 g soil) of 0.1 and 0.2 cm were recovered at 15-30 and 30-60 cm depths, respectively, in cotton fields near Marana, Arizona in June, 1979; none were recovered at depths of 0-15 and 60-90 cm. Maximum VSL occurred from August into November. These were 0.5, 0.4, 1.0, and 0.6 cm at depths of 0-15, 15-30, 30-60, and 60-90 cm, respectively. By April, 1980, VSL at the same depths were 0.05, 0.2, 0.3, and 0.05 cm, respectively. All infected cotton roots from depths of 15-45 cm in August contained viable strands, generally fewer roots contained viable strands in other months and at 0-15 cm depths.

Phymatotrichum omnivorum was isolated from tissues of infected cotton roots in August but not from rotted roots after October.

Inoculations of cotton plants with strands were unsuccessful. However, 46% of the plants became diseased when naturally infected roots were used as inoculum in the field and 50%-100% were infected when sclerotia or infected sorghum seeds were used as inoculum in the greenhouse. Sclerotia from field or laboratory cultures germinated only from attached broken strands.

CHAPTER 1

INTRODUCTION

Phymatotrichum omnivorum (Shear) Dug. is a serious, soil-borne plant pathogen in the southwestern United States and northern Mexico. The fungus produces a root-rot disease (commonly referred to as Phymatotrichum root-rot) of over 2300 spp. of dicotyledonous plants (16). Although P. omnivorum has been studied since 1888 (12), aspects of the life cycle of the pathogen are still not well understood.

Phymatotrichum omnivorum produces strands and sclerotia. Sclerotia develop exclusively from maturing strands. Otherwise, the importance or role of strands in the life cycle of the fungus is not known. Information regarding strands primarily relates to their survival on the surface of roots or in rotting root tissue. However, there are two reports concerning strand pathogenicity. Dana (1) claims to have successfully inoculated a single cotton plant using strands of P. omnivorum. Neal and McLean (9) were unsuccessful in inoculating cotton plants with strands in laboratory studies.

Several individuals have investigated the survival of strands on infected cotton roots. Wheeler (20) demonstrated that strands can survive for 12 months on infected cotton roots in Arizona. McNamara et. al. (7, 8) reported finding viable strands on decayed cotton roots in Texas fields fallow for 5 years. However, quantitative information on the survival and pathogenicity of strands is not available.

Strands form on the exterior of roots, but only fungal hyphae penetrate the internal tissues. Isolation of P. omnivorum from the interior of infected, but living roots was successfully completed by Taubenhause and Ezekiel (18). Because they could not isolate the fungus from decayed roots, they concluded that P. omnivorum can survive in infected cotton root tissue but not in decayed root tissue. Taubenhause and Kellough (19) also recovered P. omnivorum from recently killed cotton roots, but not from those dead longer than 26 days.

Infected roots containing living tissues with strands have successfully been used as inoculum. Strands growing from these roots colonized and incited disease (10, 18, 19). However, inoculations using decayed roots seldom resulted in disease (10, 18, 19), presumably due to a lack of strand production from the roots.

The sclerotial stage of P. omnivorum was first described by King and Loomis (4) in 1929. Sclerotia have been recovered from cotton fields (3, 8, 11, 16, 18), particularly in pockets at depths of 30-60 cm (3, 16). McNamara recovered few sclerotia from many infection centers, but many sclerotia from some centers. Few sclerotia were found in cotton fields in Texas (8) and Arizona (16). Many sclerotia were recovered from other cotton fields in Texas (8, 11, 18) and Arizona (16). Rogers (15) reported that as many as 2 million sclerotia occurred per acre-foot of soil in fields continuously in cotton in Texas.

Phymatotrichum omnivorum also has been reported to form smooth or "horsehair" strands (1, 8, 18). While they have been suggested to serve as survival propagules, little is known about their function.

Ratliffe (14) suggested that a saprophytic stage of P. omnivorum occurred on dead cotton roots in Texas. Others (11, 18, 19) believe a saprophytic stage does not exist.

The present study is concerned with the distribution and viability of strands in cotton fields, changes in their populations with time, and their possible role in the initiation of Phymatotrichum root-rot.

CHAPTER 2

MATERIALS AND METHODS

Recovery and Viability of *Phymatotrichum omnivorum* from Soil and Cotton Roots

Although Wheeler (15) and McNamara et. al. (7) showed that strands can survive for at least 1 year on infected roots, no studies have been made concerning the numbers of strands and their distribution in infested soils. Therefore, recovery and viability studies were conducted to determine:

1. the populations of strands that occur at various depths in infested soils and how these populations vary with time.
2. the occurrence and viability of *P. omnivorum* on roots and in root tissues.

Recovery from Soil

The vertical distribution of strands in root-rot circles was determined by taking soil cores using a hydraulically operated, coring device (Bull Soil Corer) mounted on the back of a pickup truck. The apparatus is capable of taking cores 5 cm in diameter to a depth of 1.8 m (Fig. 1). The truck was driven directly into root-rot circles for core sampling (Fig. 2).

Collections were made monthly from June, 1979 through April, 1980 from two infested cotton fields near Marana, Arizona. A collection consisted of 3-6 cores taken from within a particular root-rot



Figure 1. Soil core taken with a "Bull Soil Corer".



Figure 2. Truck mounted with a "Bull Soil Corer".

circle in each of the two fields. The soil in one field was a Gila silt loam (Fig. 3) and in the other Pima clay loam (Fig. 4).

Immediately after a core was taken it 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 separate plastic bag. The samples were transported under ice, stored under refrigeration, and processed for strands and sclerotia within 7 days.

Strands and sclerotia were extracted by vigorously suspending 50 g of soil in 800 ml of water, then rapidly decanting the suspension onto a 100 mesh sieve. The extraction was repeated at least 3 times from the same 50 g sample 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 (Fig. 5) and placed on water agar amended with 200 mg/liter streptomycin sulfate and 200 mg/liter penicillin G (following autoclaving) (AB medium) to determine viability. Strands and sclerotia were examined for germination after 3 days (Fig. 6).

During the extraction process fragmentation of strands occurred. Since strands did not fragment with any consistency of length or number of fragments, strand numbers could not be used 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 sum of the viable and nonviable strand fragment lengths was also determined. In practice two 50 g samples were taken from each core segment. The strand length sums obtained from each of the two

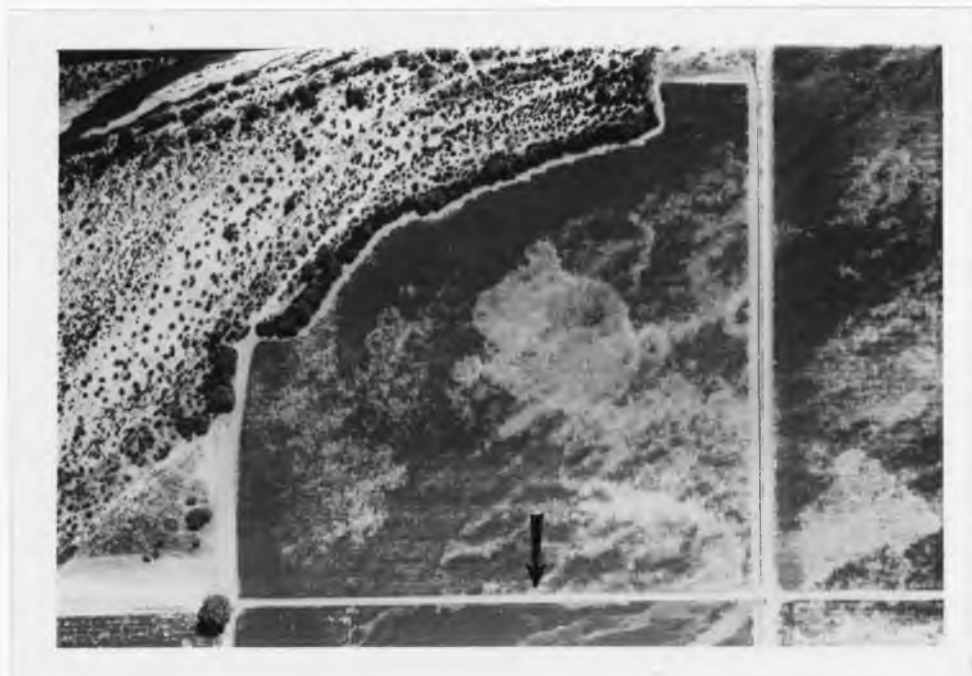


Figure 3. Soil sampling location in a cotton field near Marana, Arizona (arrow points to location).

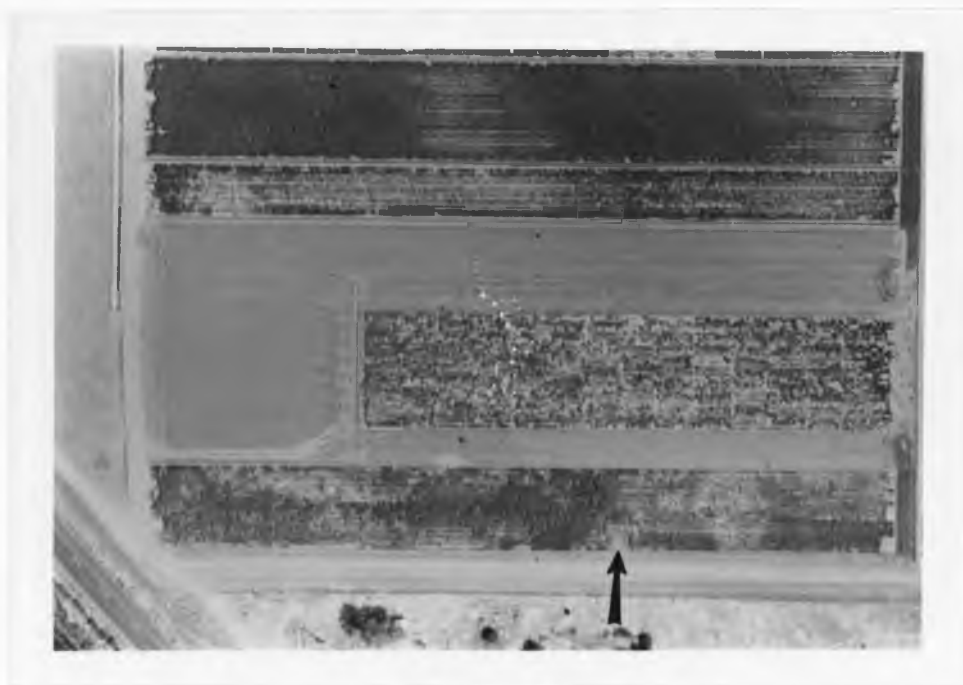


Figure 4. Soil sampling location at the University of Arizona, Marana Experimental Farm (arrow points to location).

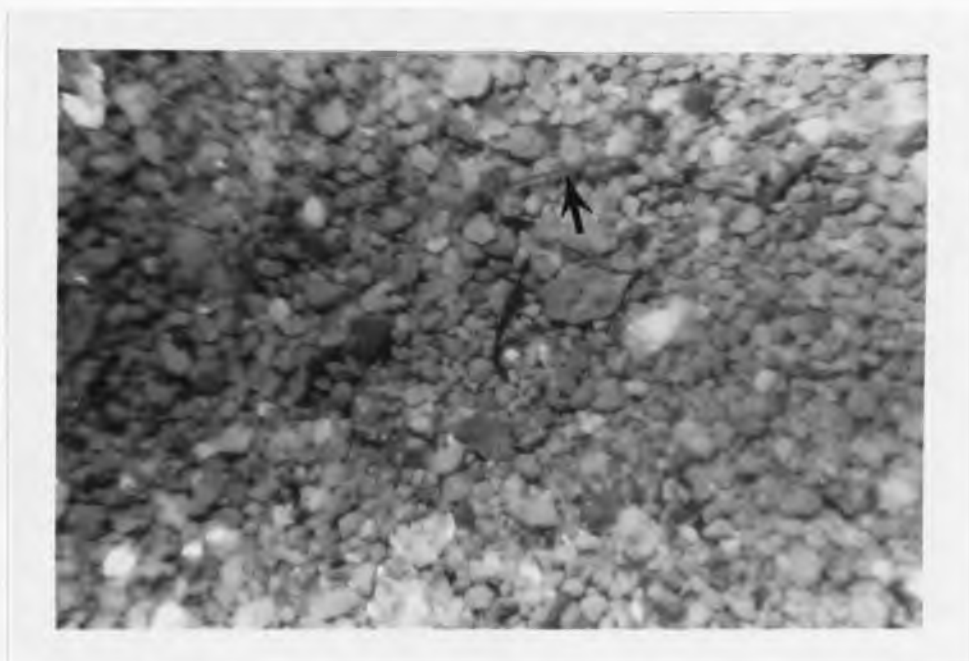


Figure 5. Phymatotrichum omnivorum strand extracted from a 50 g soil sample (arrow points to strand).



Figure 6. Germination from the broken end of a strand of Phymatotrichum omnivorum.

samples were averaged together to obtain a strand-length value per core segment. these values were then averaged together with values obtained from other core segments to define the viable strand length (VSL) or the total (viable and nonviable) strand length (TSL) per 50 g soil.

Recovery from Roots

Infected cotton roots were collected on 12 occasions from October, 1978 through February, 1980 from one root-rot circle in each of 2 separate fields near Marana, Arizona, and composited for a total of 20-50 roots. Roots 30 cm or longer in length were either pulled or dug out. 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.

Additionally, on occasion from August, 1979 through January, 1980 the same root segments used for strand determinations were used to determine if P. omnivorum could also survive internally within the root. Each root segment was stripped of cortical tissue, washed under tap water, and blotted dry. Five to six pieces of tissue were aseptically removed from the vascular and pith tissues of each segment, surface sterilized in 10% chlorox 1-3 min, blotted dry, and placed on AB medium. Isolations were made within 7 days from the date of collection. P. omnivorum growing from any of the 5-6 chips for a segment constituted a positive recovery.

Pathogenicity of Strands and Sclerotia

Production of Inoculum

A number of sources of inoculum were used in all of the field, greenhouse, and laboratory studies on pathogenicity. Some strands and sclerotia were produced in the laboratory by a soil culture method, as follows. Sorghum seeds were washed under tap water and soaked overnight. The seeds were then added to 15-cm petri plates and autoclaved 1 hr on 2 separate days. Sterile seeds were inoculated with an actively growing culture of P. omnivorum. After 1 month, 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 liter jars containing 900 g of unsterile, air-dried GSL, screened (20 mesh), adjusted to 20% moisture, and covered with a lid containing holes for aeration. The cultures were incubated at 30 C for 1 month. At this time, large numbers of mature strands and sclerotia could be wet-sieved from the soil cultures.

An alternative method for producing strands and sclerotia was also used. One-cm blocks of sorghum seed inoculum were placed on the surface of the same soil but contained in 15-cm diameter petri dishes. After 1 month at 30 C, strands and sclerotia were handpicked from the soil and used as inoculum.

Field Studies

Field plots were set up in noninfested cotton fields on the University of Arizona Experimental Farm at Marana, Arizona to experimentally determine if strands are pathogenic to mature cotton plants

of the cultivar Delta Pine 61 (DP 61). The treatments consisted of inoculating 3-month-old flowering plants 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 up to 1-2 hr before use. For each inoculation a 30-cm deep trench was dug so as to expose the healthy tap root. Twenty to 30 strands were placed on or near the exposed surface of each root at depths between 15-30 cm. The trench was then carefully filled with soil.

Infected roots (15-20 cm in length) used as inoculum were positioned parallel and adjacent to healthy tap roots, one infected root per plant. For both strand and root inoculated plants, 10 plants were used in each treatment in each of 4 replications. A check treatment consisted of merely exposing the tap roots and refilling the trench. 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 their viability.

Twenty additional plants were inoculated in the field with culturally produced strands using an alternative inoculation method. A 30-cm deep trench was dug near plants so as to expose the tap root. A small cup of soil was placed around the root at a depth of 30 cm. Twenty to 30 strands, suspended in 50 ml of water, were poured into the cup. The trench was then carefully filled with soil. Eight weeks after inoculation all plants were dug and examined for disease.

Laboratory and Greenhouse Studies

Laboratory and greenhouse studies were also initiated to determine if strands and sclerotia were pathogenic to cotton roots. Cotton plants (DP 61) were grown 5-8 wk in unsterile GSL in 15-cm pots in growth chambers maintained at 30 C. Sclerotia or strands, wet-sieved from "soil cultures", were positioned 5-cm deep on or near the tap root of each cotton plant. Treatments included inoculations with 20 strands, 1 sclerotium, 5 sclerotia, or 10 sclerotia. Six plants were used in each of 4 replications for each treatment. The roots were examined for lesion development after 30 days.

Fifty additional plants (DP 61) were grown in either unsterile or autoclave-sterile GSL in 15-cm pots in temperature boxes of growth chambers maintained at 30 C. Plants ranging in age from 5-20 wk were inoculated by placing 10-15 field or culturally produced strands on or near the tap roots of plants at depths of 2-5 cm.

To determine the longevity of viability of strands and sclerotia in unsterile soil, 10-20 strands or sclerotia were placed 3 cm below the soil surface near the roots of 5-8 wk old cotton plants (DP 61) growing in unsterile GSL in 15-cm pots in soil temperature boxes maintained at 28-30 C. Strands were removed after 0, 1, 5, and 20 days, and placed on AB medium to determine their viability. Six plants in each of 3 replications were used for each treatment. Control strands and sclerotia were placed directly on AB medium.

If strands are short-lived in soil, then the time required to induce infections is critical. To determine this 2-cm-square blocks of infested sorghum seeds were centrally buried 5-cm deep in unsterile

GSL in 15-cm pots containing 10 wk old cotton plants (DP 61) growing in soil temperature boxes adjusted to 28-30 C. The sorghum seed blocks were removed after 0, 3, 6, 9, and 12 days. Fifteen plants were used in each of 3 replications for each treatment.

Since the ability of the pathogen to utilize an energy reserve could be critical in the establishment of disease in a field situation, the germination potential of strands and sclerotia was compared. Sclerotia and strands were separately placed on unsterile GSL adjusted to 20% moisture content in 6-cm diameter petri plates and incubated at 30 C. Fifteen of each were used in each of 5 replications. These propagules were examined for new strand formation and transferred to new plates at 2-3 wk intervals.

CHAPTER 3

RESULTS

Recovery and Viability of *Phymatotrichum omnivorum* from Soil and Cotton Roots

Recovery from Soil

Soil cores were taken near the time of cotton planting (approximately mid-April) to determine initial strand populations in infested soils. On the basis of 18 cores, VSL of 0.1 and 0.2 cm occurred per 50 g soil from 15-30 and 30-60 cm depths, respectively (Figs. 8, 9). No viable strands were detected in soil from depths of 0-15 or 60-90 cm collected in June (Figs. 7, 10).

Root-rot first appeared in cotton during July in 1978 and 1979. The appearance of the disease preceded the increase in viable strand populations. Viable strand lengths increased in August, 1979 to 0.35, 1.0, and 0.1 cm for 15-30, 30-60, and 60-90 cm depths, respectively (Figs. 8, 9, 10), while VSL at 0-15 cm depths increased from 0.0 in August to 0.15 cm during September, 1979 (Fig. 7).

No new deaths from *P. omnivorum* in cotton occurred after October. Total strand populations, however, increased and peaked between September and November, 1979. Maximum values of TSL of 0.3, 0.7, 1.8, and 0.8 cm were recorded for 0-15, 15-30, 30-60, and 60-90 cm soil depth, respectively (Figs. 7, 8, 9, 10).

Viable and total strand populations begin declining in November. By the middle of April, 1980, 0.0, 0.2, 0.3, and 0.05 cm VSL were

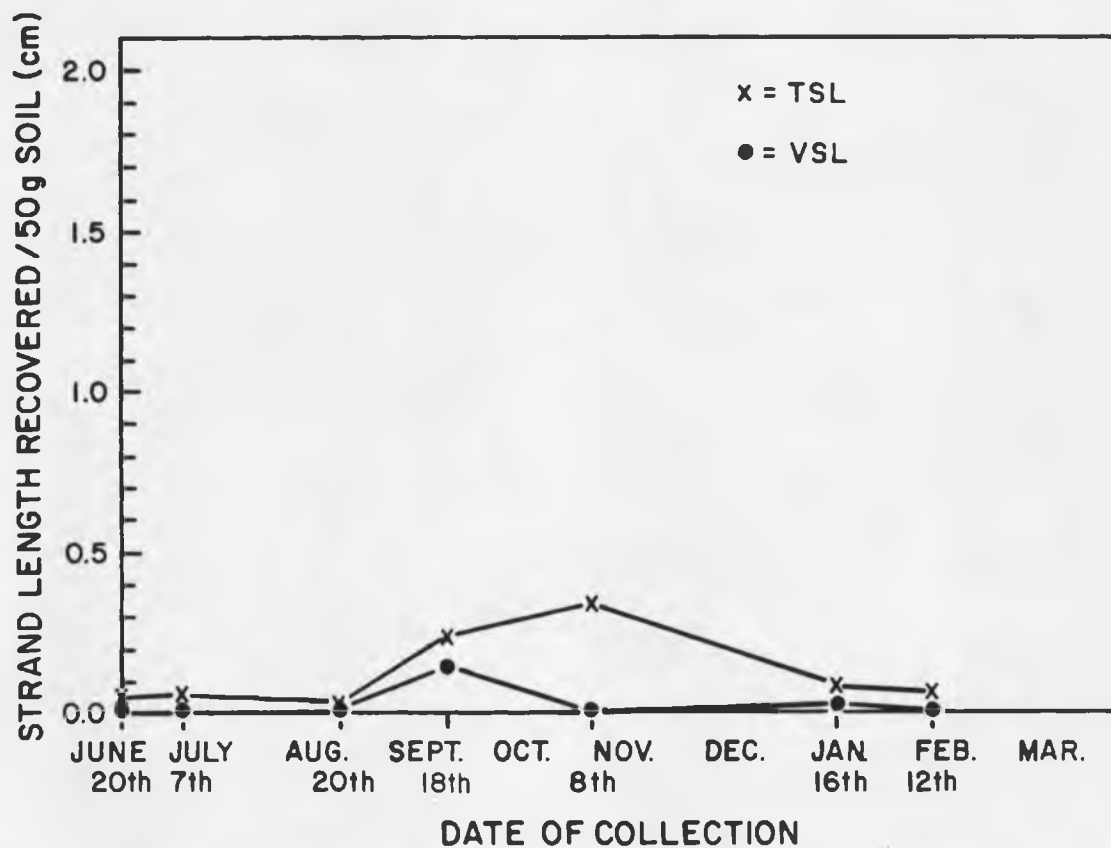


Figure 7. Total strand length (TSL) and viable strand length (VSL) recovered from 0-15-cm depths in infested cotton soils, 1979-1980.

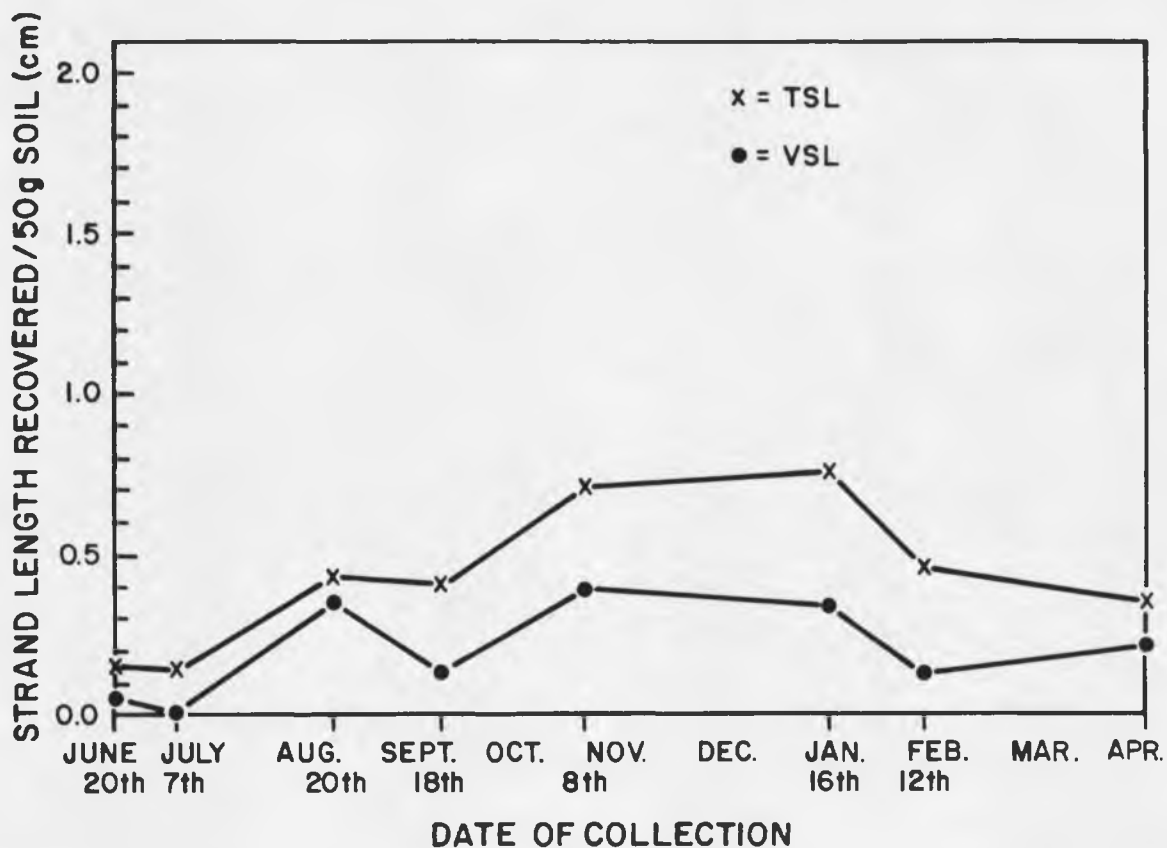


Figure 8. Total strand length (TSL) and viable strand length (VSL) recovered from 15-30-cm depths in infested cotton soils, 1979-1980.

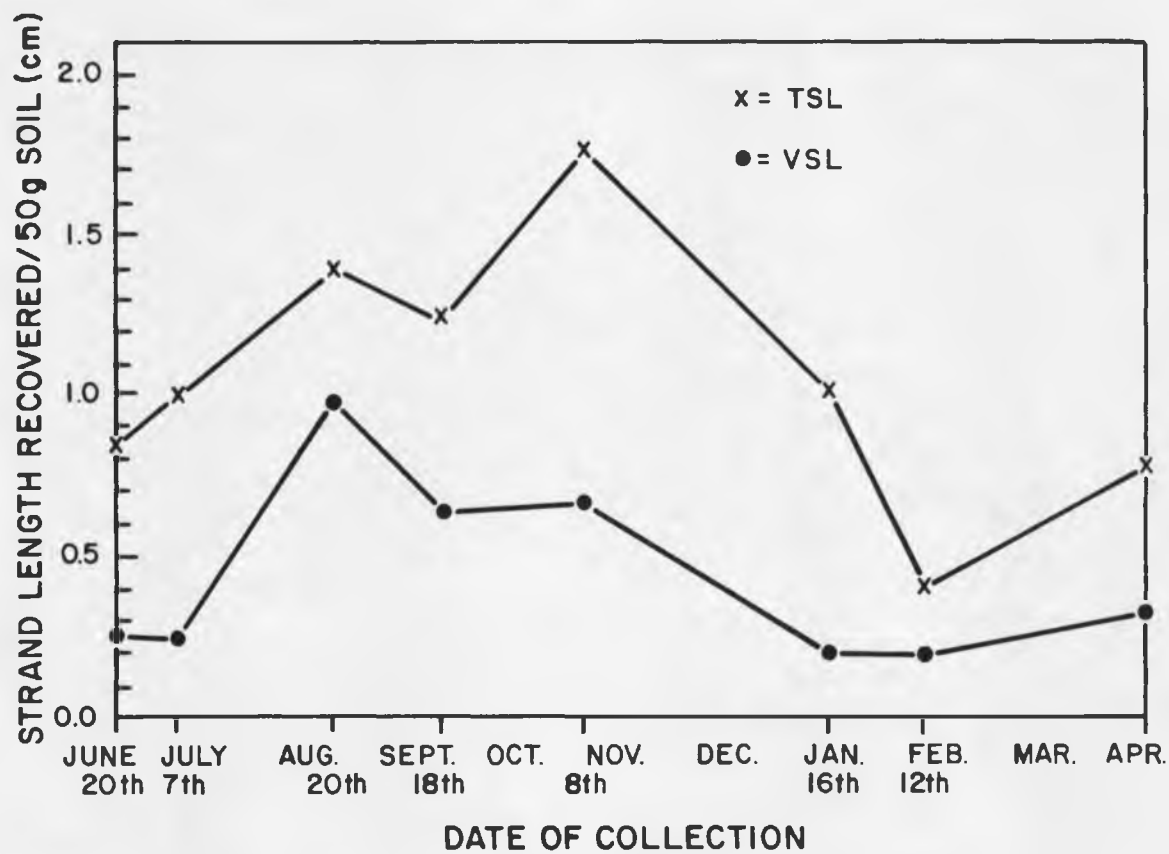


Figure 9. Total strand length (TSL) and viable strand length (VSL) recovered from 30-60-cm depths in infested cotton soils, 1979-1980.

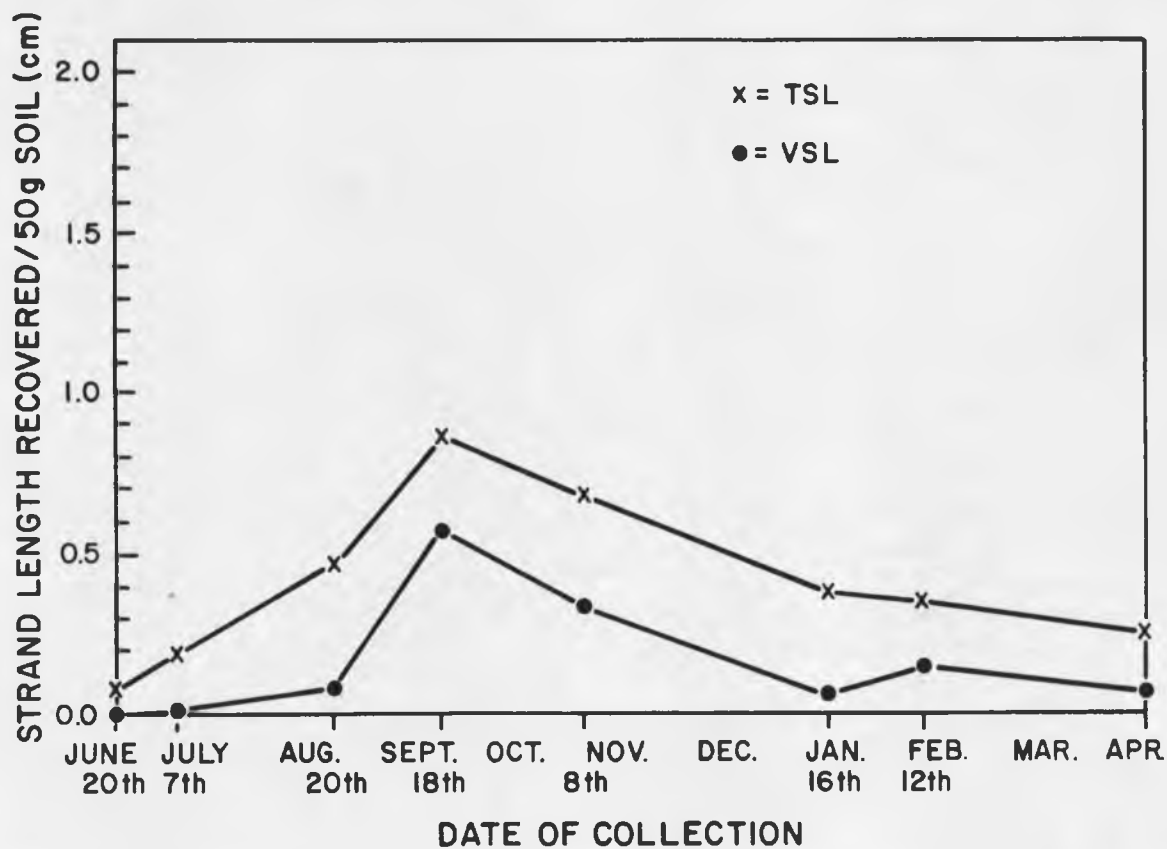


Figure 10. Total strand length (TSL) and viable strand length (VSL) recovered from 60-90-cm depths in infested cotton soils, 1979-1980.

recovered from 0-15, 15-30, 30-60, and 60-90 cm, respectively (Figs. 7, 8, 9, 10). Plots of TSL vs VSL indicate that these two indices generally are in parallel over time.

Of 60 cores taken from June, 1979 through April, 1980, 15 sclerotia were recovered of which 4 were viable. Only individual sclerotia were recovered. Each had strands attached. When placed on AB medium each viable sclerotium germinated only from the attached, broken strand fragments and not from the sclerotium itself.

Recovery from Roots

Cotton roots were collected at varying soil depths and time intervals from October 3, 1978 through January 8, 1980. The percentage of roots bearing live strands varied from 17% to 100% (Table 1). Except for the collection of August 9, 1979, roots at the 0-15-cm depth had the fewest viable strands. Generally, the greatest percentage of roots with viable strands was recovered from the 30-45 cm depth. Strand survival appeared to be highest in the late summer-early fall (Table 1).

The ability of P. omnivorum to survive within root tissues was determined with the same roots used in the strand-recovery study. Five to seven pieces of pith or stele tissues, including diseased margins, were removed from each root for the determination. During August, P. omnivorum grew from at least 1 piece from each root (Fig. 11). The recovery dropped to near 60% in September and sharply declined to 0% in mid-October (Fig 11).

Table 1. Percent of cotton roots bearing viable strands of Phymatotrichum omnivorum and the relationship of root depth to strand viability.

Collection date	Total roots	Roots at varying depths with viable strands ^a		
		0-15cm	15-30cm	30-45cm
		%	%	%
1978				
3 Oct.	53	_b	-	-
5 Oct.	51	-	92	-
18 Oct.	40	-	100	59
21 Nov.	33	-	52	59
1979				
11 Jan.	52	-	55	86
24 Jan.	22	50	77	85
9 Feb.	30	48	53	76
28 Feb.	29	34	74	96
9 Aug.	20	100	100	100
20 Aug.	19	89	100	100
18 Aug.	20	50	100	100
1980				
8 Jan.	32	17	41	-

^aViability was determined by removing 5 strands from each root surface and placing them on AB medium.

^bNo data collected.

Pathogenicity of Strands and Sclerotia

Field Studies

Ten cotton plants in each of 4 replications were inoculated in August with strands obtained from diseased roots in the field. In the same experiment 10 cotton plants in each of 4 replications were each inoculated with an infected cotton root. The cotton plants were examined after 2 months. No symptoms or signs of infection were found on any of the cotton roots inoculated with strands from laboratory cultures or from infected roots collected in the field. However, 45% of the plants inoculated with infected roots became infected or died. Strands from roots taken to the laboratory were all viable.

Laboratory and Greenhouse Studies

Strand inoculated plants were not infected by 30 days. However, 35%, 45%, and 75% of the plants respectively, inoculated with 1, 5, or 10 sclerotia became infected. No infections were observed in the roots of the 50 additional plants grown in both unsterile and autoclave sterilized GSL, inoculated with field and laboratory produced strands.

Of 50 strands and sclerotia placed on unsterile GSL in plates, 30% of the strands and 100% of the sclerotia germinated to produce new strands. Subsequent transfers of sclerotia at 2-3 wk intervals resulted in 80, 60, and 30% of the sclerotia producing new strands.

Sorghum-seed-blocks buried in 15-cm pots containing cotton, for 9 and 12 days, then removed, resulted in a 100% disease incidence. Blocks buried in the pots for 6 or 3 days resulted in 85% and 60%, respectively, of the plants becoming infected.

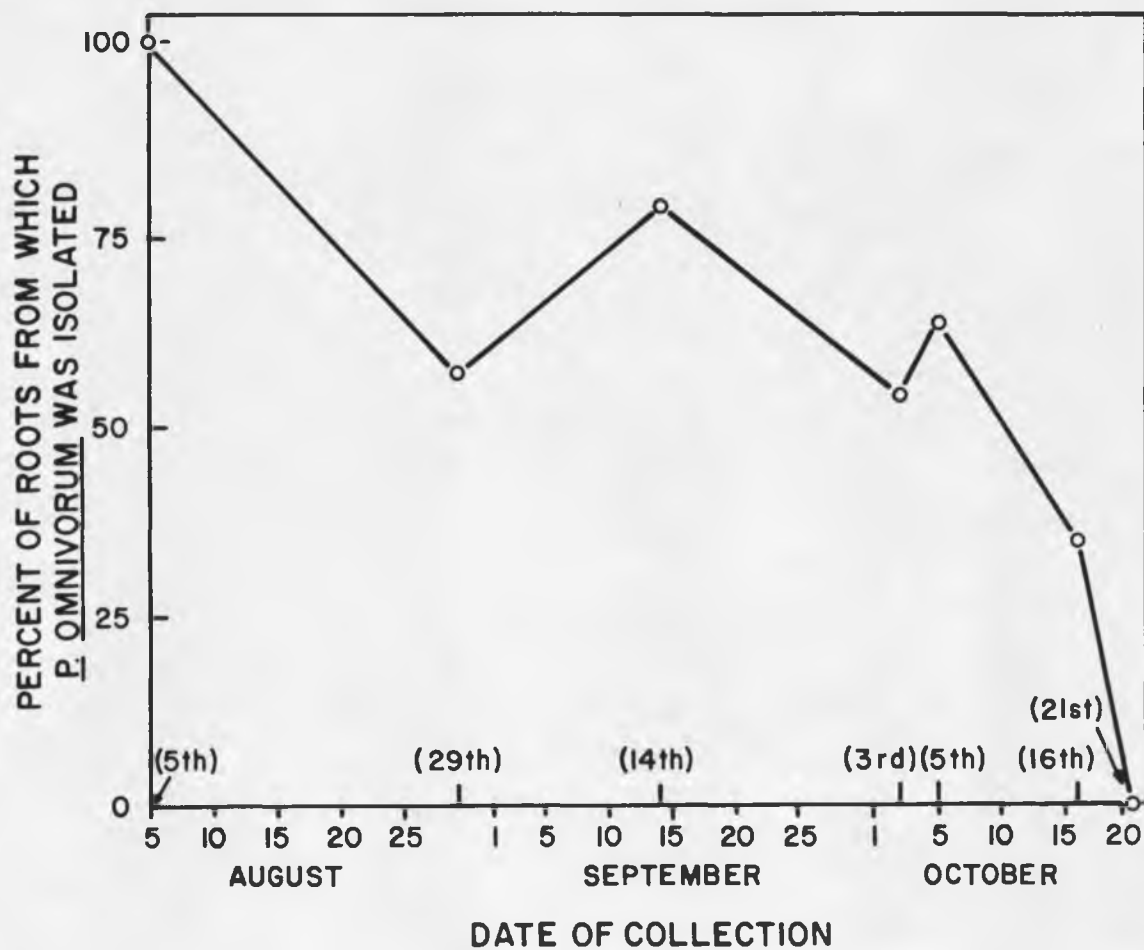


Figure 11. Percent of infected cotton roots from which *Phymatotrichum* could be isolated from pith or vascular tissues during 1979.

CHAPTER 4

DISCUSSION

Phymatotrichum root-rot appears from July to September in cotton in the Marana, Arizona, area. New infections are rare before July and after September. The greatest percentage of viable strands of P. omnivorum occurred in August through September in cotton fields in this locality, a time when disease incidence approaches maximum intensity. Thus, an increase in the population of viable strands correlates with disease incidence.

During August and September the greatest VSL occurred at depths of 30-60 cm. Fewest VSL occurred at depths of 0-15 cm. Factors which influence production and/or survival of strands at various depths are unknown.

Wheeler (20) and McNamara et. al. (7, 8) established that strands can survive at least 1 year on dead root surfaces. Recovery of strands from surfaces of infected roots in this study demonstrated 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.

Survival of P. omnivorum within root tissues was not correlated with survival of strands on root surfaces or in soil. Phymatotrichum
omnivorum was isolated from internal tissues of infected roots in

August but not in October. Thus overwintering within root tissues probably does not occur.

The ability of strands to utilize root tissues as a nutrient substrate has been reported (20) in field and laboratory studies. When roots of cotton containing strands of P. omnivorum are buried in soil, strands grow from the roots as long as living root tissues are present (18, 19). When the roots are dead, stranding does not occur.

Phymatotrichum omnivorum grows well on sterile substrates such as potato dextrose agar or autoclaved sterilized sorghum seed. However, in these studies it has been observed that P. omnivorum is very vulnerable to attack by contaminating organisms such as Penicillin spp., Aspergillis spp., and Trichoderma spp.. Phymatotrichum omnivorum in dead root tissue may be subject to destruction by other microorganisms. This would explain why P. omnivorum could not be isolated from dead roots.

Infested sorghum-seed-blocks removed after 3, 6, 9, and 12 days resulted in 60, 85, 100, and 100% disease. This suggests that Phymatotrichum may require a food base to initiate disease but once the fungus becomes established in the plant, the food base may no longer be needed. In terms of strands, the food base is the sclerotium.

The importance of the sclerotium in initiating disease was demonstrated by the lack of infectivity of strands. However, a single sclerotium attached to the strand can be used to infect plants. Thus, the minimum inoculum density of P. omnivorum appears to be a single sclerotium, which is similar to observations by Lyda (6).

On the other hand, observations on the germination of sclerotia from field and laboratory cultures (the strand extraction method also removes sclerotia) revealed that sclerotia germinate only from the broken ends of strand fragments. However, if the sclerotium is injured (rind ruptured) then germination occurs from the injury point. Thus, strands appear to function as extensions of sclerotia.

Strands growing in culture and on the surface of roots in the field are frequently observed to be interconnected. Similar interconnections probably occur among strands free in soil. Assuming that a sclerotium is required for infection, and that germination can occur from strands attached to the sclerotium, an interconnected system of strands and sclerotia would greatly increase the area occupied by P. omnivorum. However, of 60 soil cores taken in infested root-rot areas in cotton from June, 1979 through April, 1980, only 15 sclerotia were recovered of which 4 were viable. Therefore, in comparison to the populations of sclerotia, the populations of strands are very large in the vicinity of Marana, Arizona.

Strands are unique and apparently play an important role in the etiology of disease. A system of strands and sclerotia could greatly increase the area occupied by P. omnivorum and increase the efficiency of disease development.

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