Pink Root of Muskmelon and Watermelon Caused by *Phoma terrestris*

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ABSTRACT

***Phoma terrestris* was demonstrated to be the cause of pink root of cucurbits. Based on disease reaction in muskmelon roots, nine cucurbit isolates ranged from non-pathogenic to moderately virulent. The cucurbit isolates caused little or no reduction in root or foliar dry wt. during a 60 da greenhouse study. Disease reaction was similar in muskmelon, watermelon, onion, and tomato using both cucurbit and onion *P. terrestris* isolates. Based on disease reaction, pink root symptoms, and microsclerotial production, the cucurbit isolates were similar to onion isolates (ATCC 16993 and ATCC 64403). *P. terrestris* isolated from cucurbit root tissue produced pycnidia with pycnidiospores and microsclerotia on PDA, V8A, and SNA. In addition, three cucurbit isolates and one onion isolate produced microsclerotia in muskmelon roots under greenhouse conditions. Germination of microsclerotia was demonstrated for the first time suggesting a possible role of these structures in overseasoning inoculum source. Microsclerotia of *P. terrestris* were similar in appearance to those produced by *Pyrenochaeta lycopersici*, *Rhizopycnis vagum*, *Verticillium dahliae*, and *Macrophomina phaseolina*. *P. terrestris* caused minor damage to cucurbits during the 60 da duration of these studies, but the fungus caused pink root systems, browning of the roots, and produced microsclerotia in the roots. As a fungus capable of causing root lesions and debilitating the root system to some degree, *P. terrestris* may contribute to the vine decline disease complex of cucurbits.**

RESUMEN

Se demostró que *Phoma terrestris* es el agente casual de la enfermedad raíz rosada de las cucurbitaceas. Los nueve aislamientos de cucurbitaceas estudiados variaron de no patogénicos a moderadamente virulentos de acuerdo a la reacción a la enfermedad presentado en raíces de melón. Los aislamientos de cucurbitaceas causaron poco o nada de reducción en el peso seco foliar o radical durante un estudio de invernadero de 60 días. La reacción de la enfermedad fue similar en melón, sandía, cebolla y tomate cuando se usaron los aislamientos de *P. terrestris* obtenidos tanto de la cucurbitaceas como de la cebolla. Los aislamientos de cucurbitaceas fueron similares a los aislamientos de cebolla (ATCC 16993 Y ATCC 64403) en base a la reacción de la enfermedad, los síntomas de la raíz rosada, y la producción de microesclerocios. *P. terrestris* aislado de tejido radical de cucurbitaceas produjo microesclerocios cuando se cultivó en PDA, V8A y SNA. Adicionalmente, tres aislamentos obtenidos de cucurbitaceas y uno obtenido de cebolla produjeron microeclerocios en raíces de melón bajo condiciones de invernadero. Por primera vez se demostró la germinación de microesclerocios lo que sugiere un posible papel de estas estructuras como fuente de inóculo entre estaciones. La aparencia de los microesclerocios de *P. terrestris* fue similar a la de aquellos producidos por *Purenochaeta lycopersici*, *Rhyzopycnis vagnum*, *Verticillium dahlie*, y *Macrophomina phaseolina*. El hongo *P. terrestris* ocasionó poco daño a las cucurbitaceas durante los 60 días de duración de estos estudios, pero este hongo causó la coloración rosa de los sistemas radicales, necrosis de las raíces y producción de microesclerocios en estas. *P. terrestris* puede contribuir en el complejo de la enfermedad pudrición de la enredadera de las cucurbitaceas como un hongo capaz de causar lesiones en las raíces y de debilitar hasta cierto grado el sistema radical.

Additional index words. Allium cepa, Cucumis melo, Citrullus lanatas, Lycopersicon esculentum, Pyrenochaeta lycopersici, Rhizopycnis vagum, Verticillium dahliae, microsclerotia, vine decline, epidemiology

Cucurbits are an important source of agricultural income in temperate and tropical areas of the world. Intensive cultivation of these crops with inadequate rotation has resulted in an increase in both the number and severity of diseases involved in a vine decline complex and consequently has accounted for significant economic losses. 'Vine decline' is a comprehensive term for symptoms observed in cucurbits, particularly muskmelon and watermelon, exhibited as the fruit near maturity. The symptoms include yellowing and death of crown leaves which gradually radiates outward, a wilt, or vine collapse. Melons from affected vines are likely to be sunburned, prematurely ripen, and have reduced sugars (Bruton et al., 1988a; Miller et al., 1995).

Numerous fungi have been implicated or demonstrated to contribute to vine declines of cucurbits (Bruton, 1996; Bruton et al., 1988a; Miller et al., 1995). The incidence and severity of vine declines can vary greatly depending on cropping history, population of vine decline fungi, and environmental interaction. *Macrophomina phaseolina* has caused economic losses in cantaloupe in the southwestern U.S. for many years (Bruton et al., 1987). Many of the new hybrid cultivars have good to moderate tolerance to the charcoal rot fungus (Bruton and Wann, 1995). Historically, *Didymella bryoniae* does not cause extensive damage to melons in the southwestern United States (Bruton et al., 1988a). However, during the 1997 season, *D. bryoniae* destroyed much of the melon crop in the Lower Rio Grande Valley (LRGV) of Texas, primarily as a fruit rot (Miller et al., 1997). Formae speciales of *Fusarium oxysporum* have caused substantial yield losses in watermelon (Bruton et al., 1988b; Martyn & Bruton, 1989) and cantaloupe (Martyn et al., 1987), although damage is generally confined to susceptible cultivars in isolated fields. However, Monosporascus root rot/vine decline has become the number one vine decline disease of cantaloupe and honey dew melons in the LRGV of Texas (Martyn and Miller, 1996). Recently, *Acremonium cucurbitacearum*, the cause of Acremonium collapse (Garcia-Jimenez et al., 1994), was isolated from muskmelon and watermelon in the LRGV (Bruton et al., 1996) although the fungus was isolated at a low frequency (Miller and Bruton, unpublished data). *Rhizopycnis vagum* is a newly described cucurbit pathogen (Farr et al., 1998) isolated from collapsed plants in the LRGV where *Monosporascus cannonballus* is rarely isolated. It may also contribute significantly to the vine decline disease complex (Miller and Bruton, unpublished data).

In numerous observations of cucurbit roots since the early 1980's, a pink discoloration has been periodically observed on the secondary roots of muskmelon, watermelon, and occasionally squash (Bruton, personal observation). The pink discoloration on cucurbit roots was similar to pink root symptoms observed on plants infected with *Phoma terrestris* (Hess, 1962), and most thoroughly characterized on onion (Gorenz et al., 1949; Hansen, 1929; Levy and Gornik, 1981; Nelson, S. R., 1987). Symptoms of the disease caused by *P. terrestris* include pink to dark-red roots, and as the disease progresses, the roots become water soaked and eventually die. Pink root on onion is often found in conjunction with other root rot pathogens (Lacy and Roberts, 1982) and is thought to enhance secondary invasion by more aggressive pathogens, such as *Fusarium oxysporun* f. sp. *capae* (Corgan, personal communication). Consequently, the purpose of this study was to: 1) establish the etiology of pink root on cantaloupe and watermelon; 2) compare American Type Culture Collection (ATCC) onion isolates of *P. terrestris* with isolates from muskmelon and watermelon; 3) run a limited host range on other horticultural crop plants; 4) examine the potential role of microsclerotia in the epidemiology of the disease; and 5) determine the temperature for optimum growth of cucurbit isolates. Preliminary reports have been published (Bruton & Duthie, 1996; Bruton et al., 1994).

MATERIALS AND METHODS

Isolation and identification. Muskmelon and watermelon plants were collected from fields in Central Texas and southern Oklahoma during the 1992 and 1993 season and examined for pink to reddish lesions on secondary and tertiary roots. Affected roots were surface disinfested in 0.5% NaOC1 for 30-40 sec and placed on absorbent tissue and sprayed with 85% EtOH. The root segments were transferred to water agar plus 100 ppm streptomycin sulfate. The petri dishes were incubated at 25°C and examined every 24 hr for 96 hr. Each colony was transferred to Potato Dextrose Agar (PDA), V-8 Juice Agar (V-8A), and Synthetischer Nahrstoffarmer Agar (SNA) at the first opportunity and allowed to grow until sporulation. Cultures were placed under constant plant grow lights and near-ultra-violet lighting at 23 to 25°C. Isolates that did not sporulate after 30-45 days were discarded. Isolates tentatively identified as *P. terrestris* were grown on autoclaved cotton gauze to determine pink to red pigmentation as described by Watson (1961).

Epidermal tissue with microsclerotia were obtained from dried roots of cantaloupe and onion to determine microsclerotial germination. The tissue was surface disinfested in 0.5% NaOCl for 15 sec and placed on water agar. The plates were incubated in the laboratory and observed daily. In addition, healthy muskmelon and onion roots were disinfested and placed on water agar with agar plugs of *P. terrestris* isolates placed in close proximity to the roots, a modification of the technique of Biles et al. (1992). The fungus was allowed to colonize and produce microsclerotia in the root tissue. After 21 da, the root tissue was removed and microsclerotia were partially separated from root cells by gently macerating the tissue in a mortar and pestle. The suspension was incubated for 24 hr in 0.1% cellulase/distilled water. The microsclerotial/cell suspension was centrifuged (5000 rpm) and the supernatant discarded. The microsclerotia were resuspended in distilled water and incubated at 27°C. Small aliquots of the water suspension containing microsclerotia were observed daily under a compound microscope to determine microsclerotial germination. Germinating microsclerotia were photographed at 400X.

Pathogenicity tests. *P. terrestris* isolates were obtained from muskmelon and watermelon roots exhibiting pink root symptoms from several fields in Texas and Oklahoma. Two *P. terrestris* onion isolates (ATCC 64403 and ATCC 16993) were obtained from the American Type Culture Collection (APHIS Permit # 930133) for comparison with the cucurbit isolates. Inoculum was produced by adding 275 g ground oat hull (The Quaker Oats Co., Chicago, IL) to 3 L of swimming pool filter sand along with 475 ml distilled H_2O. The ingredients were thoroughly mixed and autoclaved 3 times, allowing 2 days between each autoclaving. A 1 cm plug (PDA plus mycelium) from an actively growing culture was introduced into the flask and allowed to grow under laboratory conditions. After the mycelial growth attained approximately 4-5 cm diameter, the contents were vigorously shaken and thoroughly mixed. The fungus was then allowed to fully colonize the medium for a total of 28 days. Inoculum at the rate of 25 ml volume/1500 g of mortar sand was added to 15 cm diameter standard utility

Table 1. Disease reaction of cantaloupe cv. 'Magnum 45' to cucurbit isolates of *Phoma terrestris* compared to onion isolates from ATCC.

Isolate	Host[y]	Origin	Root Disease Ratings[x]			Sclerotia[z]
			Tap	Lateral	Pink	
Control		---	1.3ab	1.1a	1.0a	-
TX3020	W	Sydney, TX	1.2a	1.1a	1.0a	-
TX3014	C	Sydney, TX	1.1a	1.2a	1.0a	-
TX3045	C	Gustine, TX	2.1c	2.2b	2.2bcd	-
ATCC 16993	O	---	1.9bc	2.2b	2.2bcd	-
TX2091	C	Sydney, TX	1.8bc	2.3b	2.1bc	+
TX3017	W	Sydney, TX	1.9bc	2.4b	2.1bc	+
OK2017	C	Lane, OK	2.0c	2.4bc	2.3bcd	-
TX3013	C	Sydney, TX	1.7abc	2.6bcd	1.7b	-
TX3000	C	Sydney, TX	2.0bc	2.7bcd	2.2bc	-
TX3019	W	DeLeon, TX	2.0c	3.1cd	2.7cd	+
ATCC 64403	O	-----	2.1c	3.1cd	2.7d	+

[x] Ratings are the mean of five replications and two studies. Values in a column followed by the same letter are not significantly different at the P=0.05 level according to Ryan-Einot-Gabriel-Welsch Multiple F Test. Root disease was evaluated on a scale from 1 (healthy) to 5 (extensive areas of discolored roots and/or absence of roots).

[y] Original host: W=watermelon; C=cantaloupe; O=onion.

[z] Presence or absence of microsclerotia.

Table 2. Disease reaction of selected horticultural crops to cucurbit and ATCC onion isolates of *Phoma terrestris* under greenhouse conditions.

Isolate	Cantaloupe		Watermelon		Onion		Tomato	
	Root Rating[y]	Sclerotia	Root Rating	Sclerotia	Root Rating	Sclerotia	Root Rating	Sclerotia
Control	1.1a	-	1.3a	-	1.5a	-	1.5a	-
ATCC16993	1.9bc	-	2.1b	+	2.5bc	+	2.3b	-
TX3019	2.0bc	+	2.0a	+	2.4bc	+	2.5b	+
ATCC64403	2.1bc	+	3.0c	+	2.9c	+	2.8b	+
TX2071	2.5c	-	2.9c	+	2.3bc	-	2.2b	+

[y] Ratings are the mean of five replications and two studies. Values in a column followed by the same letter are not significantly different at the P=0.05 level according to Ryan-Einot-Gabriel-Welsch Multiple F Test. Root disease was evaluated on a scale from 1 (healthy) to 5 (extensive areas of discolored roots and/or absence of roots).

[z] Presence or absence of microsclerotia.

greenhouse pots. The final concentration was approximately 20-25 colony forming units (CFU)/g of soil. Three to five seeds of muskmelon cv 'Magnum 45' were placed on the soil surface and approximately 2 cm sterile soil applied over the seed. Once the plants were in the cotyledon stage, all but one of the seedlings were clipped at the soil-line. Each of the nine *P. terrestris* cucurbit isolates and 2 ATCC onion isolates were tested using 5 replicates in duplicate studies. The plants were grown using normal greenhouse procedures and were fertilized weekly. At the end of 60 days, the pots were broken down by submersing the pots plus soil into water and gently allowing the soil to separate from the roots. The primary and secondary roots were rated using a system of 1 (healthy) to 5 (severely lesioned, necrotic, or discolored). The vines were separated from the root system at the soil-line and measured for length of primary runner. Isolations were made to fulfill Koch's Postulates for each of the treatments. Dry weights of roots and vines were determined.

Limited host range. The watermelon isolate TX 3019 from DeLeon, TX and the muskmelon isolate OK 2071 from Lane, OK were selected as representative of *P. terrestris* from cucurbits along with the 2 ATCC *P. terrestris* onion isolates for a limited host range study. Test plants included muskmelon cv 'Magnum 45', watermelon cv 'Allsweet', tomato cv 'Flash', and onion cv 'Stuttgart Yellow'. The same process for producing inoculum was used as before. Each isolate was tested against each plant species with 5 replications in duplicate studies. At the end of 60 days, root rot ratings (1= no disease and 5= severe disease) were made and the presence or absence of microsclerotia in the roots observed microscopically.

Effect of temperature on growth. Each of the nine cucurbit isolates were sprinkled from soil storage tubes onto PDA. After 5 da growth, each isolate was transferred to PDA and placed in growth chambers at the temperature from 12 to 37°C at 5°C increments. Radial growth was measured after 13 da.

RESULTS

Isolation and identification. Fungal isolates were transferred from the original isolations by hypal tipping as

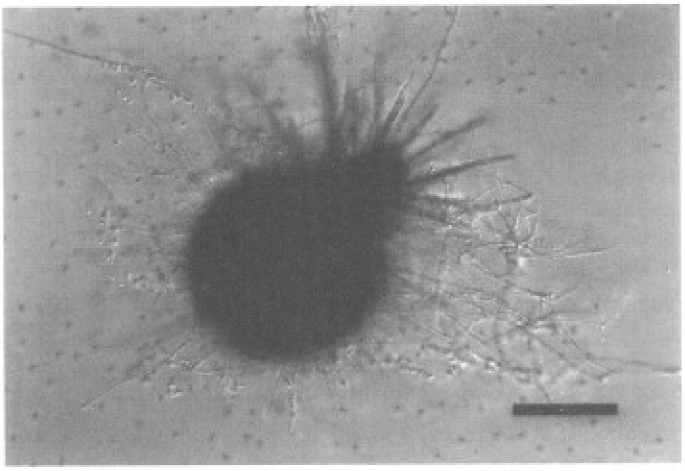

Fig. 1. Pycnidium of *Phoma terrestris* produced on SNA illustrating the setae surrounding the ostiole. Scale bar=50µm.

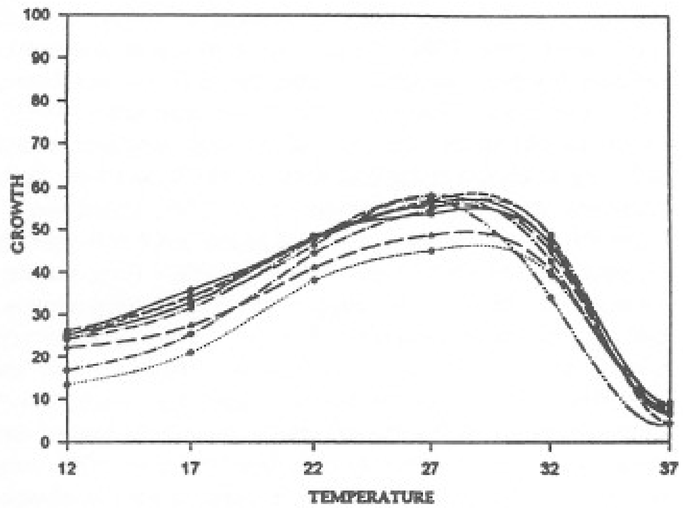

Fig. 2. Temperature growth curve of cucurbit isolates of *Phoma terrestris* on V8A.

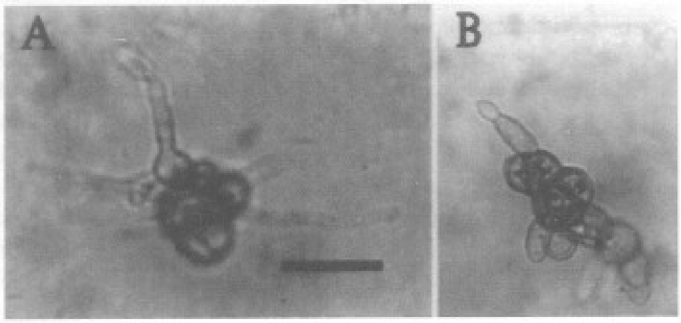

Fig. 3. Germination of microsclerotia (A-B) of *Phoma terrestris* illustrating single germ tubes from individual cells. Scale bar=10µm for both A and B.

colonies grew from the root tissue. Transfers were made for each new colony every 24 hr for 4 da. The predominant fungus isolated from the pink root lesions was a slow growing fungus exhibiting a whitish-gray appearance and imparting a red to purple pigmentation into the PDA. *Macrophomina phaseolina*, *Fusarium equiseti*, *Fusarium semitectum*, *Fusarium solani*, and *Fusarium oxysporum* were occasionally isolated from the pink root lesions. After 3 to 4 wk, the whitish-gray fungal isolates produced pycnidia on PDA, V8A, and SNA. Pycnidia produced by the isolates were globose, dark brown to black, ostiolate and sometimes multiostiolate with numerous septate setae surrounding the ostiole (Fig. 1). SNA was the preferred medium for producing pycnidia because there was little aerial mycelium and pycnidia were usually present within 3 wk. Conidia were unicellular, ovoid, biguttulate, and measuring 4.3 to 7.0 X 1.5-2.1 µm. Each of the test isolates imparted a wine red pigment to the cotton gauze. Some isolates produced microsclerotia-like structures of various shapes and sizes in the agar. Based on cultural characteristics and fungal morphology, the isolates were identified as *Phoma terrestris* E. M. Hans. [Syn. *Pyrenochaeta terrestris* (E. M. Hans.) Gorenz, J. C. Walker, & R. H. Larson] (Punithalingam and Holliday, 1973).

Microsclerotia were produced in root epidermal tissue, on aerial mycelium, and within the agar. The size of the microsclerotia were dependent on the number of individual cells aggregated together to make up the microsclerotium. Individual cells of the microsclerotium were thick-walled averaging 7 to 10 µm in diameter and darkened with age. Microsclerotia were observed germinating after 7 to 10 da in the epidermal tissue of artificially infected onion and cantaloupe root tissue that had been dried and subsequently placed on water agar. The microsclerotia incubated in sterile-distilled water were observed to germinate after 7 da. Single germ tubes (Fig. 2A-B) were produced by individual microsclerotial cells in all cases. Each cell of the microsclerotium was capable of germinating and producing one germ tube.

Effect of temperature on growth. The nine cucurbit isolates grew well at temperatures between 22 and 32°C with the greatest growth occurring at 27°C (Fig. 3). The isolates grew moderately well at 12 and 17°C but growth was minimal at 37°C.

Pathogenicity tests. The nine cucurbit isolates ranged from non-pathogenic to moderately virulent on 'Magnum 45' muskmelon (Table 1). The watermelon and muskmelon isolates were similar to the ATCC isolates with respect to disease reaction on lateral roots, tap root, and pink root pigmentation. Higher disease ratings were observed on secondary roots (1.1 to 3.1) compared to the primary root (1.2 to 2.1). During the course of these studies, it became apparent that pink discoloration alone was insufficient to establish accurate disease reaction ratings on the roots. Almost without exception, the disease ratings were higher on the lateral roots than the collective pink root rating (primary and secondary roots). There were numerous light brown lesions with no pigmentation. Isolations from both the pink root lesions and the light brown lesions yielded *P. terrestris*. There was no significant correlation ($P \leq 0.05$) between vine length, root weight, or top dry weight with disease severity ratings on the roots (data not shown). In fact, there were rarely any significant differences ($P \leq 0.05$) in vine length, root weight, and top dry weight for any isolate as compared to the non-inoculated control (data not shown).

The watermelon isolates TX 3017 and TX 3019, muskmelon isolate TX 2091, and the onion isolate ATCC 64403 produced microsclerotia in the secondary roots of muskmelon under greenhouse conditions. However, most of the cucurbit isolates did not produce microsclerotia in cantaloupe roots, including the ATCC 16993 onion isolate.

Limited host range. In the above studies, pink discoloration was not an accurate rating system for root disease reaction. Therefore, the general discoloration and pink discoloration of the roots were rated collectively. All isolates produced a pink discoloration to some degree in the roots of muskmelon, watermelon, onion, and tomato. Similar root disease ratings were recorded for a plant species regardless of the test isolate (Table 2). Maximum disease ratings of around 3.0 were not considered severe disease reactions. This is supported by the fact that there was no significant ($P \leq 0.05$) reduction in plant height, root dry weight, and top dry weight (data not shown). Microsclerotial production appears to be a highly variable attribute of *P. terrestris* isolates. Although microsclerotia were produced by all isolates in watermelon roots, microsclerotial production in the other plant species was variable.

DISCUSSION

Pink lesions have been observed on the roots of muskmelon, honeydew, watermelon, and squash roots for many years (Bruton, personal observations). However, the cause of this symptom has been the object of much speculation. *F. solani* has been suggested as the cause of pink root lesions of muskmelons (Champaco et al. 1988; Mertely et al., 1991). However, inoculation of cucurbits with *F. solani* isolates has not resulted in pink root symptoms (Bruton, unpublished data). Taubenhaus and Johnson (1917) first described pink root of onion in Texas. Since that time, much has been written on the disease as it occurs on onion (Biles et al., 1992; Coleman et al., 1992; Corgan et al., 1987; Gorenz et al., 1949; Hess and Weber, 1988). *P. terrestris* has been isolated from natural infections on numerous weeds (Tims, 1955) asparagus, bean, cabbage, carrot, cucumber, lettuce, oat, radish, sunflower, turnip, (Hess, 1962) corn, sugarcane, and sweet clover (Carvajal, 1945), and many grasses (Sprague, 1943). Kreutzer (1941) reported artificial infection of squash, cucumber, muskmelon, and numerous other crop plants using the onion pink root pathogen *P. terrestris*. He further noted pink colored lesions on the roots. Sumner et al. (1997) reported a pinkish cast to roots of cucumber and tomato in artificial inoculations with *P. terrestris*. This is the first documentation that *P. terrestris* is the cause of pink root of cucurbits in the field.

In the present study, *P. terrestris* did not cause root maceration, reduced root growth, or plant stunting in muskmelon. However, discolored roots and pink pigmentation were observed on roots of muskmelon, watermelon, onion, and tomato inoculated with *P. terrestris*. These studies were terminated after 60 days rather than plant maturity. Consequently, disease severity may have been more severe in mature plants. However, studies on onion have shown erratic results with respect to pink root and reducing yield (Kulik and Tims, 1960; Levy and Gornik, 1981). Percentage of pink root pigmentation on plant species tested in the present study was found to be inadequate for disease assessment. Other workers have also observed that pink roots do not necessarily correlate with reduced yield (Biles et al., 1992; Coleman et al., 1992; Kreutzer, 1939). Therefore, pink roots along with other pathogen related symptoms need to be considered when assessing the affect of the pathogen on yield.

A great deal of variation in pathogenicity among the cucurbit isolates of *P. terrestris* was observed in the present study. Gorenz et al. (1949) reported that onion isolates of *P. terrestris* from the same area differ greatly in pathogenicity. In addition, Sumner et al. (1997) noted virulent and avirulent isolates of *P. terrestris* on onion. In the present study, two isolates originally obtained from cucurbits were determined to be non-pathogenic to muskmelon.

The disease has been reported to be most severe at soil temperatures of 26-30°C (Gorenz et al., 1949; Walker and Tims, 1924). Planting early maturing varieties has been successful in reducing pink root severity of onion in Israel (Levy and Gornik, 1981). Yield losses in pink root susceptible varieties has been reported in south Texas (Perry and Jones, 1955) California (Hansen, 1929; Porter and Jones, 1933), Colorado (Kreutzer, 1941), and several northern states including Michigan (Lacy and Roberts, 1982), and New York (Coleman et al., 1992). Sumner et al. (1997) stated that *P. terrestris* was the primary cause of onion yield reduction in Georgia. Nelson (1987) reported that isolates from warmer environments may be more aggressive at higher temperatures. Soil temperatures considered high for onion may be relatively normal soil temperatures for cucurbits. This presents an interesting situation for the disease in cucurbits in which soil temperatures are in the 26-30°C range for a large part of the growing season. Gorenz (1949) reported that 28°C was optimum for disease development in onion under greenhouse conditions, similar to the optimum temperature for growth of cucurbit isolates in this study. Greenhouse temperatures during the present study ranged from 12 to 35°C. However, pink root on cucurbits is highly variable with little or no disease observed in some years and very prevalent in other years. During the summer of 1996, cantaloupe from New York State were observed with completely macerated and pink pigmented root systems that yielded *P. terrestris* isolates (Zitter and Bruton, unpublished data). The etiology of cucurbit pink root may be less temperature dependent than onion pink root.

Hansen (1929) first reported dark thick-walled bodies resembling chlamydospores in the roots of onion but considered them as pycnidial primordia. He did note that pycnidia were never observed. Kreutzer (1941) later noted the presence of pycnidial primordia, however, he did indicate that on one occasion pycnidia did develop from these structures in agar. These structures have been disregarded as to what role they may play in the epidemiology of the disease. Recently, Biles et al. (1992) referred to the structures as microsclerotia and noted differential production with respect to isolate. The fact that long rotations are required (5 to 6 yr) for adequate control of the pink root fungus on onion (McCoy, 1987;

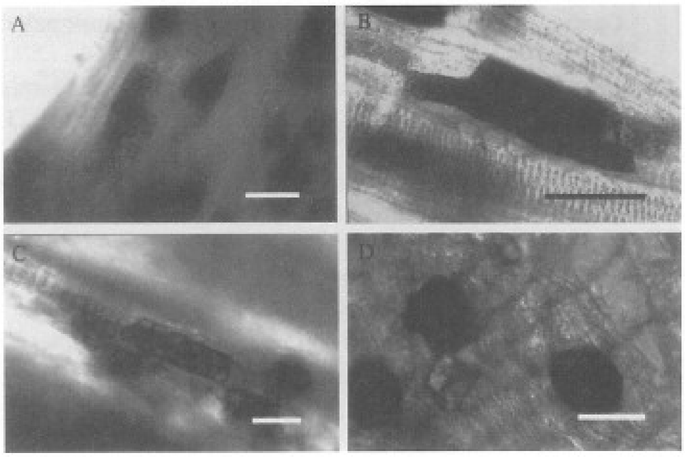

Fig. 4. Comparison of selected microsclerotial pathogens of cucurbits. (A) microsclerotia of *Phoma terrestris* in root epidermal cells of watermelon. Scale bar=50µm. (B) microsclerotia of *Pyrenochaeta lycopersici* in root epidermal cells of canteloupe. Cale bar= 100µm. (C) microsclerotia of *Rhizopycnis vagum* in root epidermal cells of cantaloupe (Miller and Bruton, unpublished). Scale bar = 50µm. (D) microsclerotia of *Macrophomina phaseolina* in root epidermal cells of watermelon. Scale bar=100µm.

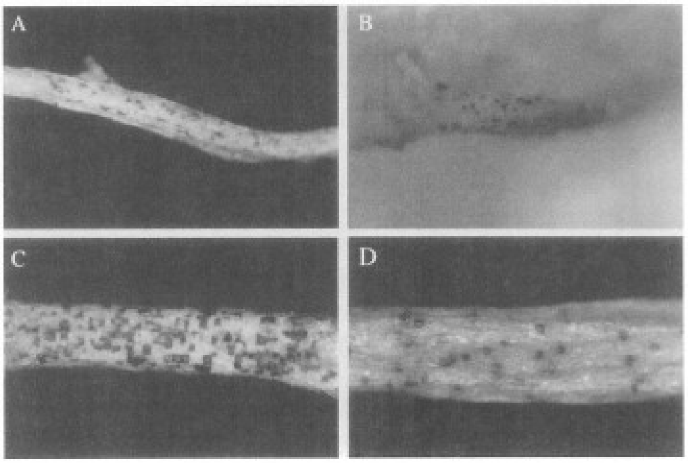

Fig. 5. Macroscopic comparison of selected microsclerotial pathogens of cucurbits. (A) *Phoma terrestris* in root epidermal cells of cantaloupe. (B) *Rhizopycnis vagum* in root epidermal cells of cantaloupe. (C) *Verticillium dahliae* in root epidermal cells of cantaloupe. (D) *Macrophomina phaseolina* in root epidermal cells of watermelon.

Taubenhaus and Mally, 1921) further suggests that a resting structure (microsclerotium) is involved as an over seasoning moculum source. Microsclerotia are consistently observed in cucurbit roots with pink root lesions under field conditions (Bruton, unpublished data). Since each cell of the microsclerotium is capable of germinating, this could increase the infection potential significantly.

P. terrestris produces microsclerotia (Fig. 4A) that are structurally similar to microsclerotia of other cucurbit pathogens. *Pyrenochaeta lycopersici* produces microsclerotia (Fig. 4B) which are considered the primary inoculum in the soil (Ball, 1979a; 1979b). *P. lycopersici*, the cause of corky root on tomato, has been artificially inoculated to cucumber (Grove and Campbell, 1987; Termohlem, 1962), muskmelon (Bruton, unpublished data; Grove and Campbell, 1987; Risser and Laugie, 1968), and squash (Grove and Campbell, 1987). Microsclerotia of *P. terrestris* are essentially identical to those produced by *P. lycopersici* in cantaloupe roots. Gubler, as cited by Grove and Campbell (1987), isolated *P. lycopersici* from naturally infected melons in California. However, the fungus has not been reported from Texas and Oklahoma. *Rhizopycnis vagum* is a recently identified fungal pathogen of cucurbits that not only produces microsclerotia (Fig. 4C) but may also impart a pink to wine red pigmentation to the roots of muskmelon and watermelon (Farr et al., 1998; Miller and Bruton, unpublished data). Each of the three fungi have similar cultural characteristics in that they: 1) are difficult to isolate with consistency, 2) grow slowly and can be overrun by saprophytes in agar, and 3) appear as a gray fluffy colonies on PDA. In contrast, *M. phaseolina* produces larger and more dense microsclerotia (Fig. 4D) that are round to oval and embedded in the epidermis and along the vascular bundles of the cucurbit root and stem. *Verticillium dahliae* is a slow growing fungus that produces microsclerotia similar to the above fungi. Each of these fungi produce microsclerotia in the epidermal cells of cucurbit roots with the exception of *M. phaseolina* which produces microsclerotia in the epidermal cells, cortex, and vascular bundles. Macroscopically, microsclerotia of *P. terrestris* (Fig. 5A), R. vagum (Fig. 5B), V. dalihae (Fig. 5C), and *M. phaseolina* (Fig. 5D) appear similar, although subtle differences exist.

Pink root, caused by *P. terrestris*, does not appear to cause detectable damage to cucurbits in Texas and Oklahoma. The fungus is never isolated with a high frequency unless isolated from pink root lesions (Bruton, unpublished data). However, pink root has occasionally been observed on more than 20% of the root system of muskmelon. The disease is widespread on watermelon and muskmelon in the Rio Grande Valley of Texas, central Texas, and Oklahoma production areas. As a fungus capable of causing root lesions and debilitating the root system to some degree, *P. terrestris* may contribute to the vine decline disease complex of cucurbits. Additional research is needed to determine the conditions necessary for *P. terrestris* infection of cucurbits, the role of microsclerotia in the epidemiology of pink root, and establish if yield reduction does occur as a result of pink root in cucurbits.

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