Peat-Based Media as a Source of *Thielaviopsis basicola* Causing Black Root Rot on Citrus Seedlings

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ABSTRACT

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Thielaviopsis basicola was identified as the cause of black root rot on citrus seedlings growing in soilless peat-based media in Florida greenhouse nurseries. Three of 14 samples of commercial bales of media and two of 12 samples of Canadian sphagnum peat yielded low densities of *T. basicola* (1–2 cfu/cm^3 of medium) when wetted and plated on selective carrot-etridiazol-nystatin medium. *T. basicola* survived in peat debris and was detected from air samples in a greenhouse that had contained infected plants 2 mo previously. Under winter conditions, where media temperatures ranged from 18 to 27 C, isolates of *T. basicola* from peat and a peat-based medium caused severe root rot on rootstock seedlings of Cleopatra mandarin; moderate root rot on Ridge Pineapple sweet orange, sour orange, and Volkamer lemon; and mild root rot on rough lemon, trifoliate orange, Carrizo citrange, and Swingle citrumelo. Propagule density in the rhizosphere of all cultivars increased from the inoculated levels in the peat-based medium. Peat-based media may act as a source of *T. basicola*, a pathogen with a wide host range.

Thielaviopsis basicola (Berk. & Broome) Ferraris (synanamorph = *Chalara elegans* Nag Raj & Kendrick) is a known root pathogen of citrus (6,20) and more than 137 other plant species including agronomic and horticultural crops (10). The fungus forms small, well-defined, brown to black lesions on citrus roots where chlamydospores occur in the cortical tissue; hence, the common name, black root rot (16). Rotting at the root tips causes sloughing of the cortex that exposes the stele and gives the root system a dry, stringy appearance. Leaf symptoms are veinal chlorosis typical of nonspecific nutritional deficiency attributable to root loss. Growth of infected seedlings is markedly reduced depending on the citrus cultivar (18).

In the winters of 1988 and 1989, severe black root rot and stunting of Cleopatra mandarin seedlings was observed in a greenhouse at the Citrus Research and Education Center, Lake Alfred, FL (CREC), and in commercial greenhouses. Sour orange, sweet orange, and Volkamer lemon were moderately affected, whereas trifoliate orange and hybrids Carrizo citrange and Swingle citrumelo were least affected. In another commercial greenhouse, transplanting and budding of affected rootstock seedlings resulted in tree mortality. In every case, seedlings were growing in peat-based media mixed with other inert ingredients (e.g., perlite, vermiculite, or polystyrene beads). In the CREC greenhouse, infestations were consistently associated with a single commercial source of medium.

Repeated recovery of *T. basicola* from affected roots of greenhouse citrus led us to conduct a survey of peat-based mixes and sources of peat to 1) determine the occurrence of this fungus in commercial media and the greenhouse environment in which citrus seedlings are grown, 2) determine whether peat was the source of the fungus in the medium, 3) evaluate the pathogenicity of isolates from potting media, and 4) confirm the susceptibility of commercial rootstock cultivars to black root rot.

MATERIALS AND METHODS

Isolation from potting media. Peat-based media were collected from containers with symptomatic citrus seedlings located in greenhouses at CREC and three commercial citrus nurseries in Central Florida. Ten cubic centimeters of medium was mixed with 90 cm^3 of sterile 0.25% water agar. One-milliliter aliquots of agar slurry were pipetted into each of five or 10 petri plates. Cooled (48 C) *T. basicola*-carrot-etridiazol-nystatin (TB-CEN) agar (13) was then added and the plates were swirled to mix the sample with the molten agar before solidification. The agar slurry was also plated onto modified pimaricin-ampicillin-rifampicin-pentachloronitrobenzene (PARP) selective medium for detection of *Phytophthora parasitica* Dastur, a commonly encountered pathogen of citrus roots in Florida (4). In some cases, samples were diluted with additional water agar to obtain countable numbers of *T. basicola* colonies. Plates were incubated at room temperature (25–27 C) for 7 days and counts were expressed as colony-forming units per cubic centimeter of potting medium.

A survey of peat-based media used in citrus nurseries was conducted in 1989. Nurserymen were asked to submit samples of media collected from sealed bales by slitting them open with a clean razor blade and removing approximately 100 cm^3 to a resealable plastic bag. At CREC, two bales from one commercial peat source were surveyed likewise. In one case, a portion of a bale was saturated with water when it was received from the supplier. A modified carrot disk assay (22) was used to isolate *T. basicola* from the saturated and dry portions of the bale. Ten-centimeter carrot pieces were washed with tap water, surface-disinfested for 1 min in 70% ethanol, and rinsed with sterile water. Carrots were aseptically split and pieces were inserted into the bale at 10 wet and 10 dry locations. After 10 days, the pieces were removed and the cut surfaces were examined under a dissecting microscope (×8 magnification) for the presence of *T. basicola* chlamydospores.

In 1990, peat samples from six different Canadian sphagnum peat bogs were obtained from each of two commercial peat companies. Aliquots of 200–300 cm^3 of peat were placed aseptically in sterile glass beakers, mixed with sterile tap water to moisten the peat (10% moisture, v/v), and the beakers were covered with foil. After 1, 2, and 3 mo of incubation at 23–27 C, 10-cm^3 aliquots were removed from each beaker and plated on TB-CEN medium as described earlier. Alternatively, 1-cm^3 aliquots of peat were placed directly in petri dishes and cooled TB-CEN agar was poured directly over the peat. In some cases, samples from a naturally infested bale of peat-based medium (2 cfu/cm^3) were plated as a positive control.

Isolation from the greenhouse environment. To determine whether *T. basicola* had become indigenous in the greenhouse at CREC, a greenhouse unit in which infestations of several hundred seedlings of different cultivars were detected in 1988 and 1989 was monitored after all plant material was removed in

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January 1990. In February, samples of potting media debris were taken from bench tops and beneath benches along the sides of the house and spread on TB-CEN plates. A central bench, which was surface-disinfested with 0.05% NaOCl 1 mo previously, was sampled by swabbing the bench top with cotton swabs moistened with sterile water. The wetted tips of the swabs were immediately streaked onto the surface of TB-CEN agar.

During March 1990, an Anderson Air Sampler loaded with TB-CEN agar plates was located on the middle bench of the greenhouse unit. Air sampling of 5, 10, and 15 min in duration was conducted on different days at about 3 p.m. when air coolers stirred the air. Air outside of the greenhouse was sampled by placing the sampler on the air intake and sampling for five half-hour periods throughout the day.

Susceptibility of citrus cultivars. Isolates of *T. basicola* obtained from infested peat-based medium (Tri) and from sphagnum peat (P3B) were tested for pathogenicity on eight cultivars of citrus and citrus hybrids. Agar disks of isolates from V8 juice agar plates (11) were used to inoculate 2-L flasks containing 1.5 L of sterile vermiculite mixed with 250 ml of half-strength V8 juice broth (11). After colonization, one part vermiculite inoculum was mixed with nine parts commercial peat-based medium to yield 1,000 cfu/cm^3. Noninfested vermiculite medium was mixed similarly for control treatments. Seven seedlings of trifoliate orange (*Poncirus trifoliata* (L.) Raf.), Ridge Pineapple sweet orange (*C. sinensis* (L.) Osbeck), sour orange (*C. aurantium* L.), Volkamer lemon (*C. volkameriana* Pasq.), rough lemon (*C. jambhiri* Lush.), Cleopatra mandarin (*C. reticulata* Blanco), Carrizo citrange (*C. sinensis* × *P. trifoliata*), and Swingle citrumelo (*C. paradisi* Macfady. × *P. trifoliata*) were planted in infested and noninfested medium in 125-cm^3 containers (Ray Leach Container Nursery, Canby, OR). Seedlings were fertilized weekly with Peters 20-20-20 (NPK) Peatlite Special (Grace-Sierra, Fogelsville, PA). Experiments were conducted from October to December 1989 with isolate Tri and from November 1990 to January 1991 with isolates Tri and P3B.

At harvest, medium from each treatment was combined among replicate containers and mixed for determination of *T. basicola* propagule density. Roots and stems were rated by grouping seedlings of all cultivars into five categories of root symptoms. Root systems showing very few or no black or sloughing fibrous roots were rated 1 and root systems with few or no remaining roots were rated 5. Fibrous roots (excluding taproot) were dried and weighed. Root rot ratings were subjected to an analysis of variance using the General Linear Models procedure (SAS, Cary, NC), and differences among cultivars were tested using Duncan's multiple range test. Similar cultivar susceptibility was obtained with isolate Tri in both experiments. The results of the 1990–1991 experiments with isolates Tri and P3B are presented.

RESULTS

Isolation from potting media. All samples of peat-based media from container-grown citrus seedlings with black root rot symptoms yielded *T. basicola* when plated on TB-CEN medium. *P. parasitica* was never detected when medium was plated on modified PARP selective medium (4). Chlamydospores in the brown to black lesions on the roots confirmed the presence of *T. basicola*. Cortical root rot appeared dry in contrast to Phytophthora root rot where affected cortical tissue appears water-soaked (4).

Of 12 nurseries surveyed for *T. basicola* in unused potting media, one sample was positive for the fungus. We also obtained one positive from each of two bales of a commercial medium associated with epidemics of black root rot in our greenhouse at CREC in 1988 and 1989. When carrot pieces were inserted into one of the bales, *T. basicola* was recovered from the wet portions of the bale but not from dry portions.

Six samples of peat were obtained from each of two suppliers of potting mixes and stored aseptically in the laboratory. One month after storage, all samples were negative; however, after 2 mo, two samples from one of the suppliers yielded 1–2 cfu of *T. basicola* per cubic centimeter of peat. At 3 and 4 mo after storage, the same two samples were positive and all others were negative. In each case, the number of *T. basicola* propagules recovered was very low (1–2/cm^3).

Isolation from the greenhouse environment. To determine whether *T. basicola* had become indigenous in the greenhouse at CREC, all plant material was removed in January 1990. One month after all plant material was removed from the CREC greenhouse, *T. basicola* was detected in 18 of 32 samples of peat debris on and under benches in areas where infested plants were located previously. *T. basicola* was also recovered in four out of four samples from the surface of a previously disinfested central bench adjacent to infested benches.

Air sampling above the benches was conducted throughout the day on 3 days in March 1990. The fungus was recovered on two of three sampling dates and propagule densities of *T. basicola* varied from 3 to 24 × 10^{-3} cfu/m^3 of air. One sampling of air outside of the greenhouse near the cooling unit intake yielded 7 × 10^{-3} cfu/m^3 of air.

Susceptibility of citrus cultivars. Initial propagule levels were approximately 1,000 cfu/cm^3 in the infested medium and 8 cfu/cm^3 in the noninfested medium attributable to natural infestation. During the experiment, shoots of seedlings did not grow in any of the treatments. In the infested treatments at harvest, propagule counts of both *T. basicola* isolates were maintained at the initial level of infestation or increased on all cultivars (Fig. 1). Populations also increased in noninfested medium from the low level at the beginning of the experiment to

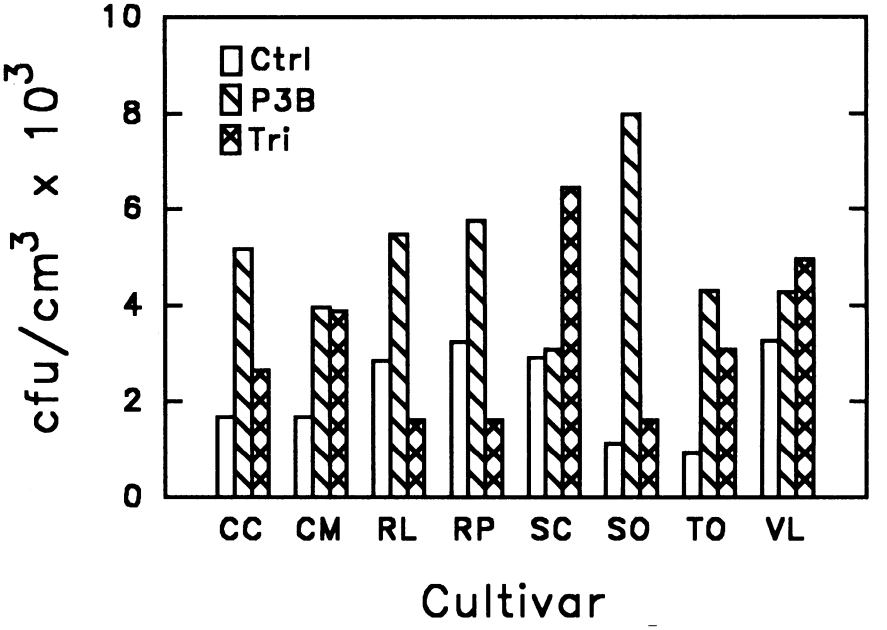

Fig. 1. Recovery of *Thielaviopsis basicola* from the rhizosphere of citrus seedlings growing for 3 mo in peat-based medium that was noninoculated (ctrl) or inoculated with isolates of *T. basicola* from peat (P3B) or infested peat-based medium (Tri) that cause black root rot. CC = Carrizo citrange, CM = Cleopatra mandarin, RL = rough lemon, RP = Ridge Pineapple sweet orange, SC = Swingle citrumelo, SO = sour orange, TO = trifoliate orange, and VL = Volkamer lemon.

>1,000 cfu/cm^3 at harvest 2 mo later.

Root rot was observed on all cultivars, even in noninfested treatments, except for trifoliate orange (Table 1). The root rot ratings among cultivars in noninfested medium were not significantly different, although Cleopatra mandarin was most affected by root rot. Inoculation with either isolate of *T. basicola* resulted in severe root rot on Cleopatra mandarin and moderate to severe root rot on sweet orange, sour orange, and Volkamer lemon. Rough lemon, Swingle citrumelo, and Carrizo citrange were mildly affected, and trifoliate orange was the least affected. The evaluation of cultivar susceptibility by visual rating was confirmed by a reduction in fibrous root weight. Cleopatra mandarin lost almost all of its roots when inoculated with *T. basicola* P3B, whereas the reduction of root weight of trifoliate orange and its hybrids, Swingle citrumelo and Carrizo citrange, was minimal.

DISCUSSION

T. basicola has been reported to be prevalent in California citrus soils (17) and capable of causing root rot on a wide range of citrus cultivars in artificial inoculations in fumigated soils (18). However, the significance of *T. basicola* as a root rot pathogen of citrus in orchard soils has not been established (16). We have now identified *T. basicola* as the cause of black root rot of citrus seedlings growing in soilless, peat-based media in Florida greenhouse nurseries.

In citrus greenhouses, Canadian sphagnum peat appears to be a source of *T. basicola* because samples of commercial peat yielded low levels of the fungus. Heretofore, peat may not have been identified as the source of *T. basicola* on susceptible crops for several reasons. First, *T. basicola* was consistently isolated from only two of 12 peats sampled and, when detected, was at a very low density. Selective TB-CEN medium was capable of detecting low propagule numbers that the carrot disk assay was not (J. H. Graham and N. H. Timmer, *unpublished*). Also, prewetting of the peat and incubating the medium for several months appeared to enhance recovery of *T. basicola*. Moisture is known to stimulate germination of chlamydospores and phialoconidia in soil, whereas drying soil inhibits germination (5).

Once *T. basicola* was introduced into a greenhouse and caused disease on stands of seedlings, the fungus survived in peat debris. Spread to noninfested areas of the greenhouse occurred via airborne propagules, presumably phialoconidia, as indicated by repeated recovery after air sampling. We assume phialoconidia, which may remain alive in soil for months at lower temperatures (7,12), survived in the greenhouse environment in the winter after infested seedlings were removed. *T. basicola* also oversummered in the greenhouse despite poor survival in soil associated with previously infested seedlings (J. H. Graham and N. H. Timmer, *unpublished*). The fungus was also detected outside of the greenhouse at the air intake, so the surrounding area could not be ruled out as a source.

Peat-based media are conducive for development of black root rot. *Ilex* cultivars became infected readily in Canadian sphagnum peat, and various ratios of bark in the medium did not suppress disease development (8). Only a reduction in pH from 6.5 to 6.0 and 5.5 decreased disease severity (8). The pH 6.5 of the medium used in our study confirms that pH near neutrality is conducive for black root rot development (1,3).

In the winter, when potting media temperatures ranged from 18 to 27 C, low inoculum densities (8 cfu/cm^3) in the noninfested treatments led to significant root rot development. No root rot developed under summer conditions when media temperatures ranged from 25 to 33 C (J. H. Graham and N. H. Timmer, *unpublished*). These observations confirmed that soil temperatures below 25 C favor the disease on citrus (19). When the medium was infested with 1,000 cfu/cm^3, root rot development was moderate to severe on most of the cultivars tested. Propagule density increased to even higher levels in the rhizosphere of all the cultivars. These populations are comparable to those detected on beans in pots (2) but are 10–100 times higher than propagule densities from crop soils (9,14).

Cleopatra mandarin was most susceptible to black root rot, followed by sweet orange, sour orange, Volkamer lemon, rough lemon, Carrizo citrange, Swingle citrumelo, and trifoliate orange. This ranking concurs with the previously reported susceptibility of citrus species and hybrids (18) and is also supported by observations in commercial greenhouses. Typically, Cleopatra mandarin was severely stunted by root rot under winter conditions, which may preclude production of winter crops of this cultivar in the presence of *T. basicola*. Trifoliate orange hybrids, Carrizo citrange and Swingle citrumelo, developed some root rot but growth was not affected. The other cultivars exhibited varying degrees of growth reduction in the greenhouse.

One of the most important considerations is the wide host range of *T. basicola*, not only on citrus cultivars but on other crop species produced in peat-based media for transplant. Isolates of the fungus from peat and peat-based media were equally pathogenic on citrus at high inoculum density, which is consistent with the lack of host specificity on other crops (15,21). The wide host range of *T. basicola* means that peat-based media may be a significant mode for introduction of the pathogen onto other susceptible greenhouse crops. Once established in a greenhouse, the pathogen could survive and produce subsequent disease cycles as observed for citrus. Thus, screening of peat or peat-based media with selective TB-CEN medium may be appropriate when a potting mix is used for crops that are highly susceptible to *T. basicola*.

Table 1. Susceptibility of citrus cultivars to *Thielaviopsis basicola* isolates from peat (P3B) and infested peat-based medium (Tri) that cause black root rot

	Root rot rating (1-5)[x]			Reduction in root weight (%)[y]	
Cultivar	Control	P3B	Tri	P3B	Tri
Cleopatra mandarin	2.3 a	5.0 a[z]	4.8 a	99	62
Sweet orange	1.8 ab	3.7 b	3.6 bc	42	37
Sour orange	2.1 a	3.6 b	3.3 bc	42	52
Volkamer lemon	1.7 ab	3.3 b	4.0 b	12	29
Rough lemon	1.8 ab	2.7 c	2.4 de	18	11
Swingle citrumelo	1.4 ab	2.6 cd	3.0 cd	4	10
Carrizo citrange	1.7 ab	2.3 cd	2.9 cd	0	0
Trifoliate orange	1.0 b	2.0 c	2.0 e	0	18

[x]Root rot ratings varied from 1 to 5 where 1 = few black and sloughing fibrous roots and 5 = complete fibrous root loss.

[y]Percent reduction in dry weight of fibrous roots vs. uninoculated control.

[z]Means (n = 7) with same letter are not significantly different (P = 0.05) by Duncan's multiple range test.

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