Vegetable Seedling Diseases Associated with Earthworm Castings Contaminated with *Phytophthora capsici* and *Pythium atrantheridium*

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**Abstract**

Earthworms and worm castings have been recommended for their beneficial effects in increasing yields and suppressing soilborne diseases. However, in a few cases, earthworm castings have been shown to harbor soilborne pathogens. The research documents that earthworm castings used as an amendment in soilless potting mixes at several organic farms in North Carolina were contaminated with *Phytophthora capsici* and several *Pythium* species. *Phytophthora capsici* and *P. atrantheridium* were subsequently isolated from rotted roots of vegetable seedlings grown in the potting mix. Commercial producers of earthworm castings should only use clean plant material to maintain earthworms and earthworm castings should be ascertained as pathogen-free before incorporation into plant growth media.

**Introduction**

Earthworm castings are an odorless, nutrient-rich, organic medium that supports a diverse microbial community (2,3,5). Castings are also rich in calcium humate, a binding agent that reduces desiccation of the castings and promotes incubation and proliferation of beneficial organisms such as *Trichoderma* spp., *Pseudomonas* spp., and mycorrhizal fungi (6,8,10,11). Worm castings have been shown to suppress Verticillium wilt of eggplant (7) and Fusarium wilt of cyclamen, iris, and tomato (8,10). Earthworms disperse propagules of both beneficial and plant pathogenic soil microorganisms (4), but less is known about the potential of earthworm castings to serve as a source of soilborne pathogens in agricultural systems (5,13). Here we report the presence of *Phytophthora* and *Pythium* species in earthworm castings and their association with disease in soilless potting mixes.

In spring 2010, four growers in North Carolina experienced problems in their organic vegetable production. The first grower noticed disease symptoms in cucumber, tomato, and pepper transplants soon after seeding. Seedlings were stunted with marginal leaf necrosis and some eventually died. Two weeks later, vegetable transplants at three other grower facilities exhibited damping-off. All growers had used the same lot of custom-blended soilless mix in their transplant product. The mix was composed of 50% peat and 50% perlite, and did not contain synthetic wetting agents or fertilizers; moreover, there were no pathogens detected in these soilless mix. The mix had also been amended with worm castings produced and purchased from same source.
A sample of the soil was initially sent to the Soil Testing Laboratory of the North Carolina Department of Agriculture (NCDA), Raleigh, NC (www.ncagr.com/agronomi/sthome.htm), for chemical analysis. The report indicated satisfactory nutrient levels. Therefore, soilborne pathogens were investigated to determine if the earthworm castings were a source for pathogens causing damping-off of the vegetables at these organic farms.

**Isolation from Diseased Plant and Worm Casting**

Traditional techniques such as media isolation and Rhododendron baiting were employed. Molecular approaches involving DNA sequence analysis and Blast search (1) were used for species identification of the pathogens. Root lesions from five diseased plants were excised and surface-sterilized in a 0.5% sodium hypochlorite solution for 5 min, placed on potato dextrose agar (PDA), and incubated for 3 days at room temperature in the dark. Isolates were initially obtained from all diseased plant roots. The representative isolates from cucumber, tomato, and pepper were named *Phytophthora* 735, 738, 739, and *Pythium* 735, 736, 739, respectively.

An indirect isolation method was used to recover oomycetes from the worm castings. Rhododendron leaves were surface-sterilized in 0.5% sodium hypochlorite for 3 min, air dried, and used as bait by cutting the leaves at the mid-vein in a herringbone pattern. Three to four cut leaves were placed into worm castings for 48 h, then set on moistened paper inside a container (length 30 cm, width 26 cm, height 10 cm) and incubated at room temperature in the dark for 3 days, to allow for potential growth of oomycetes. Each rhododendron leaf was examined daily and pieces of leaf showing discoloration were placed on PDA. Putative isolates of *Phytophthora* and *Pythium*, based on morphological characteristics, were consistently recovered from diseased roots and from the worm castings (Table 1).

<table>
<thead>
<tr>
<th>Type</th>
<th>Isolates</th>
<th>Plant</th>
<th>Accession no.</th>
<th>Species</th>
<th>Similarity (%)</th>
<th>Accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolates from plant</td>
<td><em>Phytophthora</em> 735</td>
<td>cucumber</td>
<td>GQ337919</td>
<td><em>P. capsici</em></td>
<td>100</td>
<td>DQ069293</td>
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<td></td>
<td><em>Phytophthora</em> 738</td>
<td>tomato</td>
<td>−</td>
<td><em>P. capsici</em></td>
<td>100</td>
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<tr>
<td></td>
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<td>pepper</td>
<td>−</td>
<td><em>P. capsici</em></td>
<td>100</td>
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<tr>
<td></td>
<td><em>Pythium</em> 735</td>
<td>cucumber</td>
<td>GQ337920</td>
<td><em>P. atrantheridium</em></td>
<td>100</td>
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<td>tomato</td>
<td>−</td>
<td><em>P. atrantheridium</em></td>
<td>100</td>
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<tr>
<td></td>
<td><em>Pythium</em> 739</td>
<td>pepper</td>
<td>−</td>
<td><em>P. atrantheridium</em></td>
<td>100</td>
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<tr>
<td>Isolates from worm castings</td>
<td>Cast739-1.1</td>
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<td>GQ337921</td>
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<td>Cast736-1.1</td>
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<td>−</td>
<td><em>P. spinosum</em></td>
<td>95</td>
<td>AF331090</td>
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<td></td>
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<td><em>P. spinosum</em></td>
<td>95</td>
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<tr>
<td></td>
<td>Cast738-4</td>
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<td><em>P. intermedium</em></td>
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<td><em>P. intermedium</em></td>
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<td>GQ337922</td>
<td><em>P. atrantheridium</em></td>
<td>100</td>
<td>EU109570</td>
</tr>
</tbody>
</table>

All the isolates for DNA sequence analysis listed in the table are from one farm.

x The GenBank accession numbers of *Phytophthora* and *Pythium* sequences deposited from this research.

y The GenBank accession numbers of *Phytophthora* and *Pythium* sequences in GenBank match to the sequences from this research.

The colony of *Phytophthora capsici* on PDA after 5 days culturing is white with ashen mycelium. *P. capsici* has long pedicels, sporangia are spherical to elongate with a tapering base. Sporangia have long pedicels ranging from 30 to 130 μm. Sporangia are papillate, ellipsoid, and fusiform. The lengths and widths
of sporangia range from 34 to 67 and 15 to 40 μm. *P. capsici* produces antheridia, oogonia, and oospores. Antheridia range with the diameters from 11 to 20 μm. Oogonia are spherical with diameters ranging from 22 to 52 μm. Oospores are plerotic, the wall thicknesses of oospores ranges from 3 to 6 μm, and the diameters oospores ranges from 20 to 33 μm.

The colony of *Pythium attrantheridium* on PDA after 5 days culturing is cottony with hyaline, yellowish, and well-branched mycelium. *Pythium* grew much faster than *Phytophthora*. The width of aerial hyphae is from 3 to 5 μm. There are plentiful hyphal swellings. The width of hyphal swellings is from 20 to 27 μm, Sporangia are globose with the length of 11 to 20 μm. Sporangia contain 6 to 12 zoospores. Oogonia are terminal with the diameter from 14 to 29 μm. Antheridia have a broad apical attachment with oogonia. Oospores are plerotic or aplerotic with the diameter ranging from 14 to 20 μm, the thickness of oospore walls is 2 to 3 μm. Further species identification was based on DNA sequence analysis.

**Species Identification Using DNA Sequence Analysis**

Species identification of the oomycetes was determined by comparing rRNA ITS fragment sequences. Mycelium (1-cm × 1-cm disks) from 3-day-old-cultures of *Pythium* and *Phytophthora* grown on PDA, which were ground for 1 min with a drill in the presence of 50 μl DNA extraction buffer (1M Tris pH 8, 5M NaCl, 0.5M EDTA, 10% SDS, sterile distilled water). The extract was diluted 100 fold with distilled water and 2 μl was used for PCR amplification. PCR conditions were 1 cycle of 94°C for 2 min; 30 cycles of 94°C for 1 min, 65°C for 1 min, 72°C for 3 min; and a final extension at 72°C for 10 min.

A set of primers for amplification were developed from the rRNA internal transcribed spacer (ITS) sequences. Various *Phytophthora* spp. (120 representative sequences) and *Pythium* spp. (131 representative sequences) were retrieved from GenBank (National Center for Biotechnology Information, www.ncbi.nlm.nih.gov), aligned using ClaustalX (12). Genus-specific primers were designed based on homologous regions specific to *Phytophthora* and *Pythium*, respectively. These primers were designed to target ITS1 sequences exhibiting the most variability in both genera. A 220 and 280 bp fragment was amplified in *Phytophthora* using primers (PhytoF: CGCGGTATGGTTGGCTTCGGCTGAAC and PhytoR: GCGGGTAATCCTTGGCCTGAAC) and *Pythium* using primers (PythiF: CCTCGGGAAGGATCATCATTACCAC, and PythiuR: CGAGGCTAGACATCCACTGCTG), respectively. These shorter ITS1 fragments amplified by the primers contained all the variability necessary to identify these isolates to species. The genus-specific primers were used for amplification with six different known *Pythium* and ten *Phytophthora* spp., the result showed that all the identification based on DNA sequence analysis using Blast search (1) agreed with the identification based on morphology. Isolates that were baited from the worm castings were identified as *Phytophthora capsici*, *P. attrantheridium*, *P. spinosum*, and *P. intermedium* (Table 1). However, only *P. capsici* and *P. attrantheridium* were recovered from diseased roots of the same plant for all vegetable transplants including cucumber, tomato, and pepper. The representative isolates from one farm were sequenced for species identification, after that, the rest of the isolates were identified based on the morphology compared with the representative isolates being sequenced.

Three isolates of *P. capsici* and three *P. attrantheridium* were chosen for pathogenicity test. One-month-old cucumber (cultivar Corona), tomato (cultivar Rio Colorado), and bell pepper (cultivar Camelot) seedlings were planted in the pots (four plants in each pot with two replications) with soilless mix (50% peat and 50% perlite) amended with *P. capsici* and *P. attrantheridium* at a rate of 1% (v/v contained 600 cfu/g of vermiculite). Innoculation was prepared following the procedure described earlier (9). Noninoculated controls (2 pots with 8 plants) were planted in the soilless mix without pathogens and subjected to the same conditions. The inoculation test repeated twice. Inoculated plants and un inoculated plants were kept in a greenhouse with a temperature range from 22 to 27 April 2012.
to 25°C. After 15 days, diseases symptoms from leaves and roots were observed with the similar damping-off symptoms on all inoculated plants but not on the control plants. Microorganisms were re-isolated from two symptomatic plants, which had the identical morphological features as the original isolate.

**Summary**

Based on colony morphology of isolates from diseased plants, *Phytophthora* spp. and *Pythium* spp. were suspected to be the causal agents of disease on vegetable transplants and identification which was subsequently confirmed by Blast analysis (i) of DNA of representative isolates. Furthermore, we found DNA sequences of isolates from diseased plants and worm castings were identical (Table 1). Inoculation using isolates from diseased plants or worm castings resulted in the same symptoms as observed at the farms. The same isolates could be recovered from the inoculated plants, thereby, completing Koch’s postulates.

Diseases on the vegetable transplants were caused by *P. capsici* and *P. atrantheridium*. The results suggest that the source of inoculum causing damping-off of transplants came from the worm castings the growers used at their farms. Furthermore, these pathogens remain viable and pathogenic after passage through earthworms as evidenced by the pathogenicity trials. Therefore, care should be taken in amending soil with earthworm castings. In this case, the worm castings producer used culled vegetable plants as part of his feedstock for earthworm farming and these plant tissues vegetables might have been infected with oomycete pathogens. We highly recommend that commercial worm castings producers always use clean material for earthworm farming in order to avoid contamination with soilborne plant pathogens. Moreover, we propose that worm castings be inspected before application since soilborne plant pathogens such as *Phytophthora*, *Pythium*, and *Fusarium* spp. et al. are easily spread by earthworms and worm castings.

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**Literature Cited**