Characterization of *Pythium* Species Collected from a Multiple Time-Point Sampling of Cucurbits in South Carolina

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Abstract

Species of *Pythium* cause root and stem rot in cucurbits, but no formal surveys have been conducted in the United States to identify which species are responsible. The cucurbit hosts bottle gourd, cucumber, Hubbard squash, and watermelon were transplanted in May, July, September, and November into sentinel plots in four and five different fields in 2017 and 2018, respectively, in South Carolina. Eight of the nine fields were replanted in March 2019. Isolates (600) were collected and identified by sequencing DNA of the mitochondrial cytochrome oxidase I region. The four most common species were *P. spinosum* (45.6% of all isolates), *P. myriotylum* (20.0%), *P. irregulare* (15.3%), and *P. aphanidermatum* (12.8%). *P. myriotylum* and *P. aphanidermatum* were predominantly

isolated in May, July, and September, whereas *P. spinosum* and *P. irregulare* were predominantly isolated in November and March. Isolates of *P. ultimum*, *P. irregulare*, and *P. spinosum* were more virulent than isolates of *P. myriotylum* and *P. aphanidermatum* at 25°C. Representative isolates were screened in vitro for sensitivity to three fungicides: mefenoxam, propamocarb, and oxathiapiprolin. All isolates were sensitive to mefenoxam and propamocarb, but these same isolates were insensitive to oxathiapiprolin, except those classified taxonomically in *Pythium* clade I.

Keywords: *Pythium*, cucurbits, survey, COX1, oxathiapiprolin

The family Cucurbitaceae is composed of 960 species within 115 genera. This diverse plant family ranks among the highest for numbers of species grown for human consumption (Robinson and Decker-Walters 1997). In the United States 2017 Census of Agriculture, 52,524 ha of watermelon (*Citrullus lanatus* [Thunb.] Matsum. & Nakai), 48,423 ha of cucumber (*Cucumis sativus* L.), 37,863 ha of pumpkin (*Cucurbita pepo* L.), 28,909 ha of cantaloupe (*Cucumis melo* L.), and 28,405 ha of squash (*Cucurbita maxima* Duchesne) were harvested for a total of 167,720 ha of cucurbits, making it the third largest group of vegetables in the United States after potatoes and sweet corn (USDA-NASS 2018).

Several species within the soilborne plant pathogen genus *Pythium* are known to infect cucurbits and cause root and stem rots (Deadman 2017). Aboveground symptoms of cucurbits infected by *Pythium* spp. include stunting, wilting, chlorosis, damping-off, and girdling and darkening of the lower stem at the soil line. Standing water and low temperatures increase incidences of *Pythium* diseases in cucurbit seedlings. Mature plants are also susceptible, although symptoms are less severe than on younger plants, and root systems may develop brown necrotic lesions throughout the season (Deadman 2017).

Species of *Pythium* survive in the soil as thick-walled oospores that can persist for decades (Webster and Weber 2007). As a precursor to infection, *Pythium* spp. produce motile, swimming zoospores that navigate through water films and are chemotactically attracted to plant root exudates and, especially, exudates from germinating seeds

(Martin and Loper 1999). Cultural management of *Pythium* diseases in cucurbits can be achieved by transplanting seedlings at a later growth stage, grafting to resistant cucurbit rootstocks, and planting in raised beds to reduce periods of soil saturation from heavy rainfall (Deadman 2017; Toporek and Keinath 2019). Soil fumigation with methyl bromide was once a general use control for soilborne pathogens of cucurbits, but that is no longer an available management strategy (Davis et al. 2008). The fungicides mefenoxam (FRAC code 4) and propamocarb (FRAC code 28) currently are labeled for use on cucurbits to preventatively manage *Pythium* diseases. The use of mefenoxam increased from 6 to 18% of the watermelon acreage in the United States between 2006 and 2014 (USDA-NASS 2019). Oxathiapiprolin (FRAC code 49) is a newly registered fungicide in the class piperidinyl thiazole isoxazolines, specifically targeting an oxysterol-binding protein (Pasteris et al. 2015). Oxathiapiprolin is an effective fungicide that controls many oomycete pathogens (e.g., *Phytophthora nicotianae* and *Pseudoperonospora cubensis*), but it is not labeled for control of *Pythium* spp. (Bittner and Mila 2016; Cohen 2015). Isolates of *Pythium* spp. are variably sensitive to these three fungicides (Miao et al. 2016). Isolates of several species of *Pythium* have developed resistance to propamocarb and mefenoxam, and some isolates in greenhouses are resistant to both fungicides (Moorman and Kim 2004).

Twenty-four species of *Pythium* have been reported to cause disease on species in the family Cucurbitaceae in countries around the world, although most reports have been for cucumber (Farr and Rossman 2020). The four most commonly reported species of *Pythium* infecting cucurbits are *P. aphanidermatum* (Edson) Fitzp. ($n = 65$ individual reports), *P. ultimum* Trow, ($n = 26$), *P. debaryanum* ($n = 23$), and *P. irregulare* Buisman ($n = 9$). *P. debaryanum* was not validly described in the past literature and could be a number of different species, including *P. intermedium*, *P. ultimum*, and probably *P. sylvaticum* W.A. Campb. & F.F. Hendrix (Van der Plaats-Niterink 1981). Formal surveys have not been conducted in the United States to identify the species of *Pythium* that are predominantly responsible for causing diseases on species of cucurbits. Two surveys of greenhouse cucumber in Oman identified *P. aphanidermatum* as the most prevalent species followed by *P. spinosum* Sawada (Al-Sadi et al. 2007, 2012). Likewise, the most common species recovered in cantaloupe fields sampled in Iran were *P. aphanidermatum* (71.8%), *P. ultimum* (22.3%), and *P. deliense* (5.9%). *P. aphanidermatum* and *P. ultimum*

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also have been recovered from various cucurbits in Iran, particularly *P. aphanidermatum* (Teymoori et al. 2012). Studies aimed at examining how season affects the species isolated from cucurbits are lacking; in most studies, isolates were collected at a single point in time. Recently, however, Philosoph et al. (2019) demonstrated a transition on cucumber between *P. spinosum* in cold winter months to *P. aphanidermatum* in warm summer months with mixed populations of the two species in intermediary months.

Communities of *Pythium* spp. in the soil are influenced by many factors associated with field management strategies. Incorporation of cover crops has been observed to stimulate *Pythium* spp. communities (Njoroge et al. 2008; Pankhurst et al. 1995). Soils treated with glyphosate showed an increase in *Pythium* taxa after application (Schlatter et al. 2018). Conversely, soils suppressive to *Pythium* spp. have been created using various sources of organic amendments in different agricultural systems (Dissanayake and Hoy 1999; Lumsden et al. 1987). Microbial taxa under organic compared with conventional management differ in diversity, especially under mineral compared with nonmineral fertilizer use; however, this relationship is rather complex (Hartmann et al. 2015). Martin and Loper (1999) argued *Pythium* spp. are good targets for biocontrol because of sensitivity to antagonistic microbial activity during saprophytic stages. Management strategies that increase microbial diversity would arguably have implications on *Pythium* spp. communities. Because of the cumulative effects various management strategies have on the soil microbiome and *Pythium* spp. communities, species activity and recovery may differ between organic and conventionally managed cucurbit fields.

Identification of individual species of *Pythium* was once a laborious task involving microscopic determination of morphological characters, but DNA barcodes have expedited this process (Van der Plaats-Niterink 1981). The mitochondrial cytochrome oxidase subunit 1 (COX1) and the nuclear internal transcribed spacer region of ribosomal DNA (ITS) are the sequencing targets recommended for identification of *Pythium* spp. (Robideau et al. 2011; Schroeder et al. 2013). Several recent surveys of *Pythium* spp. associated with different crops have utilized these barcode regions for reliable identification (Coffua et al. 2016; Del Castillo Múnera and Hausbeck 2016; Rojas et al. 2017a). Taxonomically, species within the genus *Pythium* have been broken up into 10 phylogenetic clades (A through J) based on ITS sequencing that correlate with differing sporangium morphology (Lévesque and De Cock 2004). *Phytopythium*, formerly *Pythium* clade K, has been described as a morphological intermediate between *Pythium* and the genus *Phytophthora* (de Cock et al. 2015).

The objectives of this research were to (i) identify *Pythium* spp. on symptomatic cucurbits planted in sentinel plots in organic and conventional fields sampled throughout the growing season, (ii) quantify virulence of *Pythium* spp. isolates on a susceptible watermelon cultivar, and (iii) screen *Pythium* spp. isolates for in vitro sensitivity to the fungicides mefenoxam, propamocarb, and oxathiapiprolin.

Materials and Methods

Sampling design. Sentinel plots planted with four different cucurbit genera and species grown from organic seeds were used to collect *Pythium* spp.: bottle gourd (*Lagenaria siceraria* [Peaceful Valley Farms, Grass Valley, CA]), cucumber (*Cucumis sativus* 'Marketmore 76' [Peaceful Valley Farms]), Hubbard squash (*Cucurbita maxima* 'Golden Hubbard' [Everwilde Farms, Sand Creek, WI]), and watermelon (*Citrullus lanatus* 'Sugar Baby' [Peaceful Valley Farms]). For plants to be transplanted at the first true leaf stage, watermelon and cucumber seeds were planted 18 days before transplanting, and bottle gourd and squash seeds were planted 14 days before transplanting. Seeds were sown into 96-cell trays containing a soilless, peat-based container mix (Fafard 3B or Metro-Mix 830; Sun Gro, Agawam, MA) if seedlings were to be planted into a conventional field and into an organic container mix (28.3 liters of Premium Organic Vegetable and Herb Mix [PROMIX, Quakertown, PA], 11.4 liters of peat, 3.8 liters of vermiculite, 3.8 liters of sand, and 0.5 liters of Nature Safe 10-2-8 All Season Fertilizer [Irving, TX]) if seedlings were to be planted into an organic field. Seedlings

were maintained in a greenhouse and watered daily until 2 days before transplanting, when they were placed in an outdoor hoop house to harden off and acclimate to ambient environmental conditions.

In each sentinel plot, plants were arranged in a randomized complete block design with 10 plants for each plant species in each of two blocks. Each sentinel plot was a single 48.7-m-long row with 0.6 m between plants. In 2017 and 2018, seedlings were transplanted on four dates during the year at 2-month intervals—May, July, September, and November—in four and five fields, respectively (Fig. 1). Plants then were monitored during a 5-week sampling period for symptoms of disease development, including stunting, wilting, chlorosis, damping-off, or girdling and darkening of the lower stem at the soil line, by visiting each field at least once per week. In 2019, eight of the nine fields were planted and sampled in March to include an earlier time in the growing season (Fig. 2). Symptoms of wilting, lower stem rot, yellowing, and stunting were considered potential indicators of infection by *Pythium* spp., and these plants were collected for isolation of the pathogen.

Culturing and storage of isolates. Isolation from symptomatic plants occurred within 24 h after collection. Roots and lower stems were rinsed under cold running tap water, surface disinfested for 30 s in 0.3% sodium hypochlorite, rinsed in sterile deionized water, and blotted dry with sterile paper towels. Four symptomatic root or lower stem pieces (5 mm in length) from each plant were evenly spaced in 90-mm-diameter Petri plates containing water agar (15 g of Bacto agar in 1 liter of deionized water) amended with rifampicin (10 mg/liter), ampicillin (250 mg/liter), and benomyl (10 mg/liter) (WA+RAB). Isolation plates were held at 23°C in the dark for 7 days and observed daily for colonies with mycelia that resembled *Pythium* spp. Single hyphae from potential colonies of *Pythium* spp. were transferred to fresh WA+RAB to avoid potential contamination. Single hyphae from these cultures were transferred to 20% V8 juice agar (V8A: 200 ml of V8 juice, 15 g of Bacto agar, 3 g of CaCO₃, and 800 ml of deionized water) for short-term storage at 23°C to confirm purity. Isolates were then stored long-term in 2-ml plastic screw-cap tubes, and each tube contained three sterile hemp seeds (Nuts.com, Cranford, NJ), 1 ml of sterile deionized water, and two 5-mm-diameter V8A plugs from the edge of an actively growing colony. All isolates of *Pythium* spp. were stored in duplicate by placing tubes in cryo/freezer boxes, which were maintained at 23°C and away from direct light.

DNA extraction and PCR. For each isolate, one 5-mm-diameter plug from an actively growing V8A culture was placed in 50 ml of sterile 20% V8 broth (per liter: 200 ml of V8 juice, 3 g of CaCO₃, and 800 ml of deionized water) in a 250-ml Erlenmeyer flask. Cultures were incubated for 7 days at 23°C in the dark; then, 50 mg of mycelium was removed, and DNA was extracted using a Synergy 2.0 Plant DNA Extraction Kit (OPS Diagnostics, Lebanon, NJ), following the manufacturer's instructions.

For each isolate of *Pythium* spp., the COX1 region of the mitochondrial DNA was amplified using the primers Fm85mod and OomCoxI-Levup (Robideau et al. 2011). The PCR reaction volume of 25 µl contained 1× GoTaq Green Master Mix (Promega, Madison, WI), 0.8 µM of each forward and reverse primer, 6.5 µl of nuclease-free water, and 2 µl of template DNA. Amplifications were performed with a PTC-200 thermal cycler (Bio-Rad, Hercules, CA) as follows: 95°C for 2 min; 35 cycles at 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min; and final extension at 72°C for 10 min. PCR products were visualized on a 1.5% agarose gel to verify amplification.

Amplicons were sequenced by Sanger sequencing in both directions using Big Dye V3.1 chemistry and run on ABI 3730XL instruments (Functional Biosciences, Madison, WI). In a few instances, the alternative reverse primer OomCoxI-Levdo was used when the amplicon generated by OomCoxI-Levup did not sequence properly. Resulting DNA sequence.ab1 files were imported to and manipulated in Geneious R11 (Geneious, Auckland, NZ). Pairwise alignments of the forward and reverse sequences were made using Geneious Alignment. Consensus sequence ends were trimmed of any regions with more than a 5% chance of an error per base. Consensus sequences

were queried in the nonredundant GenBank nucleotide database at the National Center for Biotechnology Information and compared only to verified COX1 sequences from isolates of *Pythium* spp. (Robideau et al. 2011). An isolate was identified as a specific species of *Pythium* based on 97% or greater similarity to the sequence of a verified isolate of that species. *P. ultimum* and *P. irregulare* are each

part of a species complex, the members of which are indistinguishable using either ITS or COX1 barcoding regions, so isolates were identified only by the broader species name (sensu lato) (Robideau et al. 2011). Six isolates were 97% or more similar to verified isolates of both *P. coloratum* Vaartaja (HQ708550) and *P. dissotocum* Drechsler (HQ708575); therefore, these isolates were labeled

Fig. 1. Frequency of recovery of eight species of *Pythium* (*P.*) and one species of *Phytophthora* (*Phy.*) from nine cucurbit fields in South Carolina during four sampling periods in 2017 and 2018. Columns are headed by the numbers 1 to 9, indicating different fields, with o = organically managed field and c = conventionally managed field. Data shown are the percentage of isolates of each species out of the total number of isolates recovered for each field at that sampling date. Darker shades of red indicate a higher frequency of recovery of a particular *Pythium* species than lighter shades of red. Blue indicates no recovery of any isolates of that particular *Pythium* species. Black indicates that the field was not sampled in the given month.

P. coloratum/dissotocum. Representative sequences for isolates in this survey were deposited in GenBank under accession numbers MT002462 and MT222337 to MT222935.

Fungicide sensitivity testing. Representative isolates of *Pythium* spp. from the survey were assayed for sensitivity to three oomycete-specific fungicides in their commercial formulations: mefenoxam (Ridomil Gold 4 SL, Syngenta Crop Protection, Greensboro, NC), propamocarb (Previcur Flex 6 SL, Bayer Crop Science, Research Triangle Park, NC), and oxathiapiprolin (Orondis A [A20941A], Syngenta Crop Protection). For each species of *Pythium* identified in each sample period, a maximum of three isolates were selected for screening. If possible, no two isolates of any *Pythium* species from a given sample period were selected from the same field or cucurbit species.

The fungicides were incorporated into agar media at four or five concentrations to test isolates for sensitivity. Mefenoxam (0.01, 0.03, 0.1, 0.3, and 1.0 mg of active ingredient [a.i.]/liter) and oxathiapiprolin (0.01, 0.1, 1.0, 10.0, and 100.0 mg of a.i./liter) were added to corn meal agar (CMA; per liter: 17 g of corn meal agar and 1,000 ml of deionized water). Propamocarb (0.1, 1.0, 10.0, and 100.0 mg of a.i./liter) was added to 5% clarified V8A (per liter: 50 ml of V8 juice, 15 g of Bacto agar, and 3 g of CaCO₃) due to an observed irregular growth pattern expressed by some isolates grown on CMA amended with propamocarb. Nonamended CMA and 5% clarified V8A were prepared for controls.

The experiment was a completely randomized design, conducted twice, with two replicate plates per isolate at each concentration for each fungicide and nonamended controls (Keinath 2015). CMA plugs (5-mm diameter) from 3-day-old cultures were inverted in the center of 90-mm-diameter Petri dishes, each containing 25 ml of either fungicide-amended or non-fungicide-amended medium. Cultures were incubated in the dark at 23°C for 36 h. Two perpendicular radial measurements were taken with a digital caliper from each colony at the end of the 36-h incubation period. All colony radii on fungicide-amended media of each isolate were represented as relative growth compared with growth on the nonamended CMA or 5% V8A control plates.

Virulence assay. Sixteen isolates, representing seven species of *Pythium*, were used to inoculate seedlings of the susceptible watermelon cultivar 'Estrella' (Seedway, Hall, NY) in a growth chamber (Table 1). To avoid a potential effect of host specificity, all isolates selected for screening were recovered from symptomatic watermelon seedlings during the survey. Seven Estrella seeds were sown 2.5-cm deep and evenly spaced within 15.2-cm-diameter pots filled with Metro-Mix 830. Seedlings were germinated and grown at 30°C,

45% relative humidity, and a 12-h photoperiod in a growth chamber for 7 days before inoculation. On day 7, the growth chamber temperature was lowered to 25°C, and all seedlings except the five most uniform ones were removed.

For inoculation, isolates of *Pythium* spp. were grown at 23°C in the dark on 20% V8A. Plugs (6-mm diameter) from 3-day-old cultures were placed against the seedling stem just below the container mix surface. Plugs from noncolonized 20% V8A plates were placed against seedlings in control pots. The experimental design was a randomized complete block with five replicate pots for each isolate (one per block), and each pot contained five seedlings. The experiment was conducted twice. Disease incidence data were recorded daily for 7 days as the number of plants damped-off per pot. One diseased seedling was used for isolation of each isolate of *Pythium* spp., and the COX1 region was sequenced as described previously to confirm pathogen identity. Area under the disease progress curve (AUDPC) was calculated from disease incidence data from both experiments (Shaner and Finney 1977).

Statistical analyses. Data on recovery of the total numbers of isolates of *Pythium* species, *P. spinosum*, *P. irregulare*, *P. myriotylum* Drechsler, and *P. aphanidermatum* from surveyed plants were analyzed using SAS PROC CATMOD (SAS version 9.3; SAS, Cary, NC) for categorical data, which uses a χ^2 statistic to determine treatment effects. The proportion of plants from which isolates of *Pythium* spp. were or were not recovered (a binary response) was transformed by calculating the logit, and the linear model parameters then were estimated using the maximum likelihood option. To get models that included zero values for some categories to converge (i.e., arrive at a unique solution), observed proportions were replaced with a Bayes estimator calculated as follows:

$$x = (ct + k/r) \times n / (n + k)$$

where x is the transformed proportion, ct is the count of plants with (or without) *Pythium* spp., r is 2 (for a binary response), k is a constant (which was set equal to r), and n is the total number of plants per treatment combination (Santner and Duffy 1989). Stepwise model reduction was done to improve the sensitivity of the analysis. When treatment effects were significant, contrast statements were written to compare treatment levels. Three-way interactions of field type, month, and host were examined in 2019, and four-way interactions of year, field type, month, and host were examined for combined 2017 and 2018 datasets. Significant two-way interactions were split by including nested-by-value effects for one of the factors in model statements. Proportions were generated using the predict option to back-transform logit values.

1c	2o	3c	4o	5c	6o	7c	8c	9o	
0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	<i>P. aphanidermatum</i>
0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	<i>P. myriotylum</i>
11.1	5.6	4.8	0.0	25.0		0.0	30.0	0.0	<i>P. irregulare</i>
77.8	88.9	66.7	100.0	75.0		0.0	70.0	100.0	<i>P. spinosum</i>
11.1	5.6	28.6	0.0	0.0		100.0	0.0	0.0	<i>P. ultimum</i>
0.0	0.0	0.0	0.0	5.0		0.0	0.0	0.0	<i>Phy. helicoides</i>
0.0	0.0	0.0	0.0	5.0		0.0	0.0	0.0	<i>P. coloratum / dissotocum</i>
0.0	0.0	0.0	0.0	5.0		0.0	0.0	0.0	<i>P. sylvaticum</i>
0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	<i>P. acanthicum</i>

Fig. 2. Frequency of recovery of eight species of *Pythium* (*P.*) and one species of *Phytophythium* (*Phy.*) from nine cucurbit fields in South Carolina in March 2019. Column headings: the numbers 1 to 9 identify individual fields, and letters identify field management type with o = organic and c = conventional. Data shown are the percentage of isolates of each species out of the total number of isolates recovered for each field at that sampling date. Darker shades of red indicate a higher frequency of recovery of a particular *Pythium* species than lighter shades of red. Blue indicates no recovery of any isolates of that particular *Pythium* species. Black indicates that the field was not sampled in the given month.

Data from both fungicide experiments were combined. Effective concentration (EC₅₀) values were calculated for each isolate and fungicide combination by regressing relative colony radii against the base-10 logarithm of the concentrations of each fungicide using PROC GLM in SAS (Keinath 2015). Linear regression equations were solved to calculate EC₅₀ values for each fungicide/isolate combination. EC₅₀ values for isolates screened against different fungicide concentrations were analyzed using simple linear regression (JMP

Pro version 14; SAS Institute). EC₅₀ values were set as the response variable, and species was set as the model effect variable. Only species with two or more isolates were included in this analysis. Least square means were compared using Student's *t* tests.

AUDPC values from the virulence experiment were combined and transformed to square root values to correct for nonnormality and analyzed with a mixed-model maximum likelihood analysis (SAS PROC MIXED), with *Pythium* spp. as a fixed effect and experiment and block as random effects. Residuals from analysis of variance were checked for nonnormality and inequality of variance with SAS PROC UNIVARIATE. Back-transformed least-squares means are shown, and treatment means were compared with Student *t* tests. Preplanned, single-degree-of-freedom contrasts were used to compare species collected in relatively warm and cool months.

Table 1. Virulence of 16 isolates of six species of *Pythium* and one species of *Phytophthora* inoculated onto seedlings of watermelon cultivar Estrella in a growth chamber

Species	Isolate	AUDPC value ^x
<i>P. ultimum</i>	J17-3	16.1 a ^y
<i>P. ultimum</i>	M17-56	15.1 a
<i>P. irregulare</i>	N18-50	12.8 abc
<i>P. spinosum</i>	N18-2	12.6 abc
<i>P. spinosum</i>	N18-4	12.5 abc
<i>P. myriotylum</i>	M18-1	10.0 bcd
<i>P. aphanidermatum</i>	M18-5	8.9 bcde
<i>P. irregulare</i>	N18-10	8.3 cdef
<i>P. aphanidermatum</i>	N18-5	6.9 def
<i>P. spinosum</i>	N18-13	5.9 def
<i>P. myriotylum</i>	J18-32	5.0 defg
<i>P. myriotylum</i>	M18-a8	4.6 efg
<i>P. aphanidermatum</i>	J17-23	4.6 efg
<i>P. aphanidermatum</i>	M17-42	3.6 fg
<i>P. coloratum</i> / <i>P. dissotocum</i>	N18-29	0.0 g
<i>Phytophthora helicoides</i>	J17-40	0.0 g
Preplanned comparison		<i>P</i> value
Warm-season versus cool-season species ^z	...	0.0002

^x AUDPC = area under the disease progress curve calculated from disease incidence. Incidence was determined by counting the numbers of diseased seedlings each day for 7 days in a pot containing five inoculated seedlings. The experiment was done at 25°C, 12-h photoperiod, and 45% relative humidity.

^y Mean AUDPC values followed by the same letter are not significantly different based on Fisher's protected least significant difference (*P* < 0.05).

^z *P. aphanidermatum* and *P. myriotylum* are classified as warm-season species, and *P. irregulare* and *P. spinosum* are classified as cool-season species.

Results

Field sampling. A total of 600 isolates, including eight species of *Pythium* and one species of *Phytophthora* (*Phy.*), were recovered during all sampling periods. However, 93.8% of all isolates (*n* = 563) belonged to four species: *P. spinosum* (*n* = 274), *P. myriotylum* (*n* = 120), *P. irregulare* (*n* = 92), and *P. aphanidermatum* (*n* = 77); each of these species was recovered at least once from eight of the nine fields sampled (Figs. 1 and 2). The other five species—*P. ultimum* (*n* = 17), *Phy. helicoides* (Drechsler) Abad, de Cock, Bala, Robideau, Lodhi & Lévesque (*n* = 8), *P. coloratum*/*dissotocum* (*n* = 6), *P. sylvaticum* (*n* = 4), and *P. acanthicum* Drechsler (*n* = 2)—were much less common and were recovered from one to five fields. A total of 238 isolates were recovered from watermelon seedlings, 149 isolates from cucumber seedlings, 145 isolates from Hubbard squash seedlings, and 68 isolates from bottle gourd seedlings (Fig. 3). Together, *P. spinosum*, *P. myriotylum*, *P. irregulare*, and *P. aphanidermatum* accounted for 93.7, 93.3, 93.8, and 95.6% of all isolates recovered from watermelon, cucumber, Hubbard squash, and bottle gourd, respectively. *P. acanthicum* and *Phy. helicoides* were not recovered from bottle gourd or cucumber, *P. sylvaticum* was not recovered from watermelon, and *P. ultimum* was not recovered from bottle gourd. Sixteen new associations between different cucurbit hosts were found: nine for the world and seven for the United States (Table 2).

In 2017 and 2018, there was a significant interaction of year by month (*P* = 0.0012) and year by field type (*P* = 0.0491) on the total

Fig. 3. Numbers of isolates of eight species of *Pythium* (*P.*) and one species of *Phytophthora* (*Phy.*) recovered from four different cucurbit hosts growing in nine fields in South Carolina during 2017, 2018, and 2019.

recovery of isolates of *Pythium* spp. from symptomatic cucurbits (Table 3). Total recovery was higher in May 2017 (0.57) than May 2018 (0.35) ($P = 0.0182$) and in September 2017 (0.82) than September 2018 (0.34) ($P < 0.0001$). Although there was no difference in the total recovery rates between field types within years, there was a higher total recovery in 2017 conventional fields (0.75) compared with 2018 conventional fields (0.51) ($P < 0.0001$).

In 2017 and 2018, month significantly influenced total recovery and recovery of all four major species (Table 3). Greater *P. aphanidermatum* recovery was observed in May (0.36) ($P < 0.0001$), July, (0.45) ($P = 0.0023$), and September (0.23) ($P = 0.011$) than in November (0.08) (Table 4). Greater *P. myriotylum* recovery also was observed in May (0.51) ($P < 0.0001$), July (0.51) ($P = 0.0003$), and September (0.53) ($P < 0.0001$) than in November (0.08). Greater *P. irregulare* recovery was observed in November (0.31) compared with July (0.09) ($P = 0.0011$). Greater *P. spinosum* recovery was observed in November (0.65) compared with May (0.21) ($P < 0.0001$), July (0.09) ($P < 0.0001$), and September (0.27) ($P = 0.0045$).

Table 2. New associations between cucurbit hosts and *Pythium* species recovered from diseased cucurbits in South Carolina^z

Host	<i>Pythium</i> species	New association
Bottle gourd (<i>Lagenaria siceraria</i>)	<i>P. aphanidermatum</i>	United States
	<i>P. dissotocum</i>	World
	<i>P. irregulare</i>	World
	<i>P. myriotylum</i>	World
	<i>P. spinosum</i>	World
Hubbard squash (<i>Cucurbita maxima</i>)	<i>P. sylvaticum</i>	World
	<i>P. acanthicum</i>	World
	<i>P. irregulare</i>	United States
	<i>P. myriotylum</i>	World
	<i>P. spinosum</i>	United States
	<i>P. splendens</i>	World
	<i>P. sylvaticum</i>	World
	<i>P. ultimum</i>	World
Cucumber (<i>Cucumis sativus</i>)	<i>P. irregulare</i>	United States
	<i>P. spinosum</i>	United States
	<i>P. sylvaticum</i>	United States

^z Based on Farr and Rossman (2020).

Recovery of both *P. myriotylum* ($P = 0.0298$) and *P. aphanidermatum* ($P = 0.0493$) had a significant three-way interaction of year by month by field type (Table 3). However, the effect of month was substantially greater for both species (both $P < 0.0001$), and the effects of year and field type by themselves were not significant ($P \geq 0.1415$ for field type and $P \geq 0.8001$ for year).

In 2017 and 2018, cucurbit host significantly influenced recovery of *P. aphanidermatum* ($P = 0.04$), with greater recovery from cucumber (0.28) than watermelon (0.16) (Table 3). Recovery of both *P. irregulare* ($P = 0.0144$) and *P. spinosum* ($P = 0.0205$) had a significant month by field type interaction. Across all fields sampled in November for both years, there was greater recovery of *P. irregulare* (0.46) in conventionally managed than in organically managed fields (0.16) ($P < 0.0001$) and greater recovery of *P. spinosum* in organically managed fields (0.80) than in conventionally managed fields (0.51) ($P < 0.0001$), but the two field types did not differ in other months (Fig. 1).

In March 2019, there was a significant effect of field type ($P = 0.0379$) and host ($P = 0.0427$) on total recovery of all isolates of *Pythium* spp. from symptomatic cucurbits (Table 5). Recovery was greater from organically managed (0.83) than conventionally managed fields (0.66). Total recovery rates from watermelon (0.81) were higher than those from cucumber (0.65) ($P = 0.0494$) and Hubbard squash (0.33) ($P = 0.0183$). No isolates were recovered from bottle gourd in March 2019 from any fields.

Virulence of *Pythium* species. Virulence of six species of *Pythium* and *Phy. helicoides* was based on disease progress over a 7-day period and AUDPC values for two trials combined. AUDPC values for the 16 isolates varied among species and ranged from 0 to 16.1 (Table 1). *P. coloratum/dissotocum* isolate N18-29 and *Phy. helicoides* isolate J17-40 did not cause disease in either trial. The two isolates of *P. ultimum* were more virulent than 11 other isolates, including all isolates of *P. myriotylum* and *P. aphanidermatum*, but not *P. irregulare* isolate N18-50 and *P. spinosum* isolates N18-2 and N18-4. The preplanned comparison of the warm-season species of *P. aphanidermatum* and *P. myriotylum* (mean AUDPC for seven isolates = 6.2) to the cool-season species of *P. spinosum* and *P. irregulare* (mean AUDPC for five isolates = 10.4) demonstrated that cool-season species were more virulent at 25°C than warm-season species ($P = 0.0002$; Table 1).

Significant variability in virulence, based on AUDPC values, was observed among isolates within some species of *Pythium* (Table 1). Of the three isolates of *P. spinosum*, isolate N18-13 was less virulent than isolates N18-2 and N18-4, which were equally virulent. For

Table 3. Categorical analysis of variance parameters for proportions of diseased cucurbits from which *Pythium* species were recovered in nine fields in South Carolina on four sampling dates in 2017 and 2018

Source	<i>P. aphanidermatum</i>		<i>P. irregulare</i>		<i>P. myriotylum</i>		<i>P. spinosum</i>		Total recovery	
	χ^2	<i>P</i> value	χ^2	<i>P</i> value	χ^2	<i>P</i> value	χ^2	<i>P</i> value	χ^2	<i>P</i> value
Intercept	26.12	<0.0001	64.73	<0.0001	6.05	0.01	33.02	<0.0001	0.57	0.45
Year ^v	0.23	0.63	0.54	0.46	2.16	0.14	0	0.99	16.56	<0.0001
Month ^w	35.20	<0.0001	11.69	0.01	67.20	<0.0001	93.53	<0.0001	36.74	<0.0001
Year × month	6.96	0.07	1.35	0.72	3.76	0.29	...	...	15.88	0.0012
Field type ^x	0.06	0.80	1.68	0.20	0.05	0.82	2.03	0.15	0.03	0.87
Year × field type	2.94	0.09	...	...	3.49	0.06	...	...	3.87	0.049
Month × field type	0.39	0.94	10.55	0.01	0.08	0.99	9.79	0.02	4.60	0.20
Year × month × field type	7.85	0.049	...	...	8.96	0.03	...	...	4.60	0.20
Host ^y	8.21	0.04	1.24	0.74	3.56	0.31	1.71	0.64	4.26	0.24
Month × host	... ^z	...	...	...	...	...	...	...	9.27	0.41
Field type × host	...	...	3.61	0.31	...	...	...	...	2.46	0.48
Month × field type × host	...	...	...	...	...	...	...	...	10.08	0.26
Likelihood ratio	19.27	0.99	12.01	1.00	20.27	0.98	25.68	0.98	7.11	1.00

^v 2017 or 2018.

^w May, July, September, or November.

^x Organic or conventionally managed field.

^y Bottle gourd, cucumber, Hubbard squash, or watermelon.

^z Indicates source of variation eliminated in stepwise model reduction to improve sensitivity of analysis.

three isolates of *P. myriotylum*, isolate M18-a8 was less virulent than isolate M18-1, but isolates M18a8 and J18-32 were similar in virulence. Four isolates of *P. aphanidermatum* were evaluated, and isolate M17-42 was less virulent than isolate M18-5, but each of these isolates was similar in virulence to isolates N18-5 and J17-23. There were no significant differences in virulence observed between the two isolates of *P. ultimum* nor between the two isolates of *P. irregulare*.

Fungicide sensitivity. Sixty-four representative isolates were evaluated for sensitivity to mefenoxam, 63 isolates were evaluated for sensitivity to propamocarb, and 68 isolates were evaluated for sensitivity to oxathiapiprolin—including four isolates in *Pythium* spp. clade I that were obtained from other researchers.

Mycelium growth of 64 isolates of *Pythium* spp. and *Phy. helicoides* was affected significantly by the fungicide mefenoxam based on EC₅₀ values ($P < 0.0011$). EC₅₀ values for all isolates ranged from <0.006 to 0.519 mg of a.i./liter, with significant variation observed among species (Table 6). Some isolates of *Phy. helicoides*, *Pythium coloratum/dissotocum*, *P. irregulare*, and *P. aphanidermatum* were

relatively less sensitive, with EC₅₀ values of >0.4 mg of a.i./liter. *Phy. helicoides* and *P. coloratum/dissotocum* were significantly less sensitive than *P. myriotylum*, *P. acanthicum*, *P. spinosum*, and *P. ultimum*. EC₅₀ values for five isolates of *P. ultimum* and *P. splendens* were <0.1 mg of a.i./liter. Among the 10 species, EC₅₀ values varied the most among isolates of *Phy. helicoides* (SE = 0.21), because the two isolates had EC₅₀ values of 0.09 and 0.52 mg of a.i./liter.

Mycelium growth of 63 isolates of *Pythium* spp. and *Phy. helicoides* was affected significantly by the fungicide propamocarb based on EC₅₀ values ($P < 0.0001$). There was considerable variation observed among species because mean EC₅₀ values ranged from 0.03 to 6.7 mg of a.i./liter (Table 7). Some isolates of *P. ultimum*, *P. splendens*, and *P. irregulare* were relatively less sensitive, with EC₅₀ values of ≥ 6.0 mg of a.i./liter. *P. ultimum* and *P. irregulare* were significantly less sensitive than all other species. *P. splendens* was not included in the analysis because only one isolate was assayed. *P. sylvaticum*, *P. acanthicum*, *P. myriotylum*, *P. coloratum/dissotocum*, and *Phy. helicoides* were among the most sensitive species, because all isolates had EC₅₀ values of ≤ 2.0 mg of a.i./liter, and all five

Table 4. Proportion of recovery of four *Pythium* spp. recovered from diseased cucurbits planted in nine fields in South Carolina on four sampling dates in 2017 and 2018

Month	Proportion of recovery								Total recovery	
	<i>P. aphanidermatum</i>	SE ^y	<i>P. myriotylum</i>	SE	<i>P. irregulare</i>	SE	<i>P. spinosum</i>	SE		
May	0.36 ^z	0.06	0.51	0.06	0.19	0.05	0.21	0.05	0.47	0.05
July	0.45	0.05	0.51	0.04	0.09	0.03	0.09	0.03	0.72	0.04
September	0.23	0.06	0.53	0.07	0.19	0.05	0.27	0.06	0.57	0.05
November	0.08	0.02	0.08	0.08	0.31	0.03	0.65	0.03	0.48	0.05

^y Standard error.

^z Proportions presented are back-transformed logit values.

Table 5. Categorical analysis of variance parameters for proportions of diseased cucurbits from which *Pythium* species were recovered in nine fields in South Carolina in March 2019

Source	Total recovery		<i>P. aphanidermatum</i>		<i>P. irregulare</i>		<i>P. myriotylum</i>		<i>P. spinosum</i>	
	χ^2	<i>P</i> value	χ^2	<i>P</i> value	χ^2	<i>P</i> value	χ^2	<i>P</i> value	χ^2	<i>P</i> value
Intercept	0.31	0.5762	7.30	0.0069	6.89	0.0087	7.30	0.0069	1.97	0.1601
Field type ^y	4.11	0.0427	0.20	0.65	0.30	0.58	0.20	0.65	0.48	0.49
Host ^z	8.43	0.0379	1.83	0.40	1.57	0.46	1.83	0.40	2.81	0.24
Likelihood ratio	0.85	0.65	0.05	0.97	0.04	0.98	0.05	0.97	0.62	0.73

^y Organic or conventionally managed field.

^z Bottle gourd, cucumber, Hubbard squash, or watermelon.

Table 6. Effective concentration of mefenoxam that reduced relative colony diameter by 50% (EC₅₀ values) for isolates of species of *Pythium* and one species of *Phytophythium* recovered from diseased cucurbits in South Carolina in 2017, 2018, and 2019

Species	No. of isolates	Mean EC ₅₀ value (mg of a.i./liter) ^w	SE ^x	Distribution of isolates based on EC ₅₀ values (mg of a.i./liter)					
				<0.1	0.1–0.2	0.21–0.3	0.31–0.4	0.41–0.5	0.51–0.6
<i>P. splendens</i> ^y	1	0.006	...	1	...	...	...	...	...
<i>P. ultimum</i>	4	0.012 d ^z	0.01	4	...	...	...	...	...
<i>P. spinosum</i>	11	0.092 c	0.01	2	8	1	...	...	...
<i>P. acanthicum</i>	2	0.108 bcd	0.02	1	1	...	...	...	...
<i>P. aphanidermatum</i>	14	0.112 ab	0.04	2	7	1	1	2	1
<i>P. myriotylum</i>	17	0.126 c	0.01	3	14	...	...	...	...
<i>P. sylvaticum</i> ^y	1	0.155	...	...	1	...	...	...	...
<i>P. irregulare</i>	8	0.205 ab	0.05	...	4	2	1	...	1
<i>P. coloratum/dissotocum</i>	4	0.282 a	0.06	...	1	1	1	1	...
<i>Phytophythium helicoides</i>	2	0.519 a	0.21	1	...	...	...	...	1

^w Data from two trials were combined. EC₅₀ values were calculated for each isolate by regressing relative colony radii in corn meal agar against the base-10 logarithm of the concentrations of mefenoxam. a.i. = active ingredient.

^x Standard error.

^y Not included in statistical analysis because only one isolate was assayed.

^z Mean EC₅₀ values followed by the same letter are not significantly different according to Fisher's protected least significant difference ($P < 0.05$).

species had mean EC₅₀ values of <1.0 mg of a.i./liter. *P. irregulare* isolates were the most variable (SE = 1.51) with EC₅₀ values ranging from 0.35 to 13.24 mg of a.i./liter.

Fifty-nine isolates in six taxonomic clades of the genus *Pythium* were not sensitive to the fungicide oxathiapiprolin, with relative colony diameters similar to those of the controls even at 100 mg of a.i./liter, but nine isolates in *Pythium* clade I were sensitive to this fungicide with EC₅₀ values of ≤1.05 mg of a.i./liter (Table 8). *Pythium* clade I includes the species *P. ultimum* var. *ultimum*, *P. ultimum* var. *sporangiferum*, *P. heterothallicum*, and *P. splendens*. Among the species sensitive to oxathiapiprolin, isolates of *P. ultimum* var. *ultimum* were most sensitive, and isolates of *P. heterothallicum* were least sensitive (Table 8).

Discussion

Eight species of *Pythium* and *Phy. helicoides* were recovered from four cucurbits during a 3-year survey. The most commonly isolated species of *Pythium* were *P. spinosum*, *P. irregulare*, *P. myriotylum*, and *P. aphanidermatum*. Because of the low recovery rates of the other five species, these four species should be considered the most important species on cucurbits in coastal South Carolina and could be important pathogens on cucurbits in the southeastern coastal plain. These four *Pythium* species were recovered from all four cucurbit hosts, demonstrating their ability to cause disease on four genera cultivated in the family Cucurbitaceae. More isolates were recovered from watermelon than from Hubbard squash, cucumber, and bottle gourd. Bottle gourd and interspecific hybrid squash (*Cucurbita maxima* × *C. moschata*) are used as cucurbit rootstocks to manage several other soilborne pathogens (Keinath and Hassell 2014; King et al. 2008). They have been shown to be effective rootstocks for controlling *Pythium* disease, possibly explaining the reduced disease incidence and isolate recovery on bottle gourd and Hubbard squash in this study (Al-Mawaali et al. 2012; Toporek and Keinath 2019).

P. myriotylum, *P. aphanidermatum*, and *P. irregulare* were the most abundant species collected in a survey of greenhouse floriculture crops in North Carolina (Lookabaugh et al. 2015). Likewise, *P. irregulare* and *P. aphanidermatum* were two of the main species found in a greenhouse floriculture survey in Michigan (Del Castillo Múnera and Hausbeck 2016). These four species also were recovered from bell pepper in Florida (Chellemi et al. 2000). *P. aphanidermatum* has a wide host range (Martin and Loper 1999). The recovery of these species may be explained by their ubiquitous distribution rather than specific attributes of the cucurbit hosts.

P. spinosum and *P. irregulare* were recovered primarily in November and March, months with cool weather, whereas recovery of *P. myriotylum* and *P. aphanidermatum* occurred primarily in warm months, May and July. *P. spinosum* and *P. irregulare* were never recovered in July, and, conversely, *P. myriotylum* and

P. aphanidermatum were never recovered in November or March. The fluctuation between *P. spinosum* and *P. aphanidermatum* recovery is similar to results recently described in Israel (Philosoph et al. 2019). Both species were isolated from cucumber in transitional months, but *P. aphanidermatum* and *P. spinosum* were found strictly in the warmest and coolest months, respectively. Seasonal effects also have been observed with recovery of propagules of *Pythium* spp. Ali-Shtayeh (1986) found higher numbers of colony forming units of *P. aphanidermatum* in cultivated fields in months with warmer weather. In a study on *Pythium* blight, examining sieved thatch from golf courses in Ohio, *P. aphanidermatum* propagule counts were highest in the winter and then declined in the spring (Hall et al. 1980). This was attributed to the reduction in survival of propagules as *P. aphanidermatum* became biologically active. A similar fluctuation in propagule numbers was observed in sugar beet soils in Arizona (Stanghellini et al. 1982). As a component of seasonality, temperature alone was shown to significantly influence species recovery. In New York, *P. ultimum* and *P. irregulare* were recovered frequently when the soil temperature was 21°C, but *P. oligandrum* was predominantly recovered from samples incubated at 37°C (Piecarka and Abawi 1978). Growth rate of the mycelium of *P. aphanidermatum*, *P. ultimum*, and *P. deliense* in culture at different temperatures also reflects the differences between species' environmental preferences (Teymoori et al. 2012).

The dramatic increase in numbers of isolates of *Pythium* spp. collected in November and March was a function of the criteria established to designate which plants to collect. Aboveground symptoms of stunting or wilting caused by cold temperatures are indistinguishable from those caused by *Pythium* spp., so cold injury may have led to more plants being collected. Optimal growing temperatures for cucurbits should not be lower than 18.3°C for cucumber and squash and 23.8°C for watermelon (Maynard and Hochmuth 2007). However, stunted and wilted plants collected in March and November displayed symptoms of discolored roots. Percentage recovery of *Pythium* from diseased plants was 87.4, 79, and 78% for November 2017, November 2018, and March 2019, respectively. Additionally, in the virulence assay, *P. spinosum* and *P. irregulare* isolates were among the most aggressive at 25°C. Both *P. irregulare* and *P. spinosum*, the species recovered most abundantly in cold months, have been reported previously as pathogens of different cucurbit species (Farr and Rossman 2020). *P. spinosum* also has been reported to cause dieback of mature watermelon in Florida (Hendricks and Roberts 2015).

The temperature for the virulence experiment, 25°C, represents an average temperature in spring for transplanting watermelon seedlings, based on South Carolina weather data (Keinath et al. 2019). Virulence, like recovery, can be temperature dependent within species and even within isolates of a species (Matthiesen et al. 2016).

Table 7. Effective concentration of propamocarb that reduced relative colony diameter by 50% (EC₅₀ values) for 63 isolates of nine species of *Pythium* and one species of *Phytophthora* recovered from diseased cucurbits in South Carolina in 2017, 2018, and 2019

Species	No. of isolates	Mean EC ₅₀ (mg of a.i./liter) ^w	SE ^x	Distribution of isolates based on EC ₅₀ values (mg of a.i./liter)					
				<0.1	0.1–2.0	2.1–4.0	4.1–6.0	6.1–8.0	>8.0
<i>P. sylvaticum</i> ^y	1	0.03	...	1	...	...	...	...	...
<i>P. acanthicum</i>	2	0.49 bc ^z	0.03	...	2	...	...	...	...
<i>P. myriotylum</i>	17	0.61 c	0.11	3	14	...	...	...	...
<i>P. coloratum/dissotocum</i>	4	0.74 bc	0.41	1	3	...	...	...	...
<i>Phytophthora helicoides</i> ^y	1	0.75	...	...	1	...	...	...	...
<i>P. spinosum</i>	11	2.06 b	0.41	...	5	5	1	...	...
<i>P. aphanidermatum</i>	14	2.13 b	0.18	...	9	5	...	...	...
<i>P. irregulare</i>	8	4.63 a	1.51	...	3	...	3	1	1
<i>P. splendens</i> ^y	1	6.05	...	...	...	...	...	1	...
<i>P. ultimum</i>	4	6.70 a	0.91	...	...	...	1	2	1

^w Data from two trials were combined. EC₅₀ values were calculated for each isolate by regressing relative colony radii in 20% clarified V8 agar against the base-10 logarithm of the concentrations of propamocarb. a.i. = active ingredient.

^x Standard error.

^y Not included in statistical analysis because only one isolate was assayed.

^z Means followed by the same letter are not significantly different according to Fisher's protected least significant difference ($P \leq 0.05$).

Although *P. aphanidermatum* and *P. myriotylum* were the least virulent species, this result may be due to the assay temperature. *P. myriotylum* was the most virulent species on tomato at 35°C, whereas *P. aphanidermatum* was most virulent between 27 and 35°C (Littrell and McCarter 1970). Bell pepper mortality was highest at 34°C when inoculated with *P. aphanidermatum* and *P. myriotylum* (Chellemi et al. 2000).

Variability in virulence among isolates of *Pythium* spp. has been observed previously (Del Castillo Múnera and Hausbeck 2016; Rojas et al. 2017a). The lack of disease caused by isolates of *Phy. helicoides* and *P. coloratum/dissotocum* may be due to loss of virulence during storage or weak virulence. Both of these species were infrequently isolated in the field throughout the survey. However, it is interesting that, although *P. ultimum* was the most virulent species, it was not frequently recovered. The abundance and virulence of *P. spinosum* and *P. irregulare* demonstrate the significant threat that these species pose to early season cucurbit transplants.

How early in the growing season a seedling is transplanted influences its exposure to particular species of *Pythium*. In South Carolina, typical transplanting dates range from mid-March to mid-June (Kemble et al. 2020). Thus, growers planting early will most likely encounter *P. spinosum* and *P. irregulare* with a transition into disease caused by *P. myriotylum* and *P. aphanidermatum* later in the season. Young plants growing at cool temperatures tend to mature slowly, keeping them longer in a state more susceptible to infection by *Pythium* spp. (Martin and Loper 1999). With early planting, a cucurbit crop potentially would be exposed to both cool-season and warm-season species of *Pythium* throughout its lifecycle compared with one planted later when soil is warmer. Consequently, early planting may contribute to higher overall disease.

Although seasonality appears to be a large factor influencing *Pythium* species isolation, species diversity has been shown to be a function of several other environmental and edaphic factors, such as latitude, rainfall, soil clay content, pH, field capacity, cation exchange capacity, calcium carbonate equivalent, and various minerals (e.g., calcium, magnesium, and zinc) (Broders et al. 2009; Rojas et al. 2017b; Zitnick-Anderson et al. 2017). The dominant species recovered can differ between sampling locations within a field (Bakker et al. 2017). The diversity of *Pythium* spp. recovered could also be associated with ability to grow on the medium used for isolation;

indeed *P. aphanidermatum*, *P. ultimum*, and *P. irregulare*, three of the five major species recovered in this survey, are species with fast growth rates on CMA (Jeffers and Martin 1986).

Total recovery rate did not differ significantly between conventionally or organically managed fields in 2017 and 2018 in any month; however, in 2017 and 2018, recovery of *P. irregulare* was greater in conventional fields than in organic fields, but, conversely, recovery of *P. spinosum* was greater in organic fields than in conventional fields. In March 2019, *Pythium* spp. were recovered more frequently in organic fields than in conventional fields. Because the predominant taxon recovered in March 2019 was *P. spinosum*, its frequency could have resulted in greater total recovery rates in organic fields. This study did not characterize or determine the individual management strategies used in all conventional or organic fields sampled, so it is not possible to identify reasons for the abundance of *P. irregulare* or *P. spinosum* in fields with different cultural management practices.

All isolates of *Pythium* spp. and *Phy. helicoides* tested were sensitive to mefenoxam and propamocarb in vitro, demonstrating the potential utility of these two fungicides for use in cucurbit production in South Carolina. Sensitivity to mefenoxam and the degree of variation among isolates were similar to other studies reporting EC₅₀ values for isolates in forest nurseries in Washington and alfalfa fields in Minnesota (Berg et al. 2017; Weiland et al. 2014). None of our observed EC₅₀ values reached those of resistant isolates in other studies for mefenoxam or propamocarb (Del Castillo Múnera and Hausbeck 2016; Lookabaugh et al. 2015; Moorman and Kim 2004; Weiland et al. 2014). It should be noted that the use of CMA and 5% V8A as a basal medium for fungicide sensitivity has an effect on the perceived EC₅₀ value (Shew 1984). In addition, in vitro sensitivity of an isolate to propamocarb is not always a reliable predictor for sensitivity in planta (Moorman and Kim 2004).

Oxathiapiprolin is not labeled to manage *Pythium* spp.; however, an isolate of *P. ultimum* var. *ultimum* in *Pythium* clade I was reported to be moderately sensitive to oxathiapiprolin, albeit at higher concentrations compared with other oomycetes tested (Miao et al. 2016). *Pythium* clade I species lack commonality in morphological characteristics or obvious origin (Lévesque and De Cock 2004). To validate that clade I isolates were sensitive, three isolates of *P. heterothallicum* and one isolate of *P. ultimum* var. *sporangiferum*

Table 8. Effective concentration of oxathiapiprolin that reduced relative colony diameter by 50% (EC₅₀ values) for isolates of species of *Pythium* and one species of *Phytophthora*

Clade	Species (no. of isolates)	Sensitivity	Isolate	EC ₅₀ value (mg of a.i./liter) ^w
I	<i>P. aff. ultimum</i> var. <i>ultimum</i> ^x	Sensitive	M17-56	0.15
	<i>P. aff. ultimum</i> var. <i>ultimum</i>	Sensitive	M17-47	0.16
	<i>P. aff. ultimum</i> var. <i>ultimum</i>	Sensitive	M18-a17	0.18
	<i>P. aff. ultimum</i> var. <i>ultimum</i>	Sensitive	J17-3	0.29
	<i>P. splendens</i>	Sensitive	M18-a10	0.56
	<i>P. aff. ultimum</i> var. <i>sporangiferum</i>	Sensitive	C12-99 ^y	0.56
	<i>P. heterothallicum</i>	Sensitive	C12-171 ^y	0.45
	<i>P. heterothallicum</i>	Sensitive	2_9 ^z	0.62
	<i>P. heterothallicum</i>	Sensitive	2_11 ^z	1.05
A	<i>P. aphanidermatum</i> (14)	Unaffected	Various	>100
B1	<i>P. myriotylum</i> (17)	Unaffected	Various	>100
B2	<i>P. coloratum/dissotocum</i> (4)	Unaffected	Various	>100
D	<i>P. acanthicum</i> (2)	Unaffected	Various	>100
F	<i>P. spinosum</i> (11)	Unaffected	Various	>100
	<i>P. irregulare</i> (8)	Unaffected	Various	>100
	<i>P. sylvaticum</i> (1)	Unaffected	Various	>100
K	<i>Phytophthora helicoides</i> (2)	Unaffected	Various	...

^w Data from two trials were combined. EC₅₀ values were calculated for each isolate by regressing relative colony radii in corn meal agar against the base-10 logarithm of the concentrations of oxathiapiprolin. a.i. = active ingredient.

^x Species that cannot be 100% determined using COX1 sequencing are labeled as aff., which indicates that this is the species voucher that was identified, but that does not necessarily indicate a match.

^y Isolates provided by Dr. Jamie Blair, Franklin & Marshall College. Recovered from soybean. Identified in Coffua et al. (2016) with COX1 sequencing.

^z Isolates provided by Dr. Barbara Shew, North Carolina State University. Recovered from wheat. Identified with ITS sequencing (unpublished). Both isolates have 99.8% similarity to a verified isolate of *P. heterothallicum* (HQ643562).

were obtained from other researchers. These isolates and the five isolates from the survey were sensitive to oxathiapiprolin at concentrations ≤ 1 mg of a.i./liter. The moderately sensitive species of *Pythium* were far more tolerant to oxathiapiprolin than other oomycete pathogens (Bittner and Mila 2016). The target of oxathiapiprolin is said to be the oxysterol binding protein (OSBP) (Pasteris et al. 2015). Isolates from 14 species of *Pythium* and *Phytophthora* formed separate phylogenetic clusters based on the OSBP sequences; however, the OSBPs of *P. ultimum* and *P. aphanidermatum* were similar (Miao et al. 2016). This may suggest some other mechanism of sensitivity to oxathiapiprolin in *Pythium* clade I isolates. All other isolates from eight *Pythium* species, representing five clades, and *Phy. helicoides* collected in this survey were insensitive, whereas an isolate from every described species from clade I was sensitive.

This study identified a seasonal change in recovery of *Pythium* species and *Phy. helicoides* from diseased cucurbit seedlings, variability in virulence of *Pythium* species and isolates and *Phy. helicoides* on a susceptible watermelon cultivar, and sensitivity of isolates of *Pythium* spp. and *Phy. helicoides* recovered from cucurbits to mefenoxam and propamocarb in vitro. Previously, 24 species of *Pythium* had been reported on various cultivated cucurbits, with more than half of the reports coming from *Cucumis sativus*. The geographical area covered in this survey, however, could limit broad conclusions on the overall diversity of *Pythium* species associated with cucurbits. This is the first published study of *Pythium* and *Phytophythium* species on cucurbits in South Carolina or the United States, and it is one of a few studies to consider the effect of sampling date and multiple hosts on recovery of different species. Knowing the identity and infection window of these *Pythium* species will help facilitate development of cultural management strategies and breeding efforts to manage *Pythium* species on cucurbits.

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Literature Cited

- Al-Mawaali, Q. S., Al-Sadi, A. M., Khan, A. J., Al-Hasani, H. D., and Deadman, M. L. 2012. Response of cucurbit rootstocks to *Pythium aphanidermatum*. *Crop Prot.* 42:64-68.
- Al-Sadi, A. M., Al-Ghathithi, A. G., Al-Balsuhi, Z. M., and Al-Jabri, A. H. 2012. Analysis of diversity in *Pythium aphanidermatum* populations from a single greenhouse reveals phenotypic and genotypic changes over 2006 to 2011. *Plant Dis.* 96:852-858.
- Al-Sadi, A. M., Drenth, A., Deadman, M. L., De Cock, A. W. A. M., and Aitken, E. A. B. 2007. Molecular characterization and pathogenicity of *Pythium* species associated with damping-off in greenhouse cucumber (*Cucumis sativus*) in Oman. *Plant Pathol.* 56:140-149.
- Ali-Shtayeh, M. S. 1986. Seasonal variation in population levels of *Pythium* species in irrigated and non-irrigated fields in the West Bank of Jordan and the Gaza Strip. *Trans. Brit. Mycol. Soc.* 87:503-509.
- Bakker, M. G., Moorman, T. B., Kaspar, T. C., and Manter, D. K. 2017. Isolation of cultivation-resistant oomycetes, first detected as amplicon sequences, from roots of herbicide-terminated winter rye. *Phytobiomes* 1:24-35.
- Berg, L. E., Miller, S. S., Dornbusch, M. R., and Samac, D. A. 2017. Seed rot and damping-off of alfalfa in Minnesota caused by *Pythium* and *Fusarium* species. *Plant Dis.* 101:1860-1867.
- Bittner, R. J., and Mila, A. L. 2016. Effects of oxathiapiprolin on *Phytophthora nicotianae*, the causal agent of black shank of tobacco. *Crop Prot.* 81:57-64.
- Broders, K. D., Wallhead, M. W., Austin, G. D., Lipps, P. E., Paul, P. A., and Dorrance, A. E. 2009. Association of soil chemical and physical properties with *Pythium* species diversity, community composition, and disease incidence. *Phytopathology* 99:957-967.
- Chellemi, D. O., Mitchell, D. J., Kannwischer-Mitchell, M. E., Rayside, P. A., and Rosskopf, E. N. 2000. *Pythium* spp. associated with bell pepper production in Florida. *Plant Dis.* 84:1271-1274.
- Coffua, L. S., Veterano, S. T., Clipman, S. J., Mena-Ali, J. I., and Blair, J. E. 2016. Characterization of *Pythium* spp. associated with asymptomatic soybean in southeastern Pennsylvania. *Plant Dis.* 100:1870-1879.
- Cohen, Y. 2015. The novel oomycete oxathiapiprolin inhibits all stages in the asexual life cycle of *Pseudoperonospora cubensis*—causal agent of cucurbit downy mildew. *PLoS One* 10:e0140015.
- Davis, A. R., Perkins-Veazie, P., Sakata, Y., López-Galarza, S., Maroto, J. V., Lee, S. G., Huh, Y. C., Sun, Z., Miguel, A., King, S. R., Cohen, R., and Lee, J. M. 2008. Cucurbit grafting. *Crit. Rev. Plant Sci.* 27:50-74.
- de Cock, A. W. A. M., Lodhi, A. M., Rintoul, T. L., Bala, K., Robideau, G. P., Abad, G. Z., Coffey, M. D., Shahzad, S., and Lévesque, C. A. 2015. *Phytophythium*: Molecular phylogeny and systematics. *Persoonia* 34:25-39.
- Deadman, M. L. 2017. *Pythium* and *Phytophthora* damping-off and root rot. Pages 48-50 in: *Compendium of Cucurbit Diseases and Pests*, 2nd Ed., A. P. Keinath, T. A. Zitter, and W. M. Wintermantel, eds. American Phytopathological Society, St. Paul.
- Del Castillo Múnera, J., and Hausbeck, M. K. 2016. Characterization of *Pythium* species associated with greenhouse floriculture crops in Michigan. *Plant Dis.* 100:569-576.
- Dissanayake, N., and Hoy, J. W. 1999. Organic material soil amendment effects on root rot and sugarcane growth and characterization of the materials. *Plant Dis.* 83:1039-1046.
- Farr, D. F., and Rossman, A. Y. 2020. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Retrieved April 3, 2020. <https://nt.ars-grin.gov/fungaldbases/>
- Hall, T. J., Larsen, P. O., and Schmitthenner, A. F. 1980. Survival of *Pythium aphanidermatum* in golf course turfs. *Plant Dis.* 64:1100-1103.
- Hartmann, M., Frey, B., Mayer, J., Mäder, P., and Widmer, F. 2015. Distinct soil microbial diversity under long-term organic and conventional farming. *ISME J.* 9:1177-1194.
- Hendricks, K. E., and Roberts, P. D. 2015. First report of *Pythium spinosum* as a pathogen of watermelon and in association with a dieback of watermelon in southwest Florida. *Plant Health Prog.* 16:77-79.
- Jeffers, S. N., and Martin, S. B. 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. *Plant Dis.* 70:1038-1043.
- Keinath, A. P. 2015. Baseline sensitivity of *Didymella bryoniae* to cyprodinil and fludioxonil and field efficacy of these fungicides against isolates resistant to pyraclostrobin and boscalid. *Plant Dis.* 99:815-822.
- Keinath, A. P., Coolong, T. W., Lanier, J. D., and Ji, P. 2019. Managing Fusarium wilt of watermelon with delayed transplanting and cultivar resistance. *Plant Dis.* 103:44-50.
- Keinath, A. P., and Hassell, R. L. 2014. Suppression of Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* race 2 on grafted triploid watermelon. *Plant Dis.* 98:1326-1332.
- Kemble, J. M., Meadows, I. M., Jennings, K. M., and Walgenbach, J. F., eds. 2020. Vegetable crop handbook for the southeastern United States. <http://www.vegcrophandbook.com/>
- King, S. R., Davis, A. R., Liu, W., and Levi, A. 2008. Grafting for disease resistance. *HortScience* 43:1673-1676.
- Lévesque, C. A., and De Cock, A. W. A. M. 2004. Molecular phylogeny and taxonomy of the genus *Pythium*. *Mycol. Res.* 108:1363-1383.
- Littrell, R. H., and McCarter, S. M. 1970. Effect of soil temperature on virulence of *Pythium aphanidermatum* and *Pythium myriotylum* to rye and tomato. *Phytopathology* 60:704-707.
- Lookabaugh, E. C., Ivors, K. L., and Shew, B. B. 2015. Mefenoxam sensitivity, aggressiveness, and identification of *Pythium* species causing root rot on floriculture crops in North Carolina. *Plant Dis.* 99:1550-1558.
- Lumsden, R. D., Garcia-E, R., Lewis, J. A., and Frias-T, G. A. 1987. Suppression of damping-off caused by *Pythium* spp. in soil from the indigenous Mexican chinampa agricultural system. *Soil Biol. Biochem.* 19:501-508.
- Martin, F. N., and Loper, J. E. 1999. Soilborne plant diseases caused by *Pythium* spp.: Ecology, epidemiology, and prospects for biological control. *Crit. Rev. Plant Sci.* 18:111-181.
- Matthiesen, R. L., Ahmad, A. A., and Robertson, A. E. 2016. Temperature affects aggressiveness and fungicide sensitivity of four *Pythium* spp. that cause soybean and corn damping off in Iowa. *Plant Dis.* 100:583-591.
- Maynard, D. N., and Hochmuth, G. J. 2007. Knott's Handbook for Vegetable Growers. Wiley, Hoboken, NJ.
- Miao, J., Dong, X., Lin, D., Wang, Q., Liu, P., Chen, F., Du, Y., and Liu, X. 2016. Activity of the novel fungicide oxathiapiprolin against plant-pathogenic oomycetes. *Pest Manag. Sci.* 72:1572-1577.
- Moorman, G. W., and Kim, S. H. 2004. Species of *Pythium* from greenhouses in Pennsylvania exhibit resistance to propamocarb and mefenoxam. *Plant Dis.* 88:630-632.
- Njoroge, S. M. C., Riley, M. B., and Keinath, A. P. 2008. Effect of incorporation of *Brassica* spp. residues on population densities of soilborne microorganisms and on damping-off and Fusarium wilt of watermelon. *Plant Dis.* 92:287-294.
- Pankhurst, C. E., McDonald, H. J., and Hawke, B. G. 1995. Influence of tillage and crop rotation on the epidemiology of *Pythium* infections of wheat in a red-brown earth of South Australia. *Soil Biol. Biochem.* 27:1065-1073.
- Pasteris, R. J., Hanagan, M. A., Bisaha, J. J., Finkelstein, B. L., Hoffman, L. E., Gregory, V., Shepherd, C. P., Andreassi, J. L., Sweigard, J. A., Klyaschitsky, B. A., Henry, Y. T., and Berger, R. A. 2015. The discovery of oxathiapiprolin: A new, highly-active oomycete fungicide with a novel site of action. *ACS Symp. Ser.* 1204:149-161.
- Philosoph, A. M., Dombrovsky, A., Elad, Y., Koren, A., and Frenkel, O. 2019. Insight into late wilting disease of cucumber demonstrates the complexity of the phenomenon in fluctuating environments. *Plant Dis.* 103:2877-2883.
- Pieczarka, D. J., and Abawi, G. S. 1978. Populations and biology of *Pythium* species associated with snap bean roots and soils in New York. *Phytopathology* 68:409-416.

- Robideau, G., De Cock, A. W. A. M., Coffey, M. D., Voglmayr, H., Brouwer, H., Bala, K., Chitty, D. W., D'esaulniers, N., Eggertson, Q. A., Gachon, C. M. M., Hu, C.-H., Küpper, F. C., Rintoul, T. L., Sarhan, E., Verstappen, E. C. P., Zhang, Y., Bonants, P. J. M., Ristaino, J. B., and Lévesque, C. A. 2011. DNA barcoding of oomycetes with cytochrome *c* oxidase subunit I and internal transcribed spacer. *Mol. Ecol. Resour.* 11:1002-1011.
- Robinson, R. W., and Decker-Walters, D. S. 1997. *Cucurbits*. CAB International, New York, NY.
- Rojas, J. A., Jacobs, J. L., Napieralski, S., Karaj, B., Bradley, C. A., Chase, T., Esker, P. D., Giesler, L. J., Jardine, D. J., Malvick, D. K., Markell, S. G., Nelson, B. D., Robertson, A. E., Rupe, J. C., Smith, D. L., Sweets, L. E., Tenuta, A. U., Wise, K. A., and Chilvers, M. I. 2017a. Oomycete species associated with soybean seedlings in North America—Part I: Identification and pathogenicity characterization. *Phytopathology* 107:280-292.
- Rojas, J. A., Jacobs, J. L., Napieralski, S., Karaj, B., Bradley, C. A., Chase, T., Esker, P. D., Giesler, L. J., Jardine, D. J., Malvick, D. K., Markell, S. G., Nelson, B. D., Robertson, A. E., Rupe, J. C., Smith, D. L., Sweets, L. E., Tenuta, A. U., Wise, K. A., and Chilvers, M. I. 2017b. Oomycete species associated with soybean seedlings in North America—Part II: Diversity and ecology in relation to environmental and edaphic factors. *Phytopathology* 107:293-304.
- Santner, T. J., and Duffy, D. E. 1989. *The Statistical Analysis of Discrete Data*. Springer, New York, NY. doi.org/10.1007/978-1-4612-1017-7
- Schlatter, D. C., Burke, I., and Paulitz, T. C. 2018. Succession of fungal and oomycete communities in glyphosate-killed wheat roots. *Phytopathology* 108:582-594.
- Schroeder, K. L., Martin, F. N., De Cock, A. W. A. M., Okubara, P. A., and Paulitz, T. C. 2013. Molecular detection and quantification of *Pythium* species: Evolving taxonomy, new tools, and challenges. *Plant Dis.* 97:4-20.
- Shaner, G., and Finney, R. E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology* 67: 1051-1056.
- Shew, H. D. 1984. In vitro growth response of *Phytophthora parasitica* var. *nicotianae* isolates to metalaxyl. *Phytopathology* 68:764-766.
- Stanghellini, M. E., Von-Bretzel, P., Kronland, W. C., and Jenkins, A. D. 1982. Inoculum densities of *Pythium aphanidermatum* in soils of irrigated sugarbeet fields in Arizona. *Phytopathology* 72:1481-1485.
- Teymoori, S., Shahri, M. H., Rahnama, K., and Afzali, H. 2012. Identification and pathogenicity of *Pythium* species on sugar beet in Khuzestan Province of Iran. *J. Crop Prot.* 1:239-247.
- Toporek, S., and Keinath, A. P. 2019. Resistance of seedless watermelon grafted onto cucurbit rootstocks to *Pythium* stem rot, 2018. *Plant Dis. Manag. Rep.* 13:V159.
- United States Department of Agriculture, National Agricultural Statistics Service (USDA-NASS). 2018. *Vegetables 2017 Summary*. https://www.nass.usda.gov/Publications/AgCensus/2017/Full_Report/Volume_1_Chapter_1_US/st99_1_0036_0036.pdf
- United States Department of Agriculture, National Agricultural Statistics Service (USDA-NASS). 2019. 2018 Vegetable chemical use, pesticides, watermelons. https://www.nass.usda.gov/Data_and_Statistics/Pre-Defined_Queries/2018_Vegetables/index.php.
- Van der Plaats-Niterink, A. J. 1981. Monograph of the genus *Pythium*. *Stud. Mycol.* 21:1-242.
- Webster, J., and Weber, R. 2007. *Introduction to Fungi*. Cambridge University Press, Cambridge, U.K. doi.org/10.1017/CBO9780511809026
- Weiland, J. E., Santamaria, L., and Grünwald, N. J. 2014. Sensitivity of *Pythium irregulare*, *P. sylvaticum*, and *P. ultimum* from forest nurseries to mefenoxam and fosetyl-al, and control of *Pythium* damping-off. *Plant Dis.* 98:937-942.
- Zitnick-Anderson, K. K., Norland, J. E., del Río Mendoza, L. E., Fortuna, A.-M., and Nelson, B. D. 2017. Probability models based on soil properties for predicting presence-absence of *Pythium* in soybean roots. *Microb. Ecol.* 74: 550-560.