Annuals and Herbaceous Perennials Tolerant or Resistant to Phytophthora Species in the Landscape[1]

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Abstract

Plants of one or two cultivars of 16 annuals and 14 herbaceous perennials were evaluated based on desirability and anecdotal evidence of resistance to Phytophthora root or crown rot. Six plant cultivars served as susceptible controls. Three landscape beds were established in North Carolina and each was infested with three species of *Phytophthora*: *P. nicotianae*, *P. drechsleri*, and *P. tropicalis*. Plants were regularly rated for disease incidence and symptomatic plants were assayed to determine the presence of *Phytophthora* species. Ten cultivars of annuals and seven cultivars of herbaceous perennials did not exhibit symptoms of Phytophthora root or crown rot or other disease throughout the season (June 4 to October 15, 2018). *Phytophthora* spp. were recovered from seven and six cultivars of the evaluated annuals and herbaceous perennials, respectively. *Phytophthora nicotianae*, *P. drechsleri*, or *P. cryptogea* were recovered from a susceptible host in each landscape bed. P. tropicalis was recovered from one plant cultivar evaluated. *Phytophthora cryptogea* was recovered from three plant cultivars, although this species was not intentionally introduced in the landscape beds. We identified 22 plant cultivars within 13 herbaceous plant species that grew vigorously in landscape beds infested with species of *Phytophthora*.

Index words: bedding plants, disease resistance, herbaceous perennials, landscape plants, *Phytophthora nicotianae*, *Phytophthora drechsleri*, *Phytophthora tropicalis*.

Species used in this study: yarrow (*Achillea millefolium* L. 'Desert Eve Red'), fernleaf yarrow (*Achillea filipendulina* Lam. 'Moonshine Yellow'), angelonia (*Angelonia angustifolia* Benth. 'ArchAngel Pink', 'Serenita White'), annual vinca (*Catharanthus roseus* (L.) G. Don 'Cora Apricot', 'Cora Strawberry', 'Pacifica Raspberry'), celosia (*Celosia argentea* L. 'New Look'), tickseed (*Coreopsis auriculata* L. 'Nana', 'Yellow Jethro Tull'), purple coneflower (*Echinacea purpurea* (L.) Moench 'Cheyenne Spirit', 'PowWow Wild Berry'), blanket flower (*Gaillardia* x *grandiflora* Hort. 'Goblin', 'Mesa Bi-color'), Barberton daisy (*Gerbera jamesonii* Bolus ex Hooker f. 'Crazy Daisy'), verbena (*Glandularia canadensis* 'Homestead Purple'), dusty miller (*Jacobaea maritima* (L.) Pelser & Meijden 'Silver Dust'), New Guinea impatiens (*Impatiens hawkeri* W.Bull 'Hamony', 'Sunpatiens Compact Orchid', 'Sunpatiens Lilac'), sweet potato vine (*Ipomoea batatas* (L.) Lam. 'Ace of Spades', 'Bright Idea Tri-color'), West Indian lantana (*Lantana camara* L. 'Miss Huff'), lantana (*Lantana* x *hybrida* 'New Gold'), shasta daisy (*Leucanthemum* x *superbum* (Bergmans ex J.W. Ingram) Bergmans ex Kent. 'Becky', 'Snow Lady'), bee balm (*Monarda didyma* L. 'Petite Delight', 'Jacob Cline'), ornamental grass (*Panicum virgatum* L. 'Rotstrahlbusch', 'Shenandoah'), geranium (*Pelargonium x hortorum* L.H. Bailey (pro. sp.) 'Bullseye Cherry', Calliope Dark Red'), calibrachoa (*Petunia* x *calibrachoa* 'Super Cal'), petunia (*Petunia x hybrida* (Hooker) Vilmorin 'Easy Wave Red', 'Easy Wave White', 'Wave Purple', 'Yellow Madness', Violet Picotee'), annual phlox (*Phlox drummondii* Hook. 'Intensia Red Hot', 'Phlox Star'), garden phlox (*Phlox paniculata* L. 'Amethyst True Gal'), black-eyed susan (*Rudbeckia hirta* L. 'Indian Summer', 'Prairie Sun'), mealy blue sage (*Salvia farinacea* Benth. 'Victoria Blue'), African marigold (*Tagetes erecta* L. 'Inca Yellow', 'Proud Yellow'), French marigold (*Tagetes patula* L. 'Disco Mix', 'Disco Yellow'), narrowleaf zinnia (*Zinnia angustifolia* Kunth. 'Star Orange', 'Star White'), *Phytophthora nicotianae* Breda de Haan, *Phytophthora cryptogea* Pethybr. and Laff, *Phytophthora drechsleri* Tucker, *Phytophthora tropicalis* Aragaki and J.Y. Uchida, zinnia (*Zinnia elegans* Jacq. 'Magellan Orange').

Significance to the Horticulture Industry

This research provides knowledge of 22 plant cultivars within 13 herbaceous plant species that can be successfully cultivated in *Phytophthora*-infested soils for ornamental plant producers, landscapers, and the home gardener. This study also identified ornamental plants that are not ideal candidates for infested beds due to their susceptibility to other common diseases. Future investigations should re-evaluate plants that did not perform well due to abiotic issues. Additional plant cultivars exist that also need to be evaluated in order to provide stakeholders with as many options as possible for areas infested with *Phytophthora* species.

Introduction

Phytophthora (de Bary 1876) is a genus of soil-inhabiting oomycetes that attack a broad range of economically important crops including vegetables, small and tree fruits, herbaceous and woody ornamentals, forest trees, and field crops (Erwin and Ribeiro 1996, Patel et al. 2016). In general, *Phytophthora* species cause root, crown, or stem rots, but foliar blight can also occur on some plant species (Banko and Stefani 2000, Lamour et al. 2003).

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Symptoms in landscape plants can vary depending on the plant species, but generally include loss of older leaves, decline in plant vigor and growth, branch dieback, crown rot, root rot, wilting, chlorosis, plant collapse, and death (Banko and Stefani 2000). The disease is favored by excessive moisture (Banko and Stefani 2000) and can be particularly devastating and difficult to control if it becomes established.

In North Carolina, *Phytopthora nicotianae* Breda de Haan (formerly *P. parasitica* [Dastur] G.M. Waterh.), *Phytophthora drechsleri* Tucker, and *Phytophthora tropicalis* Aragakia and J.Y. Uchida are commonly found attacking ornamental plants (Hwang and Benson 2005), but the susceptibility of many popular bedding plants is still largely unknown and some are suspected to be resistant (Creswell et al. 2011). In this study, disease resistance is defined as the ability of the plant to suppress growth of the pathogen on or in the plant. Fungicide applications can provide some disease suppression (Hagan et al. 1996), but this practice involves frequent applications that are costly and not applicable to gardeners and landscapers who grow these plants on a small scale. Therefore, obtaining the latest research and knowledge of resistant plant species to *Phytophthora* species, and understanding the susceptibility of these plants to other common diseases, would make disease avoidance possible in landscape settings and provide an economically and environmentally sustainable approach. The objective of this study was to evaluate one or two cultivars of 16 annuals and 14 herbaceous perennials for resistance to Phytophthora root or crown rot and for susceptibility to other common diseases that may occur.

Materials and Methods

Plant selection. In spring 2018, bedding plant taxa were selected for evaluation based on availability, popularity, anecdotal or experimental evidence of resistance to *Phytophthora* species (Banko and Stefani 2000, Creswell et al. 2011, Hagan and Akridge 2001, M. Yelanich, Van Wingerden International, personal communication) and evidence of resistance to other diseases (Beckerman and Lerner 2009). Four replicates of 16 annual species and 14 herbaceous perennial species were selected (Table 1). An additional four plant species and six cultivars known to be susceptible to *Phytophthora* species were selected as susceptible controls: Barberton daisy (*Gerbera jamesonii* Bolus ex Hooker) ('Crazy Daisy'), dusty miller (*Jacobaea maritima* [L.] Pelser and Meijden) ('Silver Dust'), annual vinca (*Catharanthus roseus* [L.] G. Don) ('Pacifica Raspberry'), and petunia (*Petunia x hybrida* [Hooker] Vilmorin) ('Purple Wave', 'Easy Wave White', 'Easy Wave Red') (Olson and Benson 2011, Hao et al. 2010).

Experimental design. Three landscape beds [each approximately 18.6 m^2 (200 sq ft)] were established at three research stations (one bed per location) in western and central North Carolina [Mountain Horticultural Crops Research Station (MHCREC), Mills River, NC; Mountain Research Station (MRS), Waynesville, NC; and Piedmont Research Station (PRS), Salisbury, NC] in uncultivated areas that had no previous agricultural use. Beds were tilled before establishing the bed frame and then filled with a 1:1 blend of compost (blend of pine soil conditioner with mushroom, dairy, and leaf composts) and screened, enriched top soil (1:1 blend of compost and sandy-loam topsoil). Each bed was divided into four rectangular quadrants as if a "cross" was drawn over the bed. Each quadrant was a mirror image of the adjacent quadrants [each 4.65 m^2 (50 sq ft)] and plants were laid out in the same pattern in each quadrant. The taller plants were placed towards the center of the bed and shorter plants placed toward the edge of the bed. The bed at PRS was planted on May 15, 2018, the bed at MRS was planted on May 17, 2018, and the bed at MHCREC was planted on May 18, 2018. Plants were allowed to establish for two weeks before inoculation. Pendulum® 2G granular herbicide (BASF, Ludwigshafen, Germany) was applied as a pre-emergent after planting to each bed (PRS: May 25, 2018; MHCREC: May 31, 2018; MRS: June 1, 2018) at a rate of 112 $kg{\cdot}ha^{-1}$ (100 $lb{\cdot}A^{-1}$) with a handheld spreader to suppress weeds. Care was taken to pass a hand over each plant to dislodge any herbicide granules. One plant of each of 45 cultivars was planted in each quadrant of the bed so that there were four plants of each cultivar in each bed except for *Impatiens hawkeri* 'Sunpatiens Compact Lilac' (n=9; 3 plants per bed) and *Rudbeckia hirta* 'Indian Summer' (n=11; 3 or 4 plants per bed). Therefore, there were twelve plants of each cultivar, collectively, among the three beds, except for the two cultivars where there were only nine or eleven plants in total. Plants were spaced at 30 to 46 cm (12-18 in) between each cultivar and pine bark mulch was spread on the bed surfaces to ensure adequate soil moisture and to suppress weeds.

Selection of Phytophthora spp. strains for inoculation. Two isolates each of *P. nicotianae* (17-008, 17-036), *P. drechsleri* (16-041, 17-025), and *P. tropicalis* (16-043, 17-072) were selected from a collection of isolates from bedding plants in North Carolina. If the mating type was unknown, isolates were grown on 5% clarified V8 agar (CV8) (V8® juice, Campbell Soup Company, Camden, NJ, USA; water; calcium bicarbonate, Sigma-Aldrich, St. Louis, MO, USA) (Ferguson and Jeffers 1999) and challenged with an isolate of *P. nicotianae* of known mating type A1 (18-020) for 7 to 14 days at 22 C (72 F) (Tooley et al. 1989). Isolates were characterized as mating type A1 if no oospores were formed or A2 if oospores were produced.

DNA isolation, polymerase chain reaction, and sequencing. The identification of the selected isolates studied were confirmed before inoculation. Pure cultures of *Phytophthora* species were grown on 5% CV8 agar for 3 to 5 days and a single agar plug (5 mm diameter) was transferred to a petri plate containing 10% CV8 broth. Cultures were allowed to grow at room temperature for 3 to 5 days into thick mycelial mats and then collected by vacuum filtration and rinsed with distilled water through Fisherbrand plain filter paper (Fisher Scientific, Waltham, MA, USA) (Klassen et al. 1987). The mycelium was stored in 2 ml microcentrifuge tubes at -20 C (-4 F) until processed.

Table 1. Mean disease ratings from four replicate plots in each of three landscape beds in North Carolina (MRS, MHCREC, and PRS) from June to October 2018.

		Location and Week[z]											
		MRS			MHCREC			PRS			Mean[y]		
Plant common name and species	Cultivar	3	6	9	3	6	9	3	6	9	3	6	9
African marigold (*Tagetes erecta*)	Inca Yellow	0[x]	1.25	2	0	0	3.75	0	1.25	3.5	0	0.8	3.1
	Proud Yellow	0	3.25	5	0	0.75	5	0	3.25	5	0	2.4	5
angelonia (*Angelonia angustifolia*)	ArchAngel Pink	0	0	0	0	0	0	0	0	0	0	0	0
	Serenita White	0	0	0	0	0	0	0	0	0	0	0	0
annual phlox (*Phlox drummondii*)	Intensia Red Hot	3	4.5	5	2.5	2.5	5	3	4.75	5	2.8	3.9	5
	Phlox Star	3.25	5	5	2	5	5	2.5	4	5	2.6	4.7	5
annual vinca (*Catharanthus roseus*)	Cora Apricot	0	0.75	1.5	0	0.25	2	0.5	0.75	2.25	0.2	0.6	1.9
	Cora Strawberry	0	0	0.5	0.25	0	2	0.5	0	1.25	0.3	0	1.3
bee balm (*Monarda didyma*)	Jacob Cline	0	0	1	0	2	2.75	0	0	1	0	0.7	1.6
	Petite Delight	0	0.75	2.25	0	1.5	3.25	0.75	2.25	4.25	0.3	1.5	3.3
black-eyed Susan (*Rudbeckia hirta*)	Indian Summer	1	3.25	4.5	0	2.25	3	0	2.75	4.25	0.3	2.8	3.9
	Prairie Sun	0.75	3	4.5	0	2.75	4	0	2.75	4.5	0.3	2.8	4.3
blanket flower (*Gaillardia grandiflora*)	Goblin	0.5	2	4.25	0	3.5	5	0.25	2	5	0.3	2.5	4.8
	Mesa Bi-Color	0.5	2.75	4	0	3.5	4.25	0.25	2.25	4.75	0.3	2.8	4.3
calibrachoa (*Petunia x calibrachoa*)	Super Cal	0	0.5	5	0	2.5	5	0.5	2.25	5	0.2	1.8	5
celosia (*Celosia argentea*)	New Look	0	0.5	2.75	0	0	0	0	0	2.25	0	0.2	1.7
fernleaf yarrow (*Achillea filipendulina*)	Moonshine	0	1	1.25	0	0.5	1.5	0	1.25	3.75	0	0.9	2.2
French marigold (*Tagetes patula*)	Disco Mix	0	0	3	0	0.5	5	0	0.25	3.75	0	0.3	3.9
	Disco Yellow	0	0	2.5	0	0	4.75	0	0	4.5	0	0	3.9
garden phlox (*Phlox paniculata*)	Amethyst Pearl True Gal	2.5	1.25	1.25	0	1.25	3.25	0	2.5	3.25	0.8	1.7	2.6
geranium (*Pelargonium x hortorum*)	Bullseye Cherry	0.5	2	4	0	0.25	2.25	1.5	2.5	2.75	0.7	1.6	3
	Calliope Dark Red	0	2.25	3.5	0	0.5	2.25	0	1.5	1.5	0	1.4	2.4
lantana (*Lantana x hybrida*)	New Gold	0	0	0	0	0	0	0	0	0	0	0	0
mealy blue sage (*Salvia farinacea*)	Victoria Blue	0	0	1.5	0	0	1.25	0	0	1	0	0	1.3
narrowleaf zinnia (*Zinnia angustifolia*)	Star Orange	0.25	0.5	0.25	0	0.5	0	0	0	0	0.1	0.3	0.1
	Star White	0.5	0	0	0.25	0	0	0.25	0.25	0.5	0.3	0.1	0.2
New Guinea impatiens (*Impatiens hawkeri*)	Harmony	0.5	0.75	2	1.25	0	2	1	0.25	2.5	0.9	0.3	2.2
	Sunpatiens Compact Lilac	0.25	0	0	1	0	0.75	1	0	0.5	0.8	0	0.4
	SunPatiens Compact Orchid	1	0	1.25	0	0	0	0.25	0	0.25	0.4	0	0.5
ornamental grass (*Panicum virgatum*)	Rotsrahlbusch	0	0	0	0	1.75	1.75	0	0	1	0	0.6	0.9
	Shenandoah	0	0	0	0	0	0	0	0	0	0	0	0
petunia (*Petunia x hybrid*)	Violet Picotee	0	4	5	0	4.25	5	1	5	5	0.3	4.4	5
	Yellow Madness	5	5	5	3.5	5	5	3.5	5	5	4	5	5
purple coneflower (*Echinacea purpurea*)	Cheyenne Spirit	0	0	0	0	0	0	0	0	0	0	0	0
	PowWow Wild Berry	0	0	0	0	0	0	0	0	0	0	0	0
Shasta daisy (*Leucanthemum x superbum*)	Becky	0	0	0	0	0.25	0	0	0	0.25	0	0.1	0.1
	Snow Lady	2.25	3	3.5	0	2	3.5	1	2.75	5	1.1	2.6	4
sweet potato vine (*Ipomoea batatas*)	Ace of Spades	0	0	0	0	0	0	0	0	0	0	0	0
	Bright Idea Tri-color	0	0	0	0	0	0	0	0	0	0	0	0
tickseed (*Coreopsis auriculata*)	Nana	0	0.25	0	0	0	0	0	0.5	0	0	0.3	0
	Yellow Jethro Tull	0	0	0	0	0.25	0	0	0	0	0	0.1	0
verbena (*Glandularia canadensis*)	Homestead Purple	0	0	0	0	0	0	0	0	0	0	0	0
West Indian lantana (*Lantana camara*)	Miss Huff	0	0	0	0	0	0	0	0	0	0	0	0
yarrow (*Achillea millefolium*)	Desert Eve Red	0	0.25	2.5	0	2	5	0	0.25	4.75	0	0.8	4.1
zinnia (*Zinnia elegans*)	Magellan Orange	0	0	0.75	0	0	1.5	0	0	1.25	0	0	1.2

[z]MRS=Mountain Research Station, Waynesville, NC; MHCREC=Mountain Horticultural Crops Research and Extension Center; PRS=Piedmont Research Station; Week= number of weeks after plants were established; Week 3 = June 29-July 2, 2018; Week 6 = August 10-14, 2018; Week 9 = October 1-3, 2018.

[y]The mean across all three locations was calculated for each week.

[x]Plants were rated every 10-21 d after establishment. The rating scale was: 0 = healthy, 0% wilting or chlorosis; 1 = slight wilting, ≤10% wilting or chlorosis; 2 = moderate wilting, 10-50% wilting or chlorosis; 3 = severe wilting, >50% wilting or chlorosis; 4 = 100% wilting or chlorosis, still alive; 5 = dead

Frozen mycelial mats were disrupted with two sterile 3-mm glass beads and shaken with a BioSpec Mini Beadbeater (Bartlesville, OK, USA) at 42 rpm for 20 s. DNA was extracted using the Omega Bio-Tek Plant DNA Kit (Norcross, GA, USA) and subjected to PCR by amplifying a portion of the internal transcribed spacer regions using primer pairs *ITS4* (5′-TCCTCCGCTTATTGATATGC-3′) and *ITS6* (5′-GAAGGTGAAGTCGTAACAAGG-3′) (Cooke and Duncan 1997, White et al. 1990, Cooke et al. 2000). In cases where ITS was non-informative, the β-tubulin region with primers *β-tubF* (5′- CGGTAACAACTGGGCCAAGG-3′) and *β-tubR* (5′-CCTGGTACTGCTGGTACTCAG-3′) (Kroon et al. 2004), and the cytochrome *c* oxidase subunit 1 region using *cox1F* (5′-TCCGTAGGTGAACCTGCGG-3′) and *cox1R* (5′-TCCTCCGCTTATTGATATGC-3′) (Martin et al. 2012) were used to differentiate between species. Amplifications were performed using a Bio-Rad iCycler Thermal Cycler (Version 4.006, Bio-Rad Laboratories, Inc, Hercules, CA, USA). Thermocycler conditions were as follows: (i) initial

denaturation at 94 C (201 F) for 2 min (ii) 35 cycles at 94 C (201 F) for 30 sec, 52 C (126 F) for 30 sec, and 72 C (162 F) for 1 min (iii) and a final extension at 72 C (162 F) for 10 minutes (Kroon et al. 2004). Successful amplification was confirmed by gel electrophoresis. PCR products were purified using the Invitrogen Quick PureLink kit (Thermo Fisher Scientific, Waltham, MA, USA). Purified products were sent to McLab Molecular Cloning Laboratories (San Francisco, CA) for Sanger sequencing and consensus contigs were aligned and trimmed using Geneious Prime software (Auckland, New Zealand). Resulting sequences were compared to accessions in GenBank and Phytophthora-ID.org (Grünwald et al. 2011) for identification.

Inoculum preparation. To prepare the inoculum, each isolate was grown on 5% CV8 agar at 22 C (72 F) for 5-7 days. Five plugs (5 mm diameter) were aseptically transferred into a sterile mixture of 10% clarified V8 juice broth and fine vermiculite (25% v:v) and maintained at 20 to 22 C (68 to 72 F) for 14 days in the dark (Ivors 2015). A sample (approximately 5 ml) of each container of infested vermiculite was grown on CV8 agar for 1-2 days at 22 C (72 F) just prior to inoculation to confirm that the organism had fully colonized the vermiculite and that no contamination had occurred. To infest the soil, five parallel, shallow trenches were dug 8-10 cm (3-4 in) into the soil and approximately 940 ml (32 fl oz) of inoculum was spread in each trench [4,700 ml (160 fl oz)] of inoculum per bed). Trenches were re-covered with soil and irrigation was initiated to provide adequate moisture for the inoculum. Beds were infested on June 1, 2018 and again on June 28, 2018 to ensure the inoculum was active and all plants were exposed to the inoculum.

Plant ratings, isolations, and pathogen identification. Plants were rated every 10 to 21 days for disease incidence and severity based on the percentage of plants showing symptoms of infection by *Phytophthora* species including root rot, crown rot, or aerial blight or disease by another pathogen (Table 1) to avoid recommending plants that are susceptible to other diseases. The disease rating scale included: 0 = healthy, not exhibiting symptoms of infection by *Phytophthora* species, 0% wilting or chlorosis; 1 = slight wilting, ≤10% wilting or chlorosis; 2 = moderate wilting, 10-50% wilting or chlorosis; 3 = severe wilting, >50% wilting or chlorosis; 4 = 100% wilting or chlorosis, still alive; 5 = dead. Statistical analyses were not conducted on the data collected as this would require each cultivar to be compared to a healthy (non-infected) control. This was not achievable due to financial constraints.

As disease symptoms progressed, symptomatic plants were removed, transported to the laboratory, and pathogen isolations were attempted to determine the presence of *Phytophthora* on roots and crown. Plants also were observed for other diseases as they occurred and were diagnosed. Plants were either diagnosed in the field based on symptoms or were sent to the North Carolina State University Plant Disease and Insect Clinic for diagnosis. Resulting diagnoses were identified to the genus level or the common name of the disease was recorded. Roots and stems were washed with tap water and were cut into pieces approximately 1 cm (0.4 in) in length and embedded onto a semi-selective medium for *Phytophthora* species, PARPH-V8A (Jeffers and Martin 1986). After 3 to 5 days of incubation at 22 C (72 F), suspected colonies of *Phytophthora* species were sub-cultured onto clarified V8 agar (CV8). Species were identified based on sporangium morphology (Gallegly and Hong 2008) after 24 h in 1.5% non-sterile soil extract solution (NS-SES) (Jeffers and Aldwinkle 1987). DNA was extracted from species that could not be identified based on morphological features by methods described above.

A soil-baiting bioassay (Ferguson and Jeffers 1999) was used to confirm the species of *Phytophthora* in the soil of each landscape bed were active in August and October 2018. Five to six soil sub-samples were compiled and maintained at 22 C (72 F) for less than four days until processed. Three sub-samples of soil [50 cm^3 (3.1 in^3)] from each bed were each placed in a plastic cup and flooded with 100 ml (3.4 fl oz) of deionized water. Six floating leaf discs of *Camellia japonica* L. and *Rhododendron catawbiense* Michx. were placed in each cup and cups were maintained at 22 C (72 F) for 48 to 72 hr. Leaf discs were then embedded onto PARPH-V8 medium and maintained at 20 C (68 F) for 3 to 10 days. Suspect colonies of *Phytophthora* species were sub-cultured and identified by morphology and DNA sequencing as described above. All resulting cultures of *Phytophthora* species were placed in storage on CV8 discs in sterile water with two hemp seeds at 22 C (72 F) in the dark.

At the end of the growing season, plants from all three landscape beds were removed from the bed and washed to dislodge soil. Final plant evaluations were conducted visually and based on the following scale: Excellent = no disease symptoms, excellent floral quality, and plants survived the entire growing season; Good = minor disease symptoms (<25% leaf area affected), good floral quality, and most of the plants survived the entire growing season; Fair = moderate disease symptoms (~50% leaf area affected) and fewer than half (<6) of the plants died before the end of growing season; and Poor = severe disease symptoms (>50% leaf area affected) and more than half (>6) of the plants died before the end of growing season. Other = more than half (>6) of the plants had abiotic or unknown issues that prevented a fair trial. On 15, 17, and 22 Oct 2018 at MRS, MHCREC, and PRS, respectively, two healthy plants of each species that remained in each bed were removed and assayed for the presence of *Phytophthora* on the roots or stem. Root pieces (1 to 3 cm length) from selected plants were embedded onto PARPH-V8 to determine the presence of *Phytophthora* species as described above. Suspected colonies of *Phytophthora* species were sub-cultured and identified based on methods outlined above.

Results and Discussion

At the end of the growing season, 17 of the suspected resistant plant cultivars did not exhibit symptoms of infection by *Phytophthora* species or other diseases and were rated as Excellent, five were rated as Good, seven were rated as Fair, eight were rated as Poor, and eight rated as Other (Tables 1 and 2). These ratings reflect overall

Table 2. Disease ratings and detected pathogens or diseases (if present) of annual and herbaceous perennial plant cultivars that were evaluated for tolerance or resistance to *Phytophthora* spp. and other diseases, when they occurred, in 2018 in artificially infested landscape beds in North Carolina. Total no. of plants = 12, except *I. hawkeri* 'Sunpatiens Compact Lilac' = 9, and *R. hirta* 'Indian Summer' = 11.

Rating[z]	Type	Plant name (*Latin name*)	Cultivar	*Phytophthora* species	No.	Other disease	No.
Excellent	Annual	angelonia (*Angelonia angustifolia*)	ArchAngel Pink	-	-	-	-
			Serenita White	-	-	-	-
		narrowleaf zinnia (*Zinnia angustifolia*)	Star Orange	-	-	-	-
			Star White	*P. nicotianae*	1	*Fusarium* sp.	1
		New Guinea impatiens (*Impatiens hawkeri*)	Sunpatiens Compact Lilac	-	-	-	-
			Sunpatiens Compact Orchid	-	-	-	-
		sweet potato vine (*Ipomoea batatas*)	Ace of Spades	-	-	-	-
			Bright Idea Tri-color	-	-	-	-
		lantana (*Lantana hybrida*)	New Gold	-	-	-	-
		West Indian lantana (*Lantana camara*)	Miss Huff	-	-	-	-
	Perennial	ornamental grass (*Panicum virgatum*)	Shenandoah	-	-	-	-
		purple coneflower (*Echinacea purpurea*	Cheyenne Spirit	-	-	-	-
			PowWow Wildberry	-	-	-	-
		Shasta daisy (*Leucanthemum superbum*)	Becky	-	-	-	-
		tickseed (*Coreopsis auriculata*)	Jethro Tull	-	-	-	-
			Nana	-	-	-	-
		verbena (*Glandularia canadensis*)	Homestead Purple	-	-	-	-
Good	Annual	annual vinca (*Catharanthus roseus*)	Cora Apricot	-	-	*Alternaria* sp.	5
			Cora Strawberry	*P. tropicalis*	1	*Alternaria* sp.	4
						Fusarium sp.	1
		New Guinea impatiens (*Impatiens hawkeri*)	Harmony	-	-	*Alternaria* sp.	1
						Rhizoctonia sp.	4
		zinnia (*Zinnia elegans*)	Magellan Orange	-	-	*Cercospora* sp.	8
						Powdery mildew	8
	Perennial	ornamental grass (*Panicum virgatum*)	Rotstrahlbusch	*P. cryptogea*	2	*Alternaria* sp.	2
						Leaf rust	4
Fair	Annual	celosia (*Celosia argentea*)	New Look	*P. cryptogea*	2	-	-
		geranium (*Pelargonium hortorum*)	Bullseye Cherry	-	-	*Alternaria* sp.	8
						Colletotrichum sp.	1
			Calliope Dark Red	-	-	*Alternaria* sp.	8
	Perennial	bee balm (*Monarda didyma*)	Jacob Cline	*P. cryptogea*	2	Powdery mildew	12
			Petite Delight	-	-	Powdery mildew	12
						Rhizoctonia sp.	1
		fernleaf yarrow (*Achillea filipendulina*)	Moonshine	*P. drechsleri*	1	-	-
		mealy blue sage (*Salvia farinacea*)	Victoria Blue	*P. cryptogea*	2	-	-
				P. drechsleri	1	-	-
Poor	Annual	African marigold (*Tagetes erecta*)	Inca Yellow	*P. drechsleri*	1	*Alternaria* sp.	4
						Cladosporium sp.	1
			Proud Yellow	-	-	*Alternaria* sp.	4
						Fusarium sp.	1
		calibrachoa (*Petunia x calibrachoa*)	SuperCal	*P. drechsleri*	1	-	-
				P. nicotianae	6		
		petunia (*Petunia hybrida*)	Violet Picotee	*P. nicotianae*	11	-	-
			Yellow Madness	*P. nicotianae*	12	-	-
	Perennial	blanket flower (*Gaillardia grandiflora*)	Goblin	*P. drechsleri*	2	*Entyloma* sp.	10
			Mesa Bi-color	*P. cryptogea*	1	*Alternaria* sp.	4
						Entyloma sp.	10
		Shasta daisy (*Leucanthemum superbum*)	Snow Lady	-	-	*Alternaria* sp.	9
Other	Annual	annual phlox (*Phlox drummondii*)	Intensia Red Hot	-	-	Abiotic	12
			Phlox Star	-	-	Abiotic	12
		French marigold (*Tagetes patula*)	Disco Mix	-	-	Abiotic	8
			Disco Yellow	-	-	Abiotic	8
	Perennials	black-eyed Susan (*Rudbeckia hirta*)	Indian Summer	-	-	*Phytopythium* sp.	1
						Powdery mildew	2
						Verticillium sp.	1
			Prairie Sun	-	-	*Verticillium* sp.	2
		garden phlox (*Phlox paniculata*)	Amethyst True Gal	-	-	*Alternaria* sp.	8
						Septoria sp.	10
		yarrow (*Achillea millefolium*)	Desert Eve Red	-	-	Abiotic	12

[z]**Disease Rating Scale**: **Excellent** = no disease symptoms, excellent floral quality, and plants survived the entire growing season; **Good** = minor disease symptoms (<25% leaf area affected), good floral quality, and most of the plants survived the entire growing season; **Fair** = moderate disease symptoms (~50% leaf area affected) and fewer than half (<6) of the plants died before the end of growing season; and **Poor** = severe disease symptoms (>50% leaf area affected) and more than half (>6) of the plants died before the end of growing season. **Other** = more than half (>6) of the plants had abiotic or unknown issues that prevented a fair trial.

disease susceptibility; however, not all plants were exposed to the same pathogens (other than species of *Phytophthora* used in this study) so conclusions drawn from susceptibility to other diseases must be considered with caution.

Of the susceptible plants, *P. nicotianae* and/or *P. drechsleri* were isolated from each of the plants known to be susceptible to *Phytophthora* species in each bed, which confirmed that the inoculum was active (Table 2). Soil bait assays also confirmed the presence of *Phytophthora* species in the beds; however, *P. tropicalis* was not detected in any of the baits or the susceptible plants. Interestingly, we detected *P. cryptogea* at MRS by soil baiting, although this species was not intentionally introduced into the bed. It is possible this species was introduced into the bed through one or more of the plants as *Phytophthora* species are known to be transported through ornamental plants (Bienapfl and Bolci 2014, Callaghan and Guest 2015, Goss et al. 2011). Plants were not assayed before planting since they appeared healthy, so it is unclear how this organism became established in the bed.

Of the suspected resistant cultivars, *Phytophthora* species were recovered from 11 plant species and 12 cultivars among all three beds: 12 cultivars at MRS, four cultivars at PRS and three cultivars at MHCREC. Numerically, a larger number of susceptible cultivars were infected by *Phytophthora* spp. at MRS than the other two beds. It is unclear why this occurred, but it may be that some underlying condition in the bed at MRS promoted disease, such as extended periods of saturated soil, a condition known to promote diseases caused by species of *Phytophthora* (Erwin and Ribeiro 1996), but soil moisture was not measured in this study. However, total rainfall for the period that each bed was planted was numerically greater at MHCREC and PRS than at MRS and was 72.0 cm (28.3 in), 70.4 cm (27.7 in), and 57.0 cm (22.5 in), respectively, and does not add evidence to suggest our theory of increased soil moisture. The cause of the numerical disparity in Phytophthora affected plants in each bed remains elusive. Of the evaluated plants, *P. nicotianae* was recovered most frequently (30/48 plants [62.5%]) and from twelve plants in all three beds of petunia 'Yellow Madness' in all three beds, eleven plants of petunia 'Violet Picotee' among all three beds, six plants of calibrachoa 'SuperCal' among all three beds, and one plant of zinnia 'Star White'. All plants had either died or were evaluated as Poor by the end of the season except for zinnia—it was evaluated as Excellent and not showing any above ground symptoms. The cultivars of petunia and calibrachoa had been suggested as potentially resistant to *Phytophthora* species, but this study confirms that this is not the case. This result is not surprising given the susceptibility of petunia and petunia hybrids, in general (Erwin and Ribeiro 1996, Hwang and Benson 2005, Olson and Benson 2011).

Of the evaluated plants, *P. drechsleri* was isolated from six plants at MRS: one plant of calibrachoa 'SuperCal', one plant of mealy blue sage 'Victoria Blue', one plant of fernleaf yarrow 'Moonshine', one plant of African marigold 'Inca Yellow', and two plants of blanket flower 'Goblin'. *Phytophthora drechsleri* was not found on any of the evaluated plants at MHCREC or PRS. Only the African marigold, calibrachoa, and fernleaf yarrow were dead at the end of the season; all others from which *P. drechsleri* was recovered were not showing symptoms. Given the low incidence of this oomycete on plants evaluated in this study, it is difficult to draw any firm conclusions. The reported host range of *P. drechsleri* is over 400 plant species (Erwin and Ribeiro 1996, Farr and Rossman 2020) and, yet, this is the first report of *P. drechsleri* on these twelve plants. As mentioned above, there may have been an unknown, underlying condition with the bed at MRS that was different from the other two beds and that induced *P. drechsleri* to cause disease on these plants, such as extended periods of saturated soil, but this was not measured in the study. Therefore, the role of *P. drechsleri* on these plants is unknown and requires further study.

Phytophthora tropicalis was only isolated from one vinca 'Cora Strawberry', but was not isolated from any of the susceptible plants. It is unknown why the low incidence of this pathogen occurred. The host range of *P. tropicalis* is less than 60 plant species (Farr and Rossman 2020), many of which are not considered ornamentals and the lack of preferred hosts is one possible explanation.

We only detected *P. cryptogea* from MRS from one plant of blanket flower 'Mesa Bicolor', two plants of mealy blue sage 'Victoria Blue', two plants of celosia 'New Look Celosia', two plants of ornamental grass 'Rotstrahlbusch', and two plants of bee balm 'Jacob Cline'. This pathogen was not intentionally introduced to our landscape beds and, yet, it was recovered from multiple plant species. It is possible it was introduced either through one of plants, as mentioned above, or spread naturally from surrounding areas. Regardless, we will evaluate more plants against this species if more studies like this one are pursued in the future.

Other common diseases not attributed to *Phytophthora* species were also observed on several plants throughout the study (Table 2) to avoid recommending plants that are susceptible to other diseases that may occur in the landscape. These pathogens were identified to genus morphologically or the common name of the disease was recorded. The fungal genera identified and the number of plants affected by each were: *Alternaria* (57), *Cercospora* (8), *Cladosporium* (1), *Entyloma* (10), *Fusarium* (3), *Phytopythium* (1), *Rhizoctonia* (5), *Septoria* (10), and *Verticillium* (3). Powdery mildew (34) and leaf rust (4) fungi also were observed. The presence of other diseases presumably caused by the fungi mentioned above prevented those plants from being rated as Excellent to avoid recommending plants that are susceptible to other diseases, except for narrowleaf zinnia 'Star White', which remained in Excellent condition. However, those plants in the Good rating category were not affected enough by the fungi listed to not recommend them as possible alternative plants in *Phytophthora* infested beds. It is not surprising that a number of other fungal genera that are known to cause disease were detected as the climate in North Carolina is generally humid and warm with frequent rainfall, which are conditions known to favor such diseases.

Plants that died prematurely or were rated as Poor due to abiotic or unknown issues included phlox 'Phlox Star' and 'Intensia Red Hot', French marigold 'Disco Mix' and 'Disco Yellow', garden phlox 'Amethyst True Gal', yarrow 'Desert Eve Red', and black-eyed Susan 'Indian Summer' and 'Prairie Sun'. Even though no species of *Phytophthora* were isolated from these plants, we could not yet recommend them until they can be re-evaluated to provide a more accurate and fair representation of their performance in *Phytophthora*-infested beds.

This study provides evidence of 22 plant cultivars within 13 herbaceous plant species that are resistant to *Phytophthora nicotianae*, *Phytophthora drechsleri*, and *Phytophthora tropicalis* and provides information of susceptibility to other diseases that may occur in the landscape. Continuation of this study to evaluate additional plant species and cultivars is needed to provide stakeholders with more options for landscape beds infested with species of *Phytophthora*.

Literature Cited

Banko, T. J. and M.A. Stefani. (2000). Evaluation of bedding plant varieties for resistance to Phytophthora. J. of Environ. Hort., 18(1): 40–44.

Beckerman, J. and B.R. Lerner. 2009 Disease-resistant annuals and perennials in the landscape. Purdue Extension. https://www.extension.purdue.edu/extmedia/bp/id-414-w.pdf. p.1-4. Accessed March 23, 2018.

Bienapfl, J. C. and Y. Balci. 2014. Movement of *Phytophthora* spp. in Maryland's nursery trade. Plant Dis. 98:134–144.

Callaghan, S. and D. Guest. 2015. Globalisation, the founder effect, hybrid Phytophthora species and rapid evolution: new headaches for biosecurity. Aust. Plant Path. 44:255–262.

Cooke, D.E.L. and J.M. Duncan. 1997. Phylogenetic analysis of *Phytophthora* species based on ITS1 and ITS2 sequences of the ribosomal RNA gene repeat. Myc. Res. 101:667–677.

Cooke, D. E., A. Drenth, J.M. Duncan, G. Wagels, and C. M. Brasier. 2000. A molecular phylogeny of Phytophthora and related oomycetes. Fungal Genetics and Biology 30:17–32.

Creswell, T., K. Ivors, M. Munster. 2011. Suggested plant species for sites with a history of *Phytophthora* root or crown rot. NC State University Cooperative Extension Publ. AG-747. P. 2–3. https://plantpathology.ces.ncsu.edu/wp-content/uploads/2013/05/AG-747_Crown-Rot.pdf. p. 1-4. Accessed March 23, 2018

Erwin, D.C. and O.K. Ribeiro. 1996. *Phytophthora* Diseases Worldwide. American Phytopathological Society, St. Paul, MN. pp. 562.

Farr, D.F. and A.Y. Rossman. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. https://nt.ars-grin.gov/fungaldatabases/. Accessed January 3, 2020.

Ferguson, A.J. and S.N. Jeffers. 1999. Detecting multiple species of *Phytophthora* in container mixes from ornamental crop nurseries. Plant Dis. 83:1129–1136.

Gallegly, M.E. and C. Hong. 2008. *Phytophthora*: Identifying species by morphology and DNA fingerprints. APS Press, St. Paul, MN, USA. 158 p.

Goss, E.M., M. Larsen, A. Vercauteren, S. Werres, K. Heungens, and N. Grünwald. 2011. *Phytophthora ramorum* in Canada: Evidence for migration within North American and from Europe. Phytopath. 101:166–171.

Grünwald, N.J., F.N. Martin, M.M. Larsen, C.M. Sullivan, C.M. Press, M.D. Coffey, E.M. Hansen, and J.L. Parke. 2011. Phytophthora-ID.org: A sequence-based *Phytophthora* identification tool. Plant Dis. 95:337–342.

Hagan, A.K. and J.R. Akridge. 2001. Survival of selected annuals and perennials in beds infested with *Phytophthora parasitica*, the causal fungus of shoot blight and root rot on vinca. Alabama Cooperative Extension Plant Pathology Series PP-509. p. 1–6.

Hagan, A.K., B. Behe, and J.R. J.R. Akridge, 1996. Control strategies for *Phytophthora* shoot blight on bedding plants. Proc. of South. Nur. Assoc. Res. Conf. Olive, J. (ed.). Vol. 41:180–183.

Hao, W., P.A. Richardson, and C.X. Hong. 2010. Foliar blight of annual vinca (*Catharanthus roseus*) caused by *Phytophthora tropicalis* in Virginia. Pl. Dis. 94: 274.

Hwang, J. and D.M. Benson. 2005. Identification, mefenoxam sensitivity, and compatibility type of *Phytophthora* spp. attacking floriculture crops in North Carolina. Plant Dis. 89:185–190.

Ivors, K. 2015. Vermiculite method for *Phytophthora* inoculum production. Protocol 02-08.1 p. 1. In: Laboratory Protocols for *Phytophthora* species. APS Press, St. Paul, MN.

Jeffers, S.N. and H.S. Aldwinkle. 1987. Enhancing detection of *Phytophthora cactorum* in naturally infested soil. Phytopath. 77:1475–1482.

Jeffers, S.N. and S.B. Martin. Comparison of two media selective for *Phytophthora* and *Pythium* species. Plant Dis. 70:1038–1043.

Klassen, G. R., S.A. McNabb, and M. W. M.W. Dick, (1987). Comparison of physical maps of ribosomal DNA repeating units in *Pythium*, *Phytophthora* and *Apodachlya*. Microbiology 133(10): 2953–2959.

Kroon, L.P.N.M., E.C.P. Verstappen, L.F.F. Kox, W.G. Flier, and P.J.M. Bonants. 2004. A rapid diagnostic test to distinguish between American and European populations of *Phytophthora ramorum*. Phytopath. 94:613–620.

Lamour, K. H., M.L. Daughtrey, D.M. Benson, J.J. Hwang, and M.K. Hausbeck. (2003). Etiology of *Phytophthora drechsleri* and *P. nicotianae* (= *P. parasitica*) diseases affecting floriculture crops. Plant Dis. 87(7):854–858.

Martin, F. N., Z.G. Abad, Y.Y. Balci, and K. Ivors. 2012. Identification and detection of Phytophthora: reviewing our progress, identifying our needs. Plant disease, 96(8): 1080–1103.

Olson, H. A. and D.M. Benson. 2011. Characterization of *Phytophthora* spp. on floriculture crops in North Carolina. Plant Dis. 95:1013–1020.

Patel, J. S., A. Vitoreli, A.J. Palmateer, A. El-Sayed, D.J. Norman, E.M. Goss, M.S. Brennan, and G.S. Ali. 2016. Characterization of *Phytophthora* spp. isolated from ornamental plants in Florida. Plant Dis. 100:500–509.

Tooley, P. W., C.D. Therrien, and D.L. Ritch. (1989). Mating type, race composition, nuclear DNA content, and isozyme analysis of Peruvian isolates of *Phytophthora infestans*. *Phytopathology*, *79*(4), 478–481.

White, T.J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. p. 315–322 In: M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White, (Eds.). PCR protocols: A guide to methods and applications. Academic Press, San Diego, CA.

Erratum

Corrections to the Journal of Environmental Horticulture article 38(3):107-113

page 109:

. . .ß-tubF (5′- CGGTAACAACTGGGCCAAGG-3′) and ß-tubR (5′- CCTGGTACTGCTGGTACTCAG-3′) . . .

should read:

. . .TUBUF2 (5′-CGGTAACAACTGGGCCAAGG – 3′) and TUBUR1 (5′-CCTGGTACTGCTGGTACTCAG-3′)

and,

cox1F (5′-TCCGTAGGTGAACCTGCGG-3′) and *cox1R* (5′- TCCTCCGCTTATTGATATGC-3′) (Martin et al. 2012)

should read:

COXF4N (5′-GTATTTCTTCTTTATTAGGTGC-3′) and COXR4N (5′-CGTGAACTAATGTTACATATAC-3′) (Kroon et al. 2004)

In Literature Cited:

Remove:

Martin, F. N., Z.G. Abad, Y.Y. Balci, and K. Ivors. 2012. Identification and detection of Phytophthora: reviewing our progress, identifying our needs. Plant disease, 96(8): 1080–1103.

Remove:

Kroon, L.P.N.M., E.C.P. Verstappen, L.F.F. Kox, W.G. Flier, and P.J.M. Bonants. 2004. A rapid diagnostic test to distinguish between American and European populations of Phytophthora ramorum. Phyto- path. 94:613–620.

Insert:

Kroon, L.P.N.M., F.T. Bakker, G.B.M. van den Bosch,P.J.M. Bonants, and W.G. Flier. 2004. Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences. Fungal Gen. Biol. 41:766-782.