

Evaluation of annual and herbaceous perennial plants for susceptibility to *Phytophthora* root and crown rot in the Southeastern United States¹

Ella R. Reeves^{*2}, Michelle S. Henson², Suzette R. Sharpe², and Inga M. Meadows²

Abstract

Annual and herbaceous perennial ornamental bedding plants are popular, high value crops in the southeastern United States. However, many of these plants are subject to root or crown rot caused by *Phytophthora* species. In North Carolina, *Phytophthora nicotianae* Breda de Haan, *Phytophthora drechsleri* Tucker, *Phytophthora cryptogea* Pethybr. & Laff., and/or *Phytophthora tropicalis* Aragakia and J.Y. Uchida cause this disease in greenhouse production systems and in the landscape. Because practical management options for landscapers and homeowners are limited, the objective of this study was to identify annual and herbaceous perennial ornamental landscape plants that perform well in *Phytophthora*-infested landscape beds at three locations in western and central North Carolina. Although landscape beds were artificially inoculated with *P. nicotianae*, *P. drechsleri*, *P. cryptogea* sensu lato, and *P. tropicalis*, *P. nicotianae* was the most frequently isolated species from symptomatic plants and was the only species confirmed to be active at all locations in both years of this study. Eighteen cultivars of annuals and twenty-one cultivars of herbaceous perennials performed well and have been recommended for *Phytophthora*-infested landscapes to growers and homeowners in the southeastern United States.

Index words: host resistance, *Phytophthora* root rot, landscape ornamentals, soilborne disease.

Significance to the Horticulture Industry

Eighteen cultivars of annuals and twenty-one cultivars of herbaceous perennials performed well in this study and have been recommended as an economically and environmentally sustainable management solution for *Phytophthora*-infested landscape beds in the southeastern United States. These results provide valuable information to growers, landscapers, and homeowners. The opportunity to advertise plants as being tolerant to *Phytophthora* root and crown rot may increase sales of these varieties and, therefore, increase profits. Additionally, the reduction of pesticide usage to prevent this disease will provide savings for landscapers and homeowners and may decrease the environmental impact of disease management. In order to strengthen recommendations, future work should re-evaluate these cultivars in additional locations in the Southeast and with additional exposure to other isolates of *Phytophthora* known to cause root and crown rot. Additionally, more cultivars should be evaluated using similar methods.

Introduction

The genus *Phytophthora* de Bary contains numerous species of soil-inhabiting plant pathogens that are distributed worldwide. They can cause disease in natural ecosystems and on a wide range of cultivated crops, including field crops, forest trees, fruits, vegetables, and herbaceous and woody ornamentals (Erwin and Ribeiro 1996, Patel et al. 2016). Commercial production of bedding

plants, including annual ornamental plants (annuals) and herbaceous perennial ornamental plants (herbaceous perennials), in North Carolina (NC) was valued at over \$202 million in 2017 (National Agricultural Statistics Service, USDA). These ornamental plants are popular in landscape beds in the southeastern United States but can suffer from disease caused by species of *Phytophthora*. In NC and elsewhere, *P. nicotianae*, *P. drechsleri*, *P. cryptogea*, and/or *P. tropicalis* have been identified as the most common causal agents of *Phytophthora* root and crown rot of ornamental plants in greenhouse production systems and landscapes (Hwang and Benson 2005, Henson et al. 2020, Guarnaccia et al. 2021, Lamour et al. 2003, Olson and Benson 2011, Patel et al. 2016). Symptoms of infection by these pathogens often arise under wet conditions and include a decline in plant vigor, wilting, root rot, crown rot, and plant dieback. Because many species of *Phytophthora* are able to survive in the soil for several years in the form of dormant resting structures such as oospores, chlamydospores, or hyphal aggregates, the disease can be difficult to manage in a landscape setting once present (Jung et al. 2018). The pathogen may be introduced when transplanting plants, by the movement of infested soil, by stream water, and/or by infested irrigation water or water run-off (Bienapfl and Balci 2014, Patel et al. 2016). Fungicides may be used to manage the disease but are costly and not practical for many small growers, landscapers, and homeowners. There is limited information available on host resistance to *Phytophthora* in ornamental plants. Several research studies have identified cultivars of one or more plant species resistant to *P. nicotianae* (Hagan and Akridge 2001, Parsons et al. 2017), but many ornamental plants are susceptible to more than one species of *Phytophthora* (Farr et al. 2021, Henson et al. 2020, Olson and Benson 2011) and resistance to one species of *Phytophthora* may or may not equate to resistance to another species. In 2018, we evaluated one to two cultivars each of 16 annuals and 14

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²Department of Entomology and Plant Pathology, NC State University, Mountain Research Station, Waynesville, NC 28786 USA.

*Corresponding author: Ella R. Reeves, ereeves2@ncsu.edu.

herbaceous perennials for their susceptibility to *Phytophthora* root and crown rot in North Carolina and identified 22 cultivars that performed well in *Phytophthora*-infested landscape beds (Henson et al. 2020). The objective of this study was to evaluate the susceptibility of additional cultivars of annuals and herbaceous perennials to *Phytophthora* root and crown rot. Knowledge gained from this work will allow growers, landscapers, and homeowners in the southeastern United States to manage this disease in a more sustainable manner.

Materials and Methods

Plant selection. In 2019 and 2020, plant species were selected and planted based on availability, anecdotal consumer demand, resistance to common plant diseases, and evidence of resistance or tolerance to *Phytophthora* root and crown rot in the landscape (Banko and Stefani 2000, Creswell et al. 2011, Henson et al. 2020). In 2019, one to three cultivars of each of 10 annual and 15 herbaceous perennial species were chosen for evaluation. In 2020, one to two cultivars each of seven annual and six herbaceous perennial species were chosen for evaluation. Six cultivars of perennial plants were left to overwinter in the landscape beds during the winter of 2019-2020 and, therefore, were not replanted but were re-evaluated throughout the 2020 growing season (Table 1, Table 2). These cultivars were chosen to overwinter due to their popularity as perennial plants in the landscape. Perennial plants chosen for removal between 2019 and 2020 were those that had already been evaluated for two years, were too unhealthy from the 2019 season to be evaluated thoroughly, or, there were other plants of greater interest to be evaluated. In both years, cultivars of three to four additional species were selected as susceptible controls [*Petunia hybrida* Vilm., *Catharanthus roseus* (L.) G. Don, *Senecio cineraria* DC., *Petunia x calibrachoa*] (Table 3).

Experimental design. Raised landscape beds established in 2018 for a similar study were used for evaluation of plants in 2019 and 2020 (Henson et al. 2020). Beds measured approximately 18.6 m² and are located at the Mountain Research Station (MRS) in Waynesville, NC; the Mountain Horticultural Crops Research and Extension Center (MHCRC) in Mills River, NC; and the Piedmont Research Station (PRS) in Salisbury, NC. Each bed contained four quadrants of equal size, each 4.65 m² (50 ft²), all cleared of residual plant material. Between May 20 and 22 of 2019, 0.45 kg (1 lb) of elemental sulfur and 0.45 kg of 21-0-0-24S (Professional Choice Premium Fertilizer, Rapid City, SD) were applied to each of the MRS and MHCRC beds, and 0.54 kg (1.2 lb) of 18-46-0 (Southern States, Hendersonville, NC) were applied to the PRS bed. With the exception of these additions, no other addition or removal of material was performed to prepare beds for planting in 2019. Plants were transplanted to beds between May 29 and June 3 of 2019. Based on results from soil analyses, 0.68 kg (1.5 lb) of 21-0-0-24S and 0.68 kg of elemental sulfur were applied to each of the MRS, MHCRC, and PRS beds between April 28 and 30 of 2020. A total of 0.11 cubic meters (4 ft³) of composted cow

manure (Garick LLC, Cleveland, OH) was applied to the MRS bed to mitigate soil compaction. With the exception of these additions, no other addition or removal of material was performed to prepare beds for planting in 2020. Plants were transplanted between June 1 and June 4, 2020. In both years, a single plant of each variety was planted in each quadrant of each bed. Plants were established in the same pattern in each quadrant, and shorter plants were planted along the outer edge of the bed while taller plants were planted in the center (Fig. 1). In 2019, plants were spaced 30 to 46 cm (12 to 18 in) between each other. In 2020, plants were spaced 14 to 46 cm (5.5 to 18 in) between each other due to the larger size of the overwintered perennials. In both years, weeds were removed by hand just prior to planting and pine bark mulch [approximately 5 to 10 cm deep (2 to 4 in)] was spread over the surface of each bed immediately after planting to suppress weeds and promote the retention of soil moisture. Soaker hoses were laid lengthwise in the bed just after planting and were approximately 0.5 m (1.6 ft) apart. Beds were watered automatically for 30 minutes every day regardless of rain events. Soil samples were collected in April from each bed and assayed for soil pH and nutrient analysis by the North Carolina Department of Agriculture. With the exception of the perennial plants, at the end of each growing season all plants were removed from the beds by hand and bare ground was covered with landscape fabric.

Inoculation. Inoculum was prepared as described by Henson et al. (2020) and consisted of two isolates each of *P. nicotianae* (17-008[A1], 17-036[A2]), *P. tropicalis* (16-043[A2], 17-072[A2]), *P. drechsleri* (16-168[A1], 17-025[A2]), and *P. cryptogea* sensu lato (20-010[A1], 20-019[A1]). All isolates were selected from a collection of *Phytophthora* spp. recovered from bedding plants in North Carolina. The isolates of *P. cryptogea* used as inoculum in this study are considered to belong to the species complex, as we did not conduct a multi-locus phylogenetic analysis to further separate these isolates into distinct species or hybrids (Mostowfizadeh-Ghalefarsa et al. 2010, Safaifarhani et al. 2015, van Poucke et al. 2021). We will refer to them in this paper as *P. cryptogea*. The mating type of each isolate was confirmed by challenging individual isolates with an isolate each of *P. nicotianae* of known mating type (A1) and *P. nicotianae* of known mating type (A2), or a single isolate each of *P. capsici* of known mating type (A1) and *P. cinnamomi* of known mating type (A2) for 7 to 14 days at 22 C (72 F) (Tooley et al. 1988). Each isolate was grown on 5% clarified V8 juice agar (cV8A) at 22 C for 5 to 7 days. Five plugs (5 mm diameter) were aseptically transferred to individual flasks containing a mixture (25% v:v) of 10% clarified V8 juice broth and coarse vermiculite (PVP Industries, Inc. North Bloomfield, OH). Flasks were incubated in the dark at 22 C for 14 days (Ivors 2015). Inoculum colonization and purity was confirmed prior to inoculation by aseptically spreading approximately 5 ml of infested vermiculite onto plates of cV8A and monitoring growth for one to two days at 22 C. Approximately 1 liter of vermiculite infested with each isolate was combined, and all eight liters were thoroughly mixed just prior to application. Beds were infested twice in

Table 1. Ratings of annual ornamental plants evaluated for susceptibility to diseases caused by species of *Phytophthora* and other pathogens in 2019 and 2020.

Common name	Latin name	Cultivar	Diagnosis ^y	Year ^x
Excellent:^z				
African marigold	<i>Tagetes erecta</i> L.	Antigua Yellow		19
Angelonia	<i>Angelonia angustifolia</i> Benth.	ArchAngel Blue		19
		Serenita White		19
Begonia	<i>Begonia semperflorens</i> Link & Otto	Cocktail Whiskey	<i>Phytophthora</i> sp.	20
Floss flower	<i>Ageratum houstonianum</i> Mill.	Blue Danube		20
		Blue Horizon		20
Lantana	<i>Lantana camara</i> L.	Miss Huff		19
Lantana	<i>Lantana camara</i> var. <i>hybrida</i> (Neubert) Moldenke	Little Lucky Peach Glow		19
		Little Lucky Pot of Gold		19
Sweet potato vine	<i>Ipomoea batatas</i> (L.) Lam.	Ace of Spades		19
		Tri-Color		19
Zinnia	<i>Zinnia angustifolia</i> Kunth	Star Orange		19
		Star White		19
Good:				
African marigold	<i>Tagetes erecta</i> L.	Antigua Orange	<i>Fusarium</i> sp.	19
Celosia	<i>Celosia cristata</i> L.	Dracula	Abiotic	19
French marigold	<i>Tagetes patula</i> L.	Janie Deep Orange	Abiotic	19
		Janie Spry	Abiotic	19
Verbena hybrid	<i>Verbena x hybrida</i> Groenland & Rümpler	Superbena Royal Chambray	Abiotic	19
Fair:				
Begonia	<i>Begonia semperflorens</i> Link & Otto	Senator Deep Rose	Unknown	20
Shasta daisy	<i>Leucanthemum x superbum</i> (Bergmans ex J.W. Ingram)	Landcaster Darling Daisy	<i>Phytophthora</i> sp.	20
	Bergmans ex Kent.			
Moss-rose	<i>Portulaca grandiflora</i> Hook.	Happy Trails Series	Unknown	20
		Happy Hour	Unknown	20
Vinca	<i>Catharanthus roseus</i> (L.) G. Don	Cora Cascade Lilac	<i>P. cryptogea</i>	19
			Leaf Spot	
		Cora Cascade Strawberry	Leaf Spot	19
Verbena hybrid	<i>Verbena x hybrida</i> Groenland & Rümpler	Lanai Upright Rose with Eye	<i>P. drechsleri</i>	19
			<i>P. nicotianae</i>	
		Quartz Pink	<i>P. cryptogea</i>	20
		Quartz Red with Eye	<i>Pythium</i> sp.	20
Poor:				
Gazania	<i>Gazania rigens</i> (L.) Gaertn.	New Day Tiger Mix	<i>P. cryptogea</i>	20
			<i>Pythium</i> sp.	
Shasta daisy	<i>Leucanthemum superbum</i> (Bergmans ex J.W. Ingram)	Lucille White	Unknown	20
	Bergmans ex Kent.			
Petunia	<i>Petunia hybrida</i> Vilm.	Night Sky	<i>P. drechsleri</i>	19
			<i>P. nicotianae</i>	
			<i>Pythium oopapillum</i>	
Verbena hybrid	<i>Verbena x hybrida</i> Groenland & Rümpler	Superbena Stormburst	<i>P. drechsleri</i>	19
			<i>P. nicotianae</i>	
			<i>P. cryptogea</i>	
Other:				
Lobelia	<i>Lobelia erinus</i> L.	White Riviera	<i>Pythium</i> sp.	20
			Unknown	
		Riviera Rose	Abiotic	20

^zRatings were assigned as follows: **Excellent:** no disease symptoms, excellent floral quality, and survived entire growing season; **Good:** minor disease symptoms (< 25% leaf area affected), good floral quality, and most plants survived the entire growing season; **Fair:** moderate disease symptoms (~ 50% leaf area affected), and less than half (< 6 plants) died before the end of the growing season; **Poor:** severe disease symptoms (> 50% leaf area affected), and more than half (> 6 plants) died before end of growing season; **Other:** more than half (> 6 plants) had abiotic, unknown, or alternative issues that prevented a fair trial of the cultivar's susceptibility to *Phytophthora* spp.

^yDiagnosis received from the PDIC or organisms isolated from the root or crown tissue. In some cases, *Phytophthora* sp., *Phytophthora* sp., and *Pythium* sp. were isolated from the roots of asymptomatic plants at the end of the growing season. For some plants, no diagnosis was made, and the cause of symptoms remains unknown. Isolates identified as *P. cryptogea* belong to the species sensu lato.

^xYear evaluated: 19 = 2019, 20 = 2020

2019; the first inoculation occurred between nine and 15 days after transplanting plants, and the second occurred 13 to 16 days after the first. Beds were also infested twice in 2020; the first inoculation occurred between 13 and 15 days after transplanting plants, and the second occurred 22 to 24 days after the first. In 2019, five parallel trenches measuring 8-10 cm (3-3.9 in) deep and spaced 2 ft. apart

were dug into each bed and 940 ml (32 fl oz) of inoculum was spread in each trench for each inoculation. Soil was placed over each trench and irrigation was initiated via a soaker hose system. The same methods were used in 2020, but the amount of inoculum spread in each trench was 1,280 ml (43 fl oz). In both years, all plants were planted within 30 cm (12 in) of trench inoculum.

Table 2. Ratings of herbaceous perennial ornamental plants evaluated for susceptibility to diseases caused by *Phytophthora* and other pathogens in 2019 and 2020.

Common name	Latin name	Cultivar ^y	Diagnosis ^x	Year ^w
Excellent:^z				
Catnip	<i>Nepeta x faassenii</i>	Kitten Around		20
Hybrid Yarrow	<i>Achillea filipendulina</i> Lam.	Moonshine		19
Ornamental sedge	<i>Carex flacca</i> Schreb.	Blue Zinger		19
	Ornamental sedge			
Ornamental sedge	<i>Carex testacea</i> Sol. Ex Boott	Prairie Fire		19
Tickseed	<i>Coreopsis auriculata</i> L.	Nana*	<i>Phytophthora</i> sp.	19,20
Purple coneflower	<i>Echinacea purpurea</i> (L.) Moench	PowWow Wild Berry		19
		Cheyenne Spirit		19
Ornamental grass	<i>Miscanthus sinensis</i> Andersson	Little Zebra		19
	Ornamental grass			
Ornamental grass	<i>Panicum virgatum</i> L.	Rotstrahlbusch		19
		Shenandoah		19
Verbena	<i>Verbena canadensis</i> (L.) Britton	Homestead Purple		19
Good:				
Black-eyed Susan	<i>Rudbeckia fulgida</i> Aiton	Goldsturm	Leaf spot	20
Bugleweed	<i>Ajuga reptans</i> L.	Burgundy Glow		20
		Catlin's Giant	Fusarium crown rot	20
Rose Mock Verbian	<i>Verbena canadensis</i> (L.) Britton	Homestead Purple	Abiotic	19
Ornamental grass	<i>Miscanthus sinensis</i> Andersson	Little Zebra*		20
Ornamental sedge	<i>Carex flacca</i> Schreb.	Blue Zinger*	Leaf spot	20
			<i>Pythium</i> sp.	
CreepingpPhlox	<i>Phlox subulata</i> L.	Fort Hill	Southern blight	20
		White Delight	Aerial blight	20
Salvia	<i>Salvia nemorosa</i> L.	Violet Profusion	<i>P. cryptogea</i>	20
			Insect	
Verbena	<i>Verbena peruviana</i> (L.) Britton	Endurascape Red	<i>Pythium</i> sp.	19
Fair:				
Black-eyed Susan	<i>Rudbeckia fulgida</i> Aiton	Little Goldstar	Southern blight	20
Purple coneflower	<i>Echinacea purpurea</i> (L.) Moench	Cheyenne Spirit	<i>Phytophthora</i> sp.	20
Ornamental grass	<i>Panicum virgatum</i> L.	Shenandoah*	Unknown	20
		Rostrahlbusch*	Unknown	20
Ornamental sedge	<i>Carex testacea</i> Sol. Ex Boott	Prairie Fire	<i>Phytophthora</i> sp.	20
Russian sage	<i>Perovskia atriplicifolia</i> Benth.	Denim'n Lace	<i>P. cryptogea</i>	20
		Crazy Blue	<i>P. cryptogea</i>	20
			<i>Phytophthora</i> sp.	
Poor:				
Catnip	<i>Nepeta x faassenii</i>	Junior Walker	<i>Phytophthora</i> sp.	20
			<i>Pythium</i> sp.	
Hybrid yarrow	<i>Achillea filipendulina</i> Lam.	Moonshine*	Abiotic	20
Yarrow	<i>Achillea x lewisii</i>	King Edward	<i>P. cryptogea</i>	19
			<i>P. nicotianae</i>	
			<i>Phytophthora</i> sp.	
			<i>Pythium</i> sp.	
Alyssum	<i>Alyssum wulfenianum</i> Willd.	Golden Spring	<i>P. drechsleri</i>	19
			<i>P. nicotianae</i>	
Other:				
Tickseed	<i>Coreopsis grandiflora</i> Hogg ex Sweet	Sunfire	Unknown	19
Tickseed	<i>Coreopsis verticillata</i> L.	Starlight	Unknown	19
Lychnis	<i>Lychnis x arkwrightii</i> Heydt.	Orange Gnome	<i>Pythium</i> sp.	19
Bee balm	<i>Monarda didyma</i> L.	Balmy	Abiotic	19
			Insect	
			Powdery mildew	
			Leaf Spot	
		Pardon My Purple	Nematodes	19
		Pardon My Cerise	Insect	19
			Powdery Mildew	
Black-eyed Susan	<i>Rudbeckia fulgida</i> Aiton	Little Goldstar	Insect	19
			Powdery Mildew	
			Southern blight	
			Abiotic	

^zRatings were assigned as follows: **Excellent:** no disease symptoms, excellent floral quality, and survived entire growing season; **Good:** minor disease symptoms (< 25% leaf area affected), good floral quality, and most survived the entire growing season; **Fair:** moderate disease symptoms (~ 50% leaf area affected), and less than half (< 6 plants) died before the end of the growing season; **Poor:** severe disease symptoms (> 50% leaf area affected), and more than half (> 6 plants) died before the end of the growing season; **Other:** more than half (> 6 plants) had abiotic, unknown, or alternative issues that prevented a fair trial of the cultivar's susceptibility to *Phytophthora* spp.

Table 2. Continued.

^yCultivar name followed by an asterisk (*) indicates a perennial plant that overwintered in each landscape bed between the growing seasons of 2019 and 2020.

^xDiagnosis received from the PDIC or organisms isolated from the root or crown tissue. In some cases, *Phytophthora* sp., *Phytophythium* sp., and *Pythium* sp. were isolated from the roots of asymptomatic plants at the end of the growing season. For some plants, no diagnosis was made, and the cause of symptoms remains unknown. Isolates identified as *P. cryptogea* belong to the species sensu lato.

^wYear evaluated: 19 = 2019, 20 = 2020.

In both years, a soil baiting assay was performed to confirm successful inoculation of landscape beds (Ferguson and Jeffers 1999). In early June and late September of 2019, and in late August of 2020, five to six soil samples were collected from throughout each bed, combined and mixed, and stored at 22 C for no more than four days. Three sub-samples (50 cm³) from each sample were placed in a plastic cup and flooded with 100 ml deionized water. Six leaf discs of each *Camellia japonica* L. (cultivar unknown) and *Rhododendron catawbiense* Michx. were placed in each cup, and cups were kept at 22 C. After 48 to 72 hours, leaf discs were retrieved from the cups and embedded into a semi-selective media containing clarified V8 juice (cV8A) as a nutrient source and amended with 5 mg pimarcin (MilliporeSigma, St. Louis, MO), 250 mg ampicillin (MilliporeSigma, St. Louis, MO), 10 mg rifamycin (MilliporeSigma, St. Louis, MO), 66.7 mg Terraclor (75% PCNB) (MilliporeSigma, St. Louis, MO), and 50 mg Hymexazol (Alfa Aesar, Tewksbury, MA) per liter (PARPH-cV8A) (Jeffers and Martin 1986). Plates were incubated in the dark at 20 C (68 F) for three to ten days and colonies resembling *Phytophthora* spp. were sub-cultured onto cV8A. Isolates were identified based on morphology and, in some cases, by DNA sequencing as described below.

Plant evaluation and diagnosis. In both years, plants were rated for disease incidence and severity on the date of inoculation and every 11 to 20 days afterwards until experiment termination. Due to adverse weather in 2020, final disease ratings occurred later than in 2019 and were 19 to 36 days after the previous rating. Disease severity was assessed using a rating scale where 0 = excellent floral

quality, and (or) no symptoms of disease caused by *Phytophthora* spp., 0% of foliage affected; 1 = good floral quality, slight to moderate wilting, less than 25% of foliage affected; 2 = fair floral quality, moderate to severe wilting, or ~50% of foliage affected; and 3 = poor floral quality, severe wilting or plant dead, or greater than 50% of foliage affected. Disease incidence and severity data was combined to rate plant performance as follows: Excellent: no disease symptoms, excellent floral quality, and all plants survived entire growing season; Good: minor disease symptoms (< 25% leaf area affected), good floral quality, and most plants survived the entire growing season; Fair: moderate disease symptoms (~ 50% leaf area affected), and less than half (< 6 plants) died before the end of the growing season; Poor: severe disease symptoms (> 50% leaf area affected), and more than half (> 6 plants) died before the end of the growing season; Other: more than half (> 6 plants) had abiotic, unknown, or alternative issues that prevented a fair trial of the cultivar's susceptibility to *Phytophthora* spp.. When assigned a disease severity rating of "3", a plant was removed from the bed and transported to the laboratory where isolation of *Phytophthora* spp. was attempted from the root and crown tissue. Plants were also observed for other diseases and were diagnosed in the field or were submitted to the NC State University Plant Disease and Insect Clinic (NCSU PDIC) for diagnosis. Because no non-inoculated (healthy) controls were evaluated, statistical analyses were not possible. In 2020, a single, asymptomatic plant of each cultivar was arbitrarily selected and removed from each bed at the final disease rating. These plants were assayed for the presence of *Phytophthora* on root tissue, as outlined below, to determine

Table 3. Disease observed on susceptible cultivars planted as controls in infested landscape beds in 2019 and 2020.

Common name	Latin name	Cultivar	Diagnosis ^z	Year ^y
Petunia	<i>Petunia hybrida</i> Vilm.	Wave Purple	<i>P. nicotianae</i> (12)	19
		EZ Wave Berry Velour	<i>Phytophthora</i> sp. (1)	
		Pretty Flora Pink	<i>P. nicotianae</i> (12)	19
Vinca	<i>Catharanthus roseus</i> (L.) G. Don	Tattoo Tangerine	<i>P. nicotianae</i> (6)	20
Dusty Miller	<i>Senecio cineraria</i> DC.	Tattoo Tangerine	<i>Phytophythium vexans</i> sp. (1)	19
		Silver Dust	<i>P. drechsleri</i> (5)	19
			<i>Phytophythium oedoehilum</i> (1)	20
Calibrachoa hybrid	<i>Petunia x calibrachoa</i>	Superbells Cherry Red	<i>P. cryptogea</i> (3)	19
			<i>P. drechsleri</i> (1)	
			<i>Phytophthora</i> sp. (1)	
		Superbells Red	<i>P. nicotianae</i> (12)	20
			<i>P. drechsleri</i> (1)	
			<i>P. cryptogea</i> (1)	
			<i>P. nicotianae</i> (12)	

^zDiagnosis received from the PDIC or organisms isolated from the root or crown tissue. Number in parentheses indicates number of isolates recovered. In some cases, *Phytophthora* sp., *Phytophythium* sp., and *Pythium* sp. were isolated from the roots of asymptomatic plants at the end of the growing season. For some plants, no diagnosis was made, and the cause of symptoms remains unknown. Isolates identified as *P. cryptogea* belong to the species sensu lato.

^yYear evaluated: 19 = 2019, 20 = 2020



Fig. 1. Landscape bed at the Mountain Horticultural Crops Research and Extension Center (MHCREC) in Mills River, NC. Bed was divided evenly into four rectangular quadrants, each containing a single replicate plant.

whether healthy-appearing plants harbored any species of *Phytophthora*. Due to funding shortages, this was not performed in 2019.

Isolation and identification of Phytophthora spp. Roots and crowns were washed free of soil and pieces measuring 1 to 3 cm in length were cut, surface disinfested in a solution of 10% bleach, and rinsed in sterile-distilled water. Pieces were blotted dry and embedded into PARPH-cV8A (Jeffers and Martin 1986). Cultures were incubated in the dark at 22 C for three to five days. Colonies resembling species of *Phytophthora* were transferred to cV8A and were identified based on morphology of sporangia after 24 hours of incubating colonized plugs in 1.5% non-sterile soil extract solution (NS-SES) (Jeffers and Aldwinkle 1987). All isolates were placed in long-term storage by transferring colonized plugs of the pathogen into 2 ml tubes containing two, twice-autoclaved hemp seeds and 1 ml of sterile distilled water. For species that could not be identified based on morphological features, identification was attempted by sequencing the internal transcribed spacer (ITS) region of the ribosomal DNA, and when necessary, the cytochrome *c* oxidase subunit 1 (COI) region of the mitochondrial DNA or the β -tubulin (β -tub) region of the nuclear DNA (Martin et al. 2012). Isolates identified as *P. cryptogea* in this study are considered to belong to the species complex, as we did not conduct a multi-locus phylogenetic analysis to further separate these isolates into distinct species or hybrids (Mostowfizadeh-Ghalefarsa et al. 2010; Safaiefarahani et al. 2015; Van Poucke et al. 2021). We will refer to them in this paper as *P. cryptogea*.

Amplification of desired genomic regions was attempted via direct polymerase chain reaction (PCR) (Grünwald et al. 2011). Pure cultures were transferred to plates of cV8A, sealed to retain humidity, and incubated in the dark at room temperature. After five to seven days, a pinhead size of aerial mycelium was collected using a sterile, 200 μ l pipette tip and transferred to a 0.5 ml microcentrifuge tube containing 9.8 μ l of nuclease-free water. This mycelial suspension was incubated at 95.9 C for five minutes and used as DNA template in polymerase chain reaction (PCR). Each PCR reaction tube was 18 μ l in volume and contained of 2.5 μ l 10X buffer, 2 μ l 50 mM $MgCl_2$, 0.5 μ l of 10 mM dNTPs, 1 μ l bovine-serum alkalase, 1 μ l each of primers

ITS6 (5' – GAAGGTGAAGTCGTAACAAGG – 3') and ITS4 (5' – TCCTCCGCTTATTGA TATGC – 3'), 0.2 μ l Platinum Taq polymerase, and 9.8 μ l of boiled mycelial solution (Cooke and Duncan 1997; Cooke et al. 2000, Grünwald et al. 2011, White et al. 1990). Cycling conditions included incubation at 94 C for 3 min, 35 cycles of: 94 C for 1 min, 55 C for 1 min, 72 C for 1 min followed by a final incubation at 72 C for 10 minutes. For amplification of the COI region, primers COXF4N (5' – GTATTCTTCTTTATTAGGTGC – 3') and COXR4N (5' – CGTGAACATAATGTTACATATAC – 3') were used in place of ITS6 and ITS4, and cycling conditions included incubation at 94 C for 2 m, 35 cycles of: 94 C for 30 s, 52 C for 30 s, 72 C for 1 m followed by a final incubation at 72 C for 10 minutes (Kroon et al. 2004). For amplification of the β -tubulin (β -tub) region, primers TUBUF2 (5' – CGGTAACAACCTGGGCCAAGG – 3') and TUBUR1 (5' – CCTGGTACTGCTGGTACTCAG – 3') were used in place of ITS6 and ITS4, and cycling conditions included incubation at 94 C for 2 m, 35 cycles of: 94 C for 30 s, 60 C for 30 s, 72 C for 1 m followed by a final incubation at 72 C for 10 minutes (Kroon et al. 2004). Amplicons were visualized by gel electrophoresis.

There were 44 isolates that did not yield quality PCR products using the direct method, so DNA was extracted from these isolates using a kit. A single, 5-mm diameter colonized plug was transferred from a pure, three to five-day old culture on 5% cV8A to a petri plate containing 10% cV8 broth. Cultures were incubated in the dark at room temperature for three to five days and mycelial mats were collected via vacuum filtration then stored in 2 ml cryovials at -20 C until processed. Mycelial mats were frozen in liquid nitrogen for 10 s before being disrupted with two sterile 3-mm glass beads at 42 rpm for 20 s. DNA was extracted using the Omega Bio-Tek Plant DNA Kit (Norcross, GA, USA). PCR reaction components were as explained above, but instead were 20 μ l in volume and contained of 2 μ l of DNA and 9.8 μ l of nuclease-free water. PCR cycling conditions were as outlined above.

PCR products were purified using the Invitrogen Quick PureLink kit, or ExoSAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA, USA). Purified products were Sanger sequenced in both directions at Molecular Cloning Laboratories (MCLAB) (San Francisco, CA). Consensus sequences were aligned using Geneious Prime 11.0 software (Auckland, New Zealand), and then compared to authenticated specimens (Abad et al. 2019) in GenBank (National Center for Biotechnology Information) and Phytophthora-ID.org using the BLAST algorithm (Grünwald et al. 2011) for identification.

Results and Discussion

When results from both years were combined, the performance of 18 cultivars of annuals and 21 cultivars of herbaceous perennials was rated as Good to Excellent (Tables 1 and 2). In few instances, Fusarium crown rot (*Fusarium* sp.), leaf spot (unknown cause), Pythium root rot (*Pythium* sp.) or abiotic issues were responsible for plant decline for plants rated as Good, but no species of *Phytophthora* were isolated. Of the cultivars whose

Color key for average disease severity

0.0	0.1 - 0.9	1.0 to 1.9	2.0 to 2.9	3.0	Other			
Type	Plant common name	Cultivar	Location	W4	W8	W12		
Annual	African Marigold	Antigua Orange	MHCREC					
			MRS					
			PRS					
	Antigua Yellow		MHCREC					
			MRS					
			PRS					
	Angelonia	ArchAngel Blue	MHCREC					
			MRS					
			PRS					
	Serenita White		MHCREC					
			MRS					
			PRS					
	Celosia	Dracula	MHCREC					
			MRS					
			PRS					
	French Marigold	Janie Spry	MHCREC					
			MRS					
			PRS					
	Janie Deep Orange		MHCREC					
			MRS					
			PRS					
	Lantana	Miss Huff	MHCREC					
			MRS					
			PRS					
	Little Lucky Peach Glow		MHCREC					
			MRS					
			PRS					
	Little Lucky Pot of Gold		MHCREC					
			MRS					
			PRS					
	Petunia	Night Sky	MHCREC					
			MRS					
			PRS					
	Sweet Potato Vine	Ace of Spades	MHCREC					
			MRS					
			PRS					
	Tri-Color		MHCREC					
			MRS					
			PRS					
	Verbena Hybrid	Lanai Upright Rose with Eye	MHCREC					
			MRS					
			PRS					
	Superbena Royal Chambray		MHCREC					
			MRS					
			PRS					
	Superbena Stormburst		MHCREC					
			MRS					
			PRS					
	Vince	Cora Cascade Lilac	MHCREC					
			MRS					
			PRS					
	Cora Cascade Strawberry		MHCREC					
			MRS					
			PRS					
	Star Orange		MHCREC					
			MRS					
			PRS					
	Star White		MHCREC					
			MRS					
			PRS					
Perennial	Alyssum	Golden Spring	MHCREC					
			MRS					
			PRS					
	Bee Balm	Balmy	MHCREC					
			MRS					
			PRS					
	Pardon My Purple		MHCREC					
			MRS					
			PRS					
	Pardon My Cerise		MHCREC					
			MRS					
			PRS					
	Black-eyed Susan	Little Goldstar	MHCREC					
			MRS					
			PRS					
	Orange Gnome		MHCREC					
			MRS					
			PRS					
	Little Zebra		MHCREC					
			MRS					
			PRS					
	Rostrahbusch		MHCREC					
			MRS					
			PRS					
	Shenendoah		MHCREC					
			MRS					
			PRS					
	Blue Zinger		MHCREC					
			MRS					
			PRS					
	Prairie Fire		MHCREC					
			MRS					
			PRS					
	Cheyenne Spirit		MHCREC					
			MRS					
			PRS					
	PowWow Wild Berry		MHCREC					
			MRS					
			PRS					
	Nana		MHCREC					
			MRS					
			PRS					
	Starlight		MHCREC					
			MRS					
			PRS					
	Sunfire		MHCREC					
			MRS					
			PRS					
	Endurascap Red		MHCREC					
			MRS					
			PRS					
	Homestead Purple		MHCREC					
			MRS					
			PRS					
	King Edward		MHCREC					
			MRS					
			PRS					
	Moonshine		MHCREC					
			MRS					
			PRS					

Table 4. *Phytophthora* spp. baited from the soil and detected from plants in infested landscape beds in 2019 and 2020.

Year	Species ^z	Location ^y	Detected by soil baiting	Isolated from plants
2019	<i>P. nicotianae</i>	MHCREC	+	+
		MRS	+	+
		PRS	+	+
	<i>P. cryptogea</i>	MHCREC		
		MRS	+	+
		PRS		
	<i>P. drechsleri</i>	MHCREC		+
		MRS	+	+
		PRS	+	+
	<i>P. tropicalis</i>	MHCREC		
		MRS		
		PRS		
2020	<i>P. nicotianae</i>	MHCREC	+	+
		MRS		+
		PRS		+
	<i>P. cryptogea</i>	MHCREC	+	+
		MRS	+	+
		PRS		+
	<i>P. drechsleri</i>	MHCREC		
		MRS		
		PRS		
	<i>P. tropicalis</i>	MHCREC		
		MRS		
		PRS		

^zIsolates identified as *P. cryptogea* belong to the species sensu lato.^yLocations were as follows: MHCREC: Mountain Horticultural Crops Research and Extension Center; MRS: Mountain Research Station; PRS: Piedmont Research Station.

performance was rated as Fair, seven were diagnosed with *Phytophthora* root and/or crown rot based on isolations from symptomatic tissue. A single plant of each of two cultivars was visually diagnosed with leaf spot (unknown cause), and a single plant belonging to another cultivar was visually diagnosed with southern blight [*Athelia rolfsii* (Curzi)], but for five cultivars rated as Fair, the cause of plant decline could not be identified and disease was referred to as “Unknown”. *Phytophthora* root rot and/or crown rot was determined to be the primary cause of plant decline for three cultivars of annuals and three cultivars of herbaceous perennials whose performance was rated as Poor. *Phytophthora nicotianae*, *P. drechsleri*, and/or *P. cryptogea* were isolated from at least one of these plants. *Pythium* root rot or abiotic problems were identified as the causal agents of disease of the other two cultivars in this

←
Fig. 2. Average disease severity rating of annual and herbaceous perennial ornamental plants challenged by four species of *Phytophthora* in three landscape beds in North Carolina. Rating scale as follows: 0 = excellent floral quality, and (or) no symptoms of disease caused by *Phytophthora* spp., 0% of foliage affected; 1 = good floral quality, slight to moderate wilting, less than 25% of foliage affected; 2 = fair floral quality, moderate to severe wilting, or ~50% of foliage affected; and 3 = poor floral quality, severe wilting or plant dead, or greater than 50% of foliage affected. Severity is indicated by shade of gray for each cultivar at each location. The average rating of four replicate plants recorded at three time points throughout the 2019 growing season: W4= 12 July, W8= 9 August, W12= 9 September.

Color key for average disease severity

		0.0	0.1 - 0.9	1.0 to 1.9	2.0 to 2.9	3.0	Other
Type	Plant common name	Cultivar	Location	W4	W8	W12	
Annual	African Marigold	Antigua Orange	MHCREC				
			MRS				
			PRS				
	Antigua Yellow		MHCREC				
			MRS				
			PRS				
	Angelonia	ArchAngel Blue	MHCREC				
			MRS				
			PRS				
	Serenita White		MHCREC				
			MRS				
			PRS				
	Celosia	Dracula	MHCREC				
			MRS				
			PRS				
	French Marigold	Janie Spzy	MHCREC				
			MRS				
			PRS				
	Janie Deep Orange		MHCREC				
			MRS				
			PRS				
	Lantana	Miss Huff	MHCREC				
			MRS				
			PRS				
	Little Lucky Peach Glow		MHCREC				
			MRS				
			PRS				
	Little Lucky Pot of Gold		MHCREC				
			MRS				
			PRS				
	Petunia	Night Sky	MHCREC				
			MRS				
			PRS				
	Sweet Potato Vine	Ace of Spades	MHCREC				
			MRS				
			PRS				
	Tri-Color		MHCREC				
			MRS				
			PRS				
	Verbenia Hybrid	Lanai Upright Rose with Eye	MHCREC				
			MRS				
			PRS				
	Superbena Royal Chambray		MHCREC				
			MRS				
			PRS				
	Superbena Stormburst		MHCREC				
			MRS				
			PRS				
	Vinca	Cora Cascade Lilac	MHCREC				
			MRS				
			PRS				
	Cora Cascade Strawberry		MHCREC				
			MRS				
			PRS				
	Zinnia	Star Orange	MHCREC				
			MRS				
			PRS				
	Star White		MHCREC				
			MRS				
			PRS				
Perennial	Alyssum	Golden Spring	MHCREC				
			MRS				
			PRS				
	Bee Balm	Balmy	MHCREC				
			MRS				
			PRS				
	Pardon My Purple		MHCREC				
			MRS				
			PRS				
	Pardon My Cerise		MHCREC				
			MRS				
			PRS				
	Black-eyed Susan	Little Goldstar	MHCREC				
			MRS				
			PRS				
	Lychnis	Orange Gnome	MHCREC				
			MRS				
			PRS				
	Ornamental Grass	Little Zebra	MHCREC				
			MRS				
			PRS				
	Rostrahlbusch		MHCREC				
			MRS				
			PRS				
	Shenendoah		MHCREC				
			MRS				
			PRS				
	Ornamental Sedge	Blue Zinger	MHCREC				
			MRS				
			PRS				
	Prairie Fire		MHCREC				
			MRS				
			PRS				
	Purple Coneflower	Cheyenne Spirit	MHCREC				
			MRS				
			PRS				
	PowWow Wild Berry		MHCREC				
			MRS				
			PRS				

Fig. 3. Average disease severity rating of annual and herbaceous perennial ornamental plants challenged by four species of *Phytophthora* in three landscape beds in North Carolina.

category. *Pythium* root rot (*Pythium* sp.), powdery mildew (species not identified), leaf spot (not identified), insect damage, southern blight (*Athelia rolfsii*), and parasitic nematodes caused plant decline for plants rated as Other. In 2020, all four replicate plants of Moss-Rose ‘Happy Trails Series’ and ‘Happy Hour’, Lobelia ‘White Riviera’, Gazania ‘New Day Tiger Mix’, and three of four replicate plants of Petunia ‘Pretty Flora Pink’ and Lobelia ‘Riviera Rose’ disappeared unexpectedly from the MRS bed four to six weeks after planting. It is likely that an herbivorous animal was responsible, but this cannot be confirmed. The soil pH at all locations ranged between 6.6 and 7.5 in 2019 and between 6.9 and 7.3 in 2020. Although elemental sulfur was added to each bed to lower the pH, a soil pH unfavorable for some cultivars evaluated in this study may have played a role in some of the abiotic issues observed.

The species of *Phytophthora* most frequently isolated from the roots and crowns of symptomatic plants were *P. nicotianae* (n=15/41), *P. drechsleri* (n=12/41), and *P. cryptogea* (n=10/41) (Table 4). An additional four isolates recovered from plants in this study could not be identified to species and were referred to as *Phytophthora* sp. At least one species of *Phytophthora* was recovered from the susceptible controls in both years, confirming that at least some of the inoculum was active, although *P. nicotianae* was the only species to be recovered at all locations in both years of this study (Table 3).

Mean precipitation was numerically greater in 2020 than in 2019. Total precipitation between June 1 and September 31 was 13.4 inches at the MRS, 15.2 inches at the MHCREC, and 11.6 inches at the PRS in 2019. In 2020, total precipitation over the same time period was 20.8 inches at the MRS, 23.6 inches at the MHCREC, and 17.5 inches at the PRS. Timing of disease onset and progression throughout the growing season was numerically variable by year, cultivar, and location. In 2019, at four weeks after inoculation, disease appeared on 12 cultivars at PRS but only on four cultivars at MHCREC and one cultivar at MRS (Fig. 2). For the twelve cultivars displaying symptoms of *Phytophthora* root and crown rot in the PRS bed in 2019, symptoms disappeared later in the growing season. Interestingly, this regression of symptoms was not observed on any other cultivars at any of the other locations and was not as consistent in 2020 (Fig. 3). When rating for severity of *Phytophthora* root and crown rot, nineteen cultivars in the MHCREC bed, 20 cultivars in the MRS bed, and 22 cultivars in the PRS bed had a disease severity

Rating scale as follows: 0 = excellent floral quality, and (or) no symptoms of disease caused by *Phytophthora* spp., 0% of foliage affected; 1 = good floral quality, slight to moderate wilting, less than 25% of foliage affected; 2 = fair floral quality, moderate to severe wilting, or ~50% of foliage affected; and 3 = poor floral quality, severe wilting or plant dead, or greater than 50% of foliage affected. Severity is indicated by shade of gray for each cultivar at each location. The average rating of four replicate plants recorded at three time points throughout the 2020 growing season: W4= 14 July, W8= 11 August, W12= 11 September. The asterisk (*) next to cultivar name indicates that plant was a perennial left to overwinter in each landscape bed between the growing seasons of 2019 and 2020.

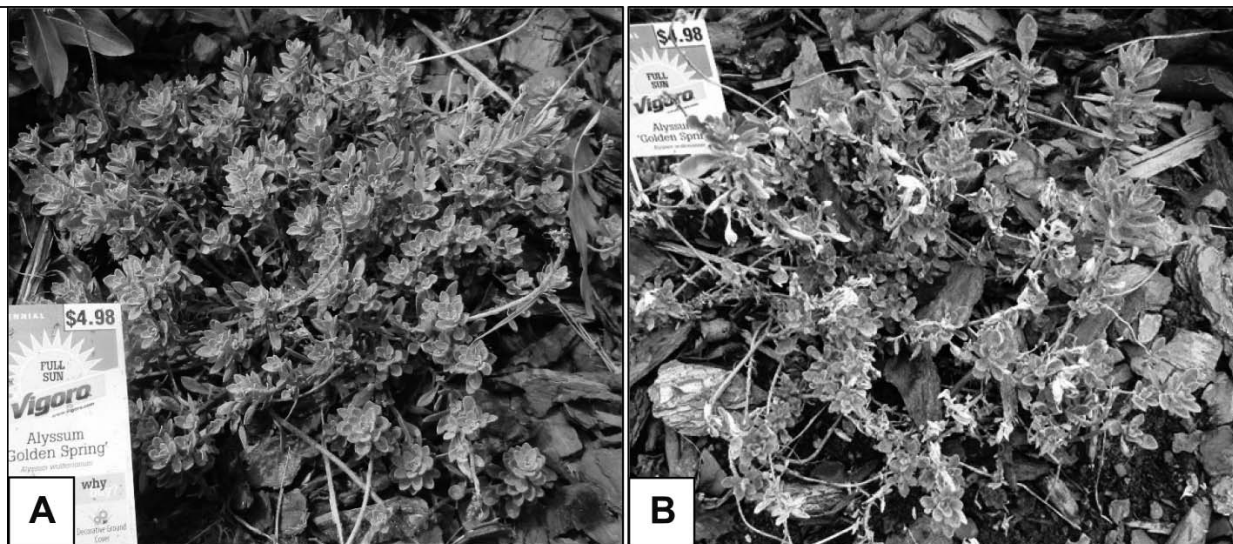


Fig. 4. Alyssum 'Golden Spring' in 2019, A: healthy plant early in the growing season, B: symptoms of *Phytophthora* root rot appeared six weeks after planting in *Phytophthora*-infested landscape bed.

rating greater than zero twelve weeks after inoculation in 2019. By the end of the growing season, all plants of petunia 'Night Sky' and *Lychnis* 'Orange Gnome' were dead at all locations. In 2020, two cultivars in the MHCRC bed, one cultivar in the MRS bed, and six cultivars in the PRS bed had a disease severity rating greater than zero four weeks after inoculation (Fig. 3). Sixteen cultivars in the MHCRC bed, 12 cultivars in the MRS bed, and 15 cultivars in the PRS bed had a disease severity rating greater than zero twelve weeks after inoculation in 2020. Death of all plants of a single cultivar at all locations was not observed in 2020.

This study identified 18 cultivars of annuals and 21 cultivars of herbaceous perennials that performed well in landscape beds infested with *Phytophthora* (Tables 1 and 2), and these cultivars have been recommended for

Phytophthora-infested landscapes to growers and homeowners in the Southeastern US in the form of an Extension publication (Henson et al. 2021). Because of the potential differences in plant exposure to *Phytophthora* spp. throughout the landscape bed, as well as differences in isolate aggressiveness, it is not appropriate to claim that these hosts are resistant to these pathogens based on the results of this study. However, the results provide preliminary evidence that some cultivars may exhibit resistance or tolerance to *Phytophthora* spp. The performance of both French Marigold 'Janie Deep Orange' and *Salvia* 'Violet Profusion' was rated as Good, but *Phytophthora* was isolated from the roots of these plants, which suggests that these cultivars may be tolerant to infection by this organism. Evidence of this has been found before; in one study, both *P. drechsleri* and *P. cryptogea*

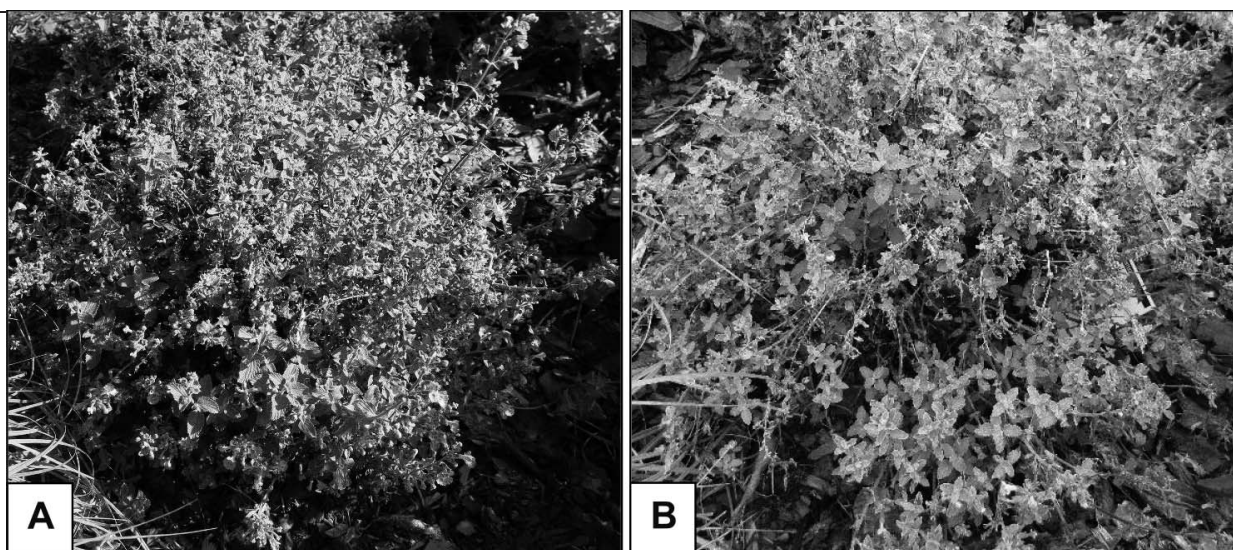


Fig. 5. Catnip 'Junior Walker' in 2020, A: healthy plant early in the growing season, B: symptoms of *Phytophthora* root rot appeared eight weeks after planting in *Phytophthora*-infested landscape bed.

were recovered from the roots of 116 out of 245 ornamental plants inoculated with these species but not exhibiting symptoms of *Phytophthora* root or crown rot (Olson and Benson 2013). Similarly, single isolates of *P. nicotianae* and *P. tropicalis* were isolated from plants rated as Excellent or Good in a study conducted in 2018 in the same landscape beds as this project (Henson et al. 2020). Colonization of roots in the absence of symptoms is known to facilitate the spread of these pathogens within the industry and in homeowner landscapes, so knowledge regarding host tolerance would be useful in preventing the inadvertent spread of this disease (Brasier 2008, Denman et al. 2007). Due to unequal exposure to the four pathogens used in the inoculum in this study, specific host-isolate interactions and the influence of cultural practices and weather conditions on disease development, future work should assess the performance of these cultivars in presence of *Phytophthora* spp. in more locations.

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