# Evaluation of annual and herbaceous perennial plants for susceptibility to Phytophthora root and crown rot in the Southeastern United States[1]

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## 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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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 $m^2$ and are located at the Mountain Research Station (MRS) in Waynesville, NC; the Mountain Horticultural Crops Research and Extension Center (MHCREC) in Mills River, NC; and the Piedmont Research Station (PRS) in Salisbury, NC. Each bed contained four quadrants of equal size, each 4.65 $m^2$ (50 $ft^2$), 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 MHCREC 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, MHCREC, and PRS beds between April 28 and 30 of 2020. A total of 0.11 cubic meters (4 $ft^3$) 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-Ghalamfarsa et al. 2010, Safaie-farahani 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 | *Tagetes erecta* L. | Antigua Yellow | | 19 |
| Angelonia | *Angelonia angustifolia* Benth. | ArchAngel Blue | | 19 |
| | | Serenita White | | 19 |
| Begonia | *Begonia semperflorens* Link & Otto | Cocktail Whiskey | *Phytopythium* sp. | 20 |
| Floss flower | *Ageratum houstonianum* Mill. | Blue Danube | | 20 |
| | | Blue Horizon | | 20 |
| Lantana | *Lantana camara* L. | Miss Huff | | 19 |
| Lantana | *Lantana camara* var. *hybrida* (Neubert) Moldenke | Little Lucky Peach Glow | | 19 |
| | | Little Lucky Pot of Gold | | 19 |
| Sweet potato vine | *Ipomoea batatas* (L.) Lam. | Ace of Spades | | 19 |
| | | Tri-Color | | 19 |
| Zinnia | *Zinnia angustifolia* Kunth | Star Orange | | 19 |
| | | Star White | | 19 |
| **Good:** | | | | |
| African marigold | *Tagetes erecta* L. | Antigua Orange | *Fusarium* sp. | 19 |
| Celosia | *Celosia cristata* L. | Dracula | Abiotic | 19 |
| French marigold | *Tagetes patula* L. | Janie Deep Orange | Abiotic | 19 |
| | | Janie Spry | Abiotic | 19 |
| Verbena hybrid | *Verbena x hybrida* Groenland & Rümpler | Superbena Royal Chambray | Abiotic | 19 |
| **Fair:** | | | | |
| Begonia | *Begonia semperflorens* Link & Otto | Senator Deep Rose | Unknown | 20 |
| Shasta daisy | *Leucanthemum x superbum* (Bergmans ex J.W. Ingram) Bergmans ex Kent. | Landcaster Darling Daisy | *Phytophthora* sp. | 20 |
| Moss-rose | *Portulaca grandiflora* Hook. | Happy Trails Series | Unknown | 20 |
| | | Happy Hour | Unknown | 20 |
| Vinca | *Catharanthus roseus* (L.) G. Don | Cora Cascade Lilac | *P. cryptogea* Leaf Spot | 19 |
| | | Cora Cascade Strawberry | Leaf Spot | 19 |
| Verbena hybrid | *Verbena* x *hybrida* Groenland & Rümpler | Lanai Upright Rose with Eye | *P. drechsleri* *P. nicotianae* | 19 |
| | | Quartz Pink | *P. cryptogea* | 20 |
| | | Quartz Red with Eye | *Pythium* sp. | 20 |
| **Poor:** | | | | |
| Gazania | *Gazania rigens* (L.) Gaertn. | New Day Tiger Mix | *P. cryptogea* *Pythium sp.* | 20 |
| Shasta daisy | *Leucanthemum superbum* (Bergmans ex J.W. Ingram) Bergmans ex Kent. | Lucille White | Unknown | 20 |
| Petunia | *Petunia hybrida* Vilm. | Night Sky | *P. drechsleri* *P. nicotianae* *Pythium oopapillum* | 19 |
| Verbena hybrid | *Verbena* x *hybrida* Groenland & Rümpler | Superbena Stormburst | *P. drechsleri* *P. nicotianae* *P. cryptogea* | 19 |
| **Other:** | | | | |
| Lobelia | *Lobelia erinus* L. | White Riviera | *Pythium* sp. Unknown | 20 |
| | | Riviera Rose | Abiotic | 20 |

[z]Ratings 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.

[y]Diagnosis received from the PDIC or organisms isolated from the root or crown tissue. In some cases, *Phytophthora* sp., *Phytopythium* 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.

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

[z]Ratings 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.**

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

[x]Diagnosis received from the PDIC or organisms isolated from the root or crown tissue. In some cases, *Phytophthora* sp., *Phytopythium* 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.

[w]Year 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 $cm^3$) 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 | *Petunia hybrida* Vilm. | Wave Purple | *P. nicotianae* (12)<br>*Phytophthora* sp. (1) | 19 |
| | | EZ Wave Berry Velour | *P. nicotianae* (12) | 19 |
| | | Pretty Flora Pink | *P. nicotianae* (6) | 20 |
| Vinca | *Catharanthus roseus* (L.) G. Don | Tattoo Tangerine | *Phytopythium vexans* sp. (1) | 19 |
| Dusty Miller | *Senecio cineraria* DC. | Silver Dust | *P. drechsleri* (5)<br>*Phytopythium oedochilum* (1) | 19<br>20 |
| Calibrachoa hybrid | *Petunia x calibrachoa* | Superbells Cherry Red | *P. cryptogea* (3)<br>*P. drechsleri* (1)<br>*Phytophthora* sp. (1) | 19 |
| | | Superbells Red | *P. nicotianae* (12)<br>*P. drechsleri* (1)<br>*P. cryptogea* (1)<br>*P. nicotianae* (12) | 20 |

[z]Diagnosis 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., *Phytopythium* 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.

[y]Year 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-Ghalamfarsa 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 ul pipette tip and transferred to a 0.5 ml microcentrifuge tube containing 9.8 ul 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 ul in volume and contained of 2.5 ul 10X buffer, 2 ul 50 mM $MgCl_2$, 0.5 ul of 10 mM dNTPs, 1 ul bovine-serum alkalase, 1 ul each of primers ITS6 (5′ – GAAGGTGAAGTCGTAACAAGG – 3′) and ITS4 (5′ – TCCTCCGCTTATTGA TATGC – 3′), 0.2 ul Platinum Taq polymerase, and 9.8 ul 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′ – GTATTTCTTCTTTATTAGGTGC – 3′) and COXR4N (5′ – CGTGAACTAATGTTACATATAC - 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′ – CGGTAACAACTGGGCCAAGG – 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 ul in volume and contained of 2 ul of DNA and 9.8 ul 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

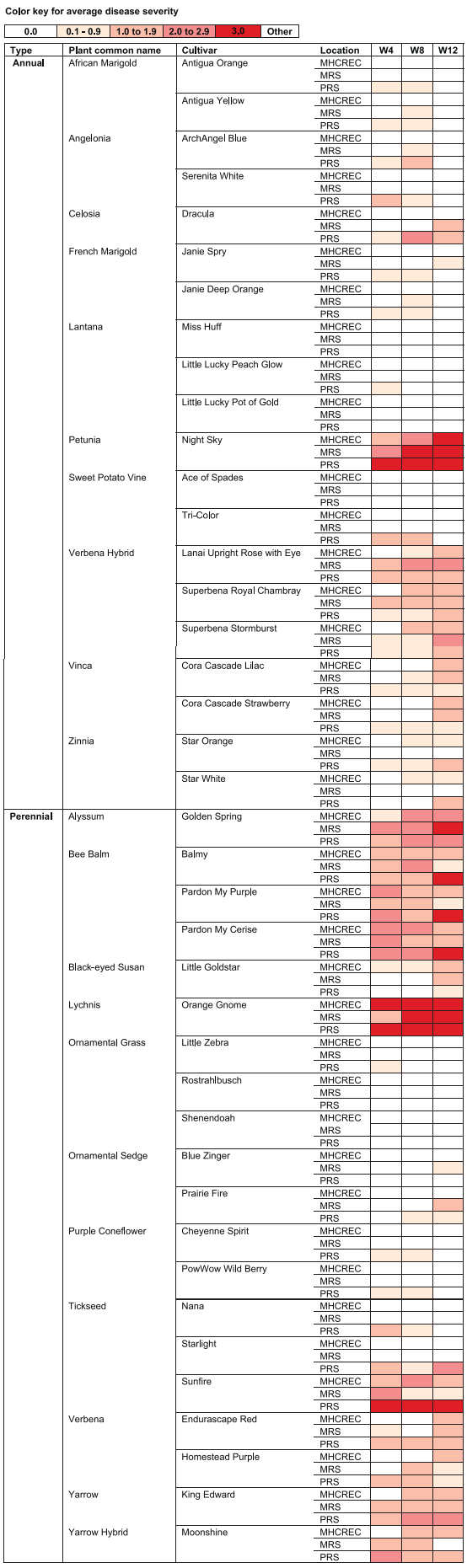


**Table 4.** ***Phytophthora*** **spp. baited from the soil and detected from plants in infested landscape beds in 2019 and 2020.**

| Year | Species[z] | Location[y] | Detected by soil baiting | Isolated from plants |
|---|---|---|---|---|
| 2019 | *P. nicotianae* | MHCREC | | |
| | | MRS | + | + |
| | | PRS | + | + |
| | *P. cryptogea* | MHCREC | | |
| | | MRS | + | + |
| | | PRS | | |
| | *P. drechsleri* | MHCREC | | + |
| | | MRS | + | + |
| | | PRS | + | + |
| | *P. tropicalis* | MHCREC | | |
| | | MRS | | |
| | | PRS | | |
| 2020 | *P. nicotianae* | MHCREC | + | + |
| | | MRS | | + |
| | | PRS | | + |
| | *P. cryptogea* | MHCREC | + | + |
| | | MRS | + | + |
| | | PRS | | + |
| | *P. drechsleri* | MHCREC | | |
| | | MRS | | |
| | | PRS | | |
| | *P. tropicalis* | MHCREC | | |
| | | MRS | | |
| | | PRS | | |

[z]Isolates identified as *P. cryptogea* belong to the species sensu lato.

[y]Locations 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. drecshleri*, 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

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**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.**

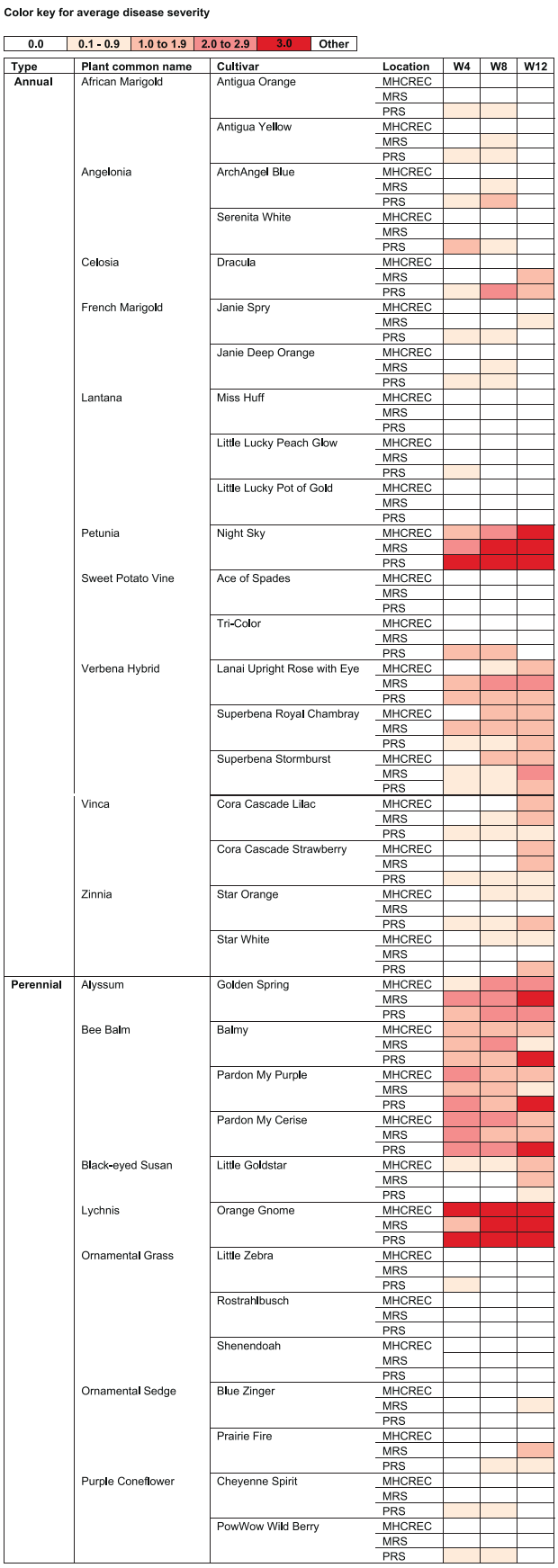


**Fig. 3. Average disease severity rating of annual and herbaceous perennial ornamental plants challenged by four species of *Phytophthora* in three landscape beds in North Carolina.**

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

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**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.**

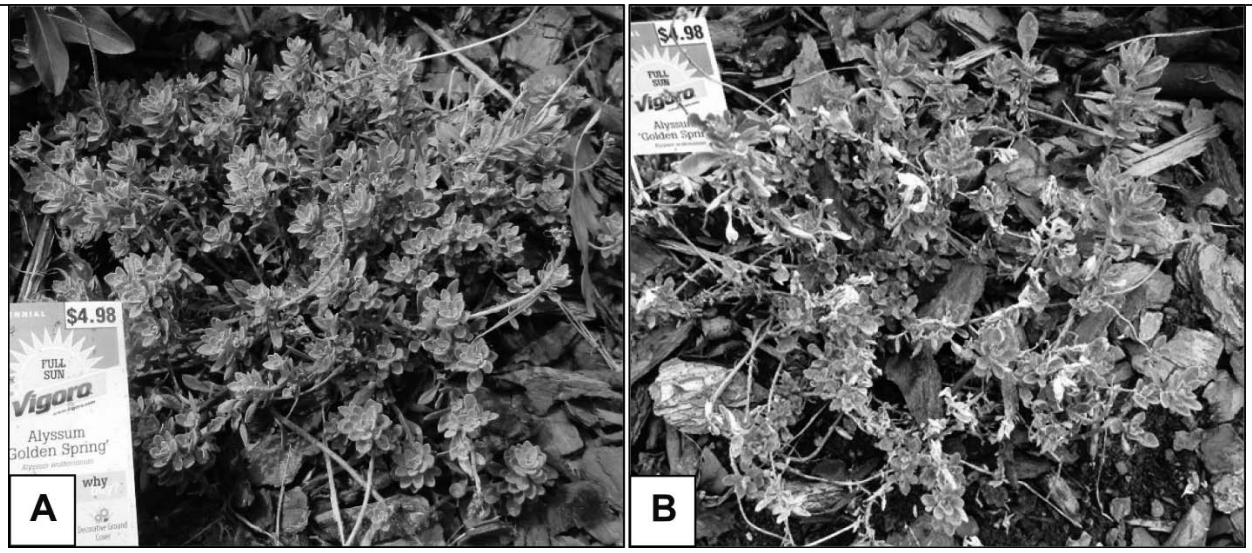


**Fig. 4. Alyssum 'Golden Spring' in 2019, A: healthy plant early in the growing season, B: symptoms of Phytophthora root rot appeared six weeks after planting in *Phytophthora*-infested landscape bed.**

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 MHCREC 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 MHCREC 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*

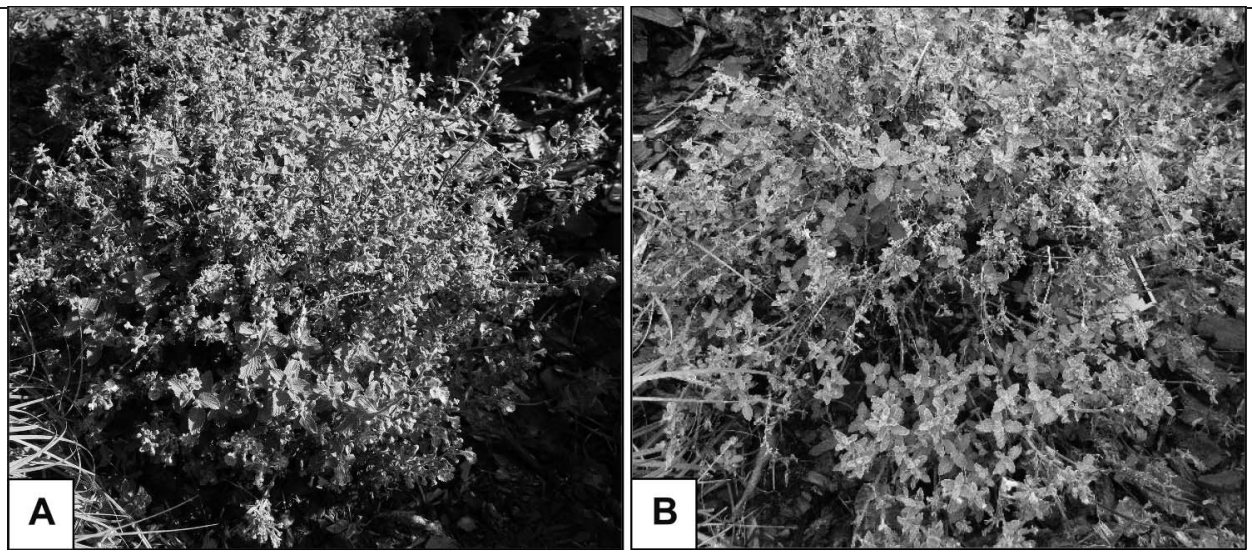


**Fig. 5. Catnip 'Junior Walker' in 2020, A: healthy plant early in the growing season, B: symptoms of Phytophthora root rot appeared eight weeks after planting in *Phytophthora*-infested landscape bed.**

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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