EVALUATION OF AERIAL BLIGHT CAUSED BY PHYTOPHTHORA ON CORA XDR AND ORNAMENTALS

by

ASHLEY DANIELLE TURNER

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MASTER OF SCIENCE

Chair of Committee,	Kevin Ong
Committee Members,	Becky Bowling
	Kimberly Cochran
	Won-Bo Shim
Interim Head of Department,	Won-Bo Shim

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ABSTRACT

Vinca plants such as Cora XDR are known to have high levels of resistance to *Phytophthora* aerial blight, and the results of this work show variable susceptibility to *Phytophthora nicotianae* in varieties and cultivars of vincas. The main objectives of this research project are the following: (i) characterize *Phytophthora* sp. 2238-2019 at the morphological and molecular level and differentiate this strain from other cultures isolated from ornamentals in Texas, (ii) conduct pathogenicity testing of *Phytophthora* isolates on Titan, Valiant and Cora XDR varieties, (iii) assess commonly applied fungicides to determine if recommended fungicide applications might be able to control *Phytophthora* sp. 2238-2019, and (iv) conduct a host plant range study. The overarching objective of this study was to characterize *Phytophthora* sp. 2238-2019 and observe its virulence on different varieties of vinca, as this isolate was shown to be pathogenic to a Cora XDR. In this study, six suspected *Phytophthora* species were obtained from the Texas Plant Disease Diagnostic Lab that had been previously isolated from *Antirrhinum majus, Catharanthus roseus (2007), Catharanthus roseus* (Cora XDR), *Dianthus caryophyllus, Catharanthus roseus (2004)* and *Viola tricolor hortensis*. Oomycete and fungal DNA was extracted from the isolates and amplified using PCR with ITS6-4 and FMPH-8 and FMPHy-10b primers. The obtained sequences were placed in NCBI BLASTn search. The resulting identity was compared to observed morphological characteristics to make identifications. Isolates were found to be unique *P. nicotianae*, with the exception of one isolate, *Phytophthora* sp. LP-2004. Pathogenicity testing on vinca showed Cora XDR vinca had a lower disease severity compared to other varieties when infected with *Phytophthora* sp. 2238, with the exception of Cora XDR 'Light Pink.' Fungicide efficacy testing revealed Stature™ (0.5x-4x), Subdue Maxx™ (0.5x-4x), and Heritage™ (4x) were found to restrict *Phytophthora* sp. 2238-

2019 colony to less than one half the diameter compared to the control plate of *Phytophthora* sp. 2238-2019. Lastly, it was found that English Lavender (*Lavandula angustifolia*), "Cora XDR Magenta Halo," and "Cora XDR Magenta," vincas were susceptible to *Phytophthora* sp. 2238-2019. Characterizing these *Phytophthora* isolates is important to diagnosticians and plant breeders of vinca for rapid and accurate diagnoses.

DEDICATION

I would like to dedicate this thesis to my family. Thank you for supporting my budding career in plant pathology. I appreciate your positivity and encouragement to pursue my passions.

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NOMENCLATURE

AUDPC	Area Under the Disease Progress Curve
Cora XDR	Cora® Extremely Disease Resistant
NCBI BLAST	National Center for Biotechnology Information Basic Local Alignment Search Tool
SC	Suspension Concentrate
WG	Water Dispersible Granule

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1. INTRODUCTION TO PHYTOPHTHORA AERIAL BLIGHT ON ORNAMENTALS

1.1 Background and Biology of *Phytophthora*

As of 2009, there were approximately 170 species and estimated 100-500 unknown *Phytophthora* species to exist (Brasier et al., 2009). Most species of *Phytophthora* are parasitic on various plant hosts. *Phytophthora* infections can cause crown rot and root rot which eventually lead to plant leaves wilting from lack of water and nutrient distribution, eventually leading to plant death. There are *Phytophthora* species that can also cause aerial blight due to the pathogen inoculum being splashed on foliar tissues when in high humidity and poor air circulation (Erwin and Ribeiro, 1996).

Phytophthora are not true fungi, but are classified as a water mold (Oomycota) (Rossman and Palm, 2006). Oomycota were previously considered fungi, but unlike fungi, Oomycota do not have chitin in the cell walls. Additionally, oomycetes are unique in that they can produce oospores, have a diploid nuclear state of vegetative mycelium, mitochondria with tubular cristae, and are able to produce two types of flagella on zoospores.

Phytophthora have coenocytic, hyaline mycelium with no or few septa. *Phytophthora* hyphae (that make up the mycelium) vary in diameter from 5 to 8 μm and can be smooth, swollen, nodose, or tuberculate forms (Erwin and Ribeiro, 1996). The sporangia produced appear translucent to light yellow under light microscopy, and can germinate in aqueous solutions or on agar by the production of germ tubes. The sporangia can detach, become airborne and release motile zoospores that can swim for hours, and the zoospores form a cyst after swimming to a suitable infection site by recognizing plant tissue exudates (Hinch and Weste, 1979). Additionally, zoospores can encyst after agitation in culture (Bartnicki-Garcia, 1973). After

recognizing water or certain plant extracts, the cyst produces germ tubes and develops hyphae to colonize host tissues under favorable conditions (Drechsler, 1930; Blackwell, 1949). Alternatively, the cyst can overwinter in infected plant debris or soil (Erwin and Ribeiro, 1996). Chlamydospores (asexual survival spores) may form terminal or intercalary (Erwin and Ribeiro, 1996).

Phytophthora sexual structures include antheridia and oogonia which can vary morphologically among species (Erwin and Ribeiro, 1996). Two mating types are required to be present together in the same plant tissue or substrate for sexual fertilization to occur and produce oospores. Following fertilization, sexual oospores form within the oogonium by a nucleus from the antheridium. An oospore that forms within the oogonium has a thick (0.5-6.0μm) inner wall (Walterhouse, 1963). The diploid oospore produces germ tubes under cool, wet conditions where sporangia can form.

Phytophthora species can be homothallic (*P. cactorum*, *P. megasperma* and *P. citricola*) or heterothallic (*P. infestans*, *P. palmivora* and *P. cinnamomi*) (Ko, 1988). Oospores in heterothallic species can form when A1 and A2 mating types grow together, and homothallic species can reproduce without the interaction of two different thalli. The advantage to heterothallic species is to allow for the recombination of genetic material, which, in turn, can result in production of different races or more virulent pathotypes (Romero and Erwin, 1969; Tooley et al., 1986; Spielman et al., 1989, 1990). Production of a pheromone or hormone-like substance by the opposite mating type can also produce oospores without hyphal contact in nearby compatible mating types (Ko, 1978).

1.2 Differentiation of *Phytophthora* Species

Phytophthora can be differentiated morphologically by observing the sporangia, hyphal swellings, chlamydospores, and oogonia/antheridia (Blackwell, 1949). In some species, new sporangiophores emerge through the base of the old sporangium from which uninucleate zoospores have been released. In other species, new sporangiophores arise just beneath the bases of the old sporangia and can produce more sporangia. Some *Phytophthora* species have distinct sporangia shapes, but sporangia size within *Phytophthora* species has been found to narrowly range depending on the host, and whether or not the sporangia are papillated (Blackwell, 1949; Waterhouse, 1963; Newhook et al., 1978; Stamps et al., 1990). The location and shape of the antheridium can vary by species (amphigynous or paragynous) (Blackwell, 1949). Location of sporangia is also another distinguishing factor in differentiating species. Erwin and Ribeiro (1996) summarized information from Waterhouse (1963), Newhook et al., (1978), and Stamps et al. (1990), and categorized the *Phytophthora* species into six groups for convenience of identification. *P. nicotianae* is Group II, and *P. drechsleri* is Group VI (Erwin and Ribeiro, 1996) (Table 1).

Table 1 *Phytophthora* Group II and VI Characteristics

	Sporangia	Oospores	Antheridia
Group II	Conspicuously papillate and show apical thickening of sporangium	Usually form when A1 and A2 mating types are paired	Amphigynous
Group VI	Non-papillate and proliferate both externally and internally	Not found	All or mostly amphigynous and may be either hetero-or homothallic

1.3 *P. nicotianae*

Originally thought of as separate species, *P. parasitica* and *P. nicotianae* are now known to be the same (Erwin and Ribeiro, 1996). *P. nicotianae* is the prevailing name (Ho and Jong, 1989; Erwin and Ribeiro, 1996). *P. nicotianae* has a wide host range for aerial blight, including but not limited to: vinca (Gill et al., 1977), poinsettia (*Euphorbia pulcherrima*) (Uchida and Aragaki 1979; Engelhard and Ploetz 1979), iris (*Iris* sp.) (Dastur, 1935), and orchid (*Paphiopedilum sp.*) (Uchida and Aragaki, 1991). *P. nicotianae* is one of the common aerial blight *Phytophthora* species attacking ornamental plants (Olson et al., 2011). *P. drechsleri* is another oomycete that can cause foliar blight on a variety of ornamental plants (Lamour et al., 2003).

1.3.1 P. nicotianae Morphology Under Microscopy and Growth Characteristics

P. nicotianae sporangia are ellipsoid, noncaducous, ovoid, pyriform, obpyriform to spherical with prominent papilla, and are known to have two papillae occur on a single sporangium (Erwin and Ribeiro, 1996). The papillate sporangia can be produced alone, or in a loose sympodium on stalks that average 375 μm in length, and range from 100 to 595 μm in length (Thomson and Hine, 1972). The sporangia average 40.18 x 28.53 μm width (ranging f11-60 μm) and 1.34 μm length (ranging 1.1-1.7 μm) (Thomson and Hine, 1972). Reticulate ridges on the surfaces of sporangia can be observed with electron and light microscopy (Khan et al., 1988). Hall (1993) reported hyphal swellings, and only 50% of 81 isolates produced chlamydospores abundantly. Chlamydospores can be terminal or intercalary with an average diameter of 33 μm (ranging 13-60 μm). Others have reported a diameter range of 20 to 60 μm (Dastur, 1913). Most isolates are heterothallic, but some can form oogonia and oospores in single

culture when inoculum is transferred from old cultures (Brasier, 1972; Tsao et al., 1985). *Phytophthora* isolated from vinca have been reported as homothallic (Schubert and Leahy, 1989). Antheridia are amphigynous and spherical or oval. The oogonia are smooth and spherical and average 26.8 μm in diameter (ranging 15-64 μm) (Hall, 1993). Oospores have been reported to average 22.6 μm (Hall, 1993), and others have reported the oospores range from 13 to 24 μm (Dastur, 1913). The minimum growth temperature for growth is 5 to 7 °C, and the maximum is 37°C. Optimum growth temperature range is 27- 32°C (Hall, 1993). Colony morphology on potato dextrose agar (PDA) are usually weblike, but on V8 agar, they can be fluffy (Erwin and Ribeiro, 1996).

1.4 Molecular and Serological Diagnostics of *Phytophthora* Species

An initial diagnosis of *Phytophthora* can be made in the field by observing symptoms of the disease followed by a positive result with a lateral flow test. If the lateral flow test is negative or not used, further analysis of host tissue by microscopic examination, culturing, and/or Enzyme Linked Immunosorbent Assay (ELISA) may be necessary to accurately confirm *Phytophthora* (Bulluck, 2006). Other common methods can be explored such as polymerase chain reaction (PCR) and deoxyribose nucleic acid (DNA) sequencing.

1.4.1 Lateral Flow Immunoassays

Lateral Flow Immunoassays (LFIA) can be used for on-site detection of plant pathogens. The device involves unidirectional flow of particles coated with specific antibodies along a nitrocellulose membrane (Hussain and Singh, 2016). If the pathogen is detected, (antibodies on the membrane bind to the antigens producing a line in a specific location. For a positive result,

both the test line and the control line must appear (Dank and Barker, 2000). There are also competitive- and inhibition- type LFIAs. LFIAs cannot provide quantification of a pathogen detected.

1.4.2 ELISA

Enzyme-linked immunosorbent assay (ELISA) is a commonly used method to detect major plant pathogens (Singh and Singh, 1995; Fang and Ramasamy, 2015). The antibodies used in ELISA to detect the pathogen have not been developed to distinguish among *Phytophthora* species (Hussain and Singh, 2016). ELISA can be used for quantitative or qualitative detection of a pathogen. Other variations of ELISA are applicable including double antibody sandwich ELISA (DAS-ELISA) (Amouzou et al., 1988).

1.4.3 PCR to Identify Phytophthora Species

Polymerase Chain Reaction (PCR) is used to identify if *Phytophthora* is present, and can be used to identify only *Phytophthora* if the primers are precise enough (Osterbauer and Trippe, 2005). There are many types of PCR that have be used for *Phytophthora* such as Nested PCR (HueiLing et al., 2006), Multiplex PCR (Li et al., 2011), Real-time PCR (Ippolito et al., 2002) and PCR-ELISA (Bailey et al., 2002). Nested PCR increases the specificity of DNA amplification by reducing non-specific amplification of DNA. This is done by having two sets of primers (outer and inner pair) for a single locus and two successive PCRs. A disadvantage of this technique is that an additional set of primers after the first run increases the chance of nonspecific contamination (Hurtado et al., 2001). Multiplex PCR is used to amplify multiple targets in a single PCR experiment. Multiple primer pairs can be used to increase diagnostic

capacity of PCR. Real-time PCR measures PCR amplification as it occurs and increases dynamic range of detection (Mumford et al., 2004; Ratti et al., 2004).

1.4.4 DNA Sequencing to Identify Phytophthora Species

A common approach to differentiating plant pathogenic fungal species is sequencing analysis of the internal transcribed spacer (ITS) region in the ribosomal (rRNA) genes (Hussain and Singh, 2016). In *Phytophthora*, the ITS regions can be informative enough to distinguish most *Phytophthora* species (Abad et al., 2019; Cooke et al., 2000; Grünwald et al., 2011). Grünwald et al. (2011) described the use of ITS and cytochrome c oxidase (Cox) spacer regions to identify *Phytophthora* species. Primers ITS6 and ITS4 can be used for amplification of the *Phytophthora* ITS region (White et al., 1990; Cooke and Duncan, 1997; Cooke et al., 2000). However, the amplification of ITS regions is not specific to *Phytophthora* and will amplify other species including *Pythium* and general fungal species (Grünwald et al., 2011).

Primers such as FMPHy-8b and FMPHy-10b are specific for amplification of *Phytophthora* spp. and will not amplify related genera (Grünwald et al., 2011). The cox genes are on the mitochondrial chromosome in *Phytophthora* (Martin et al., 2007). These genes can be used for development of species-specific markers and identification of species (Martin et al., 2004; Tooley et al., 2006). Mitochondrial genes can also be used to identify and determine phylogenetic analysis of Phytophthora (Mart et al., 2004; Tooley et al., 2006; Grünwald et al., 2011). Of the mitochondrial genes (TrnG-TrnY region, Atp9-Nad9, Cox2-Cox-1, and TrnY-Rns), that have been analyzed by Schena (2006), the Cox2-Cox-1 is the most appropriate for identification and phylogenetic studies (Hussain and Singh, 2016).

Intergenic spacer region 1 (IGS1) and intergenic spacer region 2 (IGS2) are potential alternatives to ITS regions. IGS2 was chosen to develop specific primers to detect *P. medicaginis* over ITS regions which could not differentiate *P. medicaginis* from closely related species (Liew et al., 1991). Nuclear-encoded ribosomal RNA genes can be used to design specific primers such as Ypt1 gene (White et al., 1990).

1.4.5 DNA Probes and DNA Microarray

A DNA probe is an artificially produced segment of DNA complementary to the desired gene that can be used to detect the presence of a specific set of genes. DNA probes were applied for fungal diagnostics before the widespread use of PCR such as with *P. nicotianae* detection from soil and host tissues in 1989 (Goodwin et al., 1989). DNA Microarray can be used to detect and identify multiple pathogens simultaneously to species and intra-species levels (Bodross et al., 2004; Lievens and Thomma 2005). DNA Microarrays are microscope slides that have spots of a known DNA sequence or gene printed on them; the DNA molecules on each slide act as DNA probes to detect gene expression (Bumgamer, 2013).

1.5 *Phytophthora* Aerial Blight of Vinca

Vinca (*Catharanthus roseus*), is originally from Madagascar where it prefers hot, bright sunshine and well-drained soils. Under high humidity vinca can become stressed (Mills and Jones, 1996). Environmental stress on plants can make them more vulnerable to infection and subsequent disease development (Velásquez et al., 2018). Initially, vinca breeding efforts were primarily focused on floral characteristics (e.g., color) rather than developing cultivars with improved resistance to biotic and abiotic stresses. As a result, many cultivars exhibit weak roots, and

consequently require supplemental inputs to survive production environments (UGA Extension, 2009).

Symptoms of aerial blight on vinca could develop rapidly in humid conditions and include dark brown streaks on stems, wilting of the leaves, water soaked lesions at the base of the wilted shoots, but the roots are typically not symptomatic. Aerial blight infected plants can die within 1-2 weeks post-inoculation (Lamour et al., 2003). Nirvana and Cora lines were introduced in 2007 with promising results of broad spectrum resistance to *P. nicotianae* (Thomas, 2009; Beckerman and Lerner, 2009). Cora XDR, released in 2019, offers a variety of colors and boasts high resistance to ten of the most virulent *P. nicotianae* (Thomas, 2009; Syngenta, 2022). Additionally, Valiant varieties offer intermediate resistance to aerial *Phytophthora* which means these varieties can restrict the growth and/or damage caused by a pathogen, but may exhibit a greater range of symptoms or damage compared to high resistant varieties (Agrios, 2005; PanAmerican, 2021).

To control aerial *Phytophthora* along with planting resistant varieties, annual vinca should not be planted until early summer (Mills and Jones, 1996). Mulch is recommended around plants to help prevent the splashing of the spores up on the plants. It's recommended that plants be watered from the bottom using drip irrigation or a bubbler emitter on the sprinkler system. Other control measures include disinfecting gardening tools with steam or dipping them in formalin, keeping the hose nozzle off the ground as having the nozzle on the ground could spread inoculum, and using new or sterilized containers when seeding in the greenhouse (Erwin and Ribeiro, 1996). Lastly, there are several fungicides recommended on the market to control aerial blight on ornamentals and more detail is described below.

1.6 Fungicide Applications

Fungicides labeled for control of *Phytophthora* aerial blight on ornamentals include but are not limited to: Pageant Intrinsic™ (boscalid and pyraclostrobin; FRAC groups 7 and 11, respectively), Subdue Maxx™(mefenoxam, FRAC group 4), Segway™ (cyazofamid, FRAC group 21), Stature™ (dimethomorph, FRAC group 40), and Heritage™ (azoxystrobin, FRAC group 11) (Table 2).

Table 2 Fungicides Tested Against *Phytophthora* sp. 2238-2019

Brand Name	Active Ingredient	FRAC Group	Application Rate	Frequency of Application	Reference
Heritage™	Azoxystrobin	11	4oz/100gal	7-28 days	Syngenta, 2016
Pageant Intrinsic™	Boscalid, Pyraclostrobin	7, 11	18oz/100gal	7-10 days	BASF, 2015
Segway™	Cyazofamid	21	4oz/100gal	14-28 days	FMC, 2010
Stature™ DM	Dimethomorph	40	12.8oz/100gal	10-14 days	BASF, 2006
Subdue Maxx™	Mefenoxam	4	0.5-1.0fl oz/100gal	Once	Syngenta, 2017

As of July 2021, there were no publications assessing resistance to fungicides of *P. nicotianae* strains isolated from Cora XDR plants. More studies are needed to assess the current efficacy of these fungicides. The fungicides tested in this work have different modes of action (Table 3).

Table 3 Fungicides Tested Against *Phytophthora* sp. 2238-2019: Modes of Action	
Active Ingredient(s)	Mode(s) of Action
Boscalid	Inhibits the electron transport chain by inhibiting succinate dehydrogenase (Stammler et al., 2007).
Pyraclostrobin	Inhibits the electron transport chain by stopping the electron transfer at the quinol oxidation site in the cytochrome-bc-1 complex in the pathogen (Kanungo and Joshi, 2014).
Azoxystrobin	Inhibits the electron transport chain by stopping the electron transfer at the quinone site in the cytochrome-bc-1 complex in the pathogen (Grasso et al., 2006).
Dimethomorph	Targets cell wall synthesis in oomycetes (Cohen et al., 1995).
Mefenoxam	Inhibits rRNA biosynthesis, specifically the polymerase complex I in the pathogen (Gisi and Sierotzki, 2008).
Cyazofamid	Targets the binding site of cytochrome-bc-1 (Derpmann, 2021).

Previous testing with Pageant Intrinsic™ 38WG (12oz/100gal) in 2012 showed Pageant Intrinsic™ provided statistically significant lower average aerial blight symptoms when compared to disease on untreated control plants caused by *P. nicotianae* on *C. roseus* Titan 'Blush' (Jeffers et al., 2012). The current recommended application rate of Pageant Instrinsic™ is 18oz/100gal (BASF, 2015).

Previous work by Hagan et al., (1999) applied Heritage™ 50WG to *C. roseus* 'Pacifica Punch' at various application rates for *P. nicotianae* shoot blight. Plants drenched (0.7oz/1000 sq ft) had statistically higher survival rates when compared to control plants and were similar to

survival rates of other drench application rates of this work (Hagan et al., 1999). Heritage™ 50WG at 3.0oz/100gal completely prevented plant death in 'Rose Cooler' *C. roseus* (Hausbeck et al., 2003). 'Cooler Pink' vinca was subjected to a combination of Heritage™ 50WG (2 oz/100gal) and Subdue Maxx 2MEC (1oz/100gal) and these treated plants exhibited the lowest *P. nicotianae* aerial blight disease rating on *C. roseus* 'Cooler Pink' (Hausbeck and Webster, 2006). (This effect was not statistically the lowest aerial blight disease rating as all the treated groups were statistically similar to each other with the exception of the controls). In another study, Heritage™ 50WG (0.9 oz/100gal) treated plants exhibited lower aerial blight disease severity, but not statistically significant when compared to non-treated, inoculated control plants of *C. roseus* Titan 'Blush' (Jeffers et al., 2012). Current recommended rates of Heritage™ for *Phytophthora* on ornamentals is 1-4oz every 7-28 days while symptoms persist (Syngenta, 2016). More testing is needed to determine whether Heritage™ still reduces symptoms of aerial blight as it did in 1999, 2003, and 2009 and if the aerial blight severity shown in 2012 is variety specific.

Hausbeck et al., (2003) found when *C. roseus* 'Cooler Grape' plants were treated with Subdue Maxx™ (0.5 and 1.0oz/100gal) and Stature DM™ 50WP (12.8oz/100gal), the disease severity was significantly lower for *P. nicotianae* treated plants compared to untreated inoculated control plants. Stature DM™ 50WP (12.8fl oz/100gal) and Subdue Maxx™ 21.EC (1.0z/100gal) prevented death in *C. roseus* 'Rose Cooler' *P. nicotianae* treated plants (Hausbeck et al., 2003). 'Polka Dot Pacific' vinca plants inoculated with *P. nicotianae* and treated with Stature DM™ (12.8oz/100gal) exhibited statistically lower aerial blight disease severity than untreated inoculated plants (Hausbeck and Harlan, 2005). Steddom and Kimberly (2009) found Stature DM™ (3.2-12.8oz/100gal) and Stature SC™ (6.1-12.3fl oz/100gal) significantly reduced

foliar blight caused by *P. nicotianae* on 'First Kiss Pure White' and 'First Kiss Raspberry' when compared to the untreated, inoculated control plants. Additionally, Subdue Maxx™ (1fl oz/100gal) was found to significantly reduce disease symptoms when compared to untreated, inoculated control plants in this work (Steddom and Kimberly, 2009). Jeffers et al. (2012), found Stature™ 4.2SC (6 fl oz/100gal) treated plants showed lower average foliar blight disease progress but this effect was not statistically significant when compared to control inoculated plants treated with *P. nicotianae* on *C. roseus* Titan 'Blush.' Subdue Maxx™ 2ME (1fl oz/100gal) treated plants showed statistically lower average foliar blight disease progress when compared to untreated, inoculated control plants on Titan 'Blush' in this same work (Jeffers et al., 2012). The current application for Stature™ DM against aerial *Phythopthora* is 12.8oz/100gal (BASF, 2006) and 12.25fl oz/100gal for Stature™ SC (BASF, 2011). Subdue Maxx™ is recommended at 0.5-1.0fl oz/100gal (Syngenta, 2017). Based on these works, Subdue™ and Stature™ continue to decrease *Phytophthora* aerial blight disease symptoms, but more up to date studies are needed to confirm performance.

In 2005-2006, *C. roseus* plants were sprayed with *P. nicotianae* (Palmer and Vea, 2010). and it was found Segway™ 400SC treated plants showed less aerial blight symptoms on 'Polka Dot Pacific' when compared to untreated, inoculated control plants but this effect was not statistically significant. Segway™ 400SC (3-6oz/100 gal) and Stature DM™ (6.4oz/100gal) treated plants showed no foliar blight symptoms on 'Pink Cooler' in this same work (Palmer and Vea, 2010). Segway™ is recommended at 3-6fl oz against foliar blight on ornamentals (FMC, 2010).

1.7 Research Focus and Objectives

Cora XDR are bred to be "extremely disease resistant" to *Phytophthora* aerial blight (Syngenta, 2022). Other varieties of vinca may have no resistance or have intermediate resistance like Valiant (PanAmerican, 2017). There are no current publications on Cora XDR assessing what cultivars are most susceptible to aerial blight. In 2017, there was an observation of aerial blight of vinca from *P. nicotianae*, but this study did not use Cora varieties or Cora XDR which are known to be resistant to *Phytophthora* aerial blight (Lin et al., 2017). A better understanding of which varieties are susceptible can guide breeding efforts in the right direction to create more resistant cultivars against *Phytophthora*. Furthermore, it can provide valuable information to the horticulture community as to which plants should be avoided in areas known to be problematic for aerial blight associated with *Phytophthora*. This study used *Phytophthora* isolated from: Cora XDR 'Light Pink' in 2019 (*Catharanthus roseus*)[2238-2019], periwinkle in 2004 (*Vinca minor*)[LP-2004], snapdragon in 2007 (*Antirrhinum majus*)[1756-2007], pansy in 2007 (*Viola tricolor hortensis*)[1446-2007], Dianthus (*Dianthus caryophyllus*)[1422-2007] and another vinca in 2007 (*Catharanthus roseus*)[845-2007].

The main objectives of this research project are listed below.

1.7.1 Objectives:

a. Morphological and molecular characterization of *Phytophthora* sp. 2238-2019 isolated from Cora XDR 'Light Pink' in Texas and differentiate this strain from other cultures isolated from ornamentals in Texas.

b. Pathogenicity testing: observe disease instance and severity under greenhouse conditions of these *Phytophthora* strains on Titan, Valiant and Cora XDR varieties. The purpose of which is to see if isolates other than *Phytophthora* sp. 2238-2019 can infect vinca plants.

c. Fungicide efficacy: assess commonly applied fungicides to determine if recommended fungicide application rate might be able to control *Phytophthora* sp. 2238-2019 colony growth.

d. Host plant range: observe disease incidence of common ornamental plants known to be resistant and susceptible to *Phytophthora* to assess whether additional plants are susceptible to *Phytophthora* sp. 2238-2019. Disease incidence should be recorded against *Phytophthora* sp. 2238-2019.

2. MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF PHYTOPHTHORA ISOLATES

2.1 Overview

Six suspected *Phytophthora* species were obtained from the Texas Plant Disease Diagnostic Lab in College Station, TX that had been isolated from *Antirrhinum majus* (2007), *Catharanthus roseus* (2007), *Catharanthus roseus* (Cora XDR) (2019), *Dianthus caryophyllus* (2007), *Vinca minor* (2004), and *Viola tricolor hortensis* (2007). It was hypothesized that all cultures obtained from these hosts are distinct *Phytophthora nicotianae* isolates. Oomycete and fungal DNA was extracted from the isolates and amplified using PCR with ITS6-4 and FMPH-8/FMPHy-10b primers. The amplified DNA obtained from PCR was sequenced and consensus sequences were generated. These sequences were placed in NCBI BLASTn search and PhytophtoraID.org. The result was compared to observed morphological characteristics such as size and shape of sporangia, chlamydospores, oogonia/antheridia, and colony morphology to make identifications. Isolates were found to be unique *P. nicotianae* with the exception of one isolate (LP-2004) which was more closely related to *P. capsici*. The main focus of this study was to characterize *Phytophthora* sp. 2238-2019, an isolate newly recovered from diseased Cora XDR 'Light Pink,' a resistant vinca.

2.2 Introduction

New *Phytophthora* species and hybrids continue to be discovered such as *Phytophthora obscura* in 2012 and six new *Phytophthora* species including two hybrids in 2017 (Grünwald et al., 2012 and Jung et al., 2017, respectively). Identifying and characterizing *Phytophthora* isolates can aid diagnosticians in speedy diagnoses and provide valuable information to researchers for potential new species. *Phytophthora* isolates were identified by combining the information obtained from amplifying oomycete and fungal DNA with FMPH and ITS primer sets combined with morphological characteristics obtained. The objective of this study was to document differences in morphological and molecular characteristics of *Phytophthora* isolates from ornamentals (Table 4), with a focus on *Phytophthora* sp. 2238-2019.

Table 4 *Phytophthora* Isolates, Host Plant and Isolate Reference

Host	Isolate Reference
Antirrhinum majus	*Phytophthora* sp. 1756-2007
Catharanthus roseus	*Phytophthora* sp. 845-2007
Catharanthus roseus, Cora XDR	*Phytophthora* sp. 2238-2019
Dianthus caryophyllus	*Phytophthora* sp. 1422-2007
Vinca minor	*Phytophthora* sp. LP-2004
Viola tricolor hortensis	*Phytophthora* sp. 1446-2007

2.3 Materials and Methods

2.3.1 Isolation of Phytophthora Species

The Texas Plant Disease Diagnostic Lab in College Station, TX provided isolates from vinca and other ornamentals. All isolates with the exception of *Phytophthora* sp. 2238-2019 were previously stored at room temperature (22-25°C) in mineral oil. *Phytophthora* sp. 2238-2019 was recently isolated and was taken directly from V8 plates.

2.3.2 Observation of Characteristics and Colony Morphology

All *Phytophthora* isolates were plated on hymexazol (Hx) agar which is a semi selective media (Tsao and Guy, 1977) for seven days at room temperature (22-25°C) to separate the cultures from possible *Pythium* cohabitation and aid in purifying the cultures. Once cultures were identified as pure, they were then plated on V8 agar, potato dextrose agar (PDA), and corn meal agar (CMA) by 6mm agar plugs to observe morphological characteristics (Iacob, 2016). Several media were used for observations as microorganisms can vary in their morphological characteristics depending on the culture media (Catón, 2017). Isolates were incubated at 25°C for seven days for ample fungal colonies (Matheron and Matejka, 1992). To obtain ample sporangia, V8 agar plugs were placed in sterile petri dishes and with 20% nonsterile soil extract solution (NSES) under LED light for five days at 22-25°C to produce mature structures (Leesutthiphonchai and Judelson, 2019). Some *Phytophthora* oospores were not observed until after 30 days under LED light. Each isolate was morphologically characterized by measuring the size, shape and arrangement of 100 sporangia and chlamydospores, 50 oospores/antheridium size and shape, general hyphal characteristics and size, and appearance of colony morphology on PDA and V8. Measurements were taken using a compound microscope at 400x magnification.

2.3.3 DNA Extraction and Amplification

Oomycete and fungal DNA was extracted using Zymo Fungal/Bacterial kit. ITS6, ITS4, FMPh-8, and FMPhy-10b (Cox gene) primers were used to amplify isolates (Table 5).

Table 5 Primer Sequences of ITS and Cox Gene Primers Used to Amplify *Phytophthora* Isolates

Primer	Sequence
ITS4	5'-TCCTCCGCTTATTGATATGC-3'
ITS6	5'-GAAGGTGAAGTCGTAACAAGG-3'
FMPhy-10b	5'-GCAAAAGCACTAAAAATTAAATATAA3'
FMPh-8	5'-AAGGTGTTTTTTATGGACAATGTA-3'

Fusarium proliferatum and *Bipolaris* sp. isolated from watermelon were used as outliers. *Phytophthora* isolates using FMPH-8/FMPHy-10b and ITS6/4 primers were expected to produce bands at 460-500bp and 862-941bp, respectively (Grünwald et al., 2011). The list of the primer sequences used in this study are shown above (Table 5). These primers were used during conventional polymerase chain reaction (PCR) with a thermocycler under the parameters listed in Grünwald et al., (2011) with the exception of step three for FMPHy-10b and FMPH-8 primers which was performed at 59.5°C instead of 65.5°C. Grünwald et al., (2011) described decreasing the annealing temperature by 1°C until optimal temperature is met. A temperature gradient on the thermocycler was used to reach an optional annealing temperature. An optimal anneal temperature would produce a brighter band on the gel. DNA was visualized using gel electrophoresis at 45V on a 2% agarose gel for 1-hr run time. PCR products were sent to Eton Biosciences in San Diego, CA for Sanger sequencing in both directions.

2.3.4 Generation of Consensus Sequences and Phylogenetic Tree

Sequence files were uploaded into BioEdit to generate consensus sequences (Hall, 1999). A reverse sequence was generated and aligned with the forward sequence (Antonis et al., 2003). The overhangs, which are unpaired nucleotides in the DNA sequence (Schmieder, 2010) were trimmed and the consensus sequences were placed into phytophthoraID.org and NCBI database using BLASTn algorithm to obtain a *Phytophthora* species identification. A Maximum

Likelihood phylogenetic tree with bootstrap confidence values was generated in MEGA 11 using the Hasegawa–Kishono–Yano model (Tamura, Stecher, Kumar, 2021). The tree was constructed using ITS and Cox gene sequences in MEGA 11 for all isolates except for *Pythium megacarpum, Fusarium proliferatum* and *Biopolaris sp.* sequences which used ITS gene sequence only.

2.4 Results

Morphological and Molecular Characterization

All isolates had successful amplification with *Phytophthora* specific primers FMPH-8 and FMPHy-10b with bands in the expected location of approximately 460-500 bp (Grünwald et al., 2011). *Phytophthora* sp. 845-2007 and *Phytophthora* sp. LP-2004 produced slightly larger (approx. 25-50 bp) bands than other isolates (Figure 1). Amplification with ITS primers showed bands in the expected location of approx. 862-941bp. Isolate LP-2004 produced bands slightly smaller (approx. 25-50 bp) than others using ITS primers (Figure 1).

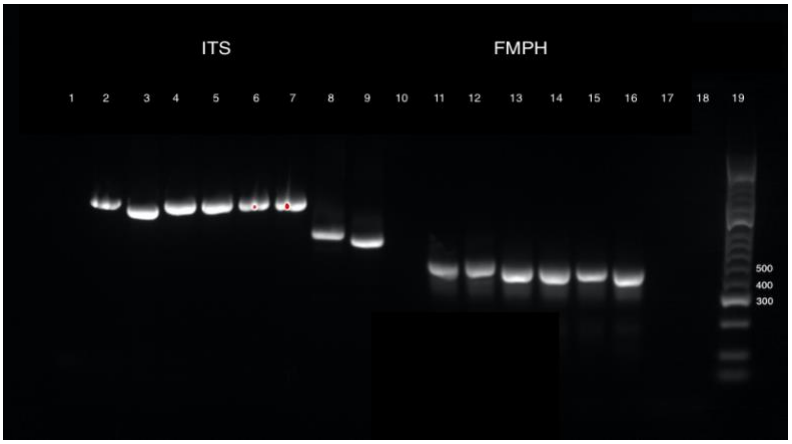

Figure 1 Amplified Products of ITS and Cox Gene Primers with *Phytophthora, Bipolaris* and *Fusarium* Isolates. DNA ladder (lane 19), **ITS6-4 amplified products** (lane 2: *Phytophthora* sp. 845-2007, lane 3: *Phytophthora* sp. LP-2004, lane 4: *Phytophthora* sp. 1446-2007, lane 5: *Phytophthora* sp. 1756-2007, lane 6: *Phytophthora* sp. 1422-2007, lane 7: *Phytophthora* sp. 2238-2019), **FMPH amplified products** (lane 11: *Phytophthora* sp. 845-2007, lane 12: *Phytophthora* sp. LP-2004, lane 13: *Phytophthora* sp. 1446-2007, lane 14: *Phytophthora* sp. 1756-2007, lane 15: *Phytophthora* sp. 1422-2007, lane 16: *Phytophthora* sp. 2238-2019). Negative control was water (lane 1 and lane 10). *Fusarium* and *Bipolaris* (lanes 8, 9, 17 and 18) respectively.

Each isolate was amplified by the primer sets and *Fusarium* and *Bipolaris* were not amplified by *Phytophthora* specific primers on the right (Figure 1). Fungi used as known controls did not correspond with any sequences in NCBI BLAST and PhytophthoraID.org databases. In addition to the sequence analysis for identification, morphological characteristics were observed to further confirm the isolated species identities.

2.4.1 Phytophthora sp. 2238-2019

Phytophthora sp. 2238 -2019 produced mostly persistent sporangia but some were caduceus. Immature sporangia at 200x total magnification and mature, irregular shaped sporangia at 400x (Figure 2). Ovoid to irregular sporangia were both papillated and non-papillated, irregularly branched and ranged from 37-40µm x 20-30µm (averaged 38.2 x 25.5µm) in diameter (Figure 2).

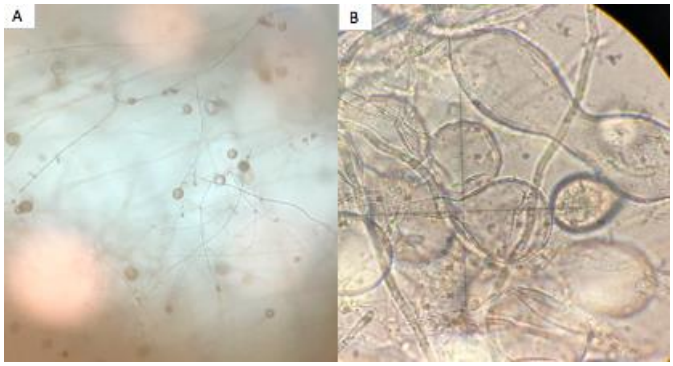

Figure 2 *Phytophthora* sp. 2238-2019 Sporangia. A) 200x sporangia B) 400x sporangia produced on V8 agar plugs after five days of growth at 25°C flooded with 20% NSES (non-sterile soil extract solution) under LED light. Viewed with a compound microscope.

Few chlamydospores were present, but they were terminal and ranged 25-36µm averaged 32.4µm (Figure 3). Smooth oospores were present on flooded V8 agar disks after 30 days (Figure 3).

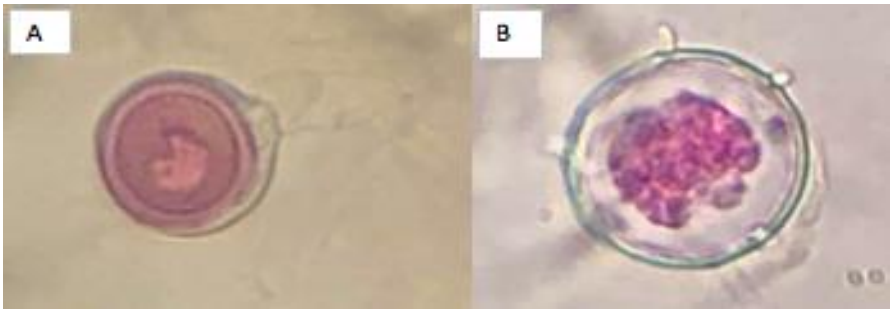

Figure 3 *Phytophthora* sp. 2238-2019 Oogonia and Chlamydospores A) Oogonia at 400x from V8 agar plugs flooded with 20% NSES incubated at 22-25°C under LED light for 30 days and B) Chlamydospore from CM agar at 400x magnification using compound microscope and dyed with 0.05 % acid fuchsin solution.

Oogonia ranged 20-22 µm and averaged 21.8µm. Single hyphal swellings were found on V8 agar with non-septated hyphae (2µm wide). When sequences were placed into the BLAST database from NCBI, the sequences showed 95.66% percent identity as *P. nicotianae*. PhytophthoraID.org identified the consensus sequence with 95% to *P. nicotianae* with Cox gene primers. With ITS6/4 primers, BLAST resulted in 88% identity to *P. nicotianae* from PhytophthoraID.org.

2.4.2 Phytophthora sp. LP-2004

Phytophthora sp. LP-2004 hyphae were found to be 3-4µm wide and not numerous on any media type (PDA, V8, Cornmeal). No oospores, chlamydospores or hyphal swellings were found. Hybrid-like shaped sporangia were present when agar plugs were flooded with NSES in light (Figure 4).

Figure 4 *Phytophthora* sp. LP-2004 Sporangia. Sporangia from V8 agar plugs after five days flooded with 20% NSES at 400x magnification viewed under compound microscope.

Papillated sporangia with elongated pedicels present (Figure 4). Ovoid papillated sporangia with elongated pedicels present ranging from 15-35µm x 8-19µm and averaged 25.46-11.04µm in diameter shown (Figure 5).

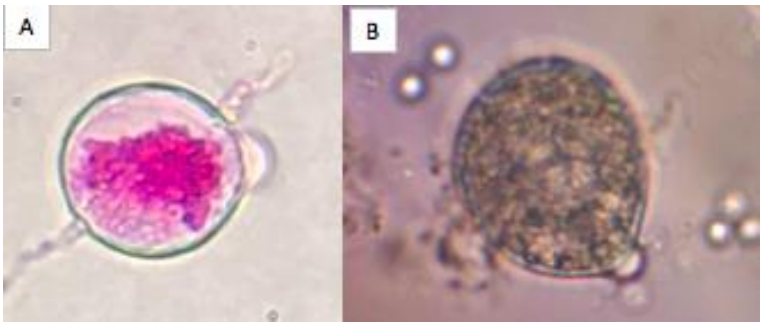

Figure 5 Papillated Sporangia of *Phytophthora* sp. LP-2004. 400x total magnification using compound microscope of sporangia. A) Papillated sporangia on CM agar stained with 0.05% acid fuchsin solution B) Papillated sporangia in 20% NSES after five days at 22-25°C under LED light.

Sporangia exit width was found to be 2µm. Sequence analysis resulted in 95.47% identity to *P. capsici* from BLAST. PhytophthoraID.org identified the consensus sequence with 95% to *P. mexicana* and *P. capsici* with Cox gene products. ITS6-ITS4 primers resulted in 100% identity to *P. capsici* in PhytopthoraID.org, and BLAST results showed 99% identity to *P. capsici.*

2.4.3 Phytophthora sp. 1756-2007

Phytophthora sp. 1756-2007 hyphae (2µm wide) were coiled and smooth. Hyphal swellings were not present. Ovoid papillated sporangia were present ranged 9-28µm x 7-19µm in averaged 15.7-9.9µm in diameter (Figure 6).

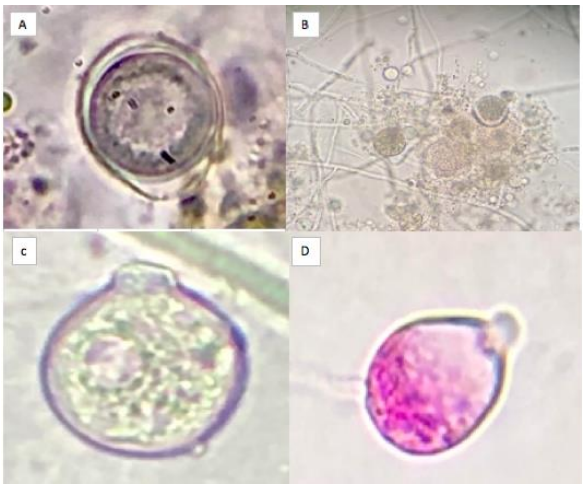

Figure 6 *Phytophthora* sp. 1756-2007 Oospores and Sporangia. A) Mature oospore at 400x total magnification from V8 agar plugs incubated in 20% NSES for 30 days under LED light at 22-25°C B) Immature oospores at 200x total magnification. Viewed under a compound microscope. C and D) Sporangia present at 400x total magnification on V8 agar plugs after five

days in 20% NSES at 22-25°C under LED light. Sporangia (right) stained with 0.05% acid fuchsin dye.

The sporangia present had exit widths 1-3µm. Oospores ranged from 6-10 µm and averaged 8.5 to 7.7µm in diameter (Figure 7). Terminal chlamydospores measuring 6-20 µm (avg 12.32) in bunches were produced on CM agar (Figure 7).

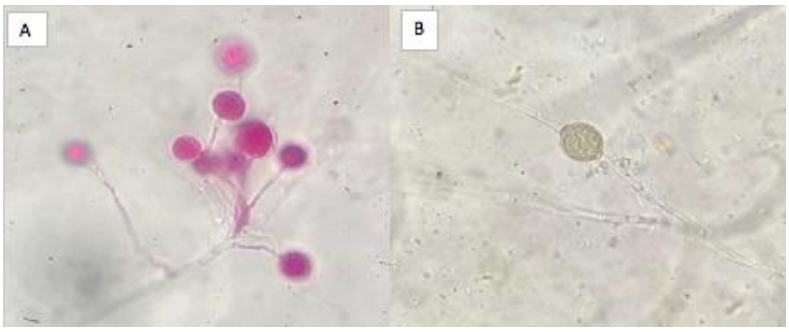

Figure 7 *Phytophthora* sp. 1756-2007 Chlamydospores. A) Clustered chlamydospore stained with 0.05% acid fuchsin dye B) Intercalary chlamydospore. Both were observed at 400x total magnification on CM agar using a compound microscope.

PhytophthoraID.org showed 99% identity to *P. nicotianae*, and in BLAST database, there was 99.47% identity to *P. nicotianae* with Cox gene primers. With ITS6-ITS4 primers, BLAST results were 94.35% identity to *P. nicotianae*, and 95% identity to *P. nicotianae* with PhytophthoraID.org.

2.4.4 Phytophthora sp. 845-2007

Phytophthora sp. 845-2007 had smooth hyphae and measured at 2-3µm wide on V8 and CM and coralloid hyphae on PDA with hyphal swellings present on PDA. Irregular, ovoid, globose and obturbinate, papillate sporangia present ranged 15-25µm x 5-22µm averaged 18.5-11.8µm (Figure 8).

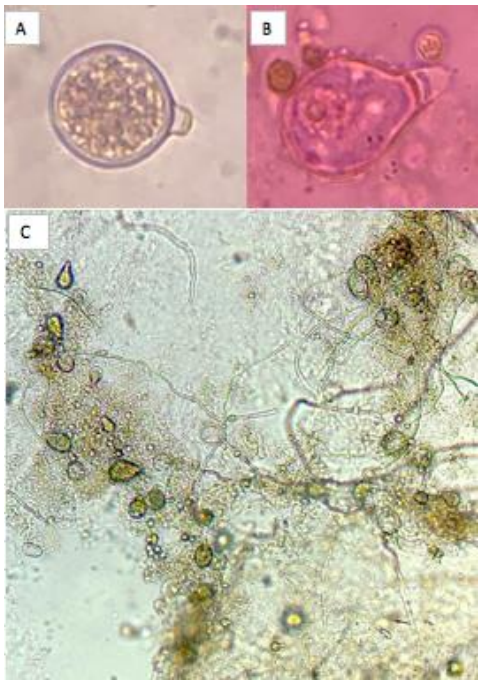

Figure 8 *Phytophthora* sp. 845-2007 Sporangia. Sporangia from V8 agar plugs in 20% NSES observed with a compound microscope after five days at 22-25°C in LED light. A) Sporangia from V8 without dye at 400x B) Sporangia with 0.05% acid fuchsin at 400x. C) Sporangia at 200x.

Sporangia exit width was 2µm. Chlamydospores in agar plugs flushed with 20% NSES after five days measured 8-13µm and averaged 10.41µm (Figure 9).

Figure 9 *Phytophthora* sp. 845-2007 Chlamydospores. Terminal chlamydospores (left) and intercalary (right) both stained with 0.05% acid fuchsin dye and observed at 400x total magnification with compound microscope from CM agar.

Oospores were not found. BLAST results indicated 97% identity to *P. nicotianae,* and PhytophthoraID.org produced 92% identity to *P. nicotianae* using Cox gene primers. ITS6-ITS4 consensus sequence in BLAST produced 99.41% identity to *P. parasitica*, and in PhytopthoraID.org identity was 99% to *P. nicotianae*.

2.4.5 Phytophthora sp. 1446-2007

Phytophthora sp. 1446-2007 produced nonpapillated and papillated sporangia found to range from 9-22µm x 6-16µm and averaged 17.5-10.6 in diameter with an exit width of 2µm (Figure 10).

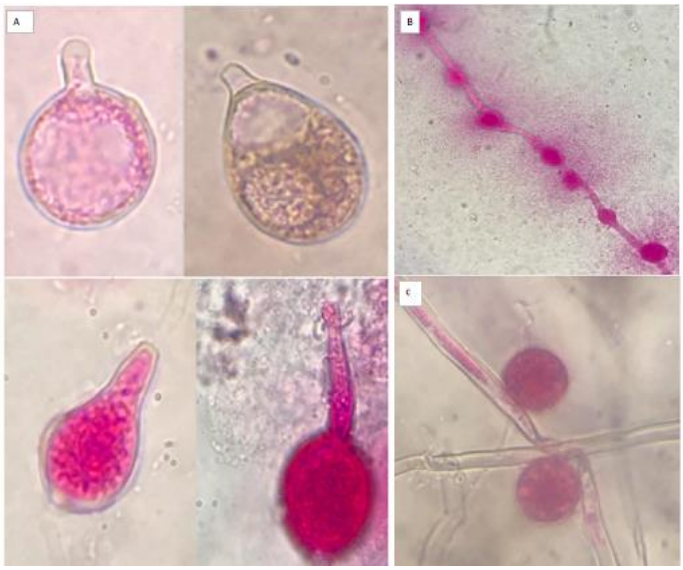

Figure 10 *Phytophthora* sp. 1446-2007 Sporangia and Chlamydospores. A) Sporangia at 400x on V8 agar plugs in 20% NSES after five days at 22-25°C under LED light B) hyphal swellings on CM C) sessile chlamydospores on CM agar.

Shapes of sporangia varied from obturbinate, obpyriform, ovoid and ellipsoid/irregular. Sessile and intercalary chlamydospores were numerous on V8 and PDA 5-11µm and averaged 8.2 µm in diameter (Figure 10). Clustered hyphal swellings were present on V8 and hyphae measured 2-3µm wide. Oospores were not found. BLAST results showed 98.4% identity to *P. nicotianae*, and 98% identity to *P. nicotianae* on PhytopthoraID.org when Cox gene products were used. PhytophthoraID.org produced 94% to *P. nicotianae* with ITS6-ITS4 products used, and 97% identity to *P. nicotianae* in BLAST.

2.4.6 Phytophthora sp.1422-2007

Phytophthora sp. 1422-2007 measured at 2-3µm wide with single and intercalary hyphal swellings present. Intercalary chlamydospores ranged from 5-11µm (avg: 8.75µm) were sparse and only observed with agar plugs in NSES (Figure 11).

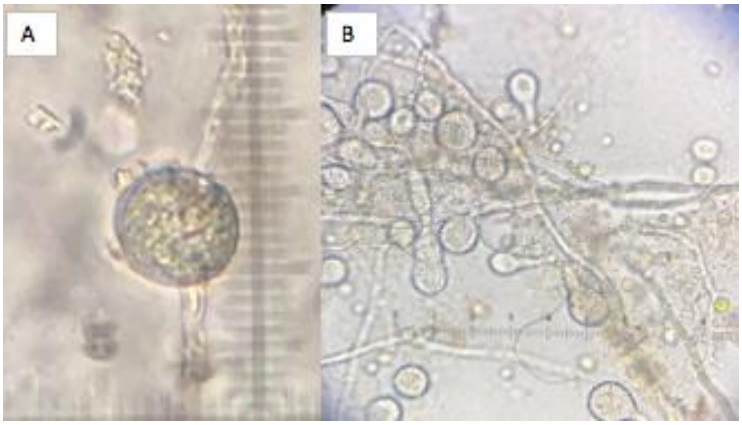

Figure 11 *Phytophthora* sp.1422-2007 Chlamydospore and Sporangia. A) intercalary chlamydospore on CM agar B) Irregular sporangia at 200x in 20% NSES. Ample sporangia observed after five days at 22-25°C under LED light.

Papillated sporangia were irregular and ranged 10-27µm x 5-19µm and averaged 17.0-19.9µm in diameter (Figure 11). There were no oospores observed. BLAST results showed 95.34% identity to *P. nicotianae*, and 95% to *P. nicotianae* on PhytophthoraID.org when Cox gene products were used. With ITS6-ITS4 products on PhytopthoraID.org 99% identity to *P. nicotianae* was found, and 99.07% identity to *P. nicotianae* with BLAST.

2.4.7 Colony Morphology Observations

PDA and V8 plates with agar plugs of the strains tested were plated for assessing colony morphology (Figures 12-13). The colony morphologies were distinct when compared to each other after 14 days.

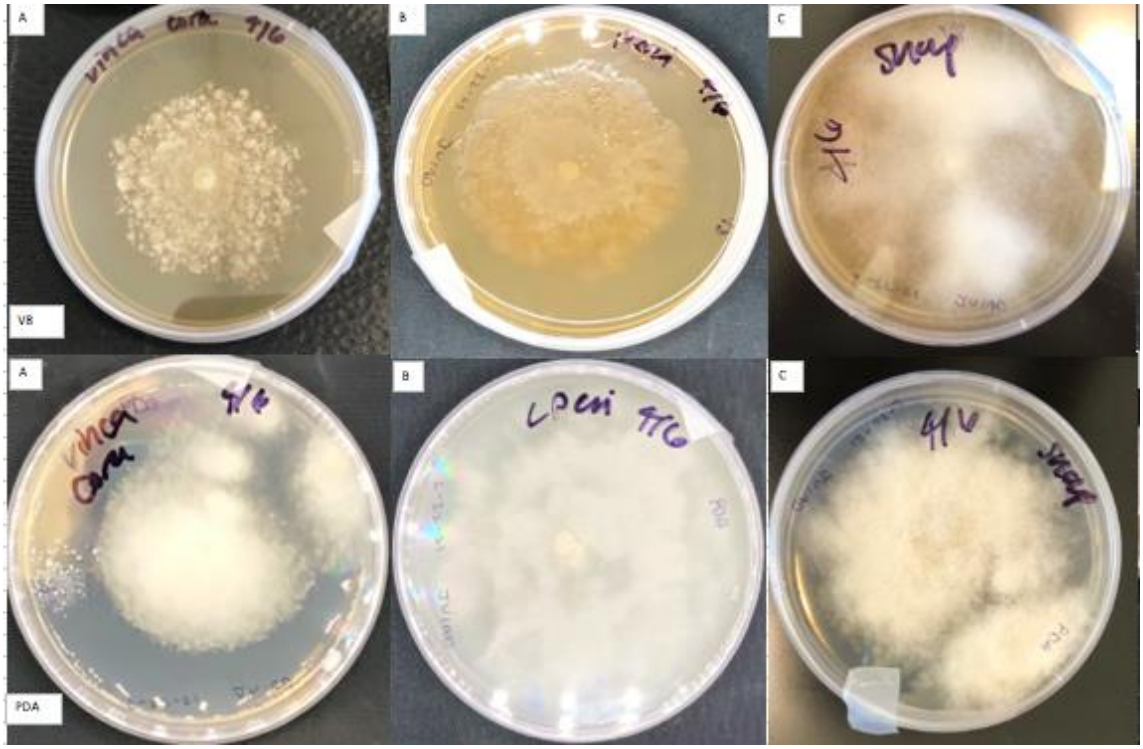

Figure 12 Colony Morphology of *Phytophthora* Isolates sp. 2238-2019, sp. LP-2004, and 1756-2007. V8 plates (top row), PDA (bottom row) day 14 at 25°C. A) *Phytophthora* sp. 2238 B) *Phytophthora* sp. LP-2004 C) *Phytophthora* 1756-2007.

.

The isolates produced fluffy mycelia on PDA after 14 days with *Phytophthora* 1756-2007 on V8 agar. *Phytophthora* LP-2004 did not produce aerial mycelia on the top of the agar on V8 plates, but tended to grow more under the agar (Figure 12). Colony morphology for *Phytophthora* sp. 845-2007, *Phytophthora* sp. 1446-2007, and *Phytophthora* sp. 1422-2007 was observed (Figure 13).

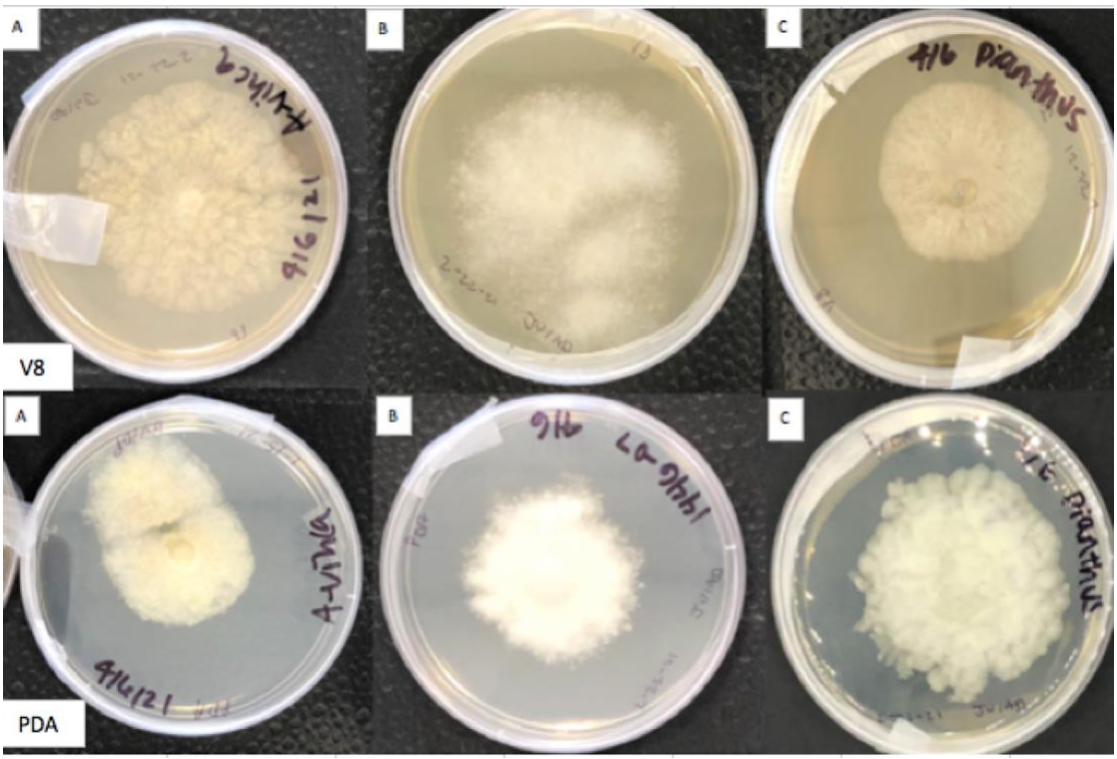

Figure 13 Colony Morphology of *Phytophthora* Isolates sp. 845-2007, sp. 1446-2007 and sp. 1422-2008. V8 plates (top row), PDA (bottom row) day 14 at 25°C. A) *Phytophthora* sp. 845-2007 B) *Phytophthora* sp. 1446-2007 C) *Phytophthora* sp. 1422-2007.

Fluffy mycelia were produced on PDA from these isolates as well. The differences in pigmentation should be noted in isolates *Phytophthora* sp. 1446-2007 and *Phytophthora* sp. 845-2007 on V8 when compared to the others on V8.

2.4.8 Phylogenetic Tree

A phylogenetic tree was generated using Cox gene sequences of *Phytophthora* isolates used in this study and ITS sequences of selected species to create an outgroup (Figure 14). In light of the location of the sequence of *Phytophthora* sp. LP-2004 in this phylogenetic analysis, *Phytophthora* sp. LP-2004 is dissimilar to the rest of the isolates tested included in this tree assembly. Sequences were aligned and trimmed using BioEdit and a fasta file was created for tree building. The Maximum Likelihood tree was generated using MEGA 11 using the Hasegawa–Kishono–Yano model.

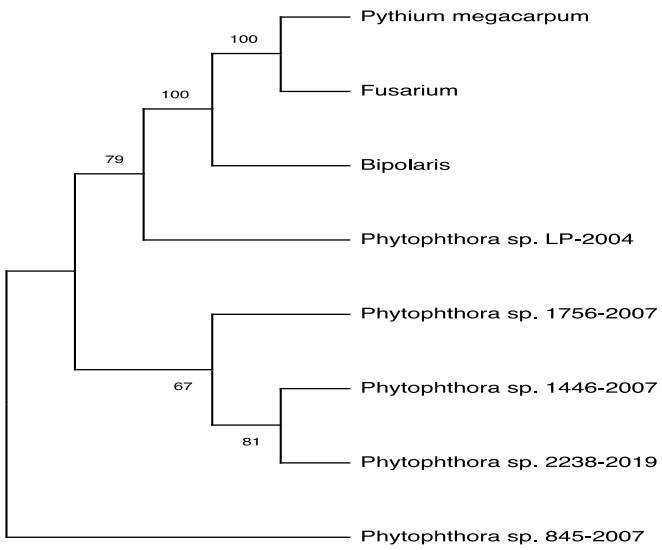

Figure 14 Phylogenetic Tree of *Phytophthora, Pythium, Bipolaris sp. and Fusarium Proliferatum* Sequences. ITS sequences of *Fusarium proliferatum, Bipolaris sp., and Pythium megacarpum* (Paul, 2000; Genbank accession number: AF203784) were used as outliers, with *Pythium megacarpum* rooting the tree. The tree was generating using MEGA11 software and bootstrap values show the proportion of times the given result was observed out of 100 iterations of the tree and can be utilitized as an indicator of confidence regarding the relatedness.

2.5 Discussion

2.5.1 Molecular Characterization

Based on the amplification of FMPH primers and ITS primers these organisms are confirmed *Phytophthora* species (Figure 1). *Phytophthora* sp. 845-2007 and *Phytophthora* sp. LP-2004 produced slightly larger bands (approximately 50 bp) than other isolates using FMPH primers (Figure 1). Amplification with ITS primers showed bands in the expected location of approx. 862-941bp (Figure 1). Variation in band size and location specific for species has been seen for *Phytophthora* isolates when using FMPh-8b and FMph-10b primers (Martin et al., 2004).

2.5.2 Morphological Characterization

P. nicotianae is one of the common aerial blight *Phytophthora* species attacking ornamental plants (Olson et al., 2011). Sporangia are ellipsoid, noncaducous, ovoid, pyriform, obpyriform to spherical with prominent papilla, and are known to have two papillae occur on a single sporangium (Erwin and Ribeiro, 1996). The papillate sporangia can be produced alone, or in a loose sympodium on stalks that average 375 μm in length, and range from 100 to 595 μm in length (Thomson and Hine, 1972). The sporangia average 40.18 x 28.53 μm width (11-60 μm) and 1.34 μm length (1.1-1.7 μm) (Thomson and Hine, 1972).

Hall (1993) reported hyphal swellings, and only 50% of 81 isolates produced chlamydospores abundantly. Chlamydospores can be terminal or intercalary with an average diameter of 33 μm (ranging 13-60 μm). Others have reported a diameter range of 20 to 60 μm (Dastur, 1913). Most isolates are heterothallic, but some can form oogonia and oospores in single culture when inoculum is transferred from old cultures (Brasier, 1972; Tsao et al., 1985).

Phytophthora isolates from vinca have been reported as homothallic (Schubert and Leahy, 1989). Antheridia are amphigynous and spherical or oval. The oogonia are smooth and spherical and average 26.8 μm in diameter (ranging 15-64 μm) (Hall, 1993). Oospores have been reported to average 22.6 μm (Hall, 1993), and others have reported the oospores range from 13 to 24 μm (Dastur, 1913). Colony morphology on potato dextrose agar (PDA) are usually weblike, but on V8 agar, they can be fluffy (Erwin and Ribeiro, 1996).

All but one *Phytophthora* isolate from this work was found to be *P. nicotianae*, and it can be concluded these *P. nicotianae* isolates are unique, as they differ in colony morphology (Figures 12-13), and morphological (Figures 2-11) characteristics present.

***Phytophthora* sp. 2238-2019** showed morphological structures consistent with known literature of *P. nicotianae* sporangia size (Erwin and Ribiero, 1996; Thomson and Hine, 1972). ***Phytophthora* sp. 2238-2019** is most similar to ***Phytophthora* sp. 1446-2007** (Figure 14). ***Phytophthora* sp. 1756-2007** showed smaller sporangia when compared to known *P. nicotianae* sporangia size (Erwin and Ribiero, 1996; Thomson and Hine, 1972), and smaller oospores than reported data (Erwin and Ribiero, 1996; Hall, 1993; Dastur, 1913) (Figure 6).

***Phytophthora* 845-2007** had smaller chlamydospores than reported literature for *P. nicotianae* (Erwin and Ribiero, 1996; Dastur, 1913) (Figure 9). ***Phytophthora* sp. 1446-2007** had smaller chlamydospores and there were sessile chlamydospores found which is uncommon for *P. nicotianae.* *P. nicotianae* chlamydospores have been more commonly reported as terminal or intercalary with an average diameter of 33 μm (ranging 13-60 μm). Others have reported a diameter range of 20 to 60 μm (Dastur, 1913). ***Phytophthora* sp. 1422-2007** (Figure 11), also showed smaller chlamydospores than reported literature for *P. nicotianae* (Erwin and Ribiero, 1996).

All other structures found for these species were consistent with reported literature for *P. nicotianae* such as oogonia which are smooth and spherical, and average 26.8 μm in diameter (15-64 μm) (G. Hall, 1993). Colony morphology observed is consistent with *P. nicotianae* for *P. nicotianae* isolates (Erwin and Ribeiro, 1996) (Figure 12-13).

The most distinct isolate was ***Phytophthora* sp. LP-2004** as the morphological characteristics observed more closely resemble *P. capsici* when compared known morphological information on *Phytophthora* species (Bowers et al., 2007; Erwin and Ribiero, 1996; Leonian, 1922). However, the observations of ***Phytophthora* LP-2004** do not completely agree with what is found in literature for *P. capsici.* Future work could assess the possibility that ***Phytophthora* LP-2004** is a hybrid species of *P. capsici* (Poucke et al., 2021). For example, a characteristic of *P. capsici* is long pedicles on sporangia (Erwin and Ribeiro, 1996). This isolate shows long pedicles, but the shape of the sporangia is highly unusual (Erwin and Ribeiro, 1996). No oospores were found which matches what is known about *P. capsici* as it is known to be predominately heterothallic (Noon and Hickman, 1974). Chlamydospores are rare in culture (Tucker, 1931) and there were no chlamydospores present from ***Phytophthora* sp. LP-2004**. Additionally, there were no hyphal swellings present which fits *P. capsici* as this organism is known to occasionally produce hyphal swellings in aqueous cultures (Erwin and Ribeiro, 1996). Colony morphology on PDA matches previous studies such as (Gangadhar, 2016). However, colony morphology of ***Phytophthora* sp. LP-2004** shown on V8 agar does not match what is found in literature, as this isolate does not produce any fluffy, aerial hyphae and grows predominantly under the agar (Bowers et al., 2007; Erwin and Ribiero, 1996; Leonian, 1922). Finally, *P. capsici* was isolated from vinca, but *P. capsici* is more generally found on pepper (*Capsicum annuum*) and cucurbits (Erwin and Ribeiro, 1996). ***Phytophthora* sp. LP-2004** is less

similar to the rest of the isolates tested, and bootstrap support values indicate low confidence in the phylogenetic placement of *Phytophthora* sp. 1756-2007 (Figure 14). Concluding, the records of observations in this study agree with the hypothesis that these *Phytophthora* isolates obtained from ornamentals are distinct *P. nicotianae*, with the exception of ***Phytophthora* sp. LP-2004** which most resembles *P. capsici* (Bowers et al., 2007; Erwin and Ribiero, 1996; Leonian, 1922). It is possible *Phytophthora* sp. LP-2004 hybridized with another *Phytophthora* able to infect vina and a *P. capsici* x *P. nicotiananae* hybrid has been produced *in vitro* by pairing in dual culture (English et al., 1999). Additional genetic work would need to be done to confirm if ***Phytophthora* sp. LP-2004** is a hybrid species (Yang et al., 2014).

3. FUNGICIDE EFFICACY TESTING ON PHYTOPHTHORA SP. 2238-2019

3.1 Overview

Phytophthora nicotianae is known to cause aerial blight of vinca. *Phytophthora* sp. 2238-2019 is believed to be a *P. nicotianae* strain. There are several fungicides recommended for aerial blight on ornamentals such as Segway™, Stature™, Subdue Maxx™, Heritage™ and Pageant Intrinsic™. A fungicide efficacy study was conducted to observe any resistance to commonly applied products and observe which product(s) are most efficient. The hypothesis is that *Phytophthora* sp. 2238-2019 mycelial growth would be most restricted on Stature™ and Subdue Maxx™ amended plates when compared to other materials tested. *Phytophthora* sp. 2238 agar plugs were placed on media amended with Segway™, Stature™, Subdue Maxx™, Heritage™ and Pageant Intrinsic.™ Mycelial growth was monitored and compared to oomycete growth on unamended plates. Stature™ and Subdue Maxx™ showed little to no growth, and the other fungicide treatments were not able to inhibit mycelial growth to less than or equal to unamended plates. The results for Stature™ and Subdue Maxx™ agree with previous efficacy studies on *P. nicotianae* (Hausbeck et al., 2003; Hausbeck and Harlan, 2005; Steddom and Kimberly, 2009).

3.2 Introduction

Fungicides are commonly used to combat disease from *Phytophthora* but can be expensive and are a potential risk to the health of the environment (De Jong and De Snoo, 2002; Van Der Werf, 1996) and frequent application can result in resistant strains (González-Tobón et al., 2019 and Schepers et al., 2018). Fungicide resistance has been reported in several *Phytophthora* species including but not limited to: *P. capsici* to pyrimorph (Pang et al., 2013), mefenoxam, fluopicolide, oxathiapiprolin, and cyazofamid (Siegenthaler and Hansen, 2021), *P. infestans* to mefenoxam (Saville et al., 2015) and *P. nicotianae* to mefenoxam (Hu, 2008; Hwang and Benson, 2007; Olsen, 2012).

P. nicotianae fungicide resistance studies have been focused on resistance to mefenoxam, the active ingredient in Subdue Maxx™. Some *Phytophthora* isolated from irrigation water have shown mefenoxam resistance (Olson et al., 2013). As of February 2022, there have not been any *in vitro* tests for *P. nicotianae* showing fungicide resistance to the active ingredients boscalid, pyraclostrobin, azoxystrobin, dimethomorph, and cyazofamid. In 2007, Hu found 26 isolates of *P. nicotianae* were highly resistant to mefenoxam, and resistant isolates showed greater infection rate and higher sporulation ability than sensitive ones. Hwang and Benson (2007) found 21% of *P. nicotianae* isolates from floriculture crops tested were insensitive to mefenoxam at either 1 or 100µg a.i./ml. Lastly, a study in 2012 using isolates obtained from ornamental plants and irrigation water found that of the 6% of isolates associated with plants found to be resistant to mefenoxam, 78% of those isolates were *P. nicotianae* (Olsen et al., 2012).

The objective of this study was to evaluate five fungicides labeled for control of *Phytophthora* aerial blight on ornamentals and observe if *Phytophthora* sp. 2238-2019 had developed resistance (Table 6). As pathogen resistance to fungicides is a major concern for the

horticultural industry, the information from this work serves to guide efforts to combat *Phytophthora* aerial blight in ornamentals.

Table 6 Fungicides Tested

Brand Name	Active Ingredient	FRAC Group	Application Rate
Heritage™	Azoxystrobin	11	4oz/100gal
Pageant Intrinsic™	Boscalid, Pyraclostrobin	7, 11	18oz/100gal
Segway™	Cyazofamid	21	4oz/100gal
Stature™ DM	Dimethomorph	40	12.8oz/100gal
Subdue Maxx™	Mefenoxam	4	0.5-1.0fl oz/100gal

3.3 Materials and Methods

3.3.1 Culture Preparation

Potato Dextrose Agar (PDA) was embedded with fungicide at 0.5x, 1x (current recommended application rate), 2x and 4x concentrations. Control plates were PDA without fungicide. *Phytophthora* sp. 2238 was grown for seven days for ample development on V8 at 25°C and one agar plug from this culture was placed in the center of fungicide-embedded plates and control plates. The diameter of *Phytophthora* sp. 2238 was measured on fungicide-embedded PDA plates over 14 days and compared to control PDA plates with no fungicides added (Siegenthaler and Hansen, 2021). There were five replicates per treatment (Siegenthaler and Hansen, 2021). The plates were arranged according to treatment group design in the incubator.

3.3.2 Data Collection and Analysis

Due to the slow growing nature of *Phytophthora* in culture, the cultures were measured every other day for 14 days, the diameter of *Phytophthora* sp. 2238-2019 colonies were taken at the x and y axes and an average was taken as outlined by Siegenthaler and Hansen (2021). The plates were marked with permanent marker on the outside growing edge to ensure the measurements were taken from the same location on the oomycete colony each time. These measurements were compared to colony diameters from unamended plates to see if *Phytophthora* sp. 2238-2019 was "sensitive" to any fungicide treatments. "Sensitive" was defined as an isolate that had inhibited mycelial growth to less than or equal to one half the average colony diameter of the unamended plates (Siegenthaler and Hansen, 2021).

3.4 Results

For days 2-8, all treatments showed significantly smaller *Phytophthora* sp. 2238-2019 colony diameters compared to unamended plates ($P<0.05$). *Phytophthora* sp. 2238-2019 mycelial growth was most restricted on Stature™ and Subdue Maxx™ amended plates. Stature™ treated plates did not show any growth of *Phytophthora* sp. 2238-2019, and Subdue Maxx™ treated plates did not show any growth until Day 10.

3.4.1 Day 2: Treatment Effects

Growth was not detected on Stature™ and Subdue Maxx™ treated plates on days 2-8. Although an increase in restriction of mycelial growth was observed for all concentrations with the exception of Segway™ (2x-4x) and Pageant™(2x), *Phytophthora* sp. 2238-2019 mycelial growth was only "sensitive" to Stature™ and Subdue Maxx™ amended media (Figure 15).

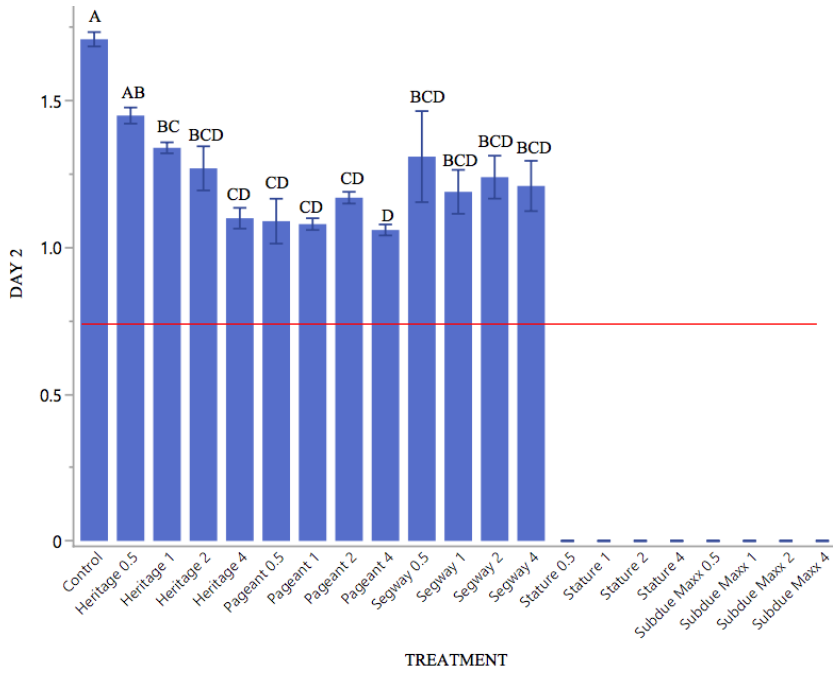

Figure 15 Day 2 Colony Diameters of *Phytophthora* sp. 2238-2019 Growth on Media Imbedded with Fungicides at 0.5x, 1x, 2x, and 4x Concentrations. Control plates were PDA and no fungicides. The red line is the minimum "sensitivity" threshold. Each error bar was constructed using 1 standard error from the mean. Data analyzed using a one-way ANOVA and Tukey test via JMP 16. Treatments sharing a group letter are not statistically different.

3.4.2 Day 4: Treatment Effects

Phytophthora sp. 2238-2019 mycelial growth was only "sensitive" to Stature™ and Subdue Maxx™ amended media. However, an increase in restriction of mycelial growth was observed for all concentrations of fungicides tested.

3.4.3 Day 6: Treatment Effects

An increase in restriction of mycelial growth was observed for all concentrations with the exception of Pageant™(1x), but *Phytophthora* sp. 2238-2019 mycelial growth was only "sensitive" to Stature™ and Subdue Maxx™ amended media (Figure 16).

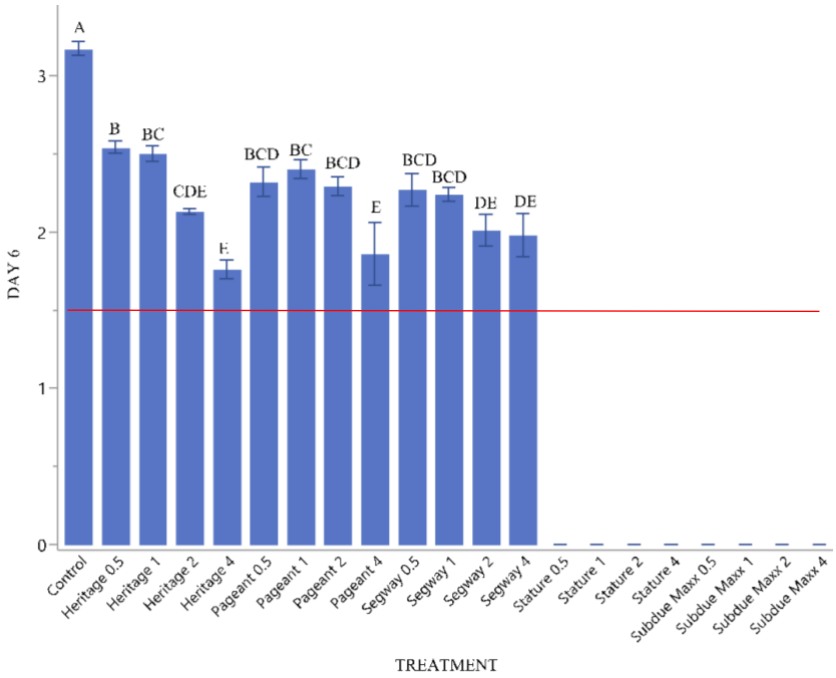

Figure 16 Day 6 Colony Diameters of *Phytophthora* sp. 2238-2019 Growth on Media Imbedded with Fungicides at 0.5x, 1x, 2x, and 4x Concentrations. Control plates were PDA and no fungicides. The red line is the minimum "sensitivity" threshold. Each error bar was constructed using 1 standard error from the mean. Data analyzed using a one-way ANOVA and Tukey test via JMP 16. Treatments sharing a group letter are not statistically different.

3.4.4 Day 8: Treatment Effects

An increase in restriction of mycelial growth was observed for all concentrations but *Phytophthora* sp. 2238-2019 mycelial growth was only "sensitive" to Stature™ and Subdue Maxx™ amended media.

3.4.5 Day 10: Treatment Effects

Stature™ treated plates did not show any *Phytophthora* growth. Most Subdue Maxx™ amended plates showed no growth but some plates did show colonies on 1x and 4x concentrations (0.8-0.85cm) (Figure 17). *Phytophthora* sp. 2238-2019 mycelial growth was "sensitive" to Stature™, Subdue Maxx™, and Heritage (4x) amended media (Figure 17). However, an increase in restriction of mycelial growth was observed for all concentrations of fungicides tested (Figure 17).

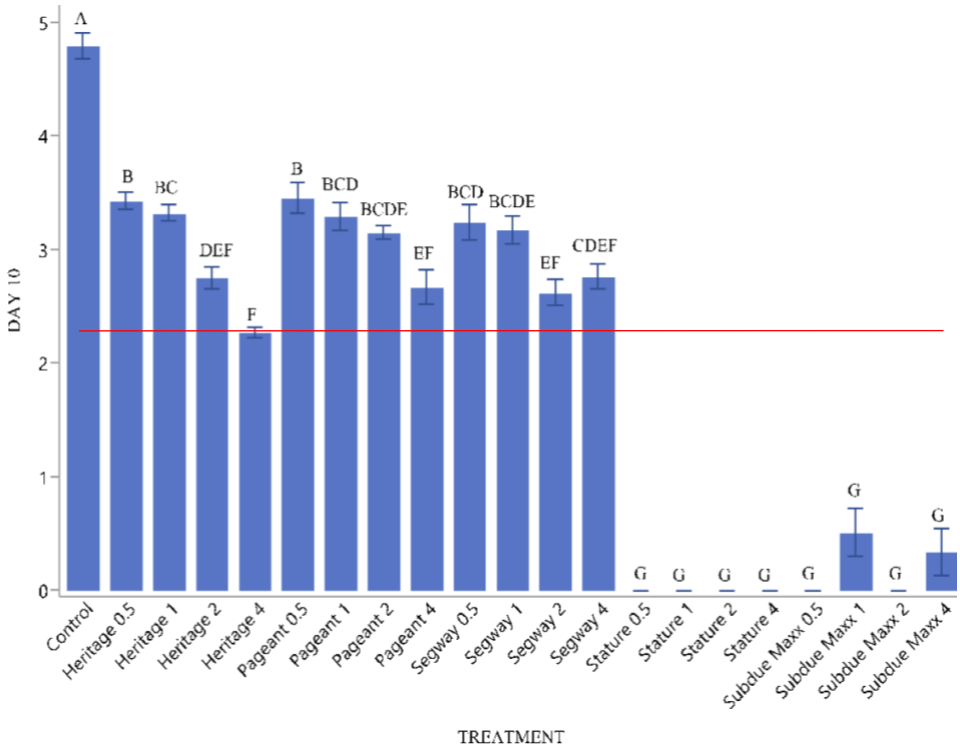

Figure 17 Day 10 Colony Diameters of *Phytophthora* sp. 2238-2019 Growth on Media Imbedded with Fungicides at 0.5x, 1x, and 4x Concentrations. Control plates were PDA and no fungicides. The red line is the minimum "sensitivity" threshold. Each error bar was constructed using 1 standard error from the mean. Data analyzed using a one-way ANOVA and Tukey test via JMP 16. Treatments sharing a group letter are not statistically different.

3.4.6 Day 12: Treatment Effects

Phytophthora sp. 2238-2019 mycelial growth was "sensitive" to Subdue Maxx™ (0.5-1x), Stature™ (0.5-1x), and Heritage at 4x. Stature™ treated plates did not show any *Phytophthora* growth. An increase in restriction of mycelial growth was observed for all concentrations of fungicides tested with the exception of Pageant Intrinsic™ at 2x (averaged 4.42 cm).

3.4.7 Day 14: Treatment Effects

Phytophthora sp. 2238-2019 mycelial growth was "sensitive" to Stature™ and Subdue Maxx™ (0.5x-4x) and Heritage™ (4x). Subdue Maxx™ showed little growth (0-1.4cm) on all fungicide concentrations (Figure 18). An increase in restriction of mycelial growth was observed for all concentrations of fungicides tested with the exception of Segway™ at 4x (averaged 3.56 cm).

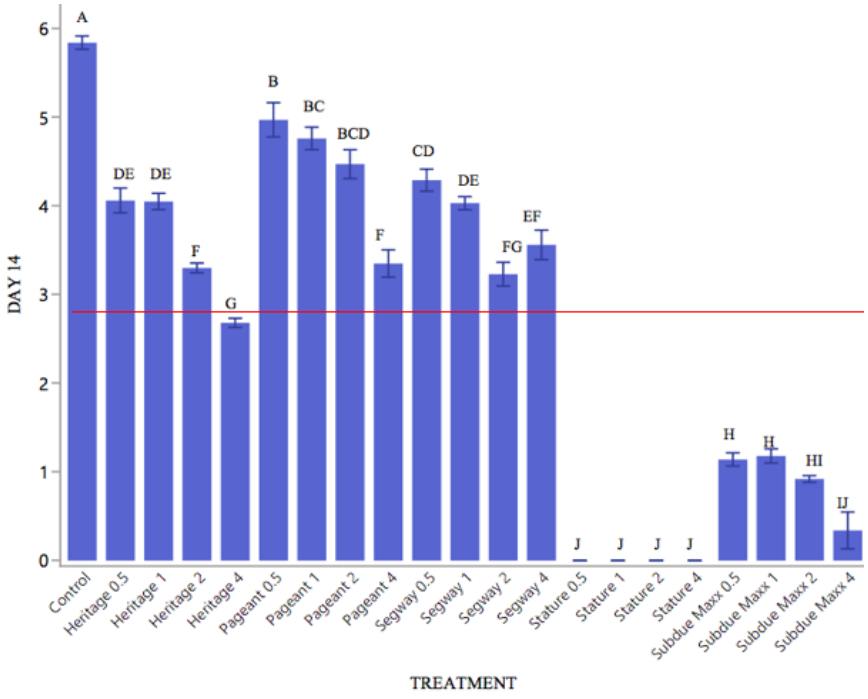

Figure 18 Day 14 Colony Diameters of *Phytophthora* sp. 2238-2019 Growth on Media Imbedded with Fungicides at 0.5x, 1x, 2x, and 4x Concentrations. Control plates were PDA and no fungicides. The red line is the minimum "sensitivity" threshold. Each error bar was constructed using 1 standard error from the mean. Data analyzed using a one-way ANOVA and Tukey test via JMP 16. Treatments sharing a group letter are not statistically different.

3.5 Discussion

Based on the data collected, *Phytophthora* sp. 2238-2019 is sensitive to Subdue Maxx™ and Stature™ (Figures 15-18). "Sensitive" is being able to reduce colony diameter of *Phytophthora* sp. 2238-2019 colony to less than or equal to one half the average control diameter (Siegenthaler and Hansen, 2021). These results may differ under greenhouse conditions on live plants. The results for Stature™ and Subdue Maxx™ agree with previous *Phytophthora* efficacy studies (Hausbeck et al., 2003; Hausbeck and Harlan, 2005; Steddom and Kimberly, 2009). The results from this experiment support the hypothesis that *Phytophthora* sp. 2238-2019 mycelial growth was most restricted when grown on Stature™ and Subdue Maxx™ embedded plates compared to average colony diameters on the other fungicide embedded plates tested. The results do not support the hypothesis that *Phytophthora* sp. 2238-2019 would be sensitive to 1x of Heritage™, Pageant Intrinsic™, and Segway™. Average colony size was shown to be smaller with increasing fungicide concentration for these treatments overtime (Figure 15-19), but *Phytophthora* sp. 2238-2019 mycelial growth was only found to be sensitive with Heritage™ 4x for Day 10, 12 and 14 observations. *Phytophthora* sp. 2238-2019 was sensitive to at Heritage™ 4x the recommended rate, but this application rate may not be feasible in the field. Based on this data, I concluded that Segway™ and Pageant Intrinsic™ perform comparably *in vitro* for *Phytophthora* sp. 2238-2019. For these fungicides to decrease average colony diameter to at least half control diameter for *Phytophthora* sp. 2238-2019, the application rates of Segway™ and Pageant Intrinsic™ would need to exceed 4x. Future work could study the effect of these application rates *in vivo*.

4. PATHOGENICITY TESTING OF PHYTOPHTHORA ISOLATES ON VINCA

4.1 Overview

Phytophthora species can cause aerial blight on vinca (*Catharanthus roseus*). The aggressiveness of *Phytophthora* isolates were assessed on current commercially available vinca cultivars. The hypothesis is that there are differences in susceptibility among vinca cultivars and varieties. Pathogenicity testing was performed using inoculation of zoospores from *Phytophthora* isolate cultures and plants were monitored for disease development. Three pathogenicity tests were conducted: Test 1 was conducted on older plants while Test 2 and 3 used seedlings. Test 1 and 2 used *Phytophthora* sp. 2238-2019, and Test 3 used several *Phytophthora* isolates separately. Test 1 revealed no significant difference in susceptibility among Cora XDR vinca, but recorded high mean area under the AUDPC on Cora XDR 'Light Pink.' Test 2 did not produce statistically relevant results due to irregular germination, but showed sporangia can be used as inoculum. For plants inoculated with *Phytophthora* sp. 2238-2019 in Test 3 on seedlings showed Cora XDR 'Light Pink' as significantly more susceptible than other Cora XDRs tested when comparing AUDPC values. Valiant 'Burgundy' and Titan 'White' showed the highest disease severity among Titan and Valiant vinca tested. Other isolates did not produce disease on vinca higher than 80 AUDPC, but there were differences among the disease severity between the *Phytophthora* isolates tested.

4.2 Introduction

P. nicotianae is a common *Phytophthora* species in nurseries with field and container production and among gardens on ornamental plants (Ahmed et al., 2012; Bienafl and Balci, 2014; Leonberger et al., 2013; Olson et al., 2011; Schwingle et al., 2007). Aerial blight reduces ornamental characteristics and marketability of numerous herbaceous ornamental plants (Erwin and Ribeiro, 1996). Chlamydospores and oospores of *P. nicotianae* can persist in plant debris and soil (Erwin and Ribeiro, 1996; Kröber, 1980), can remain in irrigation water and watersheds (Hong and Moorman, 2005; Hulvey et al., 2010), and overwinter in rhizospheres of host plants (Erwin and Ribeiro, 1996). Chlamydospores and oospores can also survive in gastrointestinal tracts and feces of various animals and could be dispersed by these vectors (Weste, 1983; Alvarez et al., 2009).

Recent studies have indicated new hosts of *Phytophthora* showing aerial blight symptoms including Dianthus (*Dianthus* spp.) in China in 2021 (Xu et al., 2021), and in 2017, aerial blight was found on Vinca, Lobelia (*Lobelia* spp.), and Calibrachoa (*Calibrachoa* spp.) in Ohio (Lin et al., 2017). As of February 2022, I am not aware of any published report demonstrating that *P. nicotianae* can infect Cora XDR varieties. The primary objective of pathogenicity testing was to determine susceptibility of common vinca varieties to *Phytophthora* sp. 2238-2019. The secondary objective of this work was to see if any of the additional *Phytophthora* isolates were pathogenic to vinca, and observe any potential differences in susceptibility of the vinca to those additional isolates tested.

4.3 Materials and Methods

Test 1: June-July 2020

4.3.1.A Plants and Experimental Design

The following plants donated from Syngenta (Gilroy, CA) and Ball (West Chicago, IL) were tested at two-months old for their susceptibility to *Phytophthora* sp. 2238-2019: Cora 'Classic Red,' Cora XDR 'Deep Strawberry,' Cora XDR 'Hotgenta,' Cora XDR 'Light Pink,' Cora XDR 'Polka Dot' and Cora XDR 'Punch.' Vinca plants, (5 replicates each), were arranged in rows according to the variety and placed into the separate control and experimental treatment areas to prevent cross contamination. Each vinca variety replicate was assigned a number and randomly placed in the same variety row in the appropriate control and experimental treatment areas in a randomized according to treatment group experimental design. To generate random placement, the plant numbers were compiled into a list and placed into "Miniwebtool." This process continued until all the replicates were in the designated variety rows. Plants were kept under greenhouse conditions, and automatic misters were set to 20 seconds every hour for Tests 1-3.

4.3.1.B Production of Test Inoculum

Based on preliminary experiments to obtain ample sporangia, sporangia were obtained from *Phytophthora* sp. 2238-2019 on V8 agar plugs after four days at 22-25°C in 20% NSES (non-sterile soil extract solution) under LED light. Plugs were then cold shocked at 4°C for six hours and two hours at 22-25°C to obtain zoospores. The agar plugs were blended in a blender and filtered with a kitchen sieve. Zoospores were diluted down with RO (reverse osmosis) water

to obtain a $1x10^6$ concentration. Zoospore solution was sprayed on the plants until runoff. Negative control plants were sprayed with RO water.

4.3.1.C Data Collection and Analysis

Disease incidence (number of plants that show aerial blight) and severity (how aggressive the aerial blight symptoms were) on day 3, 6, and 10. Area under the disease progress curve (AUDPC) was calculated from disease severity values. Plants were visually graded from 0-100% disease severity (0= no disease, 100= death). The percent values were assigned as explained previously in Parada-Rojas et al., 2019. A one-way ANOVA and Tukey test performed on the AUDPC values in JMP 16. "Cora Classic Red" was used as a positive control.

4.3.2 Test 2: Sept-October 2020

4.3.2.A Plants and Experimental Design

The following plants donated from Syngenta and Ball were tested for their susceptibility to *Phytophthora* isolates at 2.5- weeks old: Cora XDR 'Cranberry,' Cora XDR 'Deep Strawberry,' Cora XDR 'Hotgenta,' Cora XDR 'Light Pink,' Cora XDR 'Magenta Halo,' Cora XDR 'Polka Dot,' Cora XDR 'Punch,' Cora XDR 'White,' Titan 'Apricot,' Titan 'Blush,' Titan 'Burgundy,' Titan 'Icy Pink,' Titan 'Polka,' Titan 'White,' Valiant 'Apricot,' and Valiant 'Pure White.' The vinca plants, (20 varieties, variable replicates each), donated from Syngenta and Ball were arranged randomly in separate control and experimental areas in a randomized according to treatment group design. To generate random placement, the plant seedling trays were assigned a number. Numbers were compiled into a list and placed into "Miniwebtool." This process continued until all the replicates were in the designated treatment areas (control, experimental).

4.3.2.B Production of Test Inoculum

Test inoculum were produced and the plants were inoculated in a manner as previously described in Test 1, but in Test 2 sporangia instead of zoospores and the sporangia solution was diluted down with reverse osmosis (RO) water to obtain a $1x10^6$ concentration. *Phytophthora* and control solutions were sprayed in a manner illustrated in Test 1.

4.3.2.C Data Collection and Analysis

Disease incidence and severity were recorded on day 7, 10, 13, 16, 19 and 22 after inoculation. Plants were graded from 0-100% disease severity (0= no disease, 100= death). Mortality percent per variety calculated from disease severity values due to irregular replicate numbers.

4.3.3 Test 3: Pathogenicity Study Using Multiple Phytophthora Isolates

4.3.3.A Plants and Experimental Design June-July 2021

The following seeds were donated from Syngenta and Ball and tested for their susceptibility to *Phytophthora* isolates at three-weeks old: Cora 'Classic Red,' Cora XDR 'Apricot,' Cora XDR 'Cranberry,' Cora XDR 'Deep Strawberry,' Cora XDR 'Hotgenta,' Cora XDR 'Light Pink,' Cora XDR 'Magenta Halo,' Cora XDR 'Mix BK,' Cora XDR 'Orchid BK,' Cora XDR 'Polka Dot,' Cora XDR 'Punch,' Cora XDR 'White,' Titan 'Blush,' Titan 'Polka Dot,' and Titan 'Dark Red,' Titan 'White,' Valiant 'Apricot,' Valiant 'Burgundy,' Valiant 'Lilac,' Valiant 'Magenta,' Valiant 'Orchid,' Valiant 'Pure Punch,' and Valiant 'Pure White.' Vinca transplants, (23 varieties, 25 replicates each), were randomly arranged in appropriate treatment areas (control, isolate 1, isolate 2, etc.) in a randomized according to treatment group

design. (See Test 2 above for protocol). This test was conducted in a greenhouse maintained at a RH range of 80-90% and ambient temperature of 77-85°F. Cora 'Classic Red' was used as a positive control.

4.3.3.B Production of Test Inoculum

Sporangia from *Phytophthora* sp. 2238-2019, 1756-2007, 1422-2007, 845-2007, and 1446-2007 were produced following the method described in Tests 1 and 2 to obtain a $1x10^4$ concentration. *Phytophthora* and control solutions were produced and sprayed in a manner previously described in Test 1.

4.3.3.C Data Collection and Analysis

Disease incidence and severity were recorded on day 2, 3, 4, 5, 6, and 8 after inoculation. AUDPC was calculated from disease severity values. Plants were graded from 0-100% disease severity (0= no disease, 100= death). One-way ANOVA and Tukey test performed on AUDPC in JMP 16 along with a correlation coefficient on plant height and disease severity.

4.4 Results

4.4.1 Test 1

Cora and Cora XDR vinca were inoculated with *Phytophthora* sp. 2238-2019. Cora 'Classic Red,' which is known to be susceptible to aerial blight by *Phytophthora,* recorded an AUDPC value of 249. Other vinca tested were Cora XDRs, which are known to be more resistant to aerial blight by *Phytophthora* than Cora 'Classic Red.' All XDR varieties recorded AUDPC values less than 100 and were not statistically different from each other but were statistically lower than Cora 'Classic Red.' Although Cora XDR 'Hotgenta' had the lowest

disease severity and Cora XDR 'Light Pink' had the highest disease severity of the XDR's tested and these values were not significantly different than the other XDR's (Figure 19).

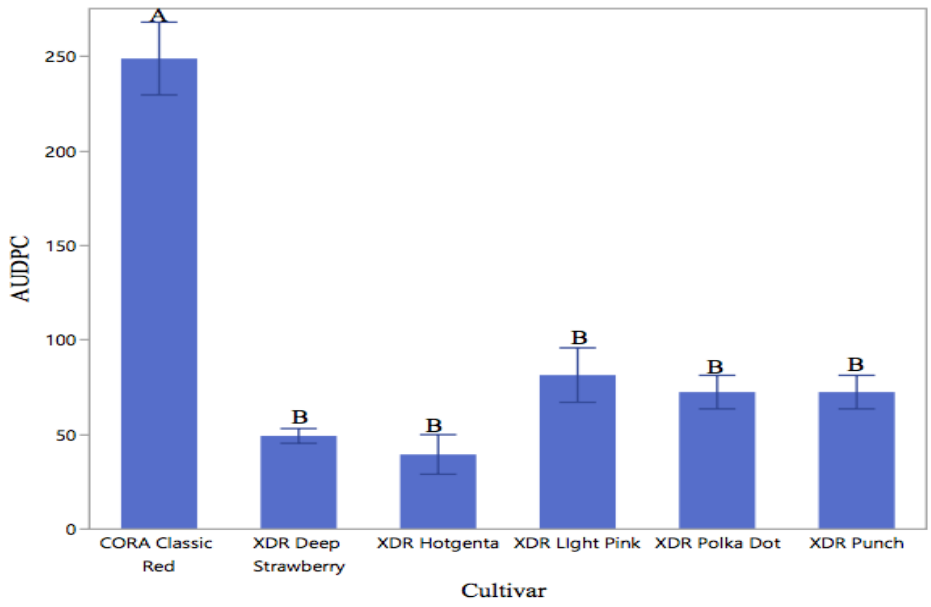

Figure 19 AUDPC per Approximately Two-Month Old Vinca Inoculated with 1x10^6 Zoospores of *Phytophthora* sp. 2238-2019 Under Greenhouse Conditions. Data analyzed with one-way ANOVA and Tukey test using JMP 16. Standard error bars constructed using one standard error from the mean. Treatments sharing a group letter are not statistically different.

4.4.2 Test 2

Differences in germination did not allow for collection of AUDPC. Therefore, percent mortality was measured based on the plants that germinated. The vinca plants showing the highest percent mortality on day 7 were different than on the final day recorded (22 days post inoculation). The varieties of vinca tested differed in progression of death over time with most Titan vincas showing more initial disease symptoms when compared to Cora, Valiant and Cora

XDR's (Figures 20A-C). No additional mortalities were observed post day 19 after inoculation as evidenced by the similar recorded rate on day 22 (Figure 20C). Titan 'White' showed consistently the highest percent mortality on the final day with *Phytophthora* sp. 2238-2019 (Figure 20C). Cora XDR 'Cranberry' and Cora XDR 'Punch' did not show any death throughout the study.

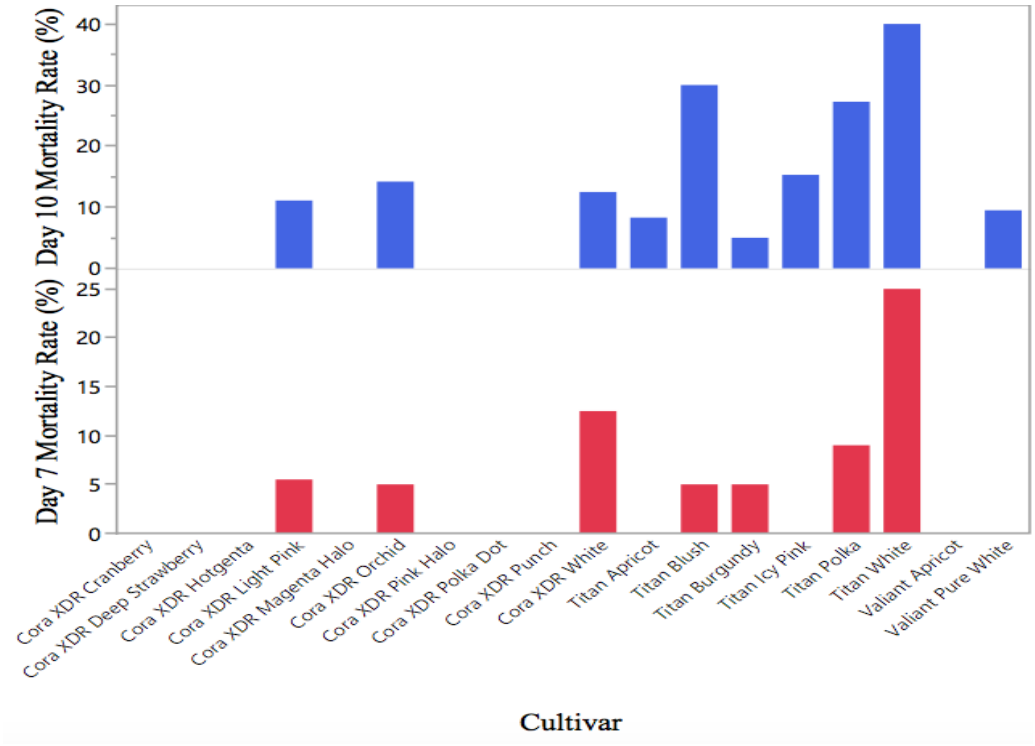

Figure 20 A) Figure 20 Day 7 and Day 10 Mortality Rate per Vinca Inoculated with 1x10^6 Sporangia of *Phytophthora* sp. 2238-2019 Under Greenhouse Conditions. Constructed using JMP 16.

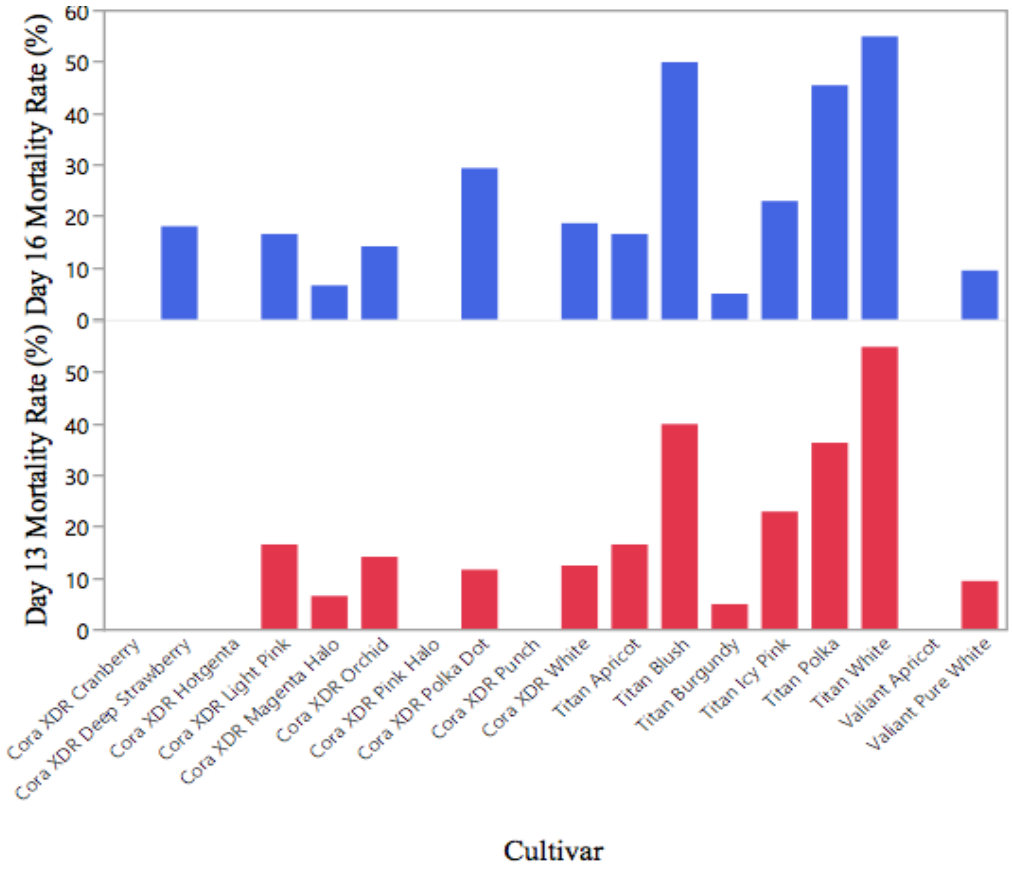

Figure 20 B) Day 13 and Day 16 Mortality Rate per Vinca Inoculated with 1x10^{6} Sporangia of *Phytophthora* sp. 2238-2019 Under Greenhouse Conditions. Constructed using JMP 16.

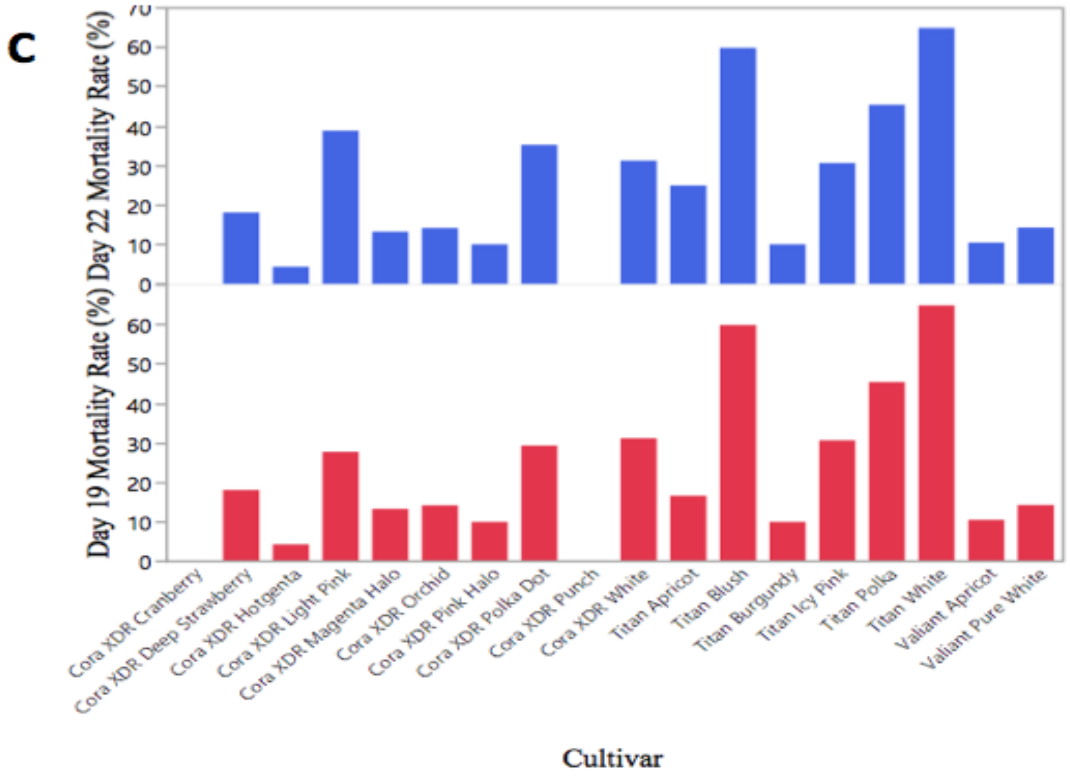

Figure 20 C) Day 19 and Day 22 Mortality Rate per Vinca Inoculated with 1x10^{6} Sporangia of *Phytophthora* sp. 2238-2019 Under Greenhouse Conditions. Constructed using JMP 16.

4.4.3 Test 3: Results Organized by Phytophthora Isolate

4.4.3.A Phytophthora sp. 2238-2019

As previously stated, Cora 'Classic Red' is known to be susceptible to aerial blight of *Phytophthora*. Cora 'Classic Red' and Cora XDR 'Light Pink' were not significantly different from each other in mean AUDPC (Figure 21). Cora 'Classic Red' recorded a mean AUDPC of 488.3 and Cora XDR 'Light Pink' recorded a mean AUDPC of 411.2. Other Cora XDRs showed significantly lower mean AUDPC from Cora 'Classic Red' and were not significantly different from each other in mean AUDPC. Cora XDR 'Cranberry,' (mean AUDPC of 11.4), Cora XDR 'Deep Strawberry,' (mean AUDPC of 20.0), and Cora XDR 'Magenta Halo,' (mean

AUDPC of 40.66), had the lowest disease severity of the XDR's (Figure 21). Valiant 'Burgundy' had the highest overall disease severity of the cultivars tested with a mean AUDPC of 516.2 (Figure 21). Valiant 'Orchid,' (mean AUDPC of 178.6), Valiant 'Pure Punch,' (mean AUDPC of 277.2), Valiant 'Apricot,' (mean AUDPC of 278.8), Valiant 'Magenta' (mean AUDPC of 280.4), all recorded significantly less disease than the Cora 'Classic Red.' Titan 'Polka Dot,' (mean AUDPC of 342), had significantly less disease than the Cora 'Classic Red,' but the other Titan varieties were not significantly different from Cora 'Classic Red.' All plants showed AUDPC significantly higher than water inoculated plants (mean AUDPC of 0).

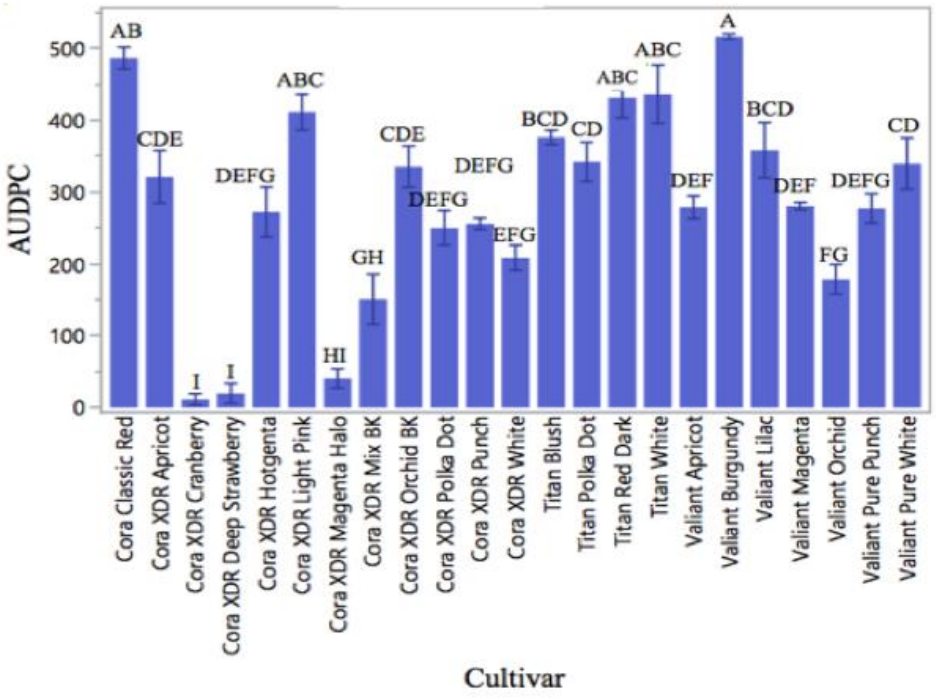

Figure 21 AUDPC per Cora XDR, Titan and Valiant Vinca Inoculated with 1x10^4 Zoospores of *Phytophthora* sp. 2238-2019 Under Greenhouse Conditions. Data analyzed by one-way ANOVA and Tukey test using JMP 16. Standard error bars constructed using one standard error from the mean. Treatments sharing a group letter are not statistically different.

4.4.3.B Phytophthora sp. 845-2007

Cora ‘Classic Red,’ (mean AUDPC of 0), did not produce disease significantly different from water inoculated plants (mean AUDPC of 0). Cora XDR ‘Light Pink,’ (mean AUDPC of 49.7), had the highest disease severity of XDR’s and was significantly greater in disease than water inoculated plants (Figure 22). Cora XDR ‘Apricot,’ (mean AUDPC of 0), Cora XDR ‘Magenta Halo’ (mean AUDPC of 0), and Cora XDR ‘Orchid’ (mean AUDPC of 0), also did not show disease significantly different from negative control. Valiant ‘Pure Punch,’ (mean AUDPC of 20.83), showed the highest disease severity of the Valiants and was significantly greater in disease than negative control, but other Valiants tested did not show disease significantly different than water inoculated plants. Titan ‘White,’ (mean AUDPC of 76.2), showed the largest disease severity of the Titans tested and was significantly greater when compared to water inoculated plants. The other varieties tested did not produce disease significantly greater than water inoculated plants.

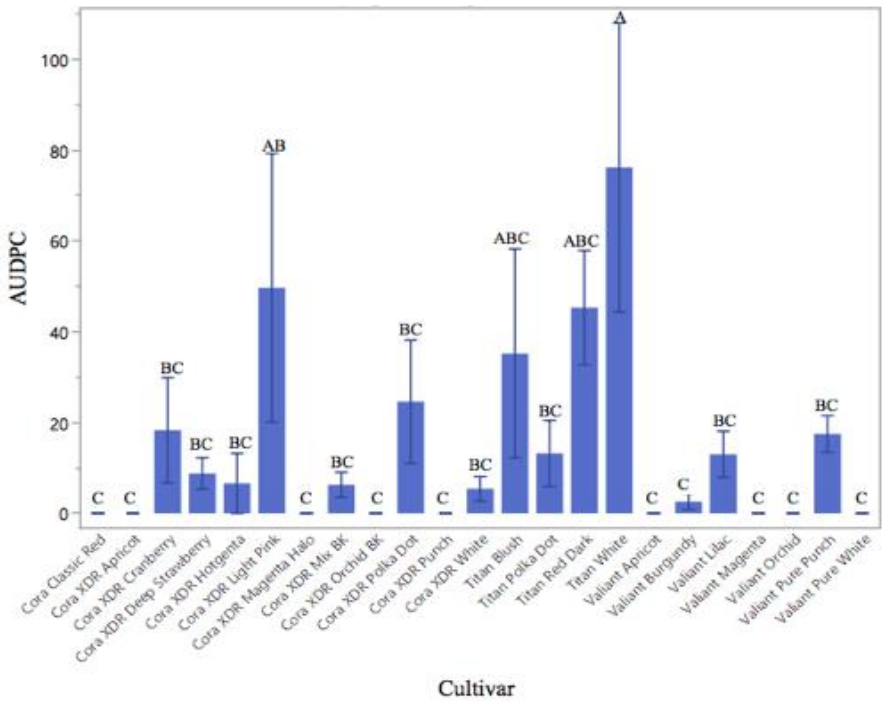

Figure 22 AUDPC per Cora XDR, Titan and Valiant Vinca Inoculated with 1x10^4 Zoospores of *Phytophthora* sp. 845-2007 Under Greenhouse Conditions. Data analyzed by one-way ANOVA and Tukey test using JMP 16. Standard error bars constructed using one standard error from the mean. Treatments sharing a group letter are not statistically different.

4.4.3.C Phytophthora sp. 1422-2007

Cora 'Classic Red,' (mean AUDPC of 1.3), did not produce disease significantly different from negative control (mean AUDPC of 0). Cora XDR 'Deep Strawberry,' (mean AUDPC of 50.7), showed a significantly greater disease severity compared to other Cora XDR's tested for this isolate (Figure 23). Other XDR's tested did not show disease significantly greater than negative control. Titan 'Blush,' (mean AUDPC of 33), showed the greatest disease severity, and Titan 'White," (mean AUDPC of 2.7), the lowest disease among Titans tested, but this was not significantly different from disease found on other Titans. Lastly, Valiant

'Burgundy,' (mean AUDPC of 16.9), showed highest disease severity and Valiant 'Pure White,' (mean AUDPC of 4.4), the lowest disease of Valiants tested, but this was not significantly different than water inoculated plants.

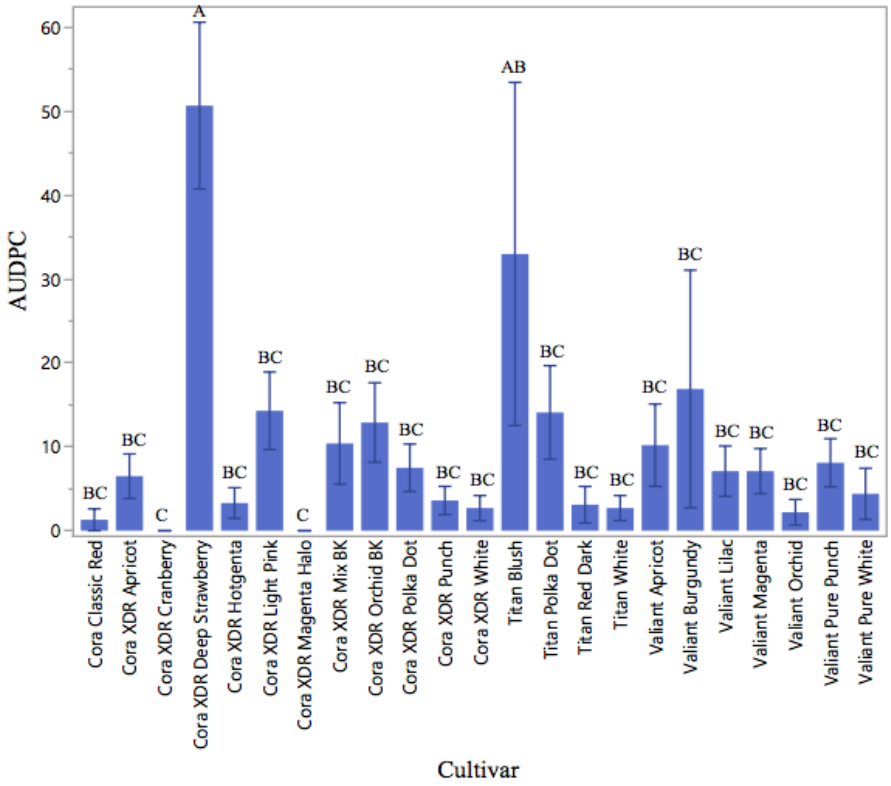

Figure 23 AUDPC per Cora XDR, Titan and Valiant Vinca Inoculated with 1x10^4 Zoospores of *Phytophthora* sp. 1422-2007 Under Greenhouse Conditions. Data analyzed by one-way ANOVA and Tukey test using JMP 16. Standard error bars constructed using one standard error from the mean. Treatments sharing a group letter are not statistically different.

4.4.3.D Phytophthora sp. 1446-2007

Upon inoculating vinca with this isolate, no disease was observed.

4.4.3.E Phytophthora sp. 1756-2007

Cora XDR 'Hotgenta,' (mean AUDPC of 28), appeared to be the only vinca cultivar tested that showed aerial blight symptoms at significantly higher numbers than water inoculated plants (mean AUDPC of 0) (Figure 24). The positive control did not appear to produce disease. It should be noted that there was a difference in plant height observed among the varieties tested. It was found that there was a correlation of -0.1751 and P<0.0001 between plant height and disease severity.

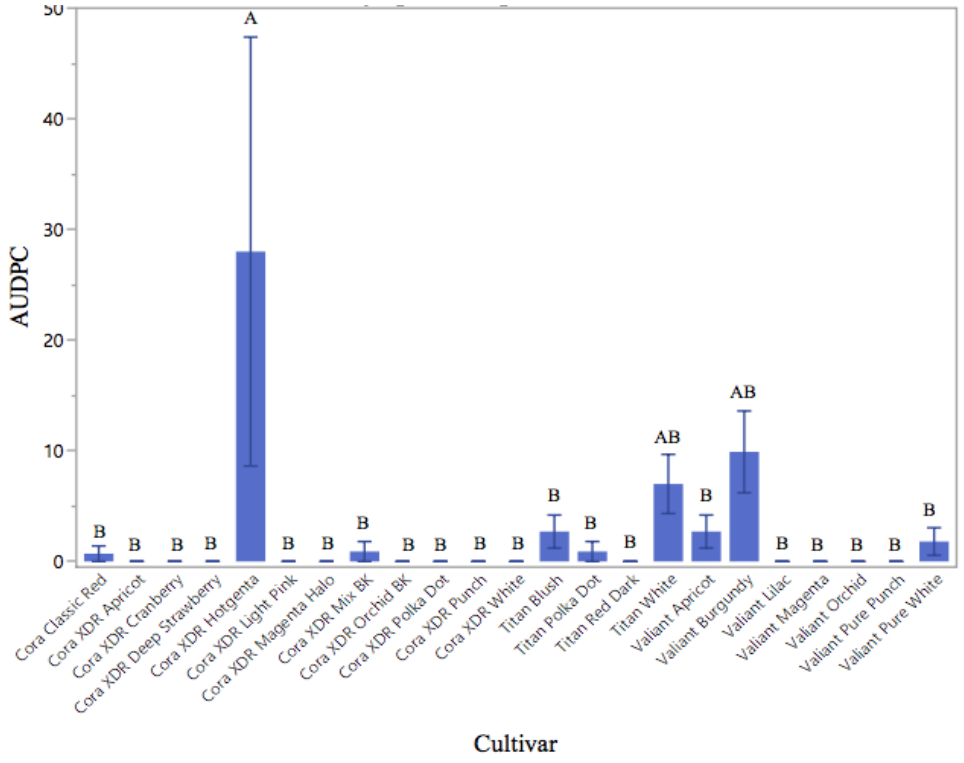

Figure 24 AUDPC per Cora XDR, Titan and Valiant Vinca Inoculated with 1x10^4 Zoospores of *Phytophthora* sp. 1756-2007 Under Greenhouse Conditions. Data analyzed by one-way ANOVA and Tukey test using JMP 16. Standard error bars constructed using one standard error from the mean. Treatments sharing a group letter are not statistically different.

4.4.3.F Phytophthora Aerial Blight Progression Between Isolates

The progression of disease *Phytophthora* sp. 2238-2019 when compared to the other isolates (Figure 25). There were differences in progression of disease among the *Phytophthora* isolates as evidenced by the photos below and AUDPC values recorded.

Figure 25 Progress of Disease on Cora XDR 'Light Pink' Vinca with *Phytophthora* Isolates. Aerial blight symptoms started on day 2 post inoculation. Progress of disease between the different isolates tested on the same vinca cultivar on day 4 post inoculation. A) Control B) *Phytophthora* sp. 1446-2007 C) *Phytophthora* sp. 1422-2007 D) *Phytophthora* sp. 845-2007 E) *Phytophthora* sp. 2238-2019 F) *Phytophthora* sp. 1756-2007.

4.5 Discussion

4.5.1 Test 1

Anything that was statistically higher or equal in disease severity to Cora 'Classic Red' is considered to have some level of susceptibility to the *Phytophthora* isolate. Therefore, all the XDR varieties tested have some level of resistance to *Phytophthora* sp. 2238-2019 and are not statistically different from each other for the varieties tested using the conditions outlined.

4.5.2 Test 2

Due to irregular germination/replicate numbers, no statistics could be performed on this data. Therefore, no reliable inferences can be made using this data. Test 2 did illustrate that sporangia can be used as an effective inoculum.

4.5.3 Test 3

4.5.3.A Phytophthora sp. 2238-2019

Most Cora XDR varieties have some level of resistance towards *Phytophthora* sp. 2238-2019 as only Cora XDR 'Light Pink' was significantly more diseased than the positive control (Figure 21). It was expected Cora XDR 'Light Pink' would show a high AUDPC for *Phytophthora* sp. 2238-2019, as *Phytophthora* sp. 2238-2019 was originally isolated from a Cora XDR 'Light Pink.' It was surprising that Valiant 'Burgundy' showed the highest disease severity as Valiant varieties have been known to have some level of resistance towards *P. nicotianae* (PanAmerican Seed, 2017). The other varieties match what is known for Valiant vinca in regards to aerial blight. Titan is not known to be resistant to aerial *P. nicotianae,* therefore most Titan varieties not being significantly different from the positive control supports the literature on

Titan being known to be resistant to *P. nicotianae* (PanAmerican Seed, 2017). Compared to the other isolates, *Phytophthora* sp. 2238-2019 caused more disease, and the progression of disease was much faster with *Phytophthora* sp. 2238-2019, when compared to the other isolates (Figure 25).

4.5.3.B Phytophthora sp. 845-2007

The positive control did not produce disease significantly greater than negative control (Figure 22). This control was previously tested against *Phytophthora* sp. 2238-2019, but had not been tested against the other varieties. All AUDPC were found to be less than 100 in isolates other than *Phytophthora* sp. 2238-2019, but if future work were to be conducted using this isolate, another vinca should be used as control.

4.5.3.C Phytophthora sp. 1422-2007

Cora XDR 'Light Pink' and Titan 'White' were the only vinca that showed significantly higher disease when compared to the negative control (Figure 23). It is interesting in both pathogenicity testing with *Phytophthora* sp. 2238-2019 and *Phytophthora* sp. 1422-2007, the Cora XDR 'Light Pink' and Titan 'White' were identified as having the high disease severity of their distinct group.

4.5.3.D Phytophthora sp. 1446-2007

No disease shown indicates these varieties are resistant to *Phytophthora* sp. 1446-2007.

4.5.3.E Phytophthora sp. 1756-2007

Cora XDR ‘Hotgenta’ was the only cultivar to have disease severity greater than negative control, but the AUDPC number was under 30 (Figure 24). This number is very low compared to isolates where cultivars were found to be susceptible to *Phytophthora* sp. 2238-2019. In addition, this is the only isolate where Cora XDR ‘Hotgenta’ showed the greatest disease severity.

The difference in severity in disease between these isolates could possibly be attributed to host specificity, as these isolates were isolated from hosts other than vinca with the exception of *Phytophthora* sp. 2238-2019 (Roy, 2017). One example of the varieties tested that showed a difference between isolates was Cora XDR ‘Light Pink’ (Figure 25). The small negative correlation between plant height and disease severity indicates the smaller the plant was, the higher the disease severity. The effect is weak because the correlation is very small. The difference in plant height was due to transplanting the vinca seedlings instead of sewing them from seed directly into the seed tray.

4.5.3.F Summary

Test 1 indicated older plants may not show differences in susceptibility among the varieties to *Phytophthora* as well as younger seedlings. This difference in susceptibility is evidenced by Cora XDR ‘Light Pink’ recorded mean AUDPC of 81.5 for two-month-old plants, and 411.2 mean AUDPC for Test 3 seedlings. While Test 2 data did not allow for interpretation of susceptibility, evidence of aerial blight by *Phytophthora* did show sporangia can be used as an effective inoculum. Additionally, it was hypothesized there would be differences in susceptibility among vinca cultivars and varieties. Test 3 indicated that there are differences in virulence among the *Phytophthora* isolates inoculated on the seedlings, as evidenced by Cora

‘Classic Red’ mean AUDPC of 488.3 when inoculated with *Phytophthora* sp. 2238-2019, and mean AUDPC of 0 when inoculated with *Phytophthora* sp. 845-2007. Lastly, Test 3 illustrated there were differences in susceptibility among vinca varieties tested to *Phytophthora* sp. 2238-2019 as evidenced by Cora XDR ‘Deep Strawberry’ mean AUDPC of 20.0 and Titan ‘White’ mean AUDPC of 436.2.

5. HOST PLANT RANGE TESTING of PHYTOPHTHORA SP. 2238-2019

5.1 Overview

Previous morphological and molecular characterization revealed *Phytophthora* sp. 2238-2019 to be *Phytophthora nicotianae*. *P. nicotianae* has a broad host range and can spread easily between infected and neighboring plants by overhead watering, high humidity and infested tools. A host plant range study was conducted in August 2021 to assess the susceptibility of current common bedding plants of known general susceptibility/resistance to *P. nicotianae* with *Phytophthora* sp. 2238-2019. The hypothesis is that *Phytophthora* sp. 2238-2019 would be able to infect most known aerial blight susceptible plants of *P. nicotianae.* *Phytophthora* sp. 2238-2019 zoospore solution was sprayed on bedding plants, and observed susceptibility/resistance every day for 12 days. Only Cora XDR 'Magenta,' Cora XDR 'Magenta Halo,' English Lavender (*Lavandula angustifolia*), and Petunia 'Laura Bush' showed symptoms of aerial blight. *Phytophthora* sp. 2238-2019 was recovered from symptomatic plant material on hymexazol (Hx) agar. Lack of symptoms for known susceptible plants could be due to host plant specificity of *Phytophthora* sp. 2238-2019.

5.2 Introduction

P. nicotianae has been reported on 255 plant genera and 90 families (Cline et al., 2008). *P. nicotianae* can cause stem, root and aerial blight (Erwin and Ribeiro, 1996). Examples of ornamentals in addition to vinca that are susceptible to *P. nicotianae* cause aerial blight include but are not limited to: poinsettia (*Euphorbia pulcherrima*) (Uchida and Aragaki 1979; Engelhard and Ploetz 1979), iris (*Iris* sp.) (Dastur, 1935), and orchid (*Paphiopedilum sp.*) (Uchida and Aragaki, 1991).

The objective of this section of the study was to determine susceptibility of common ornamental plants known to be resistant and susceptible to infection by *P. nicotianae* (stem rot, aerial blight, root rot). *Phytophthora* sp. 2238-2019 was chosen for the host plant range study because it was originally isolated from a Cora XDR 'Light Pink.' Cora XDRs are known to be resistant to most *P. nicotianae* aerial blight, therefore a better understanding of the host range of this particular isolate may help to inform further management approaches. The susceptibility of bedding plants is still largely unknown (Hanson et al, 2020; Creswell et al., 2011). Common bedding plants with known susceptibility/resistance were chosen (Table 7). Cora XDR 'Magenta' and Cora XDR 'Magenta Halo' were previously tested in this study (2020 and 2021) and found to be susceptible to *Phytophthora* sp. 2238-2019.

Table 7 Host Plant Range: Bedding Plant Susceptibility to *P. nicotianae*

Scientific Name	Common Name	Susceptible	Resistant
Lavandula angustifolia	English Lavender	collar, root (Alvarez et al., 2007); root, stem (Orlikowski and Valijuskaite, 2007); root rot (Hwang and Benson, 2005; Putnam, 1991)	
Petunia violacea	Petunia 'Laura Bush'	*Petunia* spp. (Hwang and Benson, 2005*), *Petunia hybrida* (Henson et al., 2020*)	
Verbena x *hybrida*	Verbena 'Blue Princess,' Verbena 'Homestead Purple'	*Verbena x hybrida* , (Lamour et al., 2003*), Stem rot 'Homestead Purple' (Henson et al., 2020*)	
Perovskia sp.	Russian Sage	*Perovskia* sp. (Creswell and Beckerman, 2019*)	
Salvia greggi	Salvia 'Furman's Red' Salvia 'Purple'	*Salvia* sp. (Creswell and Beckerman, 2019*)	*Salvia coccinea* 'Lady in Red,' *Salvia farinacea* 'Victoria Blue' (Banko and Stefani, 2000*).
Salvia guaranitica	Salvia 'Black and Blue'		
Salvia elegans	Pineapple Sage		
Oreganum vulgare	Italian Oregano	Oregano (Creswell and Beckerman, 2019*)	
Phlox paniculata	Phlox 'Nicky'	*Phlox paniculata* foliage blight (Drechsler et al., 2018)	
Echinacea purpurea	Purple Coneflower		(Henson et al., 2020*)
Aster oblongifolia	Hardy Aster		(Creswell and Beckerman, 2019*)
Rudbeckia hirta	Indian Summer 'Black Eyed Susan'		(Hansen et al., 2000*)
Mentha spicata	Spearmint		Spearmint (Creswell and Beckerman, 2019*)
Catharanthus roseus	Cora XDR 'Magenta,' Cora XDR 'Magenta Halo'	Aerial blight (Ch.4 of this work)	

*Disease type not specified (root, stem, aerial/foliage blight).

5.3 Materials and Methods

5.3.1 Plants and Experimental Design

Southwest Perennials from (Dallas, TX) donated approximately eight-week old plants for this study with the exception of large (approximately 1-month old) Cora XDR 'Magenta' plants which were purchased from a local nursery, and Cora XDR 'Magenta Halo' seeds were donated by Syngenta (Gilroy, CA) (Table 6). Each plant set had 10 replicates (10 control/10 experimental). Cora XDR 'Magenta' were two weeks old at time of inoculation. Both Cora XDR 'Magenta Halo' and Cora XDR 'Magenta' plants were used as positive controls. Seedling trays were arranged in separate control (RO water inoculated) and experimental treatment (test inoculum) areas in a randomized according to treatment group experimental design. Each seedling tray was assigned a name based on the genus of the plant in the tray. These plant names were compiled into a list and placed into "Miniwebtool" random word generator to generate random sets. The plants were placed into designated spots on the greenhouse bench based on the order of output from the random plant list generator. After spraying with test inoculum, plants were observed for disease incidence of aerial blight every day for 12 days. This test was conducted in a greenhouse maintained at a RH (relative humidity) range of 80-90% and ambient temperature of 77-85°F. Automated misters were set to 20 seconds every hour. Plant disease incidence (DI) was recorded with values of 0-1, with 1 showing symptoms of aerial blight and 0 showing no symptoms of aerial blight. Aerial blight symptoms explained in Parada-Rojas et al., (2019).

5.3.2 Production of Test Inoculum

Based on preliminary work to obtain ample sporangia, the sporangia from *Phytophthora* sp. 2238-2019 were produced on V8 agar plugs after four days at 22-25°C in 20% NSES (non sterile soil extract solution) under LED light. The plugs were cold shocked at 4°C for six hours and two hours at 22-25°C. The agar plugs were blended in a blender and filtered with a kitchen sieve. Zoospores were diluted down with RO water to obtain a $1x10^4$ concentration (Camacho-Tapia et al., 2016). Zoospore solution was sprayed on the plants until runoff. Negative control plants were sprayed with RO water.

5.4 Results

After 48 hours, the first symptoms of disease appeared on Cora XDR plants such as browning, dark blotches and wilt. However, most plants took longer to see symptom development (Table 8). Disease incidence was recorded for day 7 and day 12 post inoculation with zoospores (Table 8). Cora XDR plants, English Lavender, and Petunia were found to produce lesions similar to aerial blight of *P. nicotianae* such as browning and dark blotches (Figure 26) (Henson et al., 2020). *Phytophthora* sp. 2238-2019 was re-isolated on Hx plates from symptomatic plant material. The highest degree of disease incidence was seen in the larger Cora XDR 'Magenta,' (DI of 10), when compared to the other plants tested.

Table 8 *Phytophthora* sp. 2238-2019 Aerial Blight Disease Incidence on Bedding Plants

Scientific Name	Common Name	DI at Day 7	DI at Day 12
Lavendula angustifolia	English Lavender	7	8
Petunia sp.	Petunia 'Laura Bush'	0	3
Verbena x hybrida	Verbena 'Blue Princess'	0	0
	Verbena 'Homestead Purple'	0	0
Perovskia sp.	Russian Sage	0	0
Salvia greggi	Salvia 'Furman's Red'	0	0
	Salvia 'Purple'	0	0
Salvia guaranitica	Salvia 'Black and Blue'	0	0
Salvia elegans	Pineapple Sage	0	0
Oreganum vulgare	Italian Oregano	0	0
Phlox paniculata	Phlox 'Nicky'	0	0
Echinacea purpurea	Purple Coneflower	0	0
Aster oblongifolia	Hardy Aster	0	0
Rudbeckia hirta	Indian Summer' Black Eyed	0	0
	Susan	0	0
Mentha spicata	Spearmint	0	0
Catharanthus roseus	Cora XDR 'Magenta'	10	10
	Cora XDR 'Magenta Halo'	6	6

Disease incidence is out of 10 treated plants.

Additionally, Lavender and Petunia plants exhibited symptoms of environmental stress as illustrated by slight browning on control plants (Figure 26). *Phytophthora* sp. 2238-2019 was not found in browned material from control plants when plated on Hx agar.

Figure 26 Petunia, Lavender and Vinca with Aerial Blight Symptoms from *Phytophthora* sp. 2238-2019. Experimental treatments were inoculated with $1x10^4$ zoospores (left), and control plants were sprayed with water. Image shows day 12 post inoculation. A) Cora XDR 'Magenta' B) Petunia 'Laura Bush' C) English Lavender D) Cora XDR 'Magenta Halo.'

5.5 Discussion

Control plants (Cora XDR 'Magenta' and Cora XDR 'Magenta Halo') did show signs of aerial blight indicating the zoospores were viable, and any plant not showing symptoms of blight after 12 days is not susceptible to *Phytophthora* sp. 2238-2019. Most of the plants that have been reported as susceptible in the literature to *P. nicotianae* did not specifically state aerial blight susceptibility except for *Phlox paniculata* (Drechsler et al., 2018) (Table 7). *P. nicotianae* can cause stem, and root rot but only aerial blight on a few plants (Banko and Stefani et al., 2000; Lamor et al., 2000).

Lavender (Figure 26) could have been more susceptible to *Phytophthora* sp. 2238-2019 in this study due to high humidity (above 50%), as Lavender prefers well-drained soil, and other

varieties of lavender are more tolerant to hot and high humidity climates such as Spanish Lavender (*L. stoechas*) and French Lavender (*L. dendata*) (McNaughton, 2000). The plants can be seen under stress exhibiting browning on control plants (Figure 26). When plants are under stress, they are more susceptible to infections by pathogens (Desaint et al., 2020). Additionally, this is the first report of Lavender showing symptoms of aerial blight when infected with *Phytophthora* sp. 2238-2019. Diseases that have been reported caused by *P. nicotianae* include collar (Alvarez et al., 2007) root (Putnam, 1991) and stem rot (Orlikowski and Valijuskaite, 2007). Lastly, as it took longer (when compared to Lavender and vinca) for symptoms to appear (Figure 26, Table 8), it is possible more of *Petunia* may have become symptomatic if the study continued with observations. Future work could conduct another trial(s), as there was only one trial of these bedding plants conducted, further trials would need to assess the reproducibility of sensitivity/resistance to *Phytophthora* sp. 2238-2019. There are additional plants not studied that are known to be susceptible to *P. nicotianae* aerial blight (Lin et al., 2017). If additional plants that are commonly found in a nursery are found to be susceptible to *Phytophthora* sp. 2238-2019, then additional treatments precautions/cultural practices may be necessary to prevent spread.

6. CONCLUSIONS AND FUTURE WORK

Novel and hybrid *Phytophthora* strains are continuing to be identified (Bose et al., 2021, Yang et al., 2014, respectively). It is important to identify *Phytophthora* species for accurate rapid diagnosis, reporting, and plant breeding for monitoring resistance against *Phytophthora* species. This work confirmed suspected *Phytophthora* isolates as unique *Phytophthora nicotianae* species based on morphological and molecular characterization. For morphological characterization: the size, shape and appearance of sporangia, chlamydospores, oogonia/antheridia, hyphae, and appearance of colony morphology were compared to what is known for *Phytophthora* species. Identification based on molecular methods was obtained from amplified isolated DNA obtained from PCR that was sequenced and consensus sequences were generated. These sequences were placed in NCBI BLASTn search. The resulting highest percent query was compared to observed morphological characteristics such as size and shape of sporangia, chlamydospores, oogonia/antheridia, and colony morphology to make identifications. Future work can further assess how the *Phytophthora* isolates studied in this work compare genetically to each other and compare to *P. nicotianae* isolates obtained in other diagnostic clinics and/or research labs.

Pathogenicity testing revealed differences among susceptibility of vinca cultivars to *Phytophthora* sp. 2238-2019, *Phytophthora* sp. 845-2007, *Phytophthora* sp. 1422-2007, *Phytophthora* sp. 1446-2007 and *Phytophthora* sp. 1756-2007. Pathogenicity test 1 revealed two-month old plants can still be infected with zoospores of *Phytophthora* sp. 2238-2019 which may be useful for further testing, but ability to determine susceptibility of vinca varieties may be hindered as the plants age. Additionally, Test 1 revealed Cora XDR ‘Light Pink’ was not significantly different in aerial blight disease development when inoculated with *Phytophthora*

sp. 2238-2019 compared to other varieties at that age. The data obtained from Pathogenicity test 2, unfortunately, was not usable for accurate inferences because the germination was uneven, and replicate numbers were not the same for the various vinca cultivars. Test 2 revealed smaller plants can provide more information about susceptibility between the vinca cultivars when compared to test 1, and that sporangia can be used as an effective inoculum. Pathogenicity test 3 showed Titan and Valiant vinca as more likely to become infected with *Phytophthora* sp. 2238-2019, and recorded Cora XDR 'Light Pink' to have the highest disease rating of the Cora XDR's tested. Test 3 showed the Cora XDRs as having some level of resistance to *Phytophthora* sp. 2238-2019. The other *Phytophthora* isolates tested did not produce a level of disease as severe as *Phytophthora* sp. 2238-2019. Further differences between the cultivars could be illuminated if there are additional trials of *Phytophthora* sp. 2238-2019 as test 3 had variable plant height that did show a weak correlation between plant height and disease rating. It was found that there was a correlation of -0.1751 and $P<0.0001$ between plant height and disease severity using JMP 16.

In addition, commonly applied fungicides for aerial blight from *P. nicotianae* on ornamentals were tested *in vitro* against *Phytophthora* sp. 2238-2019. Based on the data collected, *Phytophthora* sp. 2238-2019 mycelial growth was most restricted on plates amended with Subdue Maxx™ and Stature™ *in vitro,* as previously defined by being able to reduce mycelial growth to less than or equal to one half the unamended treatment. The results do not support the hypothesis that *Phytophthora* sp. 2238-2019 can be sensitive to 1x with Heritage™, Pageant Intrinsic™, and Segway™. Future work can test the efficacy of these fungicides in field and greenhouse conditions against *Phytophthora* sp. 2238-2019. Lastly, a host range study was conducted to assess if *Phytophthora* sp. 2238-2019 is able to infect other plants in addition to vinca. After 12 days, Lavender, Petunia, Cora XDR 'Magenta' and Cora XDR 'Magenta Halo'

showed aerial blight symptoms and *Phytophthora* sp. 2238-2019 was re-isolated from infected plant material. Future work can address additional host plants, and conduct additional trials as there was only one host plant range trial.

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