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1 **Identification and Management of Phytophthora Aerial Blight Caused by *Phytophthora nicotianae* on**

2 ***Catharanthus roseus***

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23 **Abstract**

24 *Phytophthora nicotianae* is the most common pathogen in nurseries and gardens, infecting both woody
25 and herbaceous ornamental plants. Phytophthora aerial blight symptoms such dull water-soaked lesions
26 on shoot tips and leaf petioles, girdling on the main stem, necrosis, and wilting of annual vinca were
27 observed in a commercial greenhouse in Warren Co., Tennessee, USA in May 2016. The objective of this
28 study was to identify the causal agent of Phytophthora aerial blight and develop a fungicide
29 management recommendation for ornamental producers. Attempts to isolate the pathogen from
30 symptomatic leaf tissue were conducted and excised leaf pieces were embedded in the V8 agar
31 medium. Morphological characterization, polymerase chain reaction (PCR), sequencing, and
32 pathogenicity test of the isolate FBG2016_444 were conducted to confirm the pathogen identification.
33 The sequence identity was 100% identical to *Phytophthora nicotianae*, and a combined phylogenetic
34 tree (internal transcribed spacer [ITS]), the large subunit [LSU] of rDNA, and ras-related protein gene
35 [*Ypt*1]) grouped isolate FBG2016_444 within the clade of *P. nicotianae*. In the pathogenecity study, all
36 inoculated annual vinca plant showed Phytophthora aerial blight symptoms and *P. nicotianae* was re-
37 isolated whereas non-inoculated annual vinca plant remained symptomless. These findings confirmed *P.*
38 *nicotianae* as the causal agent of Phytophthora aerial blight of annual vinca. In addition, two rates (0.078
39 and 0.156 $mL{\cdot}L^{-1}$) and three application intervals (7, 14 and 21 days before inoculation [DBI]) of
40 oxathiapiprolin (Segovis®) were evaluated for their ability to reduce the Phytophthora aerial blight
41 severity on annual vinca plants. The control groups were positive (non-treated inoculated) and negative
42 (non-treated non-inoculated) plants. Both rates and application timings of oxathiapiprolin significantly
43 reduced Phytophthora aerial blight severity and disease progress (area under disease progress curve
44 [AUDPC]) on annual vinca plants compared to the positive control. However, 0.078 and 0.156 $mL{\cdot}L^{-1}$ of
45 oxathiapiprolin applied at 7 or 14 DBI were the most effective treatments in reducing the disease
46 severity and AUDPC on annual vinca plants. The plant growth parameters such as increase in height and

47 width, total plant weight, and root weight were not influenced by the application of oxathiapiprolin. The

48 finding reported in this study will help ornamental producers with better management of Phytophthora

49 aerial blight of annual vinca.

50

51 **Keywords:** annual vinca, application timing, chemical management, diagnosis, oxathiapiprolin,

52 Phytophthora aerial blight

53

54 **Introduction**

55 The genus *Phytophthora* is comprised of some of the most destructive oomycetes, which can cause

56 serious economic loss in crop production (Erwin and Ribeiro 1996). It is reported that more than 120

57 described species are present in the *Phytophthora* genus (Martin et al. 2014). Among the *Phytophthora*

58 species, *Phytophthora nicotianae* van Breda de Haan (syn. *P. parasitica* Dastur [Erwin and Ribeiro 1996])

59 is considered an important plant pathogen due to severe damage to crops, wide geographic distribution,

60 and diverse host range (Panabieres et al. 2016). Over 255 plant genera in 90 families are affected by

61 *P. nicotianae*, including multiple genera of economically important fruits, vegetables, forest tree, and

62 woody and herbaceous ornamental plants (Baysal-Gurel and Kabir, 2019; Panabieres et al., 2016;

63 Prigigallo et al. 2015; Yandoc et al., 2007). Symptoms of *P. nicotianae* such as root and crown rot are

64 more common, however, flowers, fruits, and foliage also infected (Erwin and Ribeiro 1996).

65 *Phytophthora nicotianae* is the most common species in nurseries (field and container

66 prroduction) and gardens attacking ornamental plants (Ahmed et al. 2012; Bienapfl and Balci 2014;

67 Leonberger et al. 2013; Olson et al. 2013; Schwingle et al. 2007). The disease caused by *P. nicotianae*

68 such as root and crown rot, and fruit and foliar blight have become a problematic disease in floriculture

69 production, reducing ornamental characteristics and marketability of numerous herbaceous annual

70 ornamental plants and perennial nursery plants (Erwin and Ribeiro 1996). This pathogen produces

71 chlamydospores and oospores as a survival structure, which facilitate to survive in plant debris and soil
72 over multiple seasons (Erwin and Ribeiro 1996; Kröber 1980), and remains in irrigation water systems
73 and watersheds (Hong and Moorman 2005; Hulvey et al. 2010). This allows it to persist for multiple
74 seasons unless contaminated materials are disinfested or removed.

75 Growers rely mainly on fungicides for the management of *Phytophthora* species and other
76 oomycete plant pathogens. Systemic fungicides such as mefenoxam and fosetyl-al are used for the
77 management of *Phytophthora nicotianae* in ornamental plants (Erwin and Ribeiro 1996; McGovern et
78 al. 2000). However, the efficacy of these fungicides has been jeopardized due to development of
79 resistant strains of species of *Phytophthora*, including *P. nicotianae* (Ferrin and Rohde 1992; Hu et al.
80 2008; Hwang and Benson 2005; Parra and Ristaino 2001). Alternatively, other means of Phytophthora
81 aerial blight management such as the use of resistant cultivars, cultural practices, soil fumigation, plant
82 extracts, and biological control agents have only provided limited control against *P. nicotianae*,
83 especially when disease pressure is high. Thus, a new active ingredient (a.i.) with a different mode of
84 action is necessary for the management of Phytophthora aerial blight in ornamental plants.

85 Oxathiapiprolin is a new fungicide belonging to the piperidinyl-thiazole-isoxazolines class (FRAC
86 code 49 [Fungicide resistance action committee 2020; Pasteris et al. 2008]), and has shown great
87 efficacy against oomycete pathogens including *P. nicotianae* (Belisle et al. 2017; Cohen 2015; Cohen et
88 al. 2017; Ji and Csinos 2015; Ji et al. 2014; Olaya et al. 2016; Qu et al. 2016). The target site of
89 oxathiapiprolin is oxysterol binding protein (OSBP), which represents the novel target site for oomycete
90 disease control (Pasteris et al. 2016). It is reported that several stages of *P. nicotianae* are sensitive to
91 oxathiapiprolin; it inhibits the zoospore release and motility, zoospore germination, sporangia
92 production and germination, and mycelium growth (Bittner and Mila 2016) and inhibits chlamydospore
93 formation of *P. nicotianae* (Gray et al. 2018).

94 Phytophthora aerial blight has been reported in annual vinca (*Catharanthus roseus* [L.] Don.) in
95 Ohio, North Carolina, California, and Florida in the United States (Erwin and Ribeiro 1996; Gill et al.
96 1977; Lin et al. 2018; McGovern et al. 2000; Olson and Benson 2011). Phytophthora aerial blight of
97 annual vinca develops rapidly and is facilitated by high soil moisture coupled with prolonged rainy
98 weather or frequent irrigation (Bowers and Locke 2004; Gill et al. 1977; McGovern et al. 2000). Foliar
99 blight symptoms appear as dull water-soaked lesions on shoot tips and leaf petioles. In later stages, dark
100 brown to black spots circle the stem. Wilting of the plant and plant death can occur within one to two
101 weeks after the first symptoms appear (Yandoc et al. 2007). The roots of the infected plants often
102 remain healthy (Erwin and Ribeiro 1996).
103 In May 2016, potted annual vinca 'Pacifica XP Deep Orchid' showed the brownish-green
104 discoloration of shoot tips and foliage in a commercial greenhose in Warren Co. Tennessee, USA with
105 disease severity and incidence reaching 50% and 60%, respectively. On plants with high disease severity,
106 stem dieback, necrosis, and wilting were evident. *Phytophthora* ImmunoStrip® test (Agdia Inc., Elkhart,
107 IN, USA) was performed as a primary screening tool using the infected foilar tissues. The positive
108 reaction indicated that the pathogen of interest was one of the *Phytophthora* species or other
109 oomycetes for all plant samples. Microscopic observation of necrotic lesion revealed globose to ovoid or
110 ellipsoid sporangia and white mycelium growth on the infected plant. Distinguishing among the
111 *Phytophthora* at the species level based on morphological characteristics is often difficult (Ippolito et al.
112 2002). However, the use of molecular analysis methods such as polymerase chain reaction (PCR) are
113 widely used and help to confirm identification of *Phytophthora* species (Bonants et al. 1997; Lacourt
114 and Duncan 1997). In addition, the *Phytophthora* genus has been supported with phylogenies generated
115 by sequence analysis of the internal transcribed spacer (ITS), beta-tubilin (b-tub) and mitochondrial
116 cytochrome c oxidase (cox) I and II regions (Cooke et al. 2000; Das et al. 2016). Therefore, we used both
117 morphological and molecular studies for the identification of *Phytophthora* species causing foliar blight

118 of annual vinca in Tennessee. In addition, we also evaluated the efficacy of different rates and
119 application timings of oxathiapiprolin (Segovis®; Syngenta, Greensboro, NC, USA) for the management of
120 Phytophthora aerial blight on annual vinca.
121
122 **Materials and methods**
123 **Isolate collection and morphological characterization.** A total of 10 annual vinca 'Pacifica XP Deep
124 Orchid' plants displaying foliar blight were sampled and a *Phytophthora* ImmunoStrip® test (Agdia Inc.,
125 Elkhart, IN, USA) revealed positive reaction. Symptomatic tissue from the edge of leaf lesions were cut
126 into small pieces (3 × 5 mm) and embedded in potato dextrose agar (PDA [Sigma-Aldrich, St. Louis, MO,
127 USA]) after surface sterilization with 70% ethanol (Sigma-Aldrich). The cultures were incubated for 5
128 days at 25°C and then hyphal tips were sub-cultured on V8 agar medium. The sub-culture plates were
129 incubated for 10-14 days at 25°C, 60% relative humidity and 12 h of light and dark. For V8 medium, 1%
130 $CaCO_3$ (98% Acros Organics, Geel, Belgium) was added to V8 juice (Campbell soup company, NJ, USA)
131 and centrifuged for 10 min at 10,000 rpm. Then, 50 ml buffered and clarified V8 juice was added to 450
132 ml deionized water (10% V8), along with 8 g agar (Sigma-Aldrich) and autoclaved at 121°C at 15 psi for
133 15 min. The subcultures grown on V8 agar medium were used for determining colony growth and
134 pigmentation. After 10 days of incubation, a portion of the mycelium (2 × 2 mm) was aseptically
135 removed from the growing culture, placed on glass slide with a sterilized scalpel and gently smeared in a
136 drop of water and covered with a coverslip for microscopic observation. The presence or absence and
137 shape and size of sporangia, papillae, and chlamydospores were examined using light microscopy (BX50;
138 Olympus, Center Valley, PA, USA). The data were recorded from 40 randomly selected sporangia,
139 papillae, and chlamydospore and their mean values and ranges were determined.
140

141 **Molecular analysis.** Isolate FBG2016_444 grown on V8 agar medium for 10 days at 25°C, 60% relative
142 humidity and 12 h of fluorescent light and used for molecular identification. The aerial mycelium of the
143 colony was dropped into a 2-mL centrifuge tube filled with 90% ethanol for DNA extraction. Total genomic
144 DNA was extracted using PowerLyzer™ Ultraclean® Microbial DNA Isolation Kit (MO-BIO Laboratories,
145 Carlsbad, CA, USA) according to the guidelines provided by the manufacturer. Following the DNA extraction,
146 the sample was stored at -20°C.

147 The ribosomal DNA internal transcribed spacer (ITS) region, the ribosomal DNA large subunit
148 (LSU) and the ras-related protein gene *Ypt*1 were amplified using primer sets – ITS5 (5'-GGA AGT AAA
149 AGT CGT AAC AAG G-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'), NL1 (5'-
150 GCATATCAATAAGCGGAGGAAAAG-3') and NL4 (5'- GGTCCGTGTTTCAAGACGG-3'), and Yph1F (5'-
151 CGACCATKGGTGTGGACTTT-3') and Yph2R (5'- ACGTTCTCMCAGGCGTATCT-3'), respectively (O'Dannell
152 1993; Schena et al. 2008; White et al. 1990). A 25 µL PCR reaction volume for each gene region was
153 prepared as follows: 2.5 µL of each primer (forward and reverse) adjusted at 10 µM concentration, 12.5
154 µL of PCR green master mix (TaqDNA polymerase, dNTPs, $MgCl_2$, and reaction buffer [GoTaq®
155 MasterMix; Promega c, Madison, WI, USA]), 5.5 µL nuclease free water, and 2.0 µL (5.0 $ng \cdot \mu L^{-1}$) DNA.
156 The amplification of the PCR product was performed using a thermal cycler (C1000 touch thermal cycler;
157 BIO-RAD Laboratories, Hercules, CA, USA). Amplification conditions were 30 cycles of 94°C for 5 min for
158 initial denaturation; 94°C for 40 s for denaturation; 55°C for ITS and LSU, and 60°C for *Ypt*1 genes
159 annealing and 72°C for 40 s for extension; and one cycle of 72°C for 5 min for the final extension. Gel
160 electrophoresis of amplified PCR products was performed on 1% agarose in 1X TBE buffer pre-stained
161 with GelRed nucleic acid (Biotium, Fremont, CA, USA) and bands were visualized under Gel Doc EZ
162 imager (BIO-RAD Laboratories). The amplified PCR products were purified using the Wizard® SV Gel and
163 PCR Clean-Up system (Promega Corporation) following the manufacturer's instructions and sent to
164 GenHunter Corporation, Nashville, TN for Sanger sequencing.

165 The sequenced regions of ITS, LSU and *Ypt*1 genes were aligned and edited using MEGA 6.0
166 software (Tamura et al. 2013). Then, the sequences of ITS, LSU and ITS, LSU and *Ypt*1 were blasted using
167 the BLASTN algorithms in the National Center for Biotechnology Information (NCBI) website. Highly
168 matching sequences from NCBI were retrieved and used as references. ClusterW was used for the
169 multiple alignment of sequences in MEGA 6.0 software and a phylogenetic tree was constructed using
170 the neighbor-joining method. Bootstrap phylogeny analysis was done with 1,000 replications to
171 statistically test the stability of the clade. Additionally, the sequence of representative isolate was
172 deposited in GenBank.
173
174 **Pathogenicity test.** Seeds of annual vinca 'Pacifica XP Deep Orchid' (Hazzard's Seed Company, Deford,
175 MI, USA) were sown into a 50-cell plug tray containing Canadian sphagnum peat (32 to 40%), vermiculite
176 and perlite (Growmix #2; Morton's Horticulture Products, McMinnville, TN, USA). The seeded annual
177 vinca tray was placed in a climate-controlled germination chamber (Stability chamber; Norlake
178 Scientific®, Hudson, WI, USA) at 27ºC and 95% relative humidity for 3 days until the radical emerged,
179 then the temperature and humidity were reduced to 24ºC and 80% relative humidity for 10 days.
180 Seedlings of annual vinca were transplanted in no.1 nursery containers, which were filled with
181 Canadian sphagnum peat, vermiculite and perlite (Growermix #2; Morton's Horticulture Products). The
182 potting mix soil was sterilized with an electric soil sterilizer tool (Soil sterilizer-Model SS-30; Pro-Grow
183 Supply Corp., Brookfield, WI, USA) at a temperature of 85ºC for 2 h before transplantation. Plants were
184 irrigated using overhead sprinklers for 2 min twice per day. Each plant was fertilized with 150 mL of
185 24N–3.5P–13.2K of micronutrient fertilizer (Miracle-Gro water-soluble all-purpose plant food; Scotts Co.,
186 Marysville, OH) and 10 g of 19N–2.1P–7.4K controlled-released fertilizer (Osmocote Pro; ICL Specialty
187 Fertilizers, Summerville, SC, USA) one week after transplantation. Transplanted annual vinca seedlings
188 did not receive any pesticides or fungicides during the entire experiment period.

189 Isolate FBG2016_444 of *Phytophthora nicotianae* collected from symptomatic annual vinca was
190 grown on V8 agar medium for 10-14 days and used for the preparation of rice grain inoculum. The rice
191 grain inoculum of *P. nicotianae* was prepared by following the protocol described by Benson and Parker
192 (2016). One month after transplantation, annual vinca were artificially inoculated by burying the four
193 rice grains colonized by *P. nicotianae* 1 cm below the surface of potting media at opposite sides of the
194 root zone of each plant. The control annual vinca plants (non-inoculated) received four autoclaved rice
195 grains per pot in the same manner. Both inoculated and non-inoculated treatment included eight single
196 -plant replications and the experiment was arranged in a completely randomized block design. The
197 absence or presence of necrotic lesions on the leaf and/or stem, and plant wilt due to *P. nicotianae* were
198 observed weekly for one month period. The pathogen was re-isolated by embedding symptomatic
199 tissue on V8 agar medium from inoculated plants. Isolation attempts were conducted from the tissue of
200 non-inoculated annual vinca plants as well.
201
202 **Efficacy of oxathiapiprolin in management of Phytophthora aerial blight of annual vinca.** The
203 greenhouse study was conducted in 2017 and 2018. The seed germination and seedling transplantation
204 of 'Pacifica XP Deep Orchid' annual vinca was conducted following the same procedure described in the
205 pathogenicity test section. One week after transplantation, each plant was fertilized with 150 mL of
206 24N–3.5P–13.2K of micronutrient fertilizer (Miracle-Gro water-soluble all-purpose plant food) and 10 g
207 of 19N–2.1P–7.4K controlled-released fertilizer (Osmocote Pro; ICL Specialty Fertilizers). The annual
208 vinca plants were watered for 2 min twice per day using an overhead irrigation system. The test plants
209 did not receive any pesticides other than the test fungicide.
210 Isolate FBG2016_444 of *Phytophthora nicotianae* was used for inoculation of annual vinca
211 plants. The plants were inoculated with *P. nicotianae*-colonized rice grain described in the pathogenicity
212 test section. The annual vinca plants were inoculated 10 weeks after the transplantation. In this study,

213 preventive drench applications of oxathiapiprolin (Segovis®; Syngenta, Greensboro, NC, USA) were
214 evaluated for the ability to manage Phytophthora aerial blight of annual vinca. Two rates (0.078 and
215 0.156 mL·L^{-1}) and three application timings (7, 14 and 21 days before inoculation [DBI]) of
216 oxathiapiprolin were studied, which generated 6 treatment variables (rate × application time). The
217 control treatment comprised of positive (non-treated, inoculated) and negative (non-treated, non-
218 inoculated) plants. As a drench application, 200 mL of treatment solution was applied to the potting mix
219 of each plant using a graduated beaker while the control plants received the same amount of sterile
220 distilled water. Eight single-plant replications per treatment were arranged in a completely randomized
221 block design.

222 The first experiment was conducted from 22 February to 28 August 2017. The seeding and
223 seedling transplantation of annual vinca were carried out on 22 February 2017 and 19 April 2017,
224 respectively. The treatments were applied on 12 (21 DBI), 19 (14 DBI) and 26 (7 DBI) June 2017. The
225 plants were inoculated on 2 July 2017 and initial height and width of plants were measured. The plants
226 were evaluated for Phytophthora aerial blight on 17 and 31 July, and 14 and 28 August 2017 and final
227 plant height, width, and fresh weight were recorded on 28 August 2017. Average temperatures for 12-
228 30 June, 1-31 July, and 1-28 August were 23.2°C (32.3/17.2°C [maximum/minimum]), 24.5°C
229 (30.9/19.77°C [maximum/minimum]), and 23.7°C (31.5/19.2°C [maximum/minimum]), respectively;
230 average relative humidity were 99.6%, 99.7%, and 99.7%, respectively.

231 The second experiment was conducted from 21 August 2017 to 18 April 2018. Seeding and
232 seedling transplantation were conducted on 21 August 2017 and 4 October 2017, respectively. The
233 annual vinca plants were treated on 1 (21 DBI), 8 (14 DBI), and 15 (7 DBI) February 2018 and inoculated
234 on 22 February 2018. The observation on Phytophthora aerial blight disease severity was performed on
235 8 and 22 March, and 5 and 18 April 2018. The initial and final height and width of plants were measured
236 on 22 February and 18 April 2018, respectively. The total plant fresh weight and fresh root weight were

237 recorded on 18 April 2018. Average temperatures for 15-28 February, 1-30 March and 1-18 April were
238 19.7°C (31.5/12.7°C [maximum/minimum]), 19.2°C (27.38/12.5°C [maximum/minimum]) and 19.4°C
239 (29.4/10.5°C [maximum/minimum]); average relative humidity were 86.0%, 84.5% and 95.3%,
240 respectively.
241

242 **Data recording.** The observation of Phytophthora aerial blight was made after the inoculation of annual
243 vinca plants at a biweekly interval for two months. Phytophthora aerial blight severity was evaluated
244 based on the percentage of foliage exhibiting symptoms using a scale of 0% to 100% foliage area
245 affected. The area under disease progress curve (AUDPC) was calculated using the formula $\sum([(x_i+x_{i-1})/2]$
246 $(t_i-t_{i-1}))$ where x_i is Phytophthora aerial blight severity rating on each evaluation date and $[t_i - t_{(i-1)}]$ is the
247 number of days between evaluations. The plant growth parameters such as increments in height and
248 width were recorded by subtracting the initial measurement from the final measurement of height and
249 width of each treatment. Total plant fresh weight and fresh root weight were recorded at the end of the
250 experiments.
251

252 **Statistical analysis.** Phytophthora aerial blight severity, AUDPC, increment in plant height and width,
253 total plant fresh weight, and fresh root weight were compared among treatments. SAS software (version
254 9.4 for Windows; SAS Institute, Cary, NC, USA) was used to statistical analysis of data. One-way analysis
255 of variance (ANOVA) was performed to partition the variance in disease severity, AUDPC, height, width,
256 total plant fresh weight, and fresh root weight into sources attributable to treatment and error. The
257 percentages of Phytophthora aerial blight in each treatment were compared using generalized linear
258 mixed model (PROC GLIMMIX) assuming a beta distribution with a logit link and means were separated
259 by Tukey studentized Range Test ($\alpha = 0.05$). AUDPC, plant width, and total plant fresh weight were log,
260 square root, and box-cox ($\lambda = 0.4$) transformed, respectively, to normalize the data; AUDPC, plant height

261 and width, total plant fresh weight, and fresh root weight were analyzed using general linear model
262 (PROC GLM) and means of the treatments were separated by Tukey studentized Range Test ($\alpha = 0.05$).
263 The data sets obtained from two repeated experiments were subjected to a two-way ANOVA analysis
264 (treatment × experiment) to check whether the variables between the experiments were significantly
265 different. The data were combined if the variability in the two experiments was not significantly
266 different at the 95% level of significance.
267

268 Results

269 **Morphological and molecular analysis.** The colony color of isolate FBG2016_444 exhibited white
270 cottony aerial mycelium growth on PDA. Numerous sporangia and chlamydospores were visible after 10
271 to 14 days of incubation on PDA medium. The chlamydospores were globose or spherical in shape and
272 measured an average of 31.7 × 30.8 µm (range: 20.7 to 39.8 × 21.6 to 37.8 µm). The chlamydospores
273 were presented intercalary or terminal in hyphae. The shape of the sporangia varied from globose to
274 ovoid, ellipsoid, or pyriform with conspicuous basal plug and were papillate and noncaducous. Mean
275 sporangia was 41.5 × 30.0 µm (range: 21.5 to 56.1 × 16.0 to 39.7 µm). Mean papillae was 5.2 × 5.3
276 (range: 2.2 to 8.4 × 2.7 to 7.3 µm). The colony growth, appearance, shape, and size of chlamydospores,
277 sporangia, and papillae of isolate FBG2016_444 derived from annual vinca were similar to the
278 morphological characteristics of *Phytophthora nicotianae* described by Erwin and Ribeiro (1996),
279 Alvarez-Rodriguez et al. (2013), and Lin et al. (2018).
280 The NCBI BLASTn result of amplified sequences of ITS, LSU and *Ypt*1 (Genbank accession nos.:
281 MW732660, MW732661, and MW748990, respectively) showed 100% similarity with the sequence of
282 *Phytophthora nicotianae* (GenBank accession nos.: MH219855 for ITS; EU080889 for LSU and MK058408
283 for *Ypt*1). In addition, a combined phylogenetic tree for ITS, LSU, and *Ypt*1 was constructed using the
284 Neighbor-joining method with bootstrap of 1,000 replications. The phylogenetic tree grouped the isolate

285 of this study within the clade of *P. nicotianae* originating from wider host range and different regions of
286 the world (Fig. 1). Therefore, the causal agent for the Phytophthora aerial blight of annual vinca was
287 identified as *P. nicotianae*.
288
289 **Pathogenicity test.** The initial symptoms of Phytophthora aerial blight, i.e., shriveled brownish-green
290 discolored water-soaked spots, were observed on the leaf of inoculated annual vinca at three to four
291 days after inoculation. Initial lesions quickly developed into typical blight symptoms (tan-brown to dark
292 brown colored spots) in 7 to 8 days whereas wilting developed after 12 to 14 days. All the inoculated
293 annual vinca plants showed the Phytophthora aerial blight symptoms whereas the non-inoculated
294 annual vinca plants remained symptom-free. *Phytophtora nicotianae* was was successfully re-isolated on
295 V8 agar medium. After 7 days of incubation, white cottony irregular mycelium growth was observed on
296 V8 agar medium and morphology of re-isolated *P. nicotianae* was identical to the original isolate. The
297 pathogen was not isolated from the non-inoculated plants.
298
299 **Efficacy of oxathiapiprolin in management of Phytophthora aerial blight of annual vinca.** There was a
300 significant interaction between treatment and experiment for disease severity ($F = 5.49$, $P < 0.001$), so
301 data for each experiment were analyzed separately. The interaction between treatment and experiment
302 was not significant for AUDPC ($F = 1.27$, $P = 0.27$), so data were combined from both experiments and
303 analyzed. In both experiments, the positive control and negative control annual vinca plants had the
304 highest and lowest final disease severity and AUDPC of Phytophthora aerial blight, respectively (Table 1).
305 All treatments significantly reduced Phytophthora aerial blight severity (Expt. 1: $F = 22.13$, $P < 0.0001$;
306 Expt. 2: $F = 76.38$, $P < 0.0001$) compared to the positive control in both experiments. There was no
307 significance difference between rates (0.078 and 0.156 $mL \cdot L^{-1}$) and application timing (7, 14 and 21 DBI)
308 of oxathiapiprolin in reducing Phytophthora aerial blight severity in annual vinca in either experiment.

309 However, 0.078 and 0.156 mL·L^{-1} of oxathiapiprolin applied 7 and 14 DBI were the most effective
310 treatments in reducing Phytophthora aerial blight severity and provided a reduction in disease not
311 statistically different than the negative control in experiment 1. All treatments significantly reduced the
312 Phytophthora aerial blight disease progress (AUDPC) ($F = 20.56$, $P < 0.0001$ [experiments combined]) on
313 treated annual vinca plants compared to the positive control (Table 1).

314 There was no significant interaction between treatments and experiments for increase in plant
315 height ($F = 0.71$, $P = 0.662$) and width ($F = 1.16$, $P = 0.329$), so the data were pooled across the
316 experiments (Table 2). There were no significant differences in terms of incremental growth in plant
317 height ($F = 0.49$, $P = 0.84$) and width ($F = 0.85$, $P = 0.55$) among the treated and non-treated annual vinca
318 plants (Table 2). The incremental growth in plant height and width ranged from 7.9 ± 0.8 cm to 9.5 ± 0.9
319 cm and 7.8 ± 1.9 cm to 10.1 ± 1.7 cm, respectively.

320 Similarly, the interaction between treatments and experiments was not significant for fresh root
321 weight ($F = 1.20$, $P = 0.307$), so data were combined from the experiments and analyzed together. In
322 both experiments, there were no significant differences in fresh root weight between the treated and
323 non-treated annual vinca plants ($F = 1.38$, $P = 0.222$). The fresh root weight of annual vinca plants
324 ranged from 5.0 ± 1.1 g to 8.9 ± 0.9 g (Table 2). The interaction of treatment and experiment was
325 significant for total plant fresh weight ($F = 3.36$ and $P = 0.0028$), so data from each experiment were
326 analyzed separately. There was significant difference between the treated and positive control annual
327 vinca plants in total plant fresh weight in experiment 1 ($F = 6.94$, $P < 0.0001$) whereas no significant
328 difference between treated and positive control was observed in experiment 2 ($F = 2.50$, $P = 0.026$). In
329 experiment 1, all treated annual vinca plants except those treated with 0.156 mL·L^{-1} of oxathiapiprolin at
330 21 DBI were statistically similar to the negative control. In experiment 2, all treated annual vinca plants
331 except those treated with 0.078 mL·L^{-1} at 7 DBI were not statistically different than the negative control;
332 however none of the treatment were significantly different than positive control.

333

334 **Discussion**

335 Based on the morphological, molecular, and pathogenecity studies the isolate of this study
336 (FBG2016_444) collected from symptomatic annual vinca leaf tissue was identified as
337 *Phytophthora nicotianae*. The sequence of the representative isolate (FBG2016_444) of annual vinca
338 was grouped in the same clade as *P. nicotianae* with high bootstrap value (100%) indicating that there
339 was no sequence difference between them. The inoculated 'Pacifica XP Deep Orchid' annual vinca
340 exhibited symptoms of Phytophthora aerial blight and *P. nicotianae* was re-isolated whereas non-
341 inoculated plants remained symptom free. Several studies have reported Phytophthora aerial blight,
342 caused by *P. nicotianae*, on annual vinca and other *Catharanthus* spp. in different states of the United
343 States (Alvarez-Rodriguez et al. 2013; Gill et al. 1977; Lamour et al. 2003; Lin et al. 2018; McGovern et
344 al. 2000; Olson and Benson 2011), India, Venezuela, Japan, and Argentina (Blanchard 1930; Katsura
345 1971; Lim et al. 2004; Malaguti 1951). *Phytophthora nicotianae* produces numerous sporangia from a
346 specialized hyphal structure called a sporangiophore. The sporangiophore can germinate directly or
347 produce zoospores depending upon the temperature and moisture (Walker and van West 2007).
348 Zoospores possess two flagella and easily disseminate through soil water, irrigation, water splash, and
349 hydroponic solutions (Stanghellini and Rasmussen 1994). Upon reaching the plant surface, the zoospore
350 germinates to develop germ tubes and penetrates the plant tissue (Ludowici et al. 2013) and blight
351 symptoms develop on aerial plant parts. In addition, *P. nicotianae* can disperse and infect plants through
352 oospores and chlamydospores (Panabieres et al. 2016). The pathogen *P. nicotianae* is hemibiotrophic in
353 nature; the pathogen relentlessly attack plants throughout the infection cycle, suppressing the host
354 defense mechanism and ultimately cause plant death (Panabieres et al. 2016)]. Therefore, Phytophthora
355 aerial blight management of annual vinca is important for ornamental producers to remain competitive
356 in the floriculture market.

357 In this study, we also examined the efficacy of rates and application timings of oxathiapiprolin
358 (Segovis®) in managing Phytophthora aerial blight of annual vinca. Phytophthora aerial blight disease
359 severity was higher in experiment 1 than experiment 2, which may have been due to the variability in
360 temperature and relative humidity. Phytophthora aerial blight disease development is favored by high
361 temperature and relative humidity (prolonged moisture), which was the condition in experiment 1. In a
362 comparative study of rates and application timings of oxathiapiprolin, we demonstrated that both rates
363 (0.078 and 0.156 $mL \cdot L^{-1}$) and three application timings (7, 14 and 21 DBI) of oxathiapiprolin lowered
364 Phytophthora aerial blight severity in treated annual vinca plants compared to the positive control.
365 However, drench applications of oxathiapiprolin at 7 or 14 DBI were the most effective treatments in
366 reducing Phytophthora aerial blight disease severity and disease progress. Oxathiapiprolin has been
367 reported to be effective against different life stages (sporangia production and germination, mycelium
368 growth, zoospore motility and germination, chlamydospore production) of *P. nicotianae* and other
369 oomycete pathogens in *in-vitro* studies (Bittner and Mila 2016; Gray et al. 2018; Miao et al. 2016b; Qu
370 et al. 2016). Similarly, field studies have shown that drip irrigation, drench, or spray application of
371 oxathiapiprolin, alone or in alternation with other fungicides, effectively reduced foliar blight caused by
372 *P. nicotianae* and AUDPC on tobacco (*Nicotiana tabacum* L. [Bittner and Mila 2017; Bittner and Mila
373 2016; Ji et al. 2014]).
374 In our present study, we did not observe a significant difference between two rates (0.078 and
375 0.156 $mL \cdot L^{-1}$) of oxathiapiprolin in reducing Phytophthora aerial blight disease severity and disease
376 progress (AUDPC) on annual vinca plants when compared within the same application timing. A similar
377 result was observed by Bittner and Mila (2016) where 0.07 kg a.i./ha and 0.28 kg a.i./ha oxathiapiprolin
378 applied at transplant were statistically similar in managing *P. nicotianae* infection on tobacco. The
379 disease pressure was low to moderate during the both experiments, which might be the reason that
380 both rates of oxathiapiprolin produced the similar efficacy result. However, the efficacy of

381 oxathiapiprolin (low and high rates) could be different under high disease pressure. For example, Ji et al.
382 (2014) observed that the higher rate of oxathiapiprolin was more effective in managing the black shank
383 than the lower rate. We observed that there was no significant difference among application timings of
384 oxathiapiprolin in reducing the disease severity when compared within the same application rate.
385 However, oxathiapiprolin (0.156 $mL \cdot L^{-1}$) applied at 7 DBI was more efficacious than oxathiapiprolin
386 (0.078 and 0.156 $mL \cdot L^{-1}$) applied at 14 and 21 DBI in reducing Phytophthora aerial blight disease progress
387 (AUDPC) on annual vinca. The half-life of oxathiapiprolin is reported to be about 8-12 days in soil (Yu et
388 al. 2017). Gray et al. (2020) studied the mobility and effectiveness of oxathiapiprolin against
389 *P. citrophthora* on citrus seedling sampled at 7, 10, 13 and 16 days after soil application. The authors
390 observed that oxathiapiprolin concentration measured in leaf extract of citrus plants were higher at 10
391 and 13 days with a higher mean inhibition zone of mycelial growth of *P. citrophthora* in a bioassay study.
392 But the concentration of oxathiapiprolin in leaves of citrus seedlings and its inhibiting capability against
393 *P. citrophthora* was reduced when sampled at 16 days after treatment. Even though systemic fungicides
394 are not rapidly degraded by weather, rainfall or dew as protectant fungicides, the concentration may be
395 reduced (to sub-lethal dose) because of the redistribution and dilution in growing plant tissue, and the
396 degradation of the active ingredient in plant tissue after a certain time period (Schilder 2010). Thus, the
397 early preventive application of systemic fungicides could fail to provide sufficient disease management
398 at the time of infection, even though they are absorbed by plants.
399 Oxathiapiprolin targets oxysterol binding protein (OSBP [Pasteris et al. 2016]). It has been
400 classified as a medium to high resistance risk fungicide (Fungicide Resistance Action Committee 2020).
401 Recent studies have shown that the mutatation caused by UV light irradiation or mycelial adaptation to
402 oxathiapiprolin could increase the occurrence of fungicide insensitive strains of oomycete pathogens su
403 ch as *P. nicotianae* (Bittner et al. 2017) and *P. capsici* (Miao et al. 2016a; Pasteris et al. 2016). However,
404 field resistance of *P. nicotianae* to oxathiapiprolin has not been reported. Fungicide resistance

405 management can be achieved by mixing or rotating two or more fungicides with different modes of
406 action and target sites (single and multi-site) instead of constant use of a single fungicide (Bika et al.
407 2020). According to Bittner et al. (2017), *P. nicotianae* insensitivity on isolates collected from tobacco
408 was due to constant exposure to sub lethal doses of oxathiapiprolin. Therefore, mixing or alternating of
409 oxathiapiprolin with other reduced risk fungicides would minimize the number of applications per
410 season and prevent the development of a resistant strain of *P. nicotianae*.
411 In summary, the causal agent for Phytophthora aerial blight of annual vinca was identified as
412 *P. nicotianae* in Tennessee. The preventive drench application of oxathiapiprolin significantly reduced
413 Phytophthora aerial blight disease severity and disease progress (AUDPC) compared to the negative
414 control; 0.078 and 0.156 $mL \cdot L^{-1}$ oxathiapiprolin applied at 7 or 14 DBI being the most effective
415 treatment. However, the application of the oxathiapiprolin did not influence the growth parameters
416 (height and width), total plant fresh weight, and root weight of the annual vinca plants. Under the
417 current circumstances, we suggest using lower rates of oxathiapiprolin when disease pressure is low to
418 moderate while the higher rate should be reserved for high disease pressure. The findings of this study
419 will help ornamental producers to better manage Phytophthora aerial blight of annual vinca.
420
421 **Acknowledgements**
422 This work was supported by the National Institute of Food and Agriculture (NIFA), United States
423 Department of Agriculture (USDA) Evans-Allen grant, under award numbers TENX-1926-CCOCP and TENX-
424 S-1083. We thank Syngenta International AG for donation of product used in this study. Mention of trade
425 name of commercial product in the publication is solely for the purpose of providing specific information
426 and does not imply recommendation or endorsement by Tennessee State University.
427

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577 concentration and dissipation in grapes and soil by ultrahigh-performance liquid
578 chromatography–tandem mass spectrometry. J. Sci. Food Agric. 97:3294-3299.

579 Table 1. Mean Phytophthora aerial blight severity and the area under disease progress curve (AUDPC) assessments, caused by

580 *Phytophthora nicotianae*, resulting from the preventive drench applications of oxathiapiprolin to annual vinca plants (*Catharanthus roseus*).

Treatment	**Rate (mL·L^{-1})**	**Application time (DBI[v])**	**Final disease severity (%)[z,y]**				**AUDPC[x,w]**	
			Expt.1		**Expt.2**			
Oxathiapiprolin	0.078	7	9.8 ± 2.1	bc[u]	1.9 ± 0.1	b[u]	90.6 ± 32.5	bc[u]
Oxathiapiprolin	0.078	14	11.4 ± 1.6	bc	2.5 ± 0.4	b	114.2 ± 32.8	b
Oxathiapiprolin	0.078	21	19.3 ± 4.1	b	2.5 ± 0.4	b	214.8 ± 70.7	b
Oxathiapiprolin	0.156	7	8.1 ± 2.4	bc	1.3 ± 0.3	b	55.6 ± 28.3	c
Oxathiapiprolin	0.156	14	12.9 ± 4.1	bc	1.4 ± 0.2	b	83.5 ± 40.1	bc
Oxathiapiprolin	0.156	21	19.3 ± 4.1	b	1.6 ± 0.2	b	224.0 ± 74.9	b
Positive control			66.1 ± 3.1	a	11.9 ± 1.3	a	801.5 ± 175.8	a
Negative control			4.9 ± 2.4	c	0.0 ± 0.0	c	51.2 ± 32.1	d
F			22.13		76.38		20.56	
df			7		7		7	
P			<.0001		<.0001		<.0001	

581 [z]Final disease severity (mean ± SE) assessed at the end of the experiments 1 and 2. The disease severity was evaluated using 0-100% scale.

582 Values are the means of eight single-plants replications for each treatment.

583 [y] Expt.1= Experiment 1 and Expt. 2 = Experiment 2.

584

585 [x]Area under disease progress curve (AUDPC) is the mean progression of disease (mean ± SE) during the experiment period for both experiments.

586 AUDPC for each treatment was calculated using the formula: $\sum([(x_i+x_{i-1})/2]\ (t_i-t_{i-1}))$ where x_i is Phytophthora aerial blight severity rating on each

587 evaluation date and $[t_i - t_{(i-1)}]$ is the number of days between evaluations. Values were the means of eight single-plant replications.

588 [w]Experiments combined.

589 [v]DBI = Days before inoculation.

590 [u]ANOVA was performed using GLIMMIX procedure (SAS 9.4). Experiment × treatment was not significant for AUDPC at $\alpha = 0.05$, so data from

591 both experiments were combined and analyzed together. Treatments means followed by the same letter within the column are not significantly

592 different at $P = 0.05$ based on Tukey's Studentized Range Test method for multiple comparison adjustment of least square means.

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599 Table 2. Growth parameters (increase in plant height and weight, total plant fresh weight and root weight) of annual vinca plants treated with

600 oxathiapiprolin; not-treated, non-inoculated (negative control); and non-treated, inoculated (positive control) groups.

Treatment	Rate ($mL \cdot L^{-1}$)	Application time (DBI[z])	Increase in height (cm)[y]		Increase in width (cm)[y]		Root weight (g)[y]		Total plant fresh weight (g)[x]			
									Expt. 1		Expt. 2	
Oxathiapiprolin	0.078	7	8.1 ± 0.9	a[w]	8.8 ± 1.7	a[w]	6.7 ± 1.5	a[w]	48.6 ± 7.2	ab[w]	16.6 ± 1.5	b[w]
Oxathiapiprolin	0.078	14	7.9 ± 0.8	a	8.7 ± 1.6	a	5.9 ± 1.2	a	45.5 ± 5.5	ab	19.8 ± 3.2	ab
Oxathiapiprolin	0.078	21	9.5 ± 0.9	a	10.1± 1.7	a	6.9 ± 1.4	a	47.9 ± 7.3	ab	19.9 ± 2.8	ab
Oxathiapiprolin	0.156	7	8.2 ± 0.9	a	7.5 ± 1.9	a	6.2 ± 1.5	a	50.6 ± 7.6	ab	24.3 ± 3.7	ab
Oxathiapiprolin	0.156	14	8.3 ± 1.0	a	8.8 ± 1.8	a	5.8 ± 1.0	a	50.2 ± 7.4	ab	19.5 ± 3.1	ab
Oxathiapiprolin	0.156	21	8.8 ± 1.1	a	9.2 ± 2.2	a	5.7 ± 1.2	a	33.1 ± 3.6	bc	23.1 ± 2.3	ab
Positive control			8.9 ± 0.8	a	8.3 ± 1.5	a	5.0 ± 1.1	a	20.7 ± 1.9	c	19.2 ± 1.9	ab
Negative control			8.3 ± 1.0	a	7.8 ± 1.9	a	8.9 ± 0.9	a	59.0 ± 2.9	a	30.6 ± 3.0	a
F			0.49		0.85		1.38		6.94		2.50	
df			7		7		7		7		7	
P			0.84		0.55		0.222		<.0001		0.0269	

601 [z]DBI = Days before inoculation.

602 [y]Experiments combined.

603 [x]Expt. 1 = Experiment 1 and Expt. 2 = Experiment 2.

604 [w]ANOVA was performed using GLM procedure (SAS 9.4). Experiment × treatment was not significant for Increase in plant height, width and root

605 weight at α = 0.05, so data from both experiments were combined and analyzed together. Treatments means followed by the same letter within

606 the column are not significantly different at P = 0.05 based on Tukey's Studentized Range Test method for multiple comparison adjustment of
607 least square means. Values are the means of eight single-plants replications for each treatment.
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621 **Figure 1.** Neighbor-joining phylogenetic tree based on the combined analysis of internal transcribed
622 spacer (ITS), the large subunit (LSU) of rDNA, and ras-related protein gene (*Ypt*1) sequences. Numbers
623 close to the branch nodes indicate 1,000 replications of bootstrap test. The FBG2016_444 code refers to
624 the isolated ID of *Phytophthora nicotianae* collected from annual vinca plant in this study. The
625 accessions number of GenBank sequences of ITS, LSU, and *Ypt*1 are used as reference materials. The
626 sequence of *Phytopythium helicoides* was used as an outgroup.

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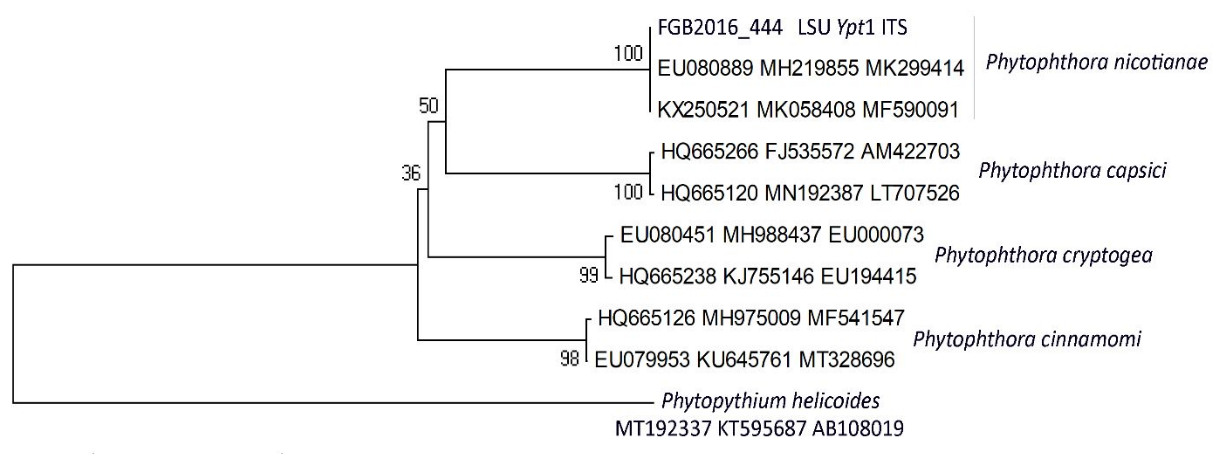

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