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# Evaluation of fungicides and biofungicide to control Phytophthora root rot (Phytophthora cinnamomi Rands) and ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) on flowering dogwoods exposed to simulated flood events

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# Evaluation of fungicides and biofungicide to control Phytophthora root rot (*Phytophthora cinnamomi* Rands) and ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) on flowering dogwoods exposed to simulated flood events

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**ABSTRACT**

Phytophthora root rot causes major economic losses in woody ornamental nurseries, especially in plants exposed to flooding. Ambrosia beetles, which attack stressed trees, are also important pests of woody plants. In this study, several products were evaluated for control of Phytophthora root rot and ambrosia beetles on containerized flowering dogwoods (*Cornus florida* L.) exposed to simulated flood events under field conditions. In two trials, preventive (7 days preflooding) or curative (1 day postflooding) sprench (combination spray and drench) treatments were applied to containerized dogwoods artificially inoculated with *Phytophthora cinnamomi* Rands. The plants were flooded by maintaining standing water for 1, 3, or 7 days. After the trials, plant growth data (dry shoot weight, dry root weight, plant height, plant width, caliper) were recorded, and roots were assessed for disease severity using a scale of 0-100% roots affected, as well as plated on PARPH-V8 medium to determine the percentage of *Phytophthora*-infected root samples. Ambrosia beetle attacks were recorded throughout the study. Longer flooding duration increased disease pressure from *Phytophthora cinnamomi* Rands. Preventive treatment of plants with Subdue MAXX consistently reduced disease severity at tested flooding durations of 1, 3, or 7 days. Preventively- (before flooding) or curatively- (after flooding) applied treatments of Empress Intrinsic, Orkestra Intrinsic, and Pageant Intrinsic reduced disease pressure at 1 and 3 days of flooding. Preventive RootShield Plus$^{+}$ treatment reduced disease pressure on plants flooded 1 day. Longer flooding periods as well as *Phytophthora cinnamomi* inoculation increased ambrosia beetle attacks on flooded dogwoods, but preventively- and curatively-applied Orkestra Intrinsic as well as preventively-applied Subdue MAXX reduced ambrosia beetle attacks compared to non-treated, inoculated controls. These treatments were effective at reducing both *Phytophthora* and ambrosia beetle damage. These results can help nursery producers successfully manage Phytophtora root rot and also reduce the risk of ambrosia beetle attacks during flood events.

Key words: *Phytophthora*; *Xylosandrus*; woody ornamental; fungicide; biofungicide; *Cornus florida*

## 1. INTRODUCTION

*Phytophthora* species cause root rot of many woody ornamental crops and resulting economic losses in nurseries are significant (Benson and Broembsen 2001; Ribeiro and Linderman 1991). Phyophthora root rot causes yellowing of leaves, wilting, and potentially plant death (Benson and Broembsen 2001). Damage from soilborne *Phytophthora* species is due to root injury resulting in impaired uptake of water and nutrients (Zentmyer 1980).

The oomycete pathogen *Phytophthora cinnamomi* Rands is distributed throughout the southeastern and Pacific regions of the United States due to the favorable conditions created by warm, wet soils (Benson and Broembsen 2001; Zentmyer 1980). Control of *P. cinnamomi* is difficult due to: (1) a wide host range including ornamental plants like *Acer*, *Cornus*, *Juglans*, *Prunus*, *Rhododendron*, and *Quercus*; (2) commonly, the aboveground portions of infected plants remain symptomless, delaying detection until late stages of the disease; (3) potential for infestation and spread of the pathogen through irrigation or runoff; (4) disease inoculum may remain viable in the soil for long periods of time; and (5) rapid inoculum production during periods of soil saturation (Erwin and Ribeiro 1996; Ribeiro and Linderman 1991; Zentmyer 1980).

Soil moisture is the most significant environmental factor affecting Phytophthora root rot development (Blaker and McDonald 1981; Jacobs and Johnson 1996; Zentmyer 1980). Disease symptoms associated with *Phytophthora* root infection increase with the period that plants are exposed to flooding, including increases of flooding duration as short as 24 h (Blaker and McDonald 1981; Wilcox and Mircetich 1985). *Phytophthora cinnamomi* growth and sporangium production increases with elevated soil moisture and is also affected by soil temperature with optimum conditions at 24 – 27 °C (Nesbitt et al. 1979). When exposed to free water in soil, sporangium production and zoospore release can occur in as little as 9 h (Benson and Broembsen 2001). With excessive irrigation or rainfall, zoospores are transported more readily throughout the soil, resulting in intensified disease severity and spread (Erwin and Ribeiro 1996).

Host susceptibility to *Phytophthora* infection is exacerbated by flooding (possibly by restricting oxygen availability to host roots), drought, salinity, heat, and frost (Blaker and

MacDonald 1981; Erwin and Ribeiro 1996; MacDonald 1984). Flooding or drought can even increase susceptibility of *Phytophthora*-resistant plants like the rhododendron cultivar 'Caroline' (Blaker and MacDonald 1981).

Adequate drainage and appropriate irrigation are often used to avoid favorable conditions for *Phytophthora* inoculum growth in the soil. However, because of rapid *P. cinnamomi* inoculum production in saturated soils, even nurseries with satisfactory drainage are at risk of *Phytophthora* infestations during flood events. Because of the difficulties in controlling *P. cinnamomi* exclusively with cultural practices, fungicides (mostly metalaxyl and fosetyl-al) are used to slow disease development and prevent the spread of the pathogen (Erwin and Ribeiro 1996; Zentmyer 1980).

Flooding also is an important stressor that induces ambrosia beetle attacks in ornamental nurseries (Ranger et al. 2013; Frank and Ranger 2016). Ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) are pests of ornamental nurseries, often associated with stressed trees that release ethanol (Ranger et al. 2013, 2015, 2016a). Ambrosia beetles also are attracted to diseased trees, including those infected with root rot pathogens (McPherson et al. 2008). Since ambrosia beetle attacks can co-occur or be induced by root rot, fungicide treatments intended to manage *Phytophthora* also could affect secondary pests like ambrosia beetles (Addesso et al. 2018).

Effective *Phytophthora* fungicides are needed due to the increased risk of infestation and spread during flooding. Since ambrosia beetles also are associated with flooded woody ornamentals, understanding fungicide effects on these secondary pests are important. The objective of this study was to evaluate preventive and curative applications of fungicides for control of *P. cinnamomi* and ambrosia beetles on containerized flowering dogwoods exposed to a simulated root flooding events of 1, 3, or 7 days. Treatments used in this study were selected partly based on a previous greenhouse study that screened fungicides and biofungicides for efficacy against Phytophthora root rot on dogwood seedlings exposed to flooding (Brown 2018).

## 2. MATERIALS AND METHODS

110 **2.1. Field study condition and design**

111 The study was conducted at the Tennessee State University Otis L. Floyd Nursery
112 Research Center (TSU-NRC), McMinnville, TN. Flowering dogwoods (*Cornus florida* L.
113 'Cherokee Princess') donated from Herd Farms Nursery (Belvidere, TN) were planted in no. 3
114 nursery containers (11.4 L, C1200, Hummert International, Earth City, MO) with Barky Beaver
115 Premium Potting Soil substrate (Barky Beaver Mulch and Soil Mix, Inc., Moss, TN consisting of
116 pine bark [77%], peat [10%], sand [10%], 0.91 kg 10-10-10 NPK fertilizer, and 2.27 kg lime
117 with an average substrate bulk density reported by the manufacturer of 248.3 kg $m^{3-1}$) on 15
118 March 2018. Plants were watered with overhead irrigation until initiation of field trials.
119 Treatments were assigned to flowering dogwoods in a randomized complete block design at four
120 nonadjacent locations (four replications) along a wooded border at TSU-NRC with 50 cm
121 spacing between plant containers (30 plants $block^{-1}$; 120 total plants).
122 Four synthetic fungicides, a biofungicide, and a biofungicide plus fertilizer program were
123 evaluated in two field trials (Table 1). The trials took place from 21 May – 25 June 2018 (Trial
124 1) and from 9 July – 10 August 2018 (Trial 2) on containerized flowering dogwoods. All
125 products were applied as a sprench application to the lower trunk and potting substrate
126 surrounding the base of the plant either preventatively 7 days before flooding or curatively 24 h
127 after flooding (Table 1). All products were applied once according to label directions at the
128 highest labeled rates in a 500 ml solution $pot^{-1}$, as this was previously determined to be the
129 volume at which the solution thoroughly wet the root zone of the plants without leaching through
130 the container (Table 1). Control treatments included non-treated, *P. cinnamomi* inoculated
131 (positive control) and non-treated, non-inoculated (negative control) plants. Control plants also
132 received 500 ml water $pot^{-1}$. Tartan Stressgard is not currently labeled for commercial nursery
133 use, but the product was provided by manufacturers for experimentation and potential future
134 label expansion.

135 For the preparation of inoculum, 25-g rice and 22-mL deionized water were autoclaved
136 for 30 min. Six 1-$cm^2$ plugs of isolate FBG201510 of *P. cinnamomi* (obtained from the culture
137 collection of Dr. Fulya Baysal-Gurel at the TSU-NRC, McMinnville, TN*;* GenBank Accession
138 No. MK099813) were mixed in each container with cooked rice and allowed to colonize rice
139 grains for 2 weeks (Holmes and Benson 1994). Flowering dogwoods were artificially inoculated

by burying four *P. cinnamomi*-colonized rice grains 1 cm below the surface of the potting substrate on opposite sides of the plant.

All treatments, including the positive and negative controls were flooded to above the root crown for 1, 3, or 7 days using well water. Flooding conditions were imposed by placing plant containers in buckets with 208 L black plastic trash bags (Warp Brothers, Chicago, IL). After 1, 3, or 7 days of flooding, the containers were allowed to drain. After draining, plants were watered as needed until the end of the experiment.

To facilitate ambrosia beetle attacks, trees were placed near wooded areas (sources of ambrosia beetle infestation). In Trial 1, flooding alone was not effective at inducing sufficient ambrosia beetle attacks (n = 21) for analysis, so ethanol lures filled with 15 mL of 95% ethanol with a release rate 16 mg $day^{-1}$ at 20°C (Standard Release ethanol lures, AgBio Inc., Westminster, CO) were attached to trees with zip ties 10 days after flooding. However, no new ambrosia beetle attacks were observed after ethanol lure deployment. So, ethanol lures were attached to trees at flood initiation in Trial 2 to enhance long range ambrosia beetle attraction at the time of optimal flood-induced host susceptibility to ambrosia beetles. In both trials, ethanol lures were kept on trees until termination of the experiment.

Maximum and minimum temperatures and rainfall amounts were monitored using the WatchDog 2700 weather station (Spectrum Technologies, Aurora, IL). In Trial 1, average maximum temperatures for 21-31 May and 1-25 June 2018 were 29.52 and 32.63°C; average minimum temperatures were 19.29 and 19.14°C; and total rainfall amounts were 4.59 and 6.50 cm, respectively. In Trial 2, average maximum temperatures for 9-31 July and 1-10 August 2018 were 31.59 and 31.78°C; average minimum temperatures were 19.75 and 20.27°C; and total rainfall amounts were 4.78 and 3.76 cm, respectively.

### 2.2. Data recording

Plants were removed from the field, and the roots were washed to remove container substrate debris at 21 (Trial 1) or 18 days (Trial 2) after the last flooding period ended. Plant height and width (maximum width; the widest part from leaf tip to leaf tip) were recorded before and after both trials. Tree caliper also was recorded at a height of ~10 cm from the base of the plant before and after each trial using a digital caliper (Mitutoyo 700-113-10 MyCal-Lite digital

169 caliper, Mitutoyo America Corporation, Aurora, IL). Shoot dry weight and root dry weight were
170 recorded after oven drying at 55 °C (VWR Signature High-Performance Horizontal Air Flow
171 Ovens 1690 Floor Model, VWR, Radnor, PA) for 7 days at the end of each trial. Also, the
172 severity of Phytophthora root rot was assessed using a scale of 0–100% roots affected at the end
173 of each trial.

174 Ambrosia beetle entrance holes were recorded throughout the trial and circled with wax
175 pencils to avoid duplicate counting. After the trial, trees with ambrosia beetle attacks were
176 dissected to recover ambrosia beetles in galleries. Ambrosia beetles were identified to species
177 using available keys (Rabaglia et al. 2006; Wood 1982).

178 *Phytophthora* infection was determined by plating five randomly selected root samples
179 (15 mm long) from the root tips of each plant (four replications per treatment) on PARPH-V8
180 medium (Ferguson and Jeffers 1999). The presence or absence of *Phytophthora* growth
181 surrounding each root sample was recorded after 2 weeks. For PARPH-V8 selective medium,
182 0.50-g $CaCO_3$ (98% Acros Organics, Geel, Belgium) was added to 50-mL V8 juice (Campbell,
183 Camden, NJ) and centrifuged for 10 min at 7,000 rpm. The buffered and clarified V8 juice was
184 added to 450-mL deionized water, along with 7.5-g agar (Sigma-Aldrich, St. Louis, MO) and
185 autoclaved for 15 min. Afterwards, 500-µL of the fungicide and antibiotics,
186 pentachloronitrobenzene (PCNB) (99% (GC) Sigma-Aldrich, St. Louis, MO) (0.63 g 50 $mL^{-1}$
187 ethanol), ampicillin (Sigma-Aldrich, St. Louis, MO) (1.25 g 50 $ml^{-1}$ ethanol), rifampicin (Sigma-
188 Aldrich, St. Louis, MO) (0.05 g 50 $mL^{-1}$ ethanol), pimaricin (2.5%) (MP Biomedicals, Santa
189 Ana, CA), and hymexazol (Sigma-Aldrich, St. Louis, MO) (250 mg 50 $mL^{-1}$ sterilized water)
190 were added to the medium (Ferguson and Jeffers 1999).

191 *Trichoderma* colonization in the RootShield Plus$^{+}$ WP-treated plants was determined
192 using dilution plating on *Trichoderma* selective medium. For *Trichoderma* selective medium,
193 0.1-g $MgSO_4$-$7H_2O$ (Sigma-Aldrich, St. Louis, MO), 0.45-g $KH_2PO_4$ (Sigma-Aldrich, St. Louis,
194 MO), 0.5-g $NH_4NO_3$ (Alfa Aesar, Tewksbury, MA), 0.075-g KCl (Sigma-Aldrich, St. Louis,
195 MO), 1.5-g dextrose (VWR, Radnor, PA), 0.01-g $FeSO_4$-$7H_2O$ (VWR, Radnor, PA), 0.01-g
196 $MnSO_4$-$H_2O$ (Fisher Scientific, Pittsburgh, PA), 0.01-g $ZnSO_4$-$7H_2O$ (Fisher Scientific,
197 Pittsburgh, PA), 0.015-g rose Bengal (Fisher Scientific, Pittsburgh, PA), 10.0-g agar (Sigma-
198 Aldrich, St. Louis, MO), and 500-mL deionized water were autoclaved for 15 min (Chung and

199 Hoitink 1990). Afterwards, the fungicides and antibiotics, 0.05-g PCNB (99% (GC) Sigma-
200 Aldrich, St. Louis, MO), 5-µL Subdue MAXX (Syngenta International AG, Basel, Switzerland),
201 0.025-g chloramphenicol (Sigma-Aldrich, St. Louis, MO), and 0.025-g streptomycin sulfate
202 (Acros Organics, Geel, Belgium) were added to the medium. For each plant, a 1-g randomly
203 selected root sample and 10-mL sterilized deionized water were ultrasonicated (Fisherbrand M-
204 Series 5.7 L Mechanical Ultrasonic Cleaning Bath, Thermo Fisher-Scientific Inc., Watham, MA)
205 for 3 min and then agitated with an incubating mini-shaker (Fisherbrand Incubating Mini-Shaker,
206 Thermo Fisher-Scientific Inc., Watham, MA) at 250 rpm for 30 min at 25°C. The particles were
207 then allowed to settle for 30 min. Dilutions of $10^{-1}$ and $10^{-2}$ were prepared and spread-plated
208 using glass beads (3-mm solid glass beads, Walter Stern, Inc., Manorhaven, NY), as well as 100-
209 µL of the undiluted sample. The numbers of *Trichoderma* colonies on plates were recorded after
210 10 days incubation in an incubator (VWR Gravity Convection Incubator, VWR, Radnor, PA) at
211 25°C. The number of colony forming units per gram of root sample was calculated from the
212 plate counts, the dilution factor and the plated volume.

213 **2.3. Statistical analysis**

214 Plant height, plant width, plant caliper, shoot dry weight, root dry weight, disease
215 severity, and percentage of root samples infected with *P. cinnamomi* were compared among
216 preventively- or curatively-applied treatments within each flood duration. Plant growth data
217 (plant height, plant width, plant caliper, dry shoot weight, dry root weight) were compared
218 among treatments using one-way Analysis of Variance (ANOVA) with SAS software, and
219 means were separated using Fisher's Least Significant Difference (LSD) test ($\alpha$=0.05) (Proc
220 GLM). The percentage of roots affected by root rot in each treatment was compared using a
221 Generalized Linear Mixed Model (GLMM) in SAS with a logit link and assuming a beta
222 distribution, and means were separated by Least Squares Means ($\alpha$=0.05) (Proc GLIMMIX).
223 The percentage of root samples with *Phytophthora* infection in each treatment was compared
224 using logistic regression in SAS with a logit link and binomial distribution, and means were
225 separated by Least Square Means ($\alpha$=0.05) (Proc GENMOD). The number of *Trichoderma*
226 colonies growing on *Trichoderma*-selective media were compared between flood duration
227 treatments with a Generalized Linear Interactive Model (GLIM) using a negative binomial
228 distribution and a log link, and means were separated by Least Squares Means ($\alpha$=0.05) (Proc

GENMOD). Mean ambrosia beetle attacks in each treatment were compared with a GLIM using a negative binomial distribution and a log link, and means were separated by Least Square Means (α=0.05) (Proc GENMOD) (Agresti 2003).

## 3. RESULTS

There were no interactions between flooding duration and treatments for any plant growth parameter (plant height, plant width, caliper, dry shoot weight) in either trial, so data were pooled across flooding durations. There were no differences among treatments for any of the plant growth parameters (Tables 2 and 3). The only difference in plant growth was in Trial 1 where plants flooded 1 or 3 days had a higher plant width ($P = 0.0021$) and dry root weight ($P = 0.0085$) than plants flooded 7 days (data not shown).

Positive control plants had the highest disease severity and highest percentage of infected root samples in both trials (Figs. 1-3). Negative control plants had lower disease severity than positive control plants and a lower percentage of infected root samples in both trials (Figs. 1-3). Positive control plants had the second most ambrosia beetle attacks among all treatments, and the negative control plants had significantly less ambrosia beetle attacks than the positive control (Fig. 5).

In Trial 1, there was an interaction between flooding duration and treatments for disease severity ($P = 0.0167$). Significant differences among treatments were observed in disease severity at flooding durations of 1 ($P = 0.0095$), 3 ($P = 0.0013$), or 7 days ($P = 0.0020$). Plants preventively-treated with Subdue MAXX or Orkestra Intrinsic had lower disease severity than the positive control plants at 1, 3, or 7 days of flooding (Fig. 1). Preventive treatments of Pageant Intrinsic or RootShield Plus$^+$ and curative treatments of Empress Intrinsic or Orkestra Intrinsic reduced disease severity compared to the positive control at 1 or 3 days of flooding, while preventive treatment of RootShield Plus$^+$ + ON-Gard 5-0-0 N-P-K (ON-Gard) treatment was effective on plants flooded 1 day (Fig. 1).

In Trial 2, there was again an interaction between flooding duration and treatments for disease severity ($P = 0.0037$). Disease severity was different among treatments at flooding

durations of 1 ($P = 0.0002$), 3 ($P = 0.0228$), or 7 days ($P = 0.0020$). As in Trial 1, Subdue MAXX-treated plants had lower disease severity than the positive control at 1, 3, or 7 days of flooding, as well as curative Empress Intrinsic treatments (Fig. 2). Preventive treatments of Orkestra Intrinsic, Pageant Intrinsic, and RootShield Plus$^+$ + ON-Gard reduced disease severity at 1 or 3 days of flooding (Fig. 2). Preventive treatment of RootShield Plus$^+$ and curative treatment of Orkestra Intrinsic reduced disease severity on plants flooded 1 day, while curative Tartan Stressgard treatment reduced disease severity at 3 days of flooding (Fig. 2).

In Trial 1, disease severity of inoculated plants increased with longer flooding duration (1 day = 26.2 ± 2.8, 3 days = 41.9 ± 3.6, 7 days = 58.3 ± 4.0; $P < 0.0001$). In Trial 2, inoculated plants flooded 1 or 3 days had lower disease severity than plants flooded 7 days (1 day = 29.5 ± 3.5, 3 days = 39.9 ± 4.6, 7 days = 57.4 ± 4.5; $P = 0.0011$).

There were no interactions between flooding duration and treatments in the percentage of selected roots with *Phytophthora* growth (Trial 1: $P = 0.5862$; Trial 2: $P = 0.8215$), so the data were pooled across flooding durations. In both trials, the percentage of infected root samples was different across treatments ($P < 0.0001$). Preventive treatments of Orkestra Intrinsic, Pageant Intrinsic, or Subdue MAXX had a lower percentage of infected root samples than the positive control in both trials, as well as curative Empress Intrinsic treatment (Fig. 3). Preventive treatment of RootShield Plus$^+$ + ON-Gard and curative treatments of Orkestra Intrinsic or Tartan Stressgard had a lower percentage of infected root samples than the positive control in Trial 2, but not Trial 1 (Fig. 3).

In inoculated plants in Trial 1, there were increasing percentage of infected root samples with increasing flood duration (1 day = 0.38 ± 0.05; 3 days = 0.56 ± 0.05; 7 days = 0.74 ± 0.04) ($P < 0.0001$). In Trial 2, inoculated plants at 1 day of flooding had a lower percentage of infected root samples than plants at 3 or 7 days of flooding (1 day = 0.41 ± 0.05; 3 days = 0.54 ± 0.04; 7 days = 0.63 ± 0.05) ($P = 0.0003$).

*Trichoderma* root colonization on RootShield Plus$^+$ treated plants was higher at 1 or 3 days of flooding compared to 7 days of flooding ($P = 0.0196$; Fig. 4). Similarly, *Trichoderma* root colonization on RootShield Plus$^+$ + ON-Gard treated plants were higher at 1 or 3 days of flooding compared to 7 days of flooding ($P = 0.0073$; Fig. 4).

Because Trial 1 had few ambrosia beetle attacks, only Trial 2 data were used for analysis. Ambrosia beetle attacks were different among treatments ($P = 0.0139$). Orkestra Intrinsic (preventive and curative), Subdue MAXX (preventive), Tartan Stressgard (curative), and the negative control had fewer ambrosia beetle attacks than the positive control (Fig. 5). Plants flooded 1 or 3 days had fewer ambrosia beetle attacks than plants flooded 7 days (1 day = 8, 3 days = 13, 7 days = 60; $P < 0.0001$). While plants were experimentally flooded, 84% of ambrosia beetle attacks occurred, while only 16% occurred within 3 days of plant removal from simulated flood event. No ambrosia beetle attacks occurred after 3 days post-flooding. Only the granulate ambrosia beetle (*Xylosandrus crassiusculus* [Motschulsky]) (n = 13) was recovered from ambrosia beetle galleries. Ambrosia beetles attacked containerized flowering dogwoods with a caliper as small as 8.7 cm, but most attacks occurred on plants above 10.0 cm (89%).

## 4. DISCUSSION

In this study, preventively- and curatively-applied fungicide treatments were effective against Phytophthora root rot, caused by *P. cinnamomi*, in containerized flowering dogwood plants during a simulated flood event; especially at shorter flood durations. Previous experiments have demonstrated that fungicide efficacy against *Phytophthora* can be affected by length of flooding period or frequency of flooding (Falloon et al. 1991; Matheron and Porchas 2000; Matheron and Porchas 2002). Preventive treatments of metalaxyl (Subdue MAXX) consistently reduced disease severity and the percentage of infected root samples, but other fungicide treatments were inconsistent among flood durations or trials.

The results from this study confirm outcomes of previous studies that Phytophthora root rot severity is associated with exposure to elevated soil moisture. Longer flooding duration increased disease severity of *Phytophthora*, likely due to enhanced zoospore production and dispersal resulting from longer exposure to free water in the soil. Also, longer flooding periods may have increased host susceptibility to the pathogen. In the *Rhododendron* cultivar 'Caroline', Blaker and MacDonald (1981) reported that *P. cinnamomi* symptoms increased with flooding periods of 24 – 48 h, while no difference in symptoms was observed in the cultivar 'Purple Splendor'. In ornamental cherry (*Prunus x yedoensis*), a combination of flooding and

315 *Phytophthora cryptogea* Pethybr. & Laff. inoculation increased mortality, defoliation, and
316 disease severity compared to inoculation alone (Jacobs and Johnson 1996). Length of flooding
317 duration affected root rot of cherry seedlings (*Prunus mahaleb* L.) inoculated with *P. cryptogea*
318 or *Phytophthora megasperma* Dreschler (Wilcox and Mircetich 1985). However, increased root
319 rot with longer flood duration was dependent on *Phytophthora* species in apple seedlings (*Malus*
320 *pumila* Mill.), with *P. cryptogea* displaying increased disease, but not *Phytophthora cactorum*
321 (Lebert & Cohn) J. Schrot. or *Phytophthora cambivora* (Petri) Buisman (Browne and Mircetich
322 1988).

323 Metalaxyl (Subdue MAXX) is a standard fungicide treatment for Phytophthora root rot
324 management on woody ornamental crops (Erwin and Ribeiro 1996; Zentmyer 1980). Preventive
325 Subdue MAXX treatment was the only treatment consistently effective across all flooding
326 durations in both trials. On asparagus (*Asparagus officinalis* L.), metalaxyl effectiveness against
327 Phytophthora rot decreased with increasing flood frequency (Falloon et al. 1991). In this study,
328 disease severity of metalaxyl-treated plants increased with longer flooding duration; but
329 metalaxyl treatment was effective compared to the positive control at all flooding durations.

330 Pyraclostrobin (Empress Intrinsic, Orkestra Intrinsic, and Pageant Intrinsic) was effective
331 as a preventive and curative treatment in this study, especially on containerized flowering
332 dogwood plants flooded < 7 days. Results were inconsistent at 7 days of flooding for preventive
333 Orkestra Intrinsic and curative Empress Intrinsic treatments. Preventive Pageant Intrinsic
334 treatments reduced *P. cinnamomi* root rot disease severity on flooded Eastern redbud (*Cercis*
335 *canadensis* L.) and tulip poplar (*Liriodendron tulipifera* L.) plants (Addesso et al. 2018).
336 Tartan Stressgard (trifloxystrobin, triadimefon) was mostly ineffective against Phytophthora root
337 rot. Strobilurin fungicides (pyraclostrobin, trifloxystrobin) increase stress tolerance in plants to
338 biotic and abiotic factors like flooding by causing changes in stress-related hormones and
339 increased antioxidative enzymatic activity (Chaves et al. 2016; Wu and Tiedemann 2001).
340 Potentially, strobilurin fungicides reduced host susceptibility to flood stress, which increased
341 effectiveness against root rot. Also, strobilurin fungicides, as well as metalaxyl, induce host
342 resistance to some pathogens, which may provide enhanced activity against *P. cinnamomi*
343 (Herms et al. 2002; Molina et al. 1998).

The biofungicide, RootShield Plus+ (*Trichoderma harzianum* Rifai strain T-22 and *T. virens* strain G-41) was only effective at shorter flooding durations in this study, possibly due to increased disease pressure. *Trichoderma* colonization on the roots of dogwoods declined in plants exposed to more than 3 days flooding, perhaps because of reduced oxygen availability, competition with *P. cinnamomi*, or deteriorating root system. Smith et al. (1990) found that *Trichoderma* species were effective against *P. cactorum* in apple plants exposed to 3 days of flooding. But, in this study *Trichoderma* species had inconsistent efficacy against *P. cinnamomi* at 3 days of flooding.

*Phytophthora* inoculation appeared to be a factor inducing ambrosia beetle attacks on flooded containerized flowering dogwoods. In other studies, non-treated, *P. cinnamomi* inoculated controls did not significantly increase ambrosia beetle attacks (Addesso et al. 2018). Discrepancies of the results between the studies could be the result of different plant species (Eastern redbud and tulip poplar vs. flowering dogwood), length of flood duration, and the use of ethanol lures to enhance ambrosia beetle response in this study. Flooding for > 3 days increased ambrosia beetle pressure in this study, probably from increased ethanol production because of increased flood stress or disease. Tree attack rates of some ambrosia beetle species have a positive correlation with ethanol dose (Reding et al. 2011; Ranger et al. 2015; Frank and Ranger 2016). Both Orkestra Intrinsic (preventive and curative) and the standard metalaxyl treatment Subdue MAXX (preventive) reduced ambrosia beetle attacks and demonstrated efficacy against *P. cinnamomi* in this study. Fungicides have demonstrated effectiveness against related ambrosia beetles and their fungi *in vitro* and *in vivo* in other studies (Addesso et al. 2018; Kagezi et al. 2015; Ranger et al. 2016b). Because all trees were inoculated with *Phytophthora* in this study, fungicide efficacy against ambrosia beetles in the absence of *Phytophthora* infection was not assessed. However, these results demonstrate that fungicides may improve protection from secondary pests like ambrosia beetles that attack infected trees.

## 5. CONCLUSIONS

Effective treatments are needed for management of Phytophthora root rot during flooding events in woody ornamental nurseries, because of the associated risk of infestation and spread of the pathogen. Longer flooding periods require higher demand for effective treatments, but also

impair control of the pathogen. Indeed, longer flooding periods increased Phytophthora root rot during this study. Likewise, longer flooding periods and *Phytophthora* inoculation of flooded trees increased ambrosia beetle attacks. Preventive or curative fungicide treatments have potential to be included as part of an integrated management strategy for Phytophthora root rot on woody ornamentals. The biofungicide RootShield Plus$^{+}$ was only effective against Phytophthora root rot at shorter flooding durations. Mefenoxam was particularly effective as a preventive treatment for *P. cinnamomi* and also reduced secondary ambrosia beetle attacks.

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## Tables

**Table 1.** Fungicides, biofungicide, and fertilizer used in this study.

| Treatment[a] | Application rate | | Product group | Manufacturer[b] |
|---|---|---|---|---|
| | ml liter$^{-1}$ | g liter$^{-1}$ | | |
| Empress Intrinsic | 0.47 | | Strobilurin | BASF |
| ON-Gard 5-0-0 | 5 | | Fertilizer | Bioworks |
| Orkestra Intrinsic | 0.78 | | Strobilurin, succinate dehydrogenase inhibitor | BASF |
| Pageant Intrinsic | | 1.35 | Strobilurin, succinate dehydrogenase inhibitor | BASF |
| RootShield Plus$^{+}$ WP | | 0.60 | Biofungicide | Bioworks |
| Subdue MAXX | 0.16 | | Phenylamide | Syngenta |
| Tartan Stressgard | 3.12 | | Strobilurin, triazole | Bayer |

[a] Active ingredients (% A.I.): Empress Intrinsic = pyraclostrobin (23.3%); ON-Gard 5-0-0 = 5% total nitrogen; Orkestra Intrinsic = pyraclostrobin (21.26%), fluxapyroxad (21.26%); Pageant Intrinsic = pyraclostrobin (12.8%), boscalid (25.2%); RootShield Plus$^{+}$ = *Trichoderma harzianum* Rifai strain T-22 (1.15%), *T. virens* strain G-41 (0.61%); Subdue MAXX = mefenoxam (22%); Tartan Stressgard = trifloxystrobin (4.17%), triadimefon (20.86%).

[b] BASF=BASF Corporation, Florham Park, NJ; Bayer=Bayer AG, Monheim an Rhein, Germany; Bioworks=Bioworks Inc., Victor, NY; Syngenta=Syngenta International AG, Basel, Switzerland

**Table 2.** Mean (± SE) plant growth data of flooded flowering dogwoods preventively- or curatively-treated with fungicides, biofungicide, or fertilizer (Trial 1).

| Treatment | Plant Height (cm) | Plant Width (cm) | Caliper (cm) | Dry shoot Weight (g) | Dry root Weight (g) |
|---|---|---|---|---|---|
| Orkestra (preventive) | 75.5 ± 2.5 | 37.5 ± 2.2 | 9.8 ± 0.3 | 28.7 ± 2.4 | 38.9 ± 6.5 |
| Pageant (preventive) | 75.1 ± 2.0 | 35.6 ± 2.2 | 9.6 ± 0.3 | 30.2 ± 2.3 | 31.0 ± 3.4 |
| RootShield Plus+ (preventive) | 73.3 ± 2.8 | 35.0 ± 1.7 | 9.8 ± 0.3 | 29.0 ± 2.2 | 36.0 ± 4.9 |
| RootShield Plus+ + ON-Gard (preventive) | 77.0 ± 2.2 | 34.9 ± 2.3 | 9.3 ± 0.4 | 29.1 ± 2.8 | 28.3 ± 5.3 |
| Subdue MAXX (preventive) | 71.3 ± 3.1 | 36.2 ± 2.8 | 9.3 ± 0.4 | 27.4 ± 2.8 | 41.9 ± 10.7 |
| Empress (curative) | 73.9 ± 2.6 | 33.3 ± 1.8 | 9.4 ± 0.4 | 29.1 ± 2.9 | 32.3 ± 4.9 |
| Orkestra (curative) | 76.8 ± 3.0 | 32.7 ± 2.0 | 9.1 ± 0.3 | 26.8 ± 1.8 | 30.2 ± 5.2 |
| Tartan (curative) | 75.1 ± 2.3 | 39.3 ± 1.7 | 9.8 ± 0.4 | 34.9 ± 4.0 | 44.0 ± 9.7 |
| Positive control[a] | 72.9 ± 2.0 | 37.4 ± 2.9 | 9.9 ± 0.3 | 29.2 ± 2.3 | 36.3 ± 8.0 |
| Negative controll[a] | 75.9 ± 2.4 | 35.9 ± 3.1 | 9.7 ± 0.3 | 29.2 ± 2.1 | 32.2 ± 9.6 |
| *F* | 0.55 | 0.87 | 0.67 | 0.76 | 0.63 |
| df | 8 | 8 | 8 | 8 | 8 |
| *P* | 0.8136 | 0.5450 | 0.7206 | 0.6351 | 0.7522 |
| CV | 11.52 | 21.33 | 12.44 | 30.15 | 65.73 |

[a] Control treatments included the non-treated, *P. cinnamomi* inoculated (positive control) and non-treated, non-inoculated (negative control) plants.

516 **Table 3.** Mean (± SE) plant growth data of flooded flowering dogwoods preventively- or
517 curatively-treated with fungicides, biofungicide, or fertilizer (Trial 2).

| Treatment | Plant Height (cm) | Plant Width (cm) | Caliper (cm) | Dry shoot Weight (g) | Dry root Weight (g) |
|---|---|---|---|---|---|
| Orkestra (preventive) | 78.0 ± 2.2 | 35.1 ± 2.4 | 9.9 ± 0.2 | 31.2 ± 2.0 | 45.3 ± 5.5 |
| Pageant (preventive) | 85.9 ± 2.4 | 35.3 ± 1.9 | 10.1 ± 0.3 | 36.3 ± 2.5 | 54.4 ± 7.2 |
| RootShield Plus+ (preventive) | 77.4 ± 3.8 | 27.9 ± 2.3 | 9.7 ± 0.5 | 27.2 ± 3.4 | 47.8 ±15.5 |
| RootShield Plus+ + ON-Gard (preventive) | 75.6 ± 2.6 | 31.6 ± 2.0 | 9.6 ± 0.3 | 30.2 ± 3.0 | 54.5 ±12.6 |
| Subdue MAXX (preventive) | 75.4 ± 2.4 | 30.1 ± 1.5 | 10.2 ± 0.3 | 32.2 ± 3.0 | 41.0 ± 7.5 |
| Empress (curative) | 78.8 ± 3.3 | 30.9 ± 2.1 | 9.5 ± 0.3 | 29.8 ± 2.6 | 41.8 ± 6.8 |
| Orkestra (curative) | 76.5 ± 3.7 | 31.9 ± 1.6 | 9.4 ± 0.3 | 30.6 ± 2.4 | 42.6 ± 6.4 |
| Tartan (curative) | 79.3 ± 2.2 | 35.0 ± 2.3 | 10.2 ± 0.3 | 31.0 ± 2.0 | 56.8 ± 5.9 |
| Positive control[a] | 74.5 ± 3.1 | 30.7 ± 1.6 | 10.3 ± 0.5 | 32.0 ± 3.5 | 45.3 ± 7.0 |
| Negative control[a] | 81.0 ± 3.6 | 37.2 ± 3.3 | 9.9 ± 0.4 | 35.7 ± 2.9 | 43.8 ± 8.3 |
| *F* | 1.30 | 1.74 | 0.88 | 0.73 | 0.47 |
| df | 8 | 8 | 8 | 8 | 8 |
| *P* | 0.2563 | 0.1011 | 0.5379 | 0.6656 | 0.8738 |
| CV | 13.14 | 21.90 | 12.10 | 30.48 | 63.09 |

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519 [a] Control treatments included the non-treated, *P. cinnamomi* inoculated (positive control) and
520 non-treated, non-inoculated (negative control) plants.

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## Figure Captions

**Fig. 1.** Disease severity (mean ± SE) of flowering dogwoods preventively- or curatively-treated with fungicides, biofungicide, or fertilizer and flooded 1, 3, or 7 days (Trial 1). For root rot disease severity, each plant was evaluated using a scale of 0−100% roots affected. Control treatments included the non-treated, *P. cinnamomi* inoculated (positive control) and non-treated, non-inoculated (negative control) plants. Asterisks beside bars represent significant differences in disease severity within a flooding duration compared to the positive control (1 day: $F = 3.28$, df = 8, $P = 0.0095$, CV = 64.41; 3 days: $F = 4.58$, df = 8, $P = 0.0013$, CV = 50.99; 7 days: $F = 4.28$, df = 8, $P = 0.0020$, CV = 40.96; $\alpha = 0.05$, Least Squares Means).

**Fig. 2.** Disease severity (mean ± SE) of flowering dogwoods preventively- or curatively-treated with fungicides, biofungicide, or fertilizer and flooded 1, 3, or 7 days (Trial 2). For root rot disease severity, each plant was evaluated using a scale of 0−100% roots affected. Control treatments included the non-treated, *P. cinnamomi* inoculated (positive control) and non-treated, non-inoculated (negative control) plants. Asterisks beside bars represent significant differences in disease severity within a flooding duration compared to the positive control (1 day: $F = 5.95$, df = 8, $P = 0.0002$, CV = 70.90; 3 days: $F = 2.76$, df = 8, $P = 0.0228$, CV = 68.89; 7 days: $F = 4.28$, df = 8, $P = 0.0020$, CV = 46.74; $\alpha = 0.05$, Least Squares Means).

**Fig. 3.** Percentage of infected root samples (mean ± SE) of flowering dogwoods preventively- or curatively-treated with fungicides, biofungicide, or fertilizer and flooded 1, 3, or 7 days. Five root samples were randomly selected from root tips of each plant and plated on selective media to examine presence or absence of *P. cinnamomi* growth. Control treatments included the non-treated, *P. cinnamomi* inoculated (positive control) and non-treated, non-inoculated (negative control) plants. Asterisks beside bars represent significant differences in disease severity within a flooding duration compared to the positive control (Trial 1: $\chi^2 = 35.42$, df = 8, $P < 0.0001$, CV = 50.02; Trial 2: $\chi^2 = 38.33$, df = 8, $P < 0.0001$, CV = 52.05; $\alpha = 0.05$, Least Squares Means).

**Fig. 4.** *Trichoderma* (mean ± SE) colony counts of root samples on *Trichoderma*-selective medium of RootShield Plus$^+$-treated plants flooded 1, 3, or 7 days. For plants treated with RootShield Plus$^+$ or the RootShield Plus$^+$ + ON-Gard program, undiluted root samples, as well as dilutions of $10^{-2}$ and $10^{-4}$ were plated on *Trichoderma*-selective medium, and the colonies were counted after 10-days incubation. Data were pooled across trials. Letters above bars represent significant differences *Trichoderma* colonies among flooding durations (RootShield Plus$^+$: $\chi^2$ = 7.86, df = 2, $P$ = 0.0196, CV = 96.72; RootShield Plus$^+$ + ON-Gard: $\chi^2$ = 9.84, df = 2, $P$ = 0.0073, CV = 85.31; $\alpha$ = 0.05, Least Squares Means).

**Fig. 5.** Ambrosia beetle attacks (mean ± SE) on flowering dogwoods preventively- or curatively-treated with fungicides, biofungicide, or fertilizer and flooded 1, 3, or 7 days (only Trial 2). Data were pooled across flooding durations. Control treatments included the non-treated, *P. cinnamomi* inoculated (positive control) and non-treated, non-inoculated (negative control) plants. Different letters beside bars indicate significantly different mean ambrosia beetle attacks among treatments ($F$ = 20.74, df = 9, $P$ = 0.0139, CV = 132.72; $\alpha$ = 0.05, Least Squares Means).

## Figures

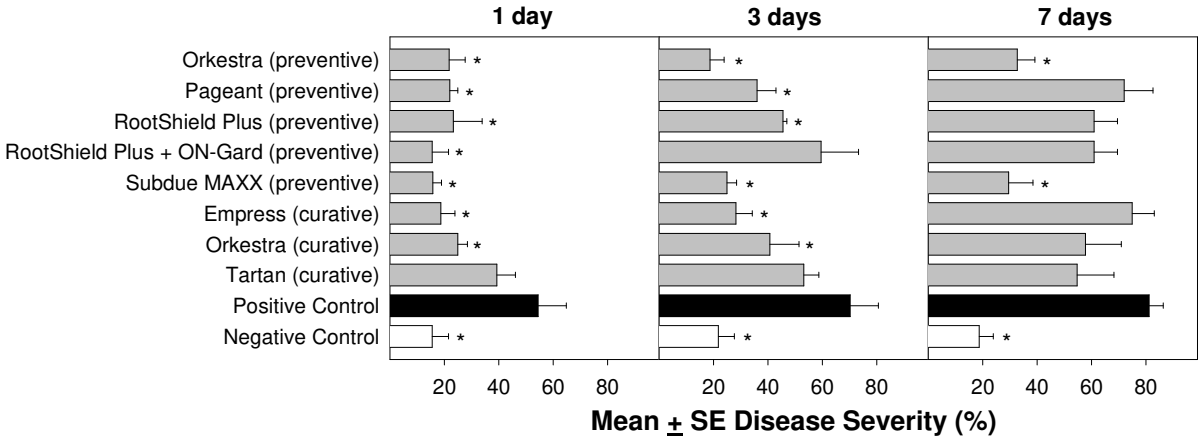



**Fig. 1.** Disease severity (mean ± SE) of flowering dogwoods preventively- or curatively-treated with fungicides, biofungicide, or fertilizer and flooded 1, 3, or 7 days (Trial 1). For root rot disease severity, each plant was evaluated using a scale of 0–100% roots affected. Control treatments included the non-treated, *P. cinnamomi* inoculated (positive control) and non-treated, non-inoculated (negative control) plants. Asterisks beside bars represent significant differences in disease severity within a flooding duration compared to the positive control (1 day: $F = 3.28$, df = 8, $P = 0.0095$, CV = 64.41; 3 days: $F = 4.58$, df = 8, $P = 0.0013$, CV = 50.99; 7 days: $F = 4.28$, df = 8, $P = 0.0020$, CV = 40.96; $\alpha = 0.05$, Least Squares Means).

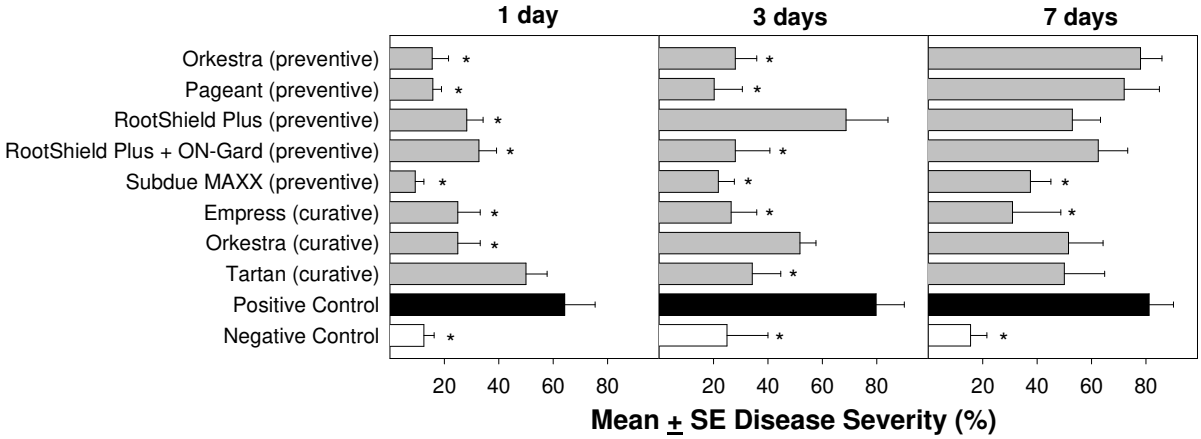
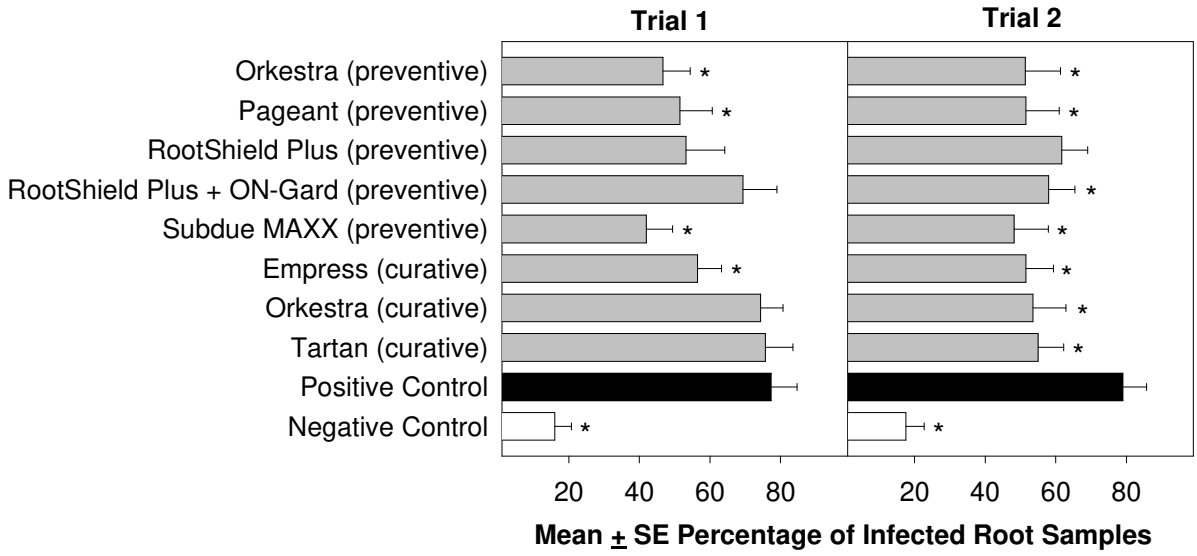



**Fig. 2.** Disease severity (mean ± SE) of flowering dogwoods preventively- or curatively-treated with fungicides, biofungicide, or fertilizer and flooded 1, 3, or 7 days (Trial 2). For root rot disease severity, each plant was evaluated using a scale of 0–100% roots affected. Control treatments included the non-treated, *P. cinnamomi* inoculated (positive control) and non-treated, non-inoculated (negative control) plants. Asterisks beside bars represent significant differences in disease severity within a flooding duration compared to the positive control (1 day: $F = 5.95$, df = 8, $P = 0.0002$, CV = 70.90; 3 days: $F = 2.76$, df = 8, $P = 0.0228$, CV = 68.89; 7 days: $F = 4.28$, df = 8, $P = 0.0020$, CV = 46.74; $\alpha = 0.05$, Least Squares Means).




606

607 **Fig. 3.** Percentage of infected root samples (mean ± SE) of flowering dogwoods preventively- or
608 curatively-treated with fungicides, biofungicide, or fertilizer and flooded 1, 3, or 7 days. Five
609 root samples were randomly selected from root tips of each plant and plated on selective media
610 to examine presence or absence of *P. cinnamomi* growth. Control treatments included the non-
611 treated, *P. cinnamomi* inoculated (positive control) and non-treated, non-inoculated (negative
612 control) plants. Asterisks beside bars represent significant differences in disease severity within
613 a flooding duration compared to the positive control (Trial 1: $\chi^2 = 35.42$, df = 8, $P < 0.0001$, CV
614 = 50.02; Trial 2: $\chi^2 = 38.33$, df = 8, $P < 0.0001$, CV = 52.05; $\alpha = 0.05$, Least Squares Means).

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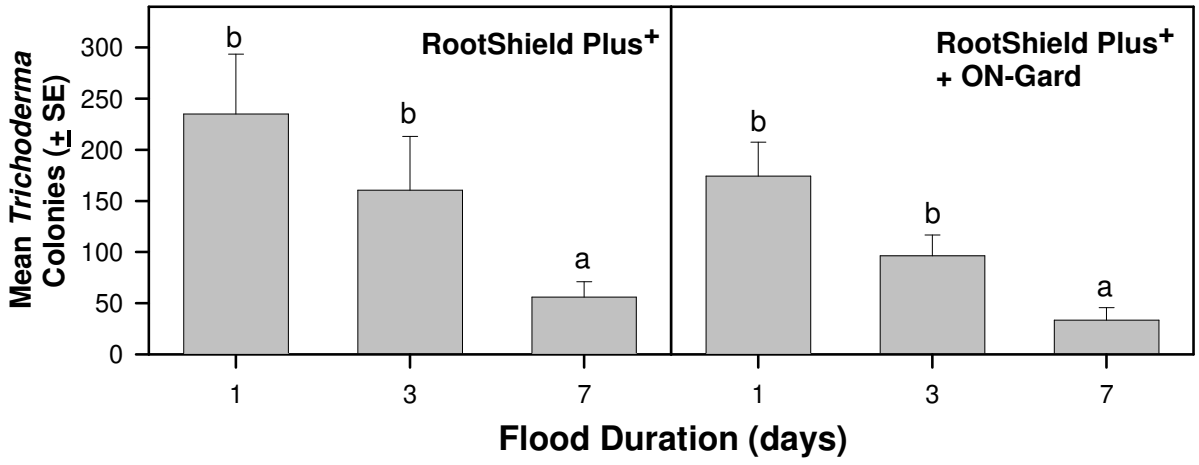


618

619 **Fig. 4.** *Trichoderma* (mean ± SE) colony counts of root samples on *Trichoderma*-selective
620 medium of RootShield Plus$^+$-treated plants flooded 1, 3, or 7 days. For plants treated with
621 RootShield Plus$^+$ or the RootShield Plus$^+$ + ON-Gard program, undiluted root samples, as well as
622 dilutions of $10^{-2}$ and $10^{-4}$ were plated on *Trichoderma*-selective medium, and the colonies were
623 counted after 10-days incubation. Data were pooled across trials. Letters above bars represent
624 significant differences *Trichoderma* colonies among flooding durations (RootShield Plus$^+$: $\chi^2$ =
625 7.86, df = 2, $P$ = 0.0196, CV = 96.72; RootShield Plus$^+$ + ON-Gard: $\chi^2$ = 9.84, df = 2, $P$ =
626 0.0073, CV = 85.31; $\alpha$ = 0.05, Least Squares Means).

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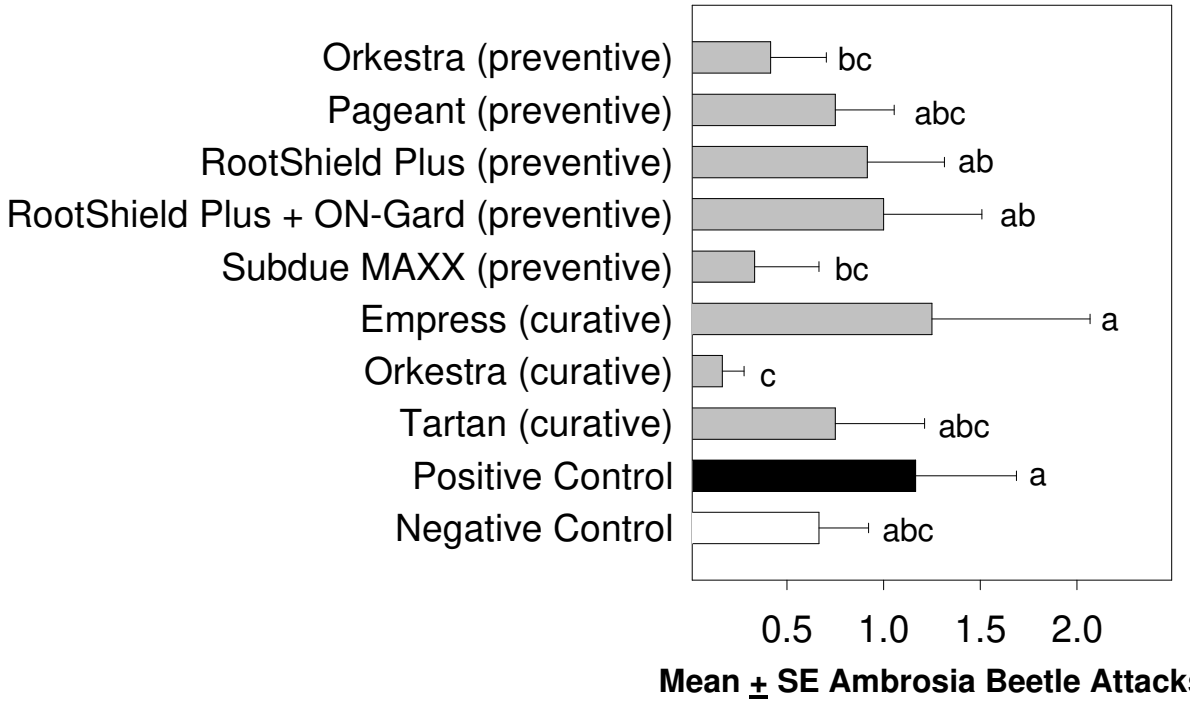


630

631 **Fig. 5.** Ambrosia beetle attacks (mean ± SE) on flowering dogwoods preventively- or
632 curatively-treated with fungicides, biofungicide, or fertilizer and flooded 1, 3, or 7 days (only
633 Trial 2). Data were pooled across flooding durations. Control treatments included the non-
634 treated, *P. cinnamomi* inoculated (positive control) and non-treated, non-inoculated (negative
635 control) plants. Different letters beside bars indicate significantly different mean ambrosia beetle
636 attacks among treatments ($F = 20.74$, df = 9, $P = 0.0139$, CV = 132.72; $\alpha = 0.05$, Least Squares
637 Means).

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639