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Effect of bacteria isolates in Powdery mildew control in flowering dogwoods (*Cornus florida* L.)

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Abstract

Five bacterial isolates collected from dogwood leaves were evaluated for powdery mildew control in shadehouse and greenhouse environments by using foliar sprays and/or root drenching. Two isolates displayed superior bioactivity and suppressed powdery mildew similar to conventional fungicide thiophanate methyl (Cleary's 3336F®). The two bacteria disrupted powdery mildew spore germination and ruptured spore membranes causing spore lyses. Bacterial filtrates without bacterial cells were also effective in suppressing powdery mildew and disrupting spore germination and suggested the involvement of secondary metabolites. The two biocontrol agents (BCAs) colonized roots endophytically and promoted plant growth.

Keywords: *Ornamentals, fungicides, Oidium spp.; Erysiphe (Sect. Microsphaera) pulchra; biological control.*

1. Introduction

Powdery mildew caused by *Erysiphe* (Sect. *Microsphaera*) *pulchra* (Cook & Peck, Braun & Takamatsu) is one of the most economically important diseases affecting dogwood production in the southeastern USA (**Leigh et al., 1998; McRitchie, 1994. Mmbaga, 1998; Mmbaga, 2000; Windham, 1994**). The disease causes stunted growth, premature defoliation, reduced esthetic value and overall decline of infected trees (**Chartfield and Rose, 1996; Mmbaga, 1998; Mmbaga, 2000; Daughtrey and Hagan, 2001; Smith, 1999**). Current management of this disease is primarily with fungicides (**Windham, 1994; Mmbaga, 2000; Mmbaga and Sheng, 1999**), but this has increased production costs (**Li et al., 2009**) and caused environmental concerns (**Mmbaga and Sheng, 1999**). Many small nurseries have ceased or terminated dogwood production because profit margins were not sufficient to

cover fungicide and labor costs associated with routine fungicide applications every two weeks from May to October (**Li et al 2010**). In addition to cost, chemical fungicides also harm non-target organisms that play a role in protecting plants against pathogens (**Kiss, 2003; Elad, 2000; Elad *et al.*, 1996**) and pose health hazards to humans, other animals including wildlife as a result of accidental exposures to the toxic chemicals. Biological agents for controlling powdery mildew will offer alternative disease management options that are presumably safer, sustainable, and less damaging to the environment than traditional chemical fungicides (**Elad, 2000; Elad *et al.*, 1996; Kiss, 2003; Mmbaga et al., 2008; Mmbaga and Sauvé, 2009**).

Bacteria belonging to the genera *Bacillus*, *Agrobacterium*, *Pseudomonas* and *Streptomyces* have previously been used to control plant diseases (**Garderner and Fravel, 2002**). A combination of biological control and other user-friendly methods such as resistant cultivars (**Hartman et al., 2003**) and cultural practices that reduce pathogen proliferation may substantially reduce the amount of fungicide used in powdery mildew control in nursery production of dogwood. Filamentous fungi, bacteria and yeast isolated from dogwood foliage in the wild have shown potential in controlling dogwood powdery mildew (**Mmbaga et al., 2008; Mmbaga and Sauvé, 2009**). Although some biological agents are often isolated from plant leaves as epiphytes, some have been reported to have an endophytic phase and interact with their host in symbiotic and other types of relationships **(Nejad and Johnson, 2000; Mmbaga and Sauvé, 2009; Pal et al 2006).** The objective of this study was to confirm the efficacy of selected bacterial isolates in powdery mildew disease control, assess their ability to colonize plants through the roots and their potential mechanism of action.

2. Materials and methods.

2.1 Efficacy of bacterial isolates in powdery mildew disease control

Four sets of experiments were conducted to (1) evaluate five isolates previously isolated from the wild **(Mmbaga et al., 2008)** and stored for two years at -80ºC, (2) evaluate isolates (B17A and B17B) that showed superior bioactivity, (3) evaluate method of application as foliar sprays and root drenching, and (4) assess the potential mechanism of action on powdery mildew spore germination. All experiments were repeated ones.

2.1.1. Bacterial isolates inoculum preparation, plant material for biological control of powdery mildew.

All bacterial isolates used in this study were previously isolated from dogwood leaves collected from two natural and uncultivated forest habitats (**Mmbaga et al., 2008**). These isolates had been stored at -80°C in 15% aqueous glycerol and were revived and grown in nutrient agar (NA) and visually checked for purity.

Pure cultures were grown in nutrient broth containing 1.0 g meat extract; 1.0 g yeast extract; 5.0 g peptone; and 5.0 g sodium chloride per L. After 24 hours growth in nutrient broth (NB), cells were pelleted by centrifugation, washed twice in sterile water and then re-suspended in sterile water containing 0.05% Tween 20. Bacterial suspensions of each isolate were adjusted to a concentration of 5.0×10^9 cells ml^{-1} and used as inoculum. Experimental plants consisted of 5-month old seedlings grown in 3.75 L (1 gallon) containers using Morton's Nursery Mix consisting of 1:1:1 sand:bark:loam (Morton's Horticultural Supplies Inc., McMinnville. TN). Plants were fertilized in early May using water-soluble Miracle-Gro™, consisting of 18-24-16 (Nitrogen:Phosphorus:Potasium) at the rate of 18 g per 3780 mL (w/v) and with controlled-release fertilizer (Nutricote Total™ 18-6-8), applied at the rate of 12 g per container. Fungicide Cleary's 3336™ F (41.25% thiophanate-methyl, Cleary Chemical Corp., Dayton, NJ) was used as a positive control at the recommended rate of 1.56 ml/L and water was used as the non-treated control. Greenhouse environment was maintained at 26 ± 3ºC and shadehouses

were covered with 65% shade cloth. Powdery mildew treatments were applied at 7-10 days intervals and their efficacy in suppressing powdery mildew severity was evaluated using a disease rating scale of 0-5 where, 0 = no disease symptoms, 1 = 1-10%, 2 = 11-25%, 3 = 26-50%, 4 = 51-75% and 5 = 75-100% of foliage covered with powdery mildew symptoms.

2.1.2. Efficacy of five bacterial BCA isolates after two years in cold storage

This study was conducted in greenhouse environment to compare five bacterial isolates with a fungicide positive control and non-treated, water control. Foliar spray method was used to apply treatments and plants were allowed to air dry for approximately 1 h and then placed in an incubation chamber maintained at 100% RH for 24 h to allow establishment of the biological control agents on plant surfaces prior to powdery mildew infection. Inoculation with powdery mildew pathogen was by using spore settling tower technique whereby spores from previously infected plants were blown into the air above test plants and allowed to float down onto test plants as described in **Mmbaga et al., 2008**. A continuous supply of air-borne powdery mildew inoculum was from previously infected plants scattered in the experimental area; powdery mildew control treatment was repeated every 7-10 days.

2.1.3. Evaluation of two isolates B17A and B17B as BCA for powdery mildew control.

Isolate 17A that showed superior efficacy as a powdery mildew BCA after two years in cold storage was designated B17A and compared to isolate B17B that was previously selected for superior efficacy against powdery mildew. Efficacies of these two isolates were compared on susceptible (S) and moderately resistant (MR) plants in shade-house environment under 65% shade-clothe and in greenhouse environment. Susceptible plants used in this study were dogwood selections 295, 327, 400 and moderately resistant plants were selections R11 and R12. Each plant selection was replicated by six individual plants per treatment and plants were arranged in a randomized complete block design. A

continuous supply of air-borne powdery mildew inoculum was from previously infected plants scattered in the experimental area; treatments with BCA was initiated when test plants showed first disease symptoms. In addition, BCA applications were done in the evening (approx. 6:00 pm) and natural dew was the only source of free moisture for BCA plant colonization. Plants were sprayed to runoff using a hand-held atomizer for even distribution of BCA suspension.

2.1.4. Efficacy of biocontrol agents applied by foliage spray and by root drenching.

Treatments for disease control were initiated when the first disease symptoms were observed and biocontrol isolate B17A and B17B suspensions of 5.0 x 10^9 bacterial cells ml^{-1}were applied using hand-held atomizer and the foliage sprays or root drenching methods. Foliage spray treatments were done using an atomizer to deliver the inoculum uniformly and plants were sprayed to run off; root drenching was done using 20 ml inoculum per plant. Treatments were applied in late evening and dew was the only source of free moisture for BCA plant colonization as described above; treatments were repeated every 7-10 d until end of August or early September following grower practice for fungicide applications.

In order to examine roots for endophytic presence of BCA when root drenching with BCA was used, root clearing was done in 10% potassium hydroxide (KOH), acidified with 20% HCl, and stained in 0.1% toluidine blue as described by **Phillip and Hayman, (1970)** with slight modifications. The cleared roots were examined under a compound microscope; the presence of bacterial cells inside root tissues was assessed at 400X-1000X magnifications. Root colonization with biological control agents was also evaluated on plants generated from BCA inoculated seed in which seed coating with BCA was done on emerged radicles soon after germinating. In this study, surface sterilized seed were first vernalized to break seed dormancy at 37ºC as described in **Evans and Blazich (1999)**. When vernalized

seed started germinating, they were drenched with BCAs (isolates B17A and B17B) using a bacterial suspensions of 5.0 x 10^9 bacterial cells ml^{-1} and planted in sterile soil in 15 cm x 20 cm containers; the non-treated control was drenched with sterile water. A replication of four containers with three plants in each container was used for each treatment and arranged in a randomized complete block experimental design. At the end of each study, roots were harvested and cleared for microscopic observations as described above.

2.2. Effect of B17A and B17B bacterial suspensions and bacterial filtrates on spore germination

Bacterial isolates B17A and B17B grown in nutrient agar for 24 hours were used to make suspensions of 5.0 x 10^9 bacterial cells ml^{-1} in sterile water and the bacterial suspension was sprayed onto the nutrient agar plates to form a thin film; sterile water was used as a negative control. The plates were incubated for approx. 12 hours then powdery mildew conidiospores were dusted onto the treated media, with spores being in direct contact with the bacteria. Germination of the conidiospores was monitored over time using a compound microscope (Leitz Diavert inverted microscope, Germany), at 200X and 320X magnification; germination was determined by any bulge or protrusions beyond the spore walls that either formed elongated or short germ tubes (**Pal and Gardener, 2006**). Spores that germinated were counted as a percentage all spores observed per field view at 200X magnification.

In addition, detached leaves were surface sterilized with 10% Clorox bleach (0.6% sodium hypochlorite) and placed on two layers of wet paper towels in an incubation chamber, two leaves per container. The leaves were brush-inoculated with powdery mildew spores and allowed to develop colonies of 2.5 - 3.5 cm diam. with fluffy mycelia visible without magnification. The leaves were then sprayed to runoff using bacterial suspensions or filtrates of the B17A and B17B with a replication of four containers and two leaves per container for each treatment. Experimental design used a randomized

147 complete block design and sterile water was used as non-treated control. Bacterial filtrates were
148 prepared by growing isolates B17A and B17B in nutrient broth incubated on a shaker for 48 hours at 37
149 °C. The cultures were then centrifuged and filtered through 0.20 µm filters to obtain cell-free
150 supernatant filtrates. The filtrate was observed under a compound microscope at 400X-1000X
151 magnifications to confirm that they did not contain any bacterial cells before the filtrate was used to
152 spray leaves to run off.

153 ***2.3. Data analysis***

154 Disease severity and spore germination data analyses were performed using general linear
155 models procedure of SAS (SAS/STAT 1990). Multiple comparisons between pairs of means were
156 performed using a series of t-tests according to SAS procedures in PROC. ANOVA (**Gomez and**
157 **Gomez 1984, SAS/STAT 1990**). The least significant differences (LSD) were calculated according to
158 Fisher's protected LSD test at $P \leq 0.05$.

159

160 **3. RESULTS**

161 ***3.1 Efficacy of bacterial isolates in powdery mildew disease control***

162 All five bacterial isolates that were retrieved from storage suppressed powdery mildew severity
163 compared to non-treated control. Isolate B017, had superior efficacy and was statistically similar to
164 fungicide control **(Fig. 1)**. In addition to controlling powdery mildew, plants treated with isolate B017
165 grew larger than those treated with other bacteria or with conventional fungicide thiophanate methyl
166 (data not shown).

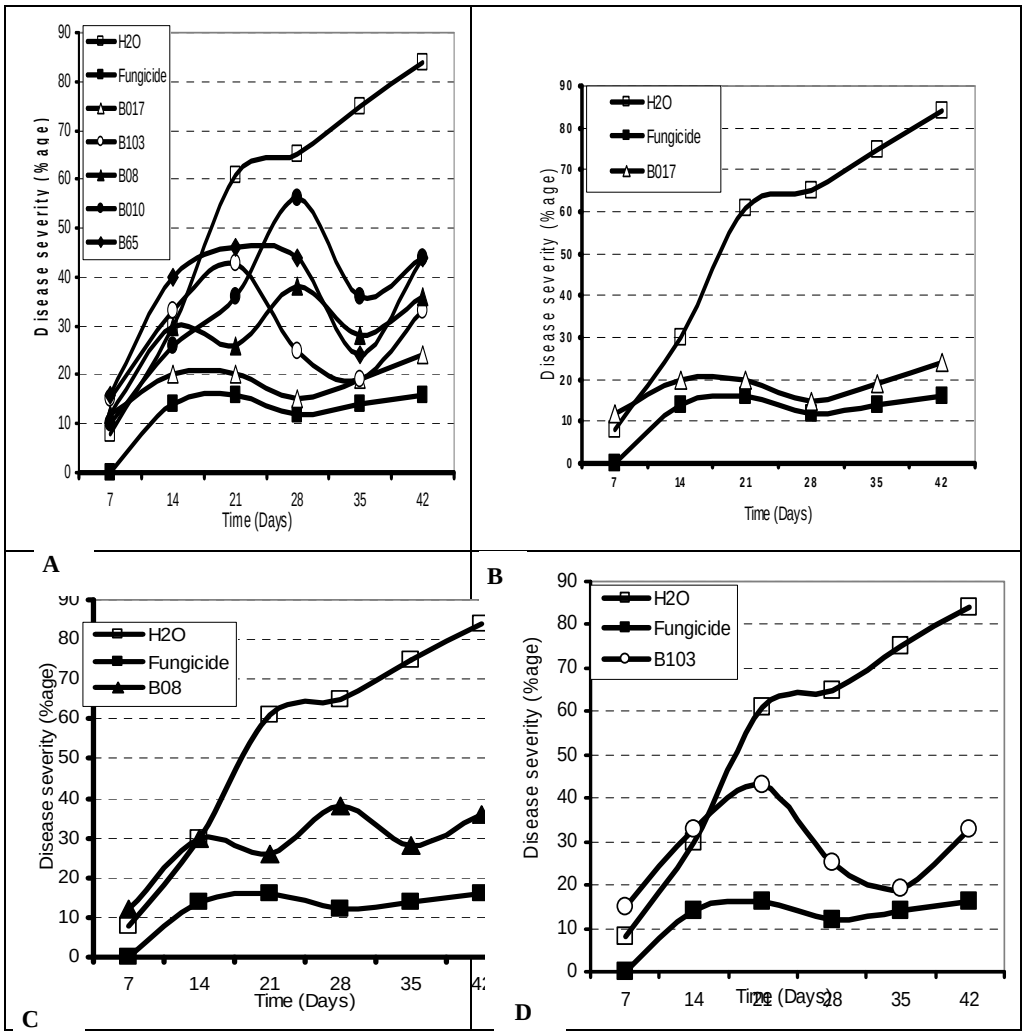

Fig. 1(A-D): Efficacy of five bacterial isolates on powdery mildew disease severity on dogwood seedlings as compared to conventional fungicide (-■-) and non-treated control (-□-); (A) All five bacterial isolates tested, and B-D individual isolates compared to conventional fungicide thiophenate methyl, and water control; (B) B017- (-Δ-); (C) B08- (-▲-); and (D) B103- (-O-).

3.1.2. Evaluation of two isolates B17A and B17B as BCA for powdery mildew control.

A comparison of B017 (B17A) and another isolate B17B showed similar efficacy in suppressing powdery mildew disease (**Fig. 2**). At the end of the study, both susceptible and moderately resistant plants treated with BCA isolates had similar disease severity and had significantly lower disease than the non-treated control at $p<0.0001$ (**Fig. 2**). The efficacy of the two BCA isolates in controlling powdery

178 mildew was statistically similar to conventional fungicide on both susceptible and moderately resistant
179 selections **(Fig. 2)**.
180
181 ***3.1.3. Efficacy of biocontrol agents applied by foliage spray and by root drenching.***
182 All plants inoculated with BCAs by either foliage sprays or root drenching developed
183 significantly less disease than the non-treated controls in both shadehouse and greenhouse experiments
184 (**Fig. 3**). However, BCA application by foliage sprays was slightly more effective than root drenching
185 (**Fig. 3**).
186

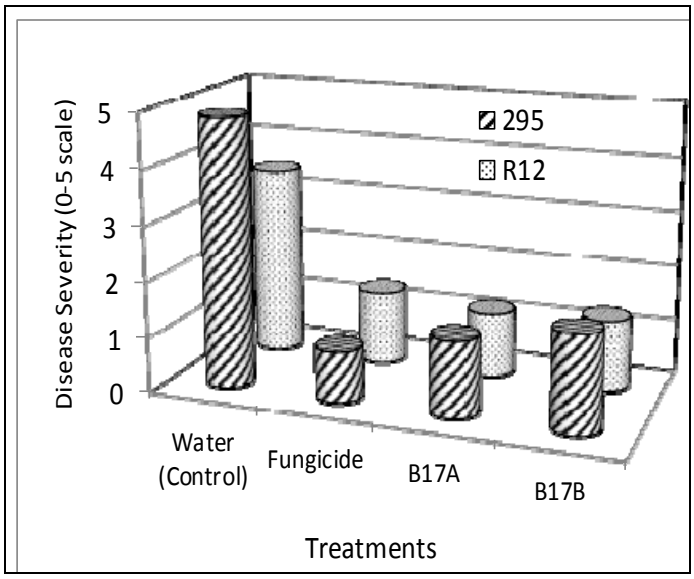

Fig. 2. Effect of two biological control agents (BCA B17A and B17B) on powdery mildew disease severity on susceptible (S) and moderately resistsant (MR) plants as shown in S-(295) and R (R12 compared to conventional fungicide thiophenate methyl and water-treated (control) by foliar sprays in greenhouse environment; disease severity was rated on a scale of 1-5 in which 1 = 1-10%, 2 = 11-25%, 3 = 26-50%, 4 = 51-75% and 5 = 75-100% of foliage covered with powdery mildew symptoms. Similar results were obtained from shadehouse environment.

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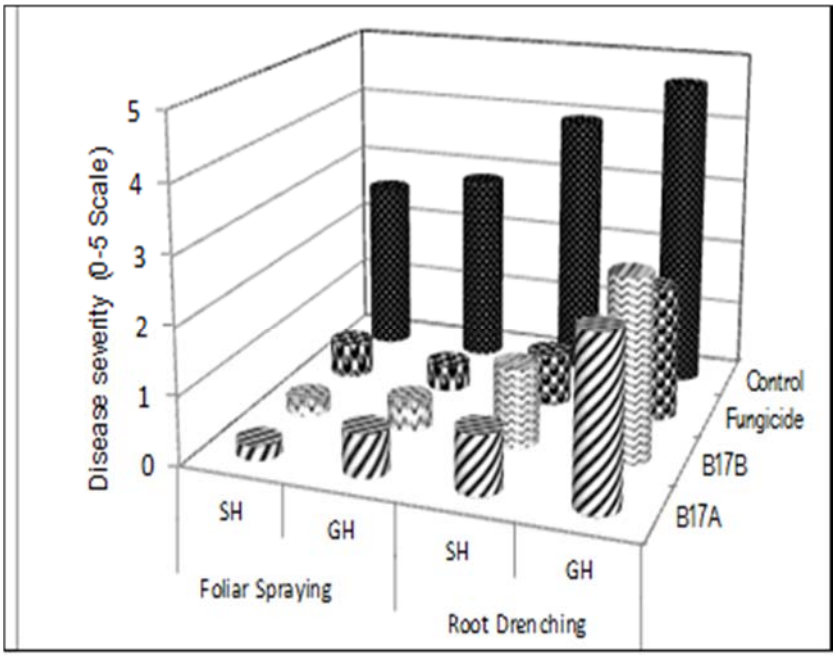

Fig. 3. Effect of two biological control agents (B17A and B17B) on powdery mildew disease severity on susceptible dogwood seedlings maintained in greenhouse (GH) and shadehouse (SH) environments where foliar sprays and root drenching method of application were compared with conventional fungicide thiophenate methyl and water-treated (control); disease severity was rated on a scale of 1-5 in which 1 = 1-10%, 2 = 11-25%, 3 = 26-50%, 4 = 51-75% and 5 = 75-100% of foliage covered with powdery mildew symptoms. Similar results were obtained in repeated experiments

Roots that had been treated with BCA bacterial suspensions showed the presence of B17A and B17B bacterial cells inside root parenchyma cells and in some xylem tissues, indicating endophytic colonization of the roots (**Fig. 4)**. Colonization of stem or leaves internal tissues was not examined.

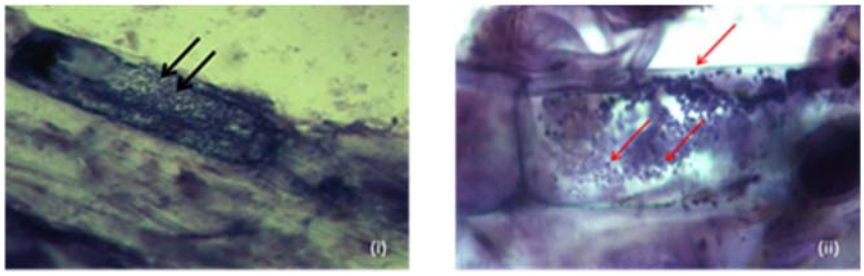

Fig. 4. Microscopic observation of Cleared dogwood roots that had been treated with two bacterial biocontrol agents (BCA), isolates B17A and B17B by root drenching showing bacterial cells inside parenchyma cells (arrows ()indicating endophytic colonization of the roots by the BCA. The roots were cleared in 10% potassium hydroxide (KOH), 1% hydrochloric acid (HCl) and counterstained in 1% toluidine blue-O.

3.2. Effect of B17A and B17B bacterial suspensions and bacterial filtrates on spore germination

Evaluation of conidiospore germination on growth media treated with B17A and B17B showed fewer spores germinated compared to the non-treated control; the BCA treated plates had significantly lower germination percentage than the water-treated controls at $p<0.0001$ when the spores had a direct

contact with the BCA and when the BCA and powdery mildew spores were in separate sections of divided plates with no physical contact between them (**Fig. 5).**

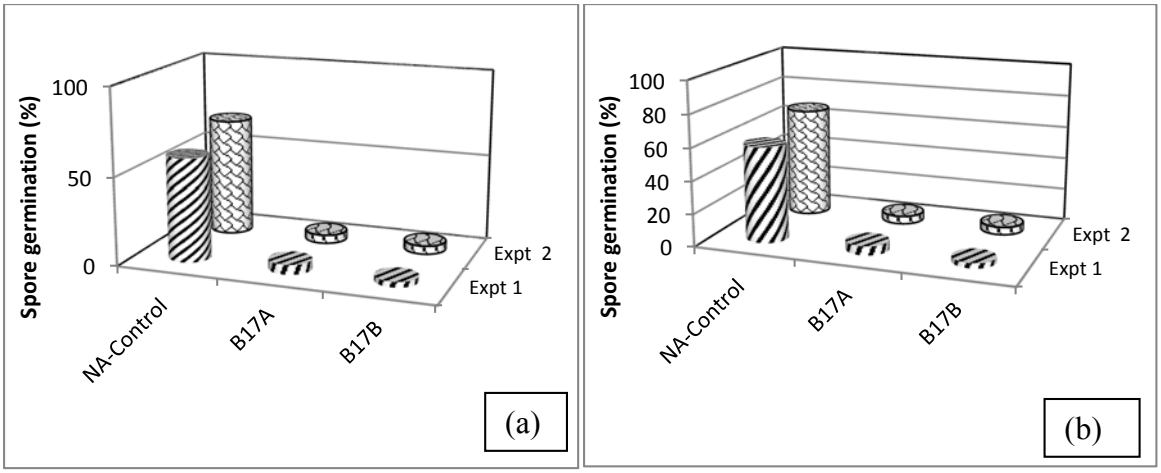

Fig. 5. Effect of biological control agents (BCAs) isolates B17A and B17B on powdery mildew conidiospores germination on nutrient agar (NA): (a) when powdery mildew spores were added on the NA media 12 hours after treatment with BCA and water treated control were in separate plate and (b) when BCA treatment and powdery mildew spores were in separate sections of divided plates with no physical contact between BCA and powdery mildew spores; water was used as (control). The experiment was repeated ones.

Some spores exposed to BCA formed germ tubes that did not continue to develop after the initial bulge stage (**Fig. 6**). At 36 hours post-inoculation, bacterial cells were observed inside powdery mildew conidiospores and some spores appeared to be lysed and bacterial cells were flowing out of the conidiospores (**Fig. 6**). Powdery mildew colonies that were sprayed with BCA suspensions and BCA filtrates with no bacterial cells stopped expanding and collapsed; spores collected from treated colonies showed lower germination percentage and had fewer germ tubes than the non-treated controls. Some spores formed germ tube initials, but the germ tubes did not elongate compared to the non-treated control; some spore lyses were also observed on BCA filtrate treatments, but not in the non-treated water control

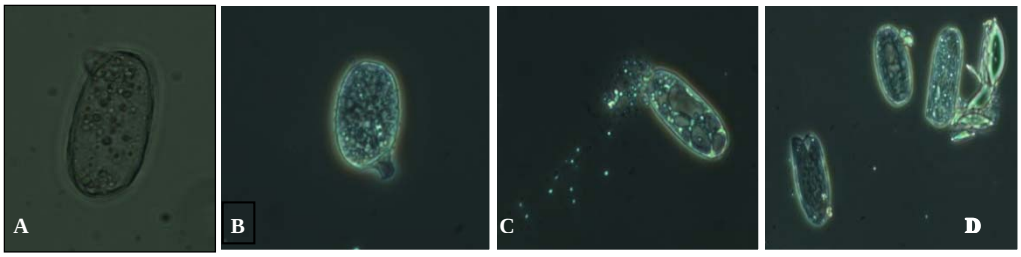

Fig. 6: Colonization of powdery mildew spores by bacterial biological control agents (A) The conidia germination showing a gem tube (Ca. 8 h after bacteria inoculation). (B) Gem tube after 15 hours and (C-D) after 36 hours showing ruptured/lysed spores.

4. DISCUSSIONS

Numerous microorganisms are naturally present on leaves of dogwoods and the majority of these are saprophytic (**Mmbaga et al., 2008; Mmbaga and Sauvé, 2009**). All five isolates tested for biocontrol activity reduced powdery mildew disease severity compared to the non-treated control (**Figs. 1-2**), but two isolates (B17A and B17B) displayed efficacy similar to that of conventional fungicides and demonstrated high potential as biological control agents (BCA). In the initial experiments, post-inoculation incubation under 100% humidity chambers was used to facilitate BCA-plant colonizations; this process facilitated the identification of isolates that had best potential as biocontrol agents. However, after this initial selection, moisture from natural dew formation was used to enhance BCA-plant colonization. It is reasonable to presume that these natural conditions were favorable to plant colonization with BCAs because the treatments were effective in suppressing powdery mildew. Results showed that the two BCAs, B17A and B17B, may provide effective alternatives to conventional fungicides in controlling powdery mildew in container-grown dogwoods in shadehouse environment **(Figs. 2-3)**.

Weekly applications of isolates B17A and B17B were as effective as a conventional fungicide thiophanate methyl, but neither the fungicide nor BCAs eliminated or stopped the disease completely. The two isolates B17A and B17B were highly effective when they were allowed to colonize plants before the pathogen was introduced and when BCA application was initiated soon after initial disease

symptoms were observed and natural colonization was used without additional moisture. These results show that the two bacterial isolates B17A and B17B offer potential microbial alternatives to conventional fungicides in powdery mildew managment. Weekly applications of the BCAs were used to maintain high bacteria populations on leaf surfaces following reports from other studies (**Lindow et. al., 2002).**

Previous studies have shown that seedling populations are more susceptible to powdery mildew than adult plants (**Li et al., 2010**); thus seedling populations make excellent test plants for studies on powdery mildew control. The two isolates were highly effective in controlling powdery mildew in seedling populations and it is reasonable to presume that they would be highly effective on adult plants. Seedling plants used in this study are also expected to have high genetic variability including variation in powdery mildew susceptibility because dogwoods are self-sterile and obligately out-crossing (open pollinated) (**Reed 1999),** thus the BCA isolates are likely to be effective on diverse populations. Although host resistance is the best method for controlling powdery mildew, none of the commercial cultivars have high resistance. Consequethly, when disease pressure is high, growers often use fungicide applications. Results from this study show that biological-based integrated disease management to control powdery mildew in nursery production of flowering dogwoods can reduce the need for conventional fungicides. Our results showed that moderately resistant plants sustained less disease severity than susceptible plants when powdery mildew was not controlled (**Fig. 2**), but both B17A and B17B BCAs were as effective on susceptible and moderately resistant plants (**Figs. 2-3**). The two BCA isolates were statistically similar to conventional fungicide in controlling powdery mildew on both susceptible and resistant selections **(Fig. 3)**.

Foliage and root inoculation methods for BCA application suppressed powdery mildew and reduced disease severity significantly compared to the non-treated control (**Fig. 3**), and the difference

between foliar sprays and root drenching methods of BCA application was not statistically significant. It is possible that root inoculation with the two BCAs suppressed powdery mildew by Induced systemic resistance (ISR), which may also include resistance to other fungal pathogens including pathogenic soil microbes (**Haas and Defago, 2005**). A slight advantage of foliage sprays over root inoculations was observed and this may be due to the direct effect of the bacterial cells on spores and on spore germination as indicated by the spore lysis, reduced spore germination, and disruption of germ tube walls (**Figs. 5-6**). Perhaps the combination of the two methods of application may be advantageous and possibly improve efficacy by providing early application in the form of seed treatment followed by foliar sprays; further study is needed to confirm the concept.

The effect of the two bacterial BCAs filtrates in sdisrupting spore germination suggests that the BCA may produce deleterious compounds or secondary metabolites as reported in other fungal disease control mechanisms from bacterial BCAs (**Zhou et al., 2007**). The effect of BCA isolates B17A and B17B on spore germination including destruction of germ tube formations suggests that antibiosis may be one of the mechanisms of action. Chemical compounds produced by the two BCA isolates have not been reported in this article, but compounds that caused lyses of spore membranes and germ tube walls may include various catabolic enzymes and some volatile compounds (**Schulz, et al., 2010)**. In addition, the BCA isolates displayed plant growth promoting properties that may be associated with mechanisms reported for other growth promoting BCAs (**Haas and Defago, 2005; Singh et al., 2003**). The mechanism of action for plant-growth promoting bacteria is not understood, but theories include induced systemic resistance in the host plant (**Haas and Defago, 2005).**

Although the two BCAs isolates were isolated from leaf epiphytes, they colonized roots and displayed endophytic colonization of treated plants. Root drenching with the BCA suppressed powdery mildew severity and promoted plant growth. The colonization process of most bacterial endophytes

291 remains largely unknown. It is reasonable to presume that soil may be their natural habitat and the
292 starting point for plant colonization and endophytic migration to the foliage, but studies on their
293 presence in the rhizosphere are needed to further explore potential for commercial application of the
294 selected BCAs **(McSpadden and Fravel, 2002**). The presence of bacterial cells inside parenchyma
295 cells and some vascular tissues of cleared roots suggested a potential avenue for introducing the BCAs
296 into dogwood plants. **Kilic and Yuen (2000), Hoffland, et al. (1995), Leeman, et al. (1995; 1996) and**
297 **Van Loon, et al. (1998)** proposed that bacterial endophytes trigger induced systemic resistance (ISR) , it
298 is likely that the two bacterial isolates are associated with ISR, but studies are required to confirm that
299 and determine other mechanisms of action.
300
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399 **Fig. 1**(A-D): Efficacy of five bacterial isolates on powdery mildew disease severity on dogwood
400 seedlings as compared to conventional fungicide (-■-) and non-treated control (-□-); (A). All five

401 bacterial isolates tested, and B-D individual isolates compared to conventional fungicide thiophenate
402 methyl, and water control; (B) B017- (-Δ-); (C) B08- (-▲-); and (D) B103- (-O-).
403 **Fig. 2.** Effect of two biological control agents (BCA B17A and B17B) on powdery mildew disease
404 severity on susceptible (S) and moderately resistsant (MR) plants as shown in S-(295) and R (R12
405 compared to conventional fungicide thiophenate methyl and water-treated (control) by foliar sprays in
406 greenhouse environment; disease severity was rated on a scale of 1-5 in which 1 = 1-10%, 2 = 11-25%,
407 3 = 26-50%, 4 = 51-75% and 5 = 75-100% of foliage covered with powdery mildew symptoms. Similar
408 results were obtained from shadehouse environment.
409 **Fig. 3.** Effect of two biological control agents (B17A and B17B) on powdery mildew disease severity on
410 susceptible dogwood seedlings maintained in greenhouse (GH) and shadehouse (SH) environments
411 where foliar sprays and root drenching method of application were compared with conventional
412 fungicide thiophenate methyl and water-treated (control); disease severity was rated on a scale of 1-5 in
413 which 1 = 1-10%, 2 = 11-25%, 3 = 26-50%, 4 = 51-75% and 5 = 75-100% of foliage covered with
414 powdery mildew symptoms. Similar results were obtained in repeated experiments
415 **Fig. 4.** Microscopic observation of Cleared dogwood roots that had been treated with two bacterial
416 biocontrol agents (BCA), isolates B17A and B17B by root drenching showing bacterial cells inside
417 parenchyma cells (arrows ()indicating endophytic colonization of the roots by the BCA. The roots were
418 cleared in 10% potassium hydroxide (KOH), 1% hydrochloric acid (HCl) and counterstained in 1%
419 toluidine blue-O.
420
421 **Fig. 5.** Effect of biological control agents (BCAs) isolates B17A and B17B on powdery mildew
422 conidiospores germination on nutrient agar (NA): (a) when powdery mildew spores were added on the
423 NA media 12 hours after treatment with BCA and water treated control were in separate plate and (b)

when BCA treatment and powdery mildew spores were in separate sections of divided plates with no physical contact between BCA and powdery mildew spores; water was used as (control). The experiment was repeated ones.

Fig. 6: Colonization of powdery mildew spores by bacterial biological control agents (A) The conidia germination showing a gem tube (Ca. 8 h after bacteria inoculation). (B) Gem tube after 15 hours and (C-D) after 36 hours showing ruptured/lysed spores.

Effect of bacteria isolates in Powdery mildew control in flowering dogwoods (*Cornus florida* L.)

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Graphic Abstract