Plant Disease • 2017 • 101:165-169 • http://dx.doi.org/10.1094/PDIS-06-16-0801-RE

Integrated Management for the Reduction of *Calonectria* Infections in Ornamental Nurseries

Alessandro Cinquerrui, Giancarlo Polizzi, Dalia Aiello, and **Alessandro Vitale,** Dipartimento di Agricoltura, Alimentazione e Ambiente, University of Catania, Via S. Sofia 100, 95123 Catania, Italy

Abstract

Chemical control represents the main effective strategy for managing Calonectria diseases in ornamental nurseries. The occurrence of fungicide-resistant strains and the European Directive on "Sustainable Use of Pesticides" has forced ornamental plant growers to establish effective integrated pest management strategies to control *Calonectria* infections. Here, three nursery experiments were performed to detect the best combinations of fungicides and biological control agents (BCA) to control both leaf spot, caused by six *Calonectria* spp. on bottlebrush and metrosideros, and stem rot, caused by *Calonectria morganii* on *Dodonaea* plants. Overall, the cyprodinil + fludioxonil mixture alone or combined with bioformulates containing *Bacillus*, *Trichoderma*, and *Streptomyces* spp. provided the best performance in reducing leaf spot and stem rot caused by *Calonectria* spp., followed by the mixture of boscalid + pyraclostrobin. Although BCA alone provided disease suppression significantly lower than the controls in most cases, these treatments were, on average, the least effective in controlling *Calonectria* infections. Otherwise, there were no significant increases in efficacy with fungicides plus BCA over fungicides alone. Thus, the application of boscalid + pyraclostrobin and cyprodinil + fludioxonil mixtures may also be used in large-scale applications to reduce Calonectria diseases because they effectively managed leaf and stem infections. Our comprehensive research applied previously acquired information on Calonectria disease management in nurseries, resulting in important data that affects integrated plans to fight these pathogens in accordance with European legislation.

In the last decade, the ornamental industry has increased its production levels worldwide (Daughtrey and Benson 2005). Because of climatic conditions, the Mediterranean basin plays an important role in the production of ornamental plants in Europe. However, producing healthy and marketable plants can be difficult, and unfavorable agronomic and phytosanitary conditions can limit plant production under nursery, field, and greenhouse conditions.

Of the phytopathological issues, diseases caused by *Calonectria* spp. such as *Calonectria pauciramosa*, *C. morganii*, *C. polizzii*, *C. mexicana*, *C. pseudomexicana*, and *C. tunisiana* are the most widespread in the Mediterranean basin, affecting many economically important crops. These plant pathogens are responsible for several different symptoms, including crown and root rot, stem rot, leaf spotting, and shoot blight, in ornamental plants (Crous 2002; Henricot and Beales 2003; Lombard et al. 2011; Pérez-Sierra et al. 2007; Polizzi and Catara 2001; Polizzi and Crous 1999; Polizzi et al. 2006, 2009; Vitale and Polizzi 2008; Vitale et al. 2013b). Consequently, adequate control strategies focused on chemical and physical measures are needed to manage *Calonectria* infections in nurseries (Vitale et al. 2013a,b). The application of fungicides is the most used approach to reduce economic losses, and many fungicides, belonging to different chemical classes, have been used to control Calonectria diseases (Aiello et al. 2013). Preventive applications of methyl benzimidazole carbamates (MBC: thiophanate-methyl) and demethylation inhibitors (DMI: prochloraz) reduce *Calonectria* infections (Aiello et al. 2013; Barnard 1984; Bertus 1976; Chase 1987; Crous 2002; Engelhard 1971; Horst and Hoitink 1968; Roos 1980, 1981).

Recently, thiophanate-methyl, fludioxonil, pyraclostrobin, trifloxystrobin, kresoxim-methyl, mancozeb, and chlorothalonil were demonstrated to reduce mycelial growth and conidial germination of *C. pseudonaviculata* under in vitro conditions (LaMondia 2014).

Although the application of fungicides such as MBC and DMI is effective in controlling Calonectria diseases, their prolonged use has led to the selection of fungicide-resistant strains (Alfenas et al. 1988; Guarnaccia et al. 2014; Vitale et al. 2009). According to the latest European Directive 2009/128/EC on the "Sustainable Use of Pesticides", modern agriculture is currently focused on biological control agent (BCA) use and on reducing fungicide applications. Environmentally friendly approaches to controlling plant diseases are being legislated. After January 2014, each member country of the European Economic Community was encouraged to adopt an integrated pest management (IPM) strategy by establishing a National Action Plan (Hillocks 2012). Directive 91/414/EEC stated that "the use of plant protection products is limited to the strict minimum necessary to maintain the pest population below the economic damage threshold", which does not mean that pesticides cannot be used but that they should be used in combination with alternative measures that reduce the required amounts of chemical compounds (Hillocks 2012). This concept is emphasized by the European Crop Protection Association, which confirms that the use of pesticides in an IPM program is not facultative because their use should ensure that the IPM programs are more economically and medically beneficial than just chemical or biological treatments (Hillocks 2012). Establishing sanitation and monitoring measures is essential to prevent plant disease development and, consequently, to obtain a satisfactory production level under a qualitative profile. However, it is equally significant to determine integrated control measures for the management of diseases found in greenhouses and nurseries (Daughtrey and Benson 2005).

Thus, this article aimed to evaluate combinations of BCA and fungicides to manage *Calonectria* infections on important ornamental hosts, thereby reducing the economic losses in ornamental nurseries in the Mediterranean basin.

Materials and Methods

Fungal isolates. Six *Calonectria* spp. were used in this study: *C. mexicana* (CBS130353 from *Dodonaea viscosa*), *C. morganii* (CBS120930 from *Callistemon* hybrid 'Rose Opal'), *Calonectria pauciramosa* (CBS130333 from *Callistemon citrinus* (Curtis) Skeels), *Calonectria polizzii* (CBS130351 from *Myrtus communis*), *C. pseudomexicana* (CBS 130354 from *Callistemon* sp.), and *C. tunisiana* (CBS130357 from *Callistemon laevis* An.) (Crous 2002; Lombard et al. 2010, 2011; Polizzi and Crous 1999).

A fresh culture of each isolate was obtained by transferring agar plugs from stock cultures onto potato dextrose agar (PDA; Oxoid, Basingstoke, UK) plates. Subsequently, 5-mm mycelial plugs, taken

Corresponding author: A. Vitale; E-mail: alevital@unict.it

Accepted for publication 29 August 2016.

from the edge of a 5-day-old colony grown on PDA, were placed onto synthetic low-nutrient agar dishes and incubated at 25°C for 21 days. A conidial suspension was prepared by adding sterile distilled water to the dishes, gently rubbing the colony surface with a sterile loop, and then filtering the solution through three layers of cheesecloth. A final spore suspension of approximately 2.5×10^5 conidia ml^{-1} was determined using a hemocytometer.

BCA and fungicides. Three experiments were performed in three greenhouses, at the geographical coordinates 37°41′50.54″ N, 15°11′37.45″ E; 37°41′50.01 N, 15°11′37.47″ E; and 37°41′55.80″, N 15°11′38.61″ E, respectively, within a nursery located in Carruba, Riposto, Catania Province, Italy. In experiments I and III, the efficacies of BCA and fungicides, applied alone or in combinations, in controlling leaf spots caused by different *Calonectria* spp. on *Callistemon* spp. and *Metrosideros* spp. were assayed, while, in experiment II, the abilities of these treatments to control stem rot caused by *Calonectria morganii* on *D. viscosa* were tested.

Experiment I. Efficacy of chemical and biological treatments in controlling Calonectria leaf spot caused by *C. morganii* on *Callistemon viminalis*. In this experiment, the capabilities of four commercial fungicides and seven microbiological formulates to control leaf spots caused by inoculations of *Calonectria morganii* on *Callistemon viminalis* were tested (Table 1). Each treatment was replicated three times in a randomized complete block design (RCBD) with 32 to 42 bottlebrush cuttings. The same number of untreated inoculated bottlebrush cuttings served as positive controls. In this experiment, BCA and fungicides were applied 24 and 3 h, respectively, before pathogen inoculation. Each treatment was performed by spraying approximately 30 ml of suspension for each replicate to run-off using a hand pump. The *C. morganii* inoculum of approximately 30 ml of conidial suspension (approximately 2.5×10^5 CFU) for each replicate was sprayed on the leaf surface of the bottlebrush using a hand-pump. After inoculation, bottlebrush cuttings were covered with plastic tunnels for 3 days and then maintained in a greenhouse at a temperature of approximately 25°C. The capabilities of the fungicides and BCA to control leaf spot symptoms were evaluated 10 days after inoculation by calculating the disease incidence of infected plants (DI_P), disease incidence of infected leaves (DI_L), and symptom severity (SS). Both DI values were calculated as percentages of cuttings or leaves showing symptoms out of the total number of plants or leaves examined × 100. However, the SS value was determined by using an empirical 1-to-5 rating scale based on the percentage of the foliar surface infected, as follows: 1 = healthy cuttings and 2 = 1 to 5, 3 = 6 to 25, 4 = 26 to 50, and 5 = more than 50% of leaf area infected. Both the average DI_L and SS values were based on 10 leaves/cutting. SS values were converted to mean disease ratings calculated as Σ (the number of diseased leaves for each single class × the disease class)/number of leaves scored. This experiment was repeated twice.

Experiment II. Efficacies of chemical, biological and integrated treatments in controlling *C. morganii* on *D. viscosa*. Different treatments consisting of chemical, biological, or combined control means were tested in this nursery experiment for their capabilities to inhibit Calonectria stem rot caused by *C. morganii* on *D. viscosa*. The scheduled spray program included 17 different treatments consisting of both tested active ingredients, which had already been reported as effective against *Calonectria* infections in nurseries (Aiello et al. 2013; Henricot and Wedgwood 2013; Henricot et al. 2008; LaMondia 2015; Vitale et al. 2012), and untested active ingredients. Thus, six commercial fungicides (including one containing the active ingredient etridiazole, active against oomycetes and also *Fusarium* spp.) and five microbiological formulates were used alone or in combination following the application rates recommended by their manufacturers (Table 1).

Treatments were arranged in an RCBD with three replications, each containing 28 *D. viscosa* seedlings. The same number of untreated inoculated seedlings served as positive controls. This experiment was performed twice.

For each trial, BCA were applied both 21 and 3 days before pathogen inoculation, whereas fungicides were applied 1 day before pathogen inoculation. The crown area of each *D. viscosa* seedling was inoculated with approximately 0.5 ml of conidial suspension (approximately 2.5×10^5 CFU) of *C. morganii* as a soil drench. Then, inoculated young seedlings were covered for 3 days under plastic tunnels and maintained at 25 ± 2°C. Biological and chemical treatments were repeated 6 and 8 days after inoculation, respectively. The application mode for the commercial formulations was the same as in the previous experiment.

Twenty-one days after pathogen inoculation, DI and SS were recorded. The former was calculated as the percentages of the number of symptomatic plants out of the total number of plants examined × 100. The SS value was calculated by measuring the length (in centimeters) of necrotic stem tissue.

Experiment III. Fungicide and BCA efficacies in controlling Calonectria leaf spot on *Metrosideros excelsa* 'Aurea' and *Callistemon* 'Captain Cook' in a nursery. The efficacies of three fungicides and three microbiological formulations, applied alone or in combinations, were tested against five species of the genus *Calonectria* (*C. pauciramosa*, *C. polizzii*, *C. pseudomexicana*, *C. tunisiana*, and *C. mexicana*) on both *Metrosideros excelsa* 'Aurea' and *Callistemon* 'Captain Cook'. The fungicides boscalid + pyraclostrobin (Signum), cyprodinil + fludioxonil (Switch), and fosetyl-Al (Aliette), and the microbiological formulates *Bacillus amyloliquefaciens* subsp. *plantarum* D747 (Amylo-X), *B. subtilis* QST713 (Serenade

Table 1. Fungicides and biological control agents employed in the experiments (Exp)

Active ingredient	Trade name	Manufacturer	Rates (g or ml/100 liters)	Formulation (%)[z]	Exp
Bacillus amyloliquefaciens subsp. *plantarum* D747	Amilo-X	Biogard	250	25 WG	I, II, III
B. subtilis QST713	Serenade Max	Bayer Crop Science	400	15.67 WP	I, II, III
Boscalid + pyraclostrobin	Signum	Basf Italia	100	26.7+6.7 WG	I, II, III
Cyprodinil + fludioxonil	Switch	Syngenta Crop Protection	80	37.5+25 WG	II, III
Etridiazole	Terrazole	Certis Europe	400	25 EC	II
Fludioxonil	Geoxe	Syngenta Crop Protection	50	50 WG	II
Fluopyram	Luna Privilege	Bayer Crop Science	50	41.66 SC	I
Fosetyl-Al	Aliette	Bayer Crop Science	300	80 WG	I, II, III
Propamocarb + fosetyl-Al	Previcur Energy	Bayer Crop Science	250	47.2+27.6 SL	I
Streptomyces griseoviridis K61	Mycostop	Bioplanet	25	33.33 WP	I, II
Thiophanate-methyl	Ranger Gold	Diachem	100	38.3 SC	II
Trichoderma asperellum TV1	Xedavir	Xeda Italia	400	2.8 WP	I
T. atroviride T11 + *T. asperellum* T25	Tusal	Certis Europe	250	0.5+0.5 WG	I, II, III
T. harzianum ICC012 + *T. viride* ICC080	Radix	Certis Europe	250	2 + 2 WP	I
T. harzianum T22	Trianum-P	Koppert Italia	100	1.15 WP	I, II

[z] Percentage of active ingredient. WG = water-dispersible granule, WP = wettable powder, SL = soluble concentration, SC = suspended concentration, and EC = emulsifiable concentration.

Max), and *Trichoderma atroviride* T11 + *T. asperellum* T25 (Tusal) were employed as commercial products at the recommended application rates (Table 1).

Each treatment was replicated three times in an RCBD using 28 metrosideros and 3 bottlebrush cuttings per replicate. The same number of untreated inoculated cuttings served as positive controls.

The application modes and times for BCA and fungicides were as in experiment I. Each *Calonectria* sp. inoculum consisted of a spray volume of approximately 25 ml for metrosideros and approximately 10 ml for bottlebrush of a conidial suspension (approximately 2.5×10^5 CFU) for each replicate. After inoculation, plants were maintained under plastic tunnels at 25 ± 2°C and approximately 70% relative humidity for 3 days.

Disease evaluation was performed as in experiment I, including adopting the same scale for SS. However, SS values were averaged using 5 and 15 leaves/plant for metrosideros and bottlebrush, respectively, and only reported to compare the fungicide efficacies across pathogens.

Statistical analysis. All of the data obtained from the in vivo experiments were subjected to an analysis of variance using the statistical package Statsoft (version 10; Statsoft Inc., Tulsa, OK) according to a parametric approach, with the factorial treatment structure and interactions with treatments arranged in an RCBD with three replicates. Because a significant effect was detected in each experiment for all of the interactions from the repeated trials, they were analyzed separately. All of the DI percentage data were transformed using arcsine ($\sin^{-1} \times$ square root x) prior to statistical analysis.

In the posthoc analyses, the mean values of DI and SS of *Calonectria* sp. infections were subsequently separated by the Newman-Keuls test ($\alpha = 0.05$).

Results

Experiment I. Fungicide and BCA efficacies in controlling *C. morganii* on *C. viminalis* in the nursery. In all of the nursery trials, some of the treatments had significant effects ($P < 0.001$) on *Calonectria* infections. Because significant treatment–trial interactions were also detected for the mean DI_P, DI_L, and SS values, individual trial results are presented in Table 2. The disease pressure was very high in the first and third trials, whereas a lower disease level was recorded in the second trial.

In all of the trials, boscalid + pyraclostrobin, fluopyram, and *B. subtilis* QST713 were the most effective treatments in controlling Calonectria leaf spot on *C. viminalis*. Under high disease pressure, these treatments showed the best performances, being significantly different than most of the other treatments. Under low disease levels, boscalid + pyraclostrobin, fluopyram, and *B. subtilis* QST713 were the only treatments that were significantly different from their relative controls and most of the remaining treatments.

The least effective treatments were *T. atroviride* T11 + *T. asperellum* T25, *T. harzianum* ICC012 + *T. viride* ICC080, *T. asperellum* TV1, and *T. harzianum* T22, and *Streptomyces griseoviridis* K61 (Table 2).

Experiment II. Efficacies of chemical, biological, and integrated treatments in controlling *C. morganii* on *D. viscosa*. Because the treatment–trial interactions were significant ($P = 0.0023$), data for each trial are shown separately in Table 3. In both trials, some treatments had significant effects on reducing stem rot infections caused by *C. morganii,* and their performances were evaluated based on the simultaneous analyses of the disease variables. In the two nursery trials, the DI values of the untreated infected plants ranged from 53.6 to 81%, while the SS values ranged from 0.81 to 1.26 cm. All of the fungicides and BCA, applied individually or in combination, had significant control effects on the stem rot of *D. viscosa* caused by *C. morganii*.

In the first trial, the cyprodinil + fludioxonil fungicide mixture applied alone or in combination with applications of bioformulation containing *Trichoderma* spp. and *Streptomyces* strains, showed the best performances in controlling both the DI and the SS of stem rot, with values of 2.4 to 6% and 0.02 to 0.06 cm, respectively. A reduction in disease was also observed for the remaining combinations of the above fungicide mixture with the remaining BCA and for thiophanate-methyl. *T. harzianum* T22, followed by etridiazole, were the least effective treatments in reducing infections caused by *C. morganii*, with values of 36.9 and 39.3% for DI, respectively, and 0.37 and 0.60 cm for SS, respectively (Table 3). An intermediate efficacy in reducing *C. morganii* infections on *Dodonaea* seedlings was detected for the remaining fungicide and BCA applications. The results of the second trial confirmed those obtained in the first, with very slight differences (Table 3). The cyprodinil + fludioxonil fungicide mixture applied alone or in combination with the tested BCA once again had the highest efficacies in reducing *C. morganii* infections on *Dodonaea* seedlings. Etridiazole showed the worst efficacy against the fungal pathogen, whereas thiophanate-methyl and boscalid + pyraclostrobin revealed a certain efficacy in controlling Dodonaea stem rot. All of the remaining treatments showed an intermediate capability to reduce *C. morganii* infections on *Dodonaea* seedlings.

Experiment III. Fungicide and BCA efficacies in controlling Calonectria leaf spot on *M. excelsa* Aurea and *Callistemon* Captain Cook in a nursery. In these nursery experiments, in which both *M. excelsa* Aurea and *Callistemon* Captain Cook were independently inoculated with *C. mexicana*, *C. pauciramosa*, *C. polizzii*, *C. pseudomexicana*, and *C. tunisiana*, some treatments (fungicide and BCA treatments alone or combined) showed significant effects on *Calonectria* sp. infections ($\alpha = 0.05$). Because the treatment–pathogen–host

Table 2. Effects of fungicides and biological control agents against leaf spot caused by *Calonectria morganii* on bottlebrush *(Callistemon viminalis*) in experiment I[z]

Treatment	First trial			Second trial			Third trial		
	DI_P	DI_L	SS	DI_P	DI_L	SS	DI_P	DI_L	SS
Boscalid + pyraclostrobin	2.3 c	0.2 c	1.00 c	1.6 c	0.4 c	1.00 c	0.0 e	0.0 d	1.00 b
Fluopyram	4.6 c	1.1 c	1.01 bc	0.0 c	0.0 c	1.00 c	1.3 de	0.1 cd	1.00 b
Bacillus subtilis QST713	10.5 c	1.8 c	1.07 bcd	1.6 c	0.2 c	1.00 c	1.3 de	0.1 cd	1.00 b
B. amyloliquefaciens subsp. *plantarum* D747	19.7 bc	2.7 c	1.03 bc	4.8 bc	1.4 bc	1.02 c	10.7 bcd	1.5 bc	1.01 b
Fosetyl-Al	17.2 bc	4.1 c	1.05 bcd	22.2 ab	11.3 a	1.21 a	5.3 cde	1.3 bcd	1.02 b
Propamocarb + fosetyl-Al	16.0 bc	5.0 bc	1.06 bcd	18.2 ab	7.0 ab	1.11 bc	25.3 b	4.5 b	1.05 b
Trichoderma atroviride T11 + *T. asperellum* T25	46.6 ab	15.0 ab	1.19 abc	14.3 ab	7.6 ab	1.11 bc	13.3 bcd	2.7 b	1.04 b
T. harzianum ICC012 + *T. viride* ICC080	51.4 ab	15.8 ab	1.19 abc	18.2 ab	6.9 ab	1.13 b	20.0 bc	3.9 b	1.04 b
T. asperellum TV1	60.8 a	20.4 a	1.26 a	5.6 bc	1.8 bc	1.03 bc	12.0 bcd	2.3 b	1.02 b
Streptomyces griseoviridis K61	69.1 a	16.9 ab	1.22 ab	11.9 ab	5.3 ab	1.05 bc	13.3 bcd	2.7 b	1.03 b
T. harzianum T22	64.4 a	15.5 ab	1.18 abcd	26.2 ab	13.4 a	1.22 a	20.0 bc	4.5 b	1.07 b
Untreated and inoculated control	83.3 a	23.9 a	1.30 a	34.1 a	13.7 a	1.25 a	78.7 a	16.9 a	1.21 a

[z] Data are the means of three replicates, each containing 32 to 42 bottlebrush cuttings. Values followed by the same letters within a column are not significantly different according to the Newman-Keuls test ($\alpha = 0.05$) for disease incidence of infected plants (%; DI_P), disease incidence of infected leaves (%; DI_L), and SS = symptom severity (centimeters; SS). Arcsine square root transformation was applied on percentage values prior to data analysis.

and treatment–host interactions were always significant, the data on treatment efficacies are presented independently for each *Calonectria* sp. (Table 4).

The infection levels induced by each *Calonectria* spp. were always significantly higher in *Callistemon* Captain Cook than in *M. excelsa* Aurea (Table 4). The boscalid + pyraclostrobin mixture provided the best performance in controlling Calonectria leaf spot, except for those caused by *C. mexicana* (Table 4), in which significant differences were not detected for SS. Similarly, the cyprodinil + fludioxonil mixture alone or applied in combination with both bioformulations containing *Bacillus* spp. significantly reduced *Calonectria* sp. infections, although with slight differences among the species examined (Table 4).

However, the biological treatments, on average, showed lower performances against Calonectria leaf spot. The lowest efficacy was detected for the bioformulation containing *T. atroviride* T11 + *T. asperellum* T25, followed by those containing *Bacillus* spp. For the chemical treatments, fosetyl-Al provided the lowest reduction in pathogen infections (Table 4).

Table 3. Effects of fungicides and biological control agents against stem infections caused by *Calonectria morganii* on *Dodonaea viscosa* in experiment II[z]

	First trial		Second trial	
Treatment	**DI**	**SS**	**DI**	**SS**
(Cyprodinil + fludioxonil) + *Streptomyces griseoviridis* K61	2.4 g	0.02 e	1.2 il	0.01 cd
Cyprodinil + fludioxonil	6 fg	0.04 e	3.6 ghi	0.02 cd
(Cyprodinil + fludioxonil) + *Trichoderma atroviride* T11 + *T. asperellum* T25	6 fg	0.05 e	0 l	0 d
(Cyprodinil + fludioxonil) + *T. harzianum* T22	6 fg	0.06 e	2.4 hi	0.03 cd
(Cyprodinil + fludioxonil) + *Bacillus amyloliquefaciens* subsp. *plantarum* D747	8.3 efg	0.06 de	3.6 ghi	0.03 cd
(Cyprodinil + fludioxonil) + *B. subtilis* QST713	8.3 efg	0.06 de	4.8 fghi	0.05 cd
Thiophanate-methyl	10.7 ef	0.07 de	2.4 hi	0.03 cd
Boscalid + pyraclostrobin	13.1 e	0.09 de	4.8 fghi	0.06 cd
Fludioxonil	14.3 de	0.11 de	6 efgh	0.06 cd
B. subtilis QST713	20.6 cd	0.21 cd	9.5 cdefg	0.09 cd
Fosetyl-Al	24.1 c	0.31 c	20.2 c	0.17 c
B. amyloliquefaciens subsp. *plantarum* D747	23.8 c	0.3 c	17.9 cd	0.15 cd
S. griseoviridis K61	24.1 c	0.34 c	8.3 defgh	0.08 cd
T. atroviride T11 + *T. asperellum* T25	29.8 c	0.35 c	10.7 cdef	0.11 cd
T. harzianum T22	36.9 b	0.37 c	13.1 cde	0.12 cd
Etridiazole	39.3 b	0.6 b	27.4 b	0.3 b
Untreated	81.0 a	1.26 a	53.6 a	0.81 a

[z] Data are the means of three replicates, each containing 28 Dodonaea seedlings. Values followed by the same letters within a column are not significantly different according to the Newman-Keuls test ($\alpha = 0.05$) for disease incidence (%; DI) and symptom severity (centimeters; SS). Arcsine square root transformation was applied on percentage values prior to data analysis.

Discussion

Calonectria diseases currently represent a serious threat to ornamental plant production in the Mediterranean basin. The potential use of single applications of BCA or fungicides for controlling Calonectria diseases has been addressed in recent articles (Aiello et al. 2013; Henricot and Wedgwood 2013; LaMondia 2015; Vitale et al. 2012) but little or no information is available regarding an IPM strategy based on combinations of fungicides and microbiological formulates. Thus, our research adds useful information, contributing to a new integrated strategy using chemical and biological means against the most widespread *Calonectria* spp. in the Mediterranean basin (i.e., *C. morganii*, *C. pauciramosa*, *C. polizzii*, *C. tunisiana*, *C. mexicana*, and *C. pseudomexicana*).

Based on our data, the cyprodinil + fludioxonil fungicide mixture could be used in fungicide spray schedules because it provided the best performance in reducing leaf and stem infections caused by different *Calonectria* spp., regardless of whether they were used alone or in combination with BCA. These results were comparable with those obtained for the boscalid + pyraclostrobin fungicide mixture. For this latter formulation, Henricot and Wedgwood (2013) recently demonstrated a good efficacy against *C. pseudonaviculata* causing blight on *Buxus* spp. Encouraging data were also obtained for fluopyram against Calonectria leaf spot on bottlebrush. Although applications of thiophanate-methyl alone reduced *Calonectria* sp. infections, the use of this fungicide is discouraged because fungicide-resistant phenotypes have been detected in Italian plant nurseries (Polizzi and Vitale 2001; Vitale et al. 2009).

BCA performances were variable depending on the host, pathogen species, symptomatology, and nursery conditions involved. Indeed, microbiological formulations containing *Bacillus* spp., *Trichoderma* spp., and *S. griseoviridis*, when applied alone, were, on average, less effective than fungicides and combined treatments in reducing *Calonectria* sp. infections. This variability was already reported for *T. harzianum* strain T22, whose effectiveness against *C. pauciramosa* was strain specific and also dependent on the treatment's timing (Vitale et al. 2012). On the other hand, variability of disease infection levels recorded within the single experiment for the efficacy evaluation

Table 4. Effects of integrated treatments in reducing severity of leaf spot caused by five *Calonectria* spp. on metrosideros and bottlebrush in experiment III[z]

	Metrosideros excelsa Aurea					*Callistemon* Captain Cook				
Treatment	***C. mex***	***C. pau***	***C. pol***	***C. pse***	***C. tun***	***C. mex***	***C. pau***	***C. pol***	***C. pse***	***C. tun***
(Cyprodinil + fludioxonil) + (*T. atroviride* T11 + *T. asperellum* T25)	1.11 ab	1.04 b	1.04 b	1.04 b	1.05 bc	1.24 c	1.13 bc	1.07 b	1.01 d	1.02 c
(Cyprodinil + fludioxonil) + *B. amyloliquefaciens* subsp. *plantarum* D747	1.05 b	1.06 b	1.05 b	1.06 b	1.05 bc	1.06 c	1.03 c	1.04 b	1.04 d	1.01 c
(Cyprodinil + fludioxonil) + *B. subtilis* QST713	1.05 b	1.06 b	1.05 b	1.04 b	1.05 bc	1.04 c	1.12 bc	1.07 b	1.01 d	1.01 c
B. amyloliquefaciens subsp. *plantarum* D747	1.08 ab	1.06 b	1.05 b	1.10 ab	1.06 bc	1.13 c	1.34 b	1.29 b	1.24 d	1.37 c
B. subtilis QST713	1.09 ab	1.04 b	1.03 b	1.05 b	1.04 bc	1.21 c	1.21 bc	1.32 b	2.11 c	1.57 c
Boscalid + pyraclostrobin	1.10 ab	1.05 b	1.03 b	1.03 b	1.02 c	1.08 c	1.04 c	1.01 b	1.01 d	1.07 c
Cyprodinil + fludioxonil	1.11 ab	1.02 b	1.04 b	1.04 b	1.04 bc	1.16 c	1.13 bc	1.07 b	1.03 d	1.29 c
Fosetyl-Al	1.15 a	1.08 b	1.03 b	1.06 b	1.05 bc	1.25 c	1.21 bc	1.10 b	1.39 d	1.24 c
T. atroviride T11 + *T. asperellum* T25	1.11 ab	1.08 b	1.05 b	1.05 b	1.09 b	1.87 b	1.16 bc	1.22 b	3.04 b	2.70 b
Untreated and inoculated control	1.14 a	1.22 a	1.25 a	1.14 a	1.22 a	4.00 a	2.26 a	3.30 a	4.46 a	4.70 a

[z] Data are the means of three replicates, each containing 28 and 3 cuttings from metrosideros and bottlebrush, respectively. *Calonectria* spp.: *Calonectria mexicana* = *C. mex*, *C. pauciramosa* = *C. pau*, *C. polizzii* = *C. pol*, *C. pseudomexicana* = *C. pse*, and *C. tunisiana* = *C. tun*. Severity symptom (SS) values followed by the same letters within a column are not significantly different according to the Newman-Keuls test ($\alpha = 0.05$). Symptom severity was calculated according to a 1-to-5 rating scale based on the percentage of infected area on the leaf surface tissues (0 to more than 50%).

of integrated control means may be related to lower temperature values and humid conditions occurring in some nursery trials. It is well known that *Calonectria* sp. infections were most prevalent under wet, warm, and humid conditions (Crous, 2002, Vitale et al. 2013b). However, there were no significant increases in efficacy when fungicides were used in combination with BCA. Furthermore, the reduced performances of bioformulations applied alone can probably be related to the extreme inoculum density of the pathogen and environmental conditions adverse to BCA but favorable to pathogens, as was reported by Daughtrey and Benson (2005). Consequently, the BCA applications as stand-alone control measures for Calonectria disease control in nursery management cannot be largely encouraged in different agronomic and phytosanitary conditions. Based on our results, the integrated management of *Calonectria* sp. infections using tested chemical and biological means under our production conditions is no better than the use of chemicals alone.

Therefore, our findings do not support the adoption under actual European legislation of IPM programs to control Calonectria diseases, although this information could be useful in producing healthy ornamental plants. However, this article could be considered the starting point for the rational control of Calonectria diseases against which future IPM advances will be evaluated and compared over time. Recommendations on the decreased use of some chemical compounds, although effective against these fungi, are also reported to reduce the development of fungicide-resistant strains (FRAC 2007; Guarnaccia et al. 2014).

An effective IPM strategy can be best achieved by testing different BCA and fungicides alone or in combination, and cannot rely on a single or limited number of commercial formulations. Future studies should be oriented to the new search of BCA or integrated means to implement management of Calonectria diseases in ornamental nurseries.

Acknowledgments

This research was supported by MIUR project number PON01_01611 (SO. PRO.ME: "Sustainable production of potted plants in Mediterranean environment"). We thank V. Guarnaccia and P. T. Formica for their technical assistance in the experimental assays.

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