Impact of *Calonectria* Diseases on Ornamental Horticulture: Diagnosis and Control Strategies

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Abstract

Diseases caused by fungi in the genus *Calonectria* pose a significant threat to the ornamental horticulture industries in Europe and the United States. *Calonectria* spp. are particularly challenging pathogens to manage in ornamental production systems and the urban landscape for multiple reasons. A high level of species diversity and poorly resolved taxonomy in the genus makes proper pathogen identification and disease diagnosis a challenge, though recent molecular phylogenetic studies have made significant advances in species delimitation. From a disease management perspective, *Calonectria* spp. produce long-lived survival structures (microsclerotia) that contaminate nursery production systems and can survive multiple years in the absence of a susceptible plant host. Latent infection of plant material is poorly

understood but likely contributes to long-distance dissemination of these fungal pathogens, including the clonal *Calonectria* spp. responsible for the global emergence of boxwood blight. Breeding for disease resistance represents a sustainable strategy for managing *Calonectria* diseases but is challenging due to the perennial nature of many ornamental plants and high levels of susceptibility in commercial cultivars. Ultimately, long-term sustainable management of *Calonectria* diseases will require an improved understanding of pathogen biology as well as integration of multiple disease management strategies.

Keywords: chemical, cultural and biological practices, disease management, etiology, fungi, ornamentals, pathogen diversity

The order Hypocreales (Ascomycota) includes approximately 2,700 fungal species from 240 genera. The ascomycete family Nectriaceae (Hypocreales) contains numerous important plant and human pathogens, as well as some species used in industrial and commercial applications (Kirk et al. 2008).

The fungal genus *Calonectria* is placed in the family Nectriaceae. This genus is distinguished from the other hypocrealean genera based on its *Cylindrocladium*-like asexual morphs that are more commonly encountered in nature than sexual morphs (Crous 2002).

The genus *Calonectria* was first introduced in 1867 by the Italian botanist Giuseppe De Notaris, generally known for his work with Cryptogamiae (*kryptos* = “hidden” + *gamein* = “to marry”), based on *Calonectria daldiniana* De Not. (type species, a later synonym of *C. pyrochroa*) collected on dead leaves of *Magnolia grandiflora* in Locarno (Switzerland) (De Notaris 1867).

The asexual genus *Cylindrocladium* was first described in 1982 by an American botanist, Andrew Price Morgan, who collected fungal

species *Cylindrocladium scoparium* from a dead pod of honey locust (*Gleditsia triacanthos*) in Ohio, United States. *Calonectria* spp. were initially reported as saprobes because the proof of pathogenicity using a *Cylindrocladium scoparium* Morgan (sexual morph *Calonectria cylindrospora* (Ellis & Everh.) Rossman, L. Lombard & Crous) isolate failed to reproduce root rot symptoms on coniferous seedlings (Graves 1915). The first demonstration of *Calonectria* spp. as plant pathogens was by Massey (1917), followed by Anderson (1919) on roses.

Currently, *Calonectria* spp. are causal agents of important diseases in tropical and subtropical climates around the world. They are polyphagous and nonhost-specific fungi (Crous 2002). In Australia, South America, South Africa, and Asia, these diseases cause extensive losses in forestry plantations (Alfenas et al. 2015; Lombard et al. 2009; Wu and Chen 2021). In the United States and Europe, these pathogens are responsible for many diseases on a wide range of ornamental plants (Lane et al. 2006; Lombard et al. 2010c; Vitale et al. 2013b). Species of *Calonectria* affect forestry, and agricultural and horticultural crops. Approximately 100 plant families and 335 plant host species are susceptible (Lombard et al. 2010c). In forestry, the majority of *Calonectria* diseases have been reported on species belonging to the families Fabaceae (*Acacia* spp.), Myrtaceae (*Eucalyptus* spp.), and Pinaceae (*Pinus* spp.) (Alfenas et al. 2015; Crous 2002; Lombard et al. 2010c). On *Eucalyptus* spp., belonging to one of the most susceptible genera, different *Calonectria* spp. cause cutting rot and damping-off in nurseries, leaf and shoot blight, and stem canker

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and defoliation in plantations (Crous 2002; Rodas et al. 2005). *Calonectria* spp. also cause important diseases on agricultural crops such as (i) *Cylindrocladium* black rot of *Arachis hypogea* (peanut) caused by *Calonectria ilicicola* Boedijn & Reitsma (asexual morph *Cylindrocladium parasiticum* Crous, M. J. Wingf. & Alfenas); (ii) red crown rot of soybean (*Glycine max*) caused and *Calonectria pyrochroa* (Desm.) Sacc. (asexual morph *Cylindrocladium ilicicola* Hawley Boedijn & Reitsma); and (iii) *Cylindrocladium* tuber rot of potato (*Solanum tuberosum*) caused by *Calonectria brassicae* (Panwar & Bohra) L. Lombard, M.J. Wingf. & Crous (Lombard et al. 2010c). On horticultural crops, *Calonectria* spp. have been widely reported from ornamental commercial nurseries, gardens, and historic landscapes, causing high yield losses (Daughtrey 2019; Vitale et al. 2013b).

Ornamental horticulture consists of floriculture crops or trees, shrubs, and vines and their products such as potted ornamental plants, cut flowers and foliage, bedding plants, and woody and herbaceous plants used in landscape horticulture. Urban horticulture or environmental horticulture is a relatively recent science associated with landscaping, gardening, and arboriculture. The worldwide annual floriculture trade was valued at approximately U.S.\$70 billion in 2015 (Misra and Ghosh 2016). In 2016, over 145 countries were participating in the trading of floriculture plants and products.

The aim of this review is to present an overview of the genus *Calonectria* and its *Cylindrocladium*-like morphs, with emphasis on species detected on ornamental plants in Europe and the United States, their global importance, and strategies for their control in nursery production and urban landscapes.

Impact of *Calonectria* Diseases

Host range and symptoms. *Calonectria* diseases represent one of the major limiting factors for ornamental horticulture crop production, especially under warm, wet, and humid environmental conditions. Several *Calonectria* spp. have been reported from urban landscapes and from commercial nurseries (Daughtrey 2019; Vitale et al. 2013b). *Calonectria pauciramosa* C. L. Schoch & Crous and *C. cylindrospora* (formerly *C. morganii* Crous, Alfenas & M. J. Wingf.) are the main species associated with ornamental crops in nurseries. *Calonectria* spp. affecting ornamental plants in Europe and the United States are reported in Table 1. The host plants reported are common species produced in nurseries or typically used in gardening and landscaping. The most common ornamental plant families affected are *Arecaceae*, *Buxaceae*, *Ericaceae*, *Myrtaceae*, *Lauraceae*, *Pinaceae*, and *Sapindaceae* (Aiello et al. 2020a; Henricot et al. 2000; Polizzi and

Table 1. Main *Calonectria* spp. and species complexes detected on ornamental plants in Europe and the United States

Species	Species complex	Host species	Locations
<i>Calonectria colhounii</i>	<i>C. colhounii</i>	<i>Metrosideros collinus</i> , <i>Howea forsteriana</i> <i>Leucospermum</i> spp., <i>Leucothoe</i> spp., <i>Callistemon</i> sp.	United States United States
<i>C. cylindrospora</i> (syn. <i>C. morganii</i>)	<i>C. cylindrospora</i>	<i>Pistacia lentiscus</i> , <i>Melaleuca acuminata</i> , <i>Callistemon</i> spp. <i>Azalea</i> hybrids <i>Calluna vulgaris</i> , <i>Erica</i> spp., <i>Rosa</i> hybrids <i>Rhododendron</i> hybrids	Italy Belgium United Kingdom Germany, The Netherlands
<i>C. hederiae</i>	<i>C. reteaudii</i>	<i>Hedera helix</i>	United Kingdom, France
<i>C. henricotiae</i>	<i>C. naviculata</i>	<i>Buxus</i> spp.	Belgium, Germany, The Netherlands, United Kingdom, Slovenia, Czech Republic
<i>C. ilicicola</i>	<i>C. kyotensis</i>	<i>Laurus nobilis</i> <i>H. forsteriana</i> , <i>Laccospadix australasica</i>	Italy United States
<i>C. indusiate</i> (syn. <i>C. theae</i>)	<i>C. colhounii</i>	<i>Strelitzia nicolai</i> , <i>Rhododendron</i> sp. <i>H. forsteriana</i> , <i>Chamaedorea</i> spp., <i>Washingtonia</i> spp., <i>Cocus nucifera</i> , <i>M. collinus</i>	United States United States
<i>C. lauri</i>	<i>C. mexicana</i>	<i>Buxus sempervirens</i> , <i>Ilex aquifolium</i> <i>L. nobilis</i>	The Netherlands, France, Belgium United Kingdom
<i>C. pacifica</i>	<i>C. kyotensis</i>	<i>Araucaria heterophylla</i>	United States
<i>C. pauciramosa</i> (syn. <i>C. polizzii</i>)	<i>C. candelabrum</i>	<i>P. lentiscus</i> , <i>Brahea armata</i> , <i>Feijoa sellowiana</i> , <i>Arbutus unedo</i> , <i>Acacia retinodes</i> , <i>Eugenia myrtifolia</i> , <i>Dodonaea viscosa</i> , <i>Agonis flexuosa</i> , <i>Metrosideros</i> spp., <i>Melaleuca</i> spp., <i>Eucalyptus</i> spp. <i>Myrtus communis</i> <i>Erica capensis</i> <i>Callistemon</i> spp. <i>Polygala myrtifolia</i> <i>Ceanothus thyrsiflorus</i> <i>P. myrtifolia</i>	Italy Italy Italy Italy, United States United States Italy, Spain, United States Italy, Spain Italy, United Kingdom Italy
<i>C. pseudomexicana</i> (syn. <i>C. tunisiana</i>)	<i>C. mexicana</i>		
<i>C. pseudonaviculata</i>	<i>C. mexicana</i> <i>C. naviculata</i>	<i>Eucalyptus globulus</i> <i>Buxus</i> spp.	Italy United Kingdom, Belgium, France, Germany, Croatia, The Netherlands, Austria, Denmark, Ireland, Italy, Slovenia, Sweden, Switzerland, Spain, Czech Republic, United States
<i>C. pteridis</i>	<i>C. pteridis</i>	<i>Sarcococca</i> spp., <i>Pachysandra</i> spp. <i>S. reginae</i> , <i>Rhododendron</i> sp., <i>Washingtonia</i> spp., <i>Howea</i> spp., <i>Chamaedorea</i> spp., <i>C. nucifera</i>	United States United States
<i>C. rumohrae</i> <i>C. spathiphylli</i>	<i>C. gracilipes</i> <i>C. spathiphylli</i>	<i>Adiantum</i> sp. <i>Spathiphyllum</i> sp.	The Netherlands Italy, Germany, United Kingdom

Catara 2001; Polizzi and Crous 1999; Polizzi et al. 2007a, b, 2009, 2012). Symptoms included leaf spot; crown, collar, and root rot; stem lesions; and shoot blight (Crous 2002; Vitale and Polizzi 2007, 2008; Vitale et al. 2008, 2009a). As a consequence of these infections, plants can show wilting, stunting, chlorosis, or defoliation (Henricot et al. 2000; Polizzi and Crous 1999; Vitale and Polizzi 2007, 2008) (Figs. 1, 2, 3, 4, and 5).

In ornamental nurseries, infections start at early stages of seedling and cutting propagation in the greenhouse (Fig. 3A), leading to severe economic losses. Cultivation systems and agronomic practices such as densely grown plants and overhead sprinkler irrigation, as well as environmental conditions such as high relative humidity and mild temperatures, are highly favorable for *Calonectria* infections during rooting or seed germination. On seedlings, symptoms appear soon after their germination and consist of water-soaked constriction at the soil line or below that can extend upward and lead to plant collapse within 1 to 2 weeks. On cuttings, infection usually starts at the lower end of the stem, which appears rotted and blackened (Fig. 3C). Swelling and cracking of necrotic areas at crown or basal stem level may develop into basal stem canker (Polizzi and Crous 1999) (Fig. 2C). The appearance of symptoms is often followed by profuse sporulation of the pathogen on infected tissue under humid conditions. In some hosts such as *Polygala myrtifolia*, *Arbutus unedo*, and *Feijoa sellowiana*, plants can be asymptomatic for several years even if latently infected, then suddenly collapse (Vitale et al. 2013b) (Figs. 2A, B, and D and 3D). Leaf spot symptoms caused by *Calonectria* spp. vary according to host species, host age, and pathogen species. Generally, they consist of small, water-soaked spots that are brown, red, or purple in color, which eventually turn light to dark brown with age and are surrounded by a red, brown, or purple border and chlorotic halo

(Fig. 1A to E). On *Melaleuca* spp., *Myrtus communis*, and *Pistacia lentiscus*, infection can cause total defoliation (Fig. 3B). On Buxaceae, boxwood blight caused by *C. pseudonaviculata* L. Lombard, M. J. Wingf. & Crous and also by *C. henricotiae* Gehesquière, Heungens, and J. A. Crouch is the major disease affecting these crops in gardens and urban landscapes (Figs. 4 and 5). The disease was reported on different species and cultivars of *Buxus* and on *Sarcococca* and *Pachysandra* genera (Daughtrey 2019; Henricot et al. 2000; LeBlanc et al. 2018). *C. henricotiae* has been detected only in Europe, whereas *C. pseudonaviculata* currently is widespread in the United Kingdom, Europe, New Zealand, Asia, Canada, and the United States, where it is present in 30 states and the District of Columbia (Hall et al. 2021). Boxwood blight has been observed to be most severe with high precipitation and high humidity during the summer (Daughtrey 2019). The characteristic symptoms on *Buxus* spp. consist of rapid leaf blighting and defoliation. After several cycles of infection with conducive environmental conditions, infected plants can show severe defoliation. Small plants sometimes die (Henricot 2006). Symptoms of leaf spot often start at the leaf tip and, under conducive conditions, the entire leaves become browned or blackened. Moreover, characteristic black, elongated streaks or cankers can begin to appear along the axis of the shoot following infection within a single season (Daughtrey 2019).

Disease cycle and pathogen dissemination. *Calonectria* spp. exhibit repeated complete infection cycles in nurseries or gardens within a single crop cycle (Fig. 6). They overwinter in soil or plant debris as assemblages of chlamydospores forming microsclerotia. Infected plant material such as leaves or stems falls to the ground, releasing these survival structures to act later as primary inoculum. Microsclerotia might survive for 15 years or more even without a

Fig. 1. Disease symptoms caused by *Calonectria* spp. on ornamental hosts. **A and B,** Leaf spots and defoliation on *Callistemon* spp. caused by *Calonectria cylindrospora*; **C,** leaf spots on *Brahea armata* caused by *C. pauciramosa*; **D,** leaf spots on *Arbutus unedo* caused by *C. pauciramosa*; and **E,** leaf spots on *Feijoa sellowiana* caused by *C. pauciramosa*.

Fig. 2. Disease symptoms caused by *Calonectria pauciramosa* on *Polygala myrtifolia* and effects of *Trichoderma harzianum* T22 treatments on plants growth. **A and B,** Chlorosis and defoliation; **C,** swelling and cracking of necrotic area at crown level; **D,** mortality; and **E,** larger size of plants treated with *Trichoderma* compared with untreated plants (on the right).

Fig. 3. Disease symptoms caused by *Calonectria* spp. on ornamental hosts. **A,** Damping-off of *Callistemon laevis* cuttings during propagation stage caused by *Calonectria cylindrospora*; **B and C,** defoliation and crown, stem, and root rot of *Myrtus communis* caused by *C. pauciramosa*; **D,** wilting of *Feijoa sellowiana* seedlings caused by *C. pauciramosa*; **E and F,** wilting, crown rot, and perithecia of *C. ilicicola* on *Laurus nobilis*; and **G,** crown rot on *Pistacia lentiscus* and perithecia of *C. pauciramosa*.

host plant (Crous 2002; Thies and Patton 1970), then germinate in the presence of a susceptible host under hot and humid conditions. In nursery production, following the primary infection and pathogen development during seedling or cutting production in the greenhouse, new inoculum production (conidia, chlamydospores, or ascospores) occurs on infected tissues. From these sites, secondary inoculum spreads through splashing or flow of water to new susceptible plants (aboveground plant parts or adjacent plants) or, through handling, to subsequent container-grown transplants or field areas. In nurseries, inoculum spreads over short distances mainly by water splash but aerial movement has also been reported (Crous 2002). Moreover, inoculum can be moved long distances on infected transplants or infested substrates. The presence of free water and high humidity, as well as plastic film for mulching and substrates from recycled and

composted materials, can promote the dissemination of *Calonectria* diseases in nurseries (Crous 2002; Noble and Roberts 2004; Vitale et al. 2013b). For *C. pseudonaviculata*, water is the main means of inoculum dispersal; movement by air is not common due to its large and sticky conidia. The use of leaf blowers seems to be one of the distribution methods of inoculum within a garden; many other potential means of human-mediated and animal-mediated dispersal have been suggested for this species (Gehesquière et al. 2016). The sale of winter holiday greenery using clippings collected from nurseries has been suggested as another route of long-distance dissemination (Daughtrey 2019).

Some *Calonectria* spp. such as *C. cylindrospora*, *C. pauciramosa*, *C. naviculata*, and *C. henricotiae* exhibit a wide temperature tolerance, growing from below 10°C to above 30°C (Crous 2002; Gehesquière et al. 2016). Others such as *C. mexicana* C. L. Schoch & Crous,

Fig. 4. Boxwood blight disease caused by *Calonectria pseudonaviculata*. **A and B**, Leaf spots; **C**, fungal sporulation on the underside of a leaf; **D**, infected leaf with the microscerotia stained in cotton blue; **E**, microscerotium in culture; **F**, protoperithecia; and **G**, conidium germinating from both cells.

Fig. 5. Château de Villandry Renaissance gardens (Grand country house on the Loire Villandry, France). **A**, Healthy garden in 2004 and **B**, blight caused by *Calonectria pseudonaviculata* on *Buxus sempervirens* 'Suffruticosa' in 2014.

Fig. 6. Illustrated disease cycle of *Calonectria* spp.

C. ilicicola, and *C. spathiphylli* El-Gholl, J. Y. Uchida, Alfenas, T. S. Schub., Alfieri & A. R. Chase are high-temperature species that grow well from 10 to 30°C or higher.

Pathogen Identification

Morphology-based diagnosis. In recent years, there has been a notable increase in the number of taxonomic studies on *Calonectria* spp., leading to changes in the nomenclature of described species and, in some cases, increasing or decreasing the number of accepted species. Currently, the genus includes 11 species complexes within the Prolate and Sphaero-Naviculate groups and 169 described species of *Calonectria* based on morphological characteristics combined with DNA sequence data (Liu et al. 2020; Lombard et al. 2010a, b).

Observation of symptoms and signs is the most reliable method to identify diseases caused by *Calonectria* spp. A characteristic white bloom of mycelium with sporulation may develop on symptomatic tissue under humid conditions, and microsclerotia are observed on infected plants with epidermal peels or by clearing and staining (Daughtrey 2019). Rapid defoliation, leaf spot (Fig. 4A and B), black streaks on the current season's shoots, and white sporulation or survival structures (Fig. 4C to G) allow a reliable field identification of boxwood blight (Daughtrey 2019). Moreover, for some species, the sexual morph (perithecia) can be detected on symptomatic tissues. Perithecia of *C. ilicicola* and *C. pauciramosa* have been observed in nurseries on Sicily (Southern Italy) on infected plants of *Laurus nobilis* (Fig. 3E and F) and *P. lentiscus* (Fig. 3G), respectively (Polizzi et al. 2012; Vitale and Polizzi 2007) (Fig. 3F and G). These structures are morphologically very similar among different

Calonectria spp., showing a characteristic shape and color which might be helpful in rapid field identification of *Calonectria* diseases.

For many years, the description of the genus *Calonectria* has been based on characteristics of a *Cylindrocladium*-like asexual morph (Brasier 1997; Taylor et al. 2000) (Fig. 7A to E). Typical cylindrical conidia and conidiophores with stipe extensions ending in vesicles can develop on infected tissue under humid field conditions or by incubating symptomatic plants at high humidity for 2 to 3 days. Morphological features of *Calonectria* spp. have been widely examined on carnation leaf agar, synthetic nutrient-poor agar, and minimal salt agar (Crous et al. 1992; Lombard et al. 2010a). Malt extract agar helps to determine optimal growth temperatures and colony color, and has been widely used to culture *Calonectria* fungi for subsequent molecular analysis (Lombard et al. 2010a; Vitale et al. 2013b).

Vesicle shape, conidium septation and dimensions, and stipe extension length as well as culture characteristics (mycelial growth and color) have been the primary taxonomic criteria used to distinguish *Calonectria* spp. (Lombard et al. 2010a; Peralilly 1991). However, in closely related species, a high degree of plasticity was noted in some of these characteristics, which makes morphology-based identification difficult (Schoch et al. 1999).

Mating strategies have been used to identify new species in genus *Calonectria* (Crous 2002; Schoch et al. 1999). Some species (e.g., *C. ilicicola*) are homothallic and produce perithecia containing viable ascospores in culture and in nature (Polizzi et al. 2012); other species (e.g., *C. pauciramosa* and *C. cylindrospora*) are heterothallic and self-sterile hermaphrodites that produce female structures called protoperithecia that must be spermatized from an opposite mating type (Fig. 7F and G) (Schoch et al. 2001). For some species such as

C. pseudonaviculata and *C. henricotiae*, no sexual morph has been observed in vitro or in vivo (Gehesquière et al. 2016; Lombard et al. 2010b), although barren perithecia have been observed in culture (Castroagudín et al. 2020b; LeBlanc et al. 2018) and on incubated leaf tissue (N. Shishkoff, unpublished data). Several difficulties associated with morphological identification have been highlighted and, currently, it is not considered sufficiently reliable to distinguish the different *Calonectria* spp. (Lombard et al. 2010b, 2011).

Before the molecular era, 37 *Calonectria* and 52 *Cylindrocladium* spp. were recognized (Crous 2002; Crous et al. 2004; Lombard et al. 2010c) and morphological features did provide some useful information for species discrimination in the *Calonectria* genus. However, the adoption of a polyphasic identification process through multilocus DNA sequence data provided further insights for identification at the species or species complex level (Chen et al. 2011; Crous et al. 2004, 2006; Lombard et al. 2010a, b, 2015a, b).

Application of molecular genetics to taxonomy. During the last decade, several studies focusing on DNA sequence comparisons and associated phylogenetic inference significantly affected the taxonomy of *Calonectria*. Initially, sequences of the internally transcribed spacer (ITS) region of ribosomal RNA allowed researchers to discriminate between *Cylindrocladium scoparium* and *C. floridanum* isolates (Jeng et al. 1997). Later studies found that the β -tubulin (*tub*) (Schoch et al. 2001) and *histone H3* (*his3*) (Kang et al. 2001) genes improved resolution of genetic differences among species of *Calonectria*. Therefore, concatenated DNA sequences of the ITS, *tub*, and *his3* gene portions were used to resolve taxonomic issues in the genus (Crous et al. 2004, 2006; Henricot and Culham 2002), and *tub* was found to be the most valuable. More recently, *translation elongation factor 1- α* (*tef1*) and *calmodulin* (*cal*) genes were adopted in *Calonectria* taxonomy studies (Crous et al. 2004; Lombard et al. 2010a, c). Lombard et al. (2010c) introduced use of the *actin* (*act*) and the 28S large subunit nonribosomal DNA (LSU) loci, and provided sequence data for all seven gene regions common to the species recognized in the genus at that time, resulting in the discovery of 7 new species, bringing the total number of accepted *Calonectria* spp. to 68. Later, Lombard et al. (2016) demonstrated a total of 141 *Calonectria* spp., and provided for all of these (except for *Calonectria hederiae* and *C. pyrochroa*) the ex-type cultures associated with DNA barcodes. The RNA polymerase II second largest subunit (*rpb2*) sequence data for 68 *Calonectria* spp. were provided by two

recent studies (Jayawardena et al. 2019; Marin-Felix et al. 2017). Overall, several molecular markers have been utilized (Jayawardena et al. 2019; Li et al. 2017; Lombard et al. 2010c, 2016; Marin-Felix et al. 2017); however, a lack of consistency has been observed. Thus, the aim of a recent major study (Liu et al. 2020) was to produce a complete source of DNA sequence data for all 169 species of *Calonectria* recognized after Marin-Felix et al. (2017) and Jayawardena et al. (2019). Liu et al. (2020) synonymized a large number of species that were indistinguishable from already known taxa, supporting the acceptance of 120 defined species of *Calonectria* grouped in 11 species complexes. The eight used markers were also evaluated individually, showing that six gene regions (*tef1*, *tub2*, *cmdA*, *his3*, *rpb2*, and *act*) provide effective barcodes for the genus, while ITS and LSU are not informative. *C. candelabrum*, *C. cylindrospora*, *C. mexicana*, and *C. naviculata* species complexes are the most relevant for pathogens of interest on ornamental plants. *C. polizzii* was reduced to synonymy with *C. pauciramosa* in the *C. candelabrum* species complex. The *C. cylindrospora* species complex includes *C. cylindrospora* sensu stricto, previously known as *C. morganii* and *Cylindrocladium scoparium*. *Calonectria mexicana* and *C. pseudomexicana* (syn. *C. tunisiana*) are also well-known ornamental plant pathogens (Aiello et al. 2020a; Liu et al. 2020; Lombard et al. 2011) which are included in the *C. mexicana* species complex. More accessible costs and techniques for obtaining high-quality genomes, as well as new resources to facilitate research on fungal biology, genetics, and virulence, have led to the first high-quality and nearly completed genome of *C. ilicicola* (Liu et al. 2021). Thus, it is assumed that the multilocus approach will be integrated with whole-genome comparison in future for the correct identification of *Calonectria* spp.

Global Emergence of the Boxwood Blight Pathogens *C. henricotiae* and *C. pseudonaviculata*

C. henricotiae and *C. pseudonaviculata* are recently diverged sister-species that cause boxwood blight on ornamental and noncultivated (forest) boxwood plants. Both pathogens were discovered as novel fungal species following emergence of boxwood blight as a new disease in the United Kingdom in the 1990s (LeBlanc et al. 2018). Following its discovery in the United Kingdom, boxwood blight was found in New Zealand, across continental Europe, and in ecosystems bordering the Black Sea. In 2011, the disease was

Fig. 7. Morphological characteristics of *Calonectria pauciramosa*. **A**, Fungal colonies resulting from isolation on artificial substrates; **B**, variability in mycelial growth among different isolates in culture on potato dextrose agar; **C** and **D**, chlamydospores forming microsclerotia on carnation leaf agar (CLA); **E**, conidiophore, cylindrical conidia, stipe, and obpyriform terminal vesicle of asexual morph on CLA; and **F** and **G**, sexual morph consisting of perithecia containing asci and ascospores on CLA.

detected in the United States. Ongoing pathogen surveys have shown that *C. henricotiae* remains confined to the United Kingdom and Europe, whereas *C. pseudonaviculata* has become the primary pathogen causing boxwood blight on other continents (Daughtrey 2019).

Initial studies using multilocus DNA sequencing and low-resolution molecular markers found little genetic diversity in *C. henricotiae* and *C. pseudonaviculata* (Castroagudín et al. 2020a; Gehesquière et al. 2016; Henricot and Culham 2002). Subsequent research showed that populations of each species harbor a single mating-type idiomorph, confirming a lack of sexual recombination (Malapi-Wight et al. 2019). Genotyping global pathogen populations using 11 simple sequence repeat (SSR) markers also found limited genetic diversity within these species but identified 14 genotypes in the primary pathogen species *C. pseudonaviculata*. Interestingly, only one common *C. pseudonaviculata* SSR genotype was present on every continent reporting boxwood blight and the majority of genotypes occurred at low frequencies in the United Kingdom and Europe (LeBlanc et al. 2019). Limited genetic diversity and lack of sexual recombination in pathogen populations suggested the boxwood blight pathogens were recently introduced, clonal fungi.

In the United States, pathogen isolates first detected on the west coast were identified as the *C. pseudonaviculata* SSR genotype found on every other continent, whereas isolates from the east coast were an SSR genotype found only in Europe and the United Kingdom (LeBlanc et al. 2019). Both of these genotypes have since been found on both coasts but the one first detected on the east coast has become the predominant pathogen in the U.S. ornamental boxwood industry (Castroagudín et al. 2020b). Subsequent sequencing identified four genetically differentiated clonal lineages in global populations of *C. pseudonaviculata*. Reconstruction of the population history of these clonal lineages showed independent origins of the two lineages in the United States linked to disease emergence in 2011. One lineage in the United States was derived from United Kingdom or European populations. The second, more common lineage in the United States was from an unknown ancestral population (LeBlanc et al. 2019). This work provided evidence that boxwood blight was introduced into the United States at least twice, once from the United Kingdom or Europe and once from an unidentified source.

Many questions remain unanswered. There is strong evidence that the boxwood blight pathogens are introduced clonal fungi; however, their native geographic range is still unknown. Identifying the geographic origins of these fungi will provide important insight into emergence of the disease in the 1990s and inform efforts to prevent future introductions. Domestic movement of the pathogens is also still poorly characterized, although they were potentially introduced via the nursery industry (Gehesquière et al. 2016). Future efforts to understand genetic changes in pathogen populations within boxwood production cycles or in nurseries in different regions, coupled with diagnostic testing of boxwood plant material, will help trace pathogen movement and reduce disease spread in the ornamental industry.

Survival of *Calonectria* spp.

Natural conditions. Viable microsclerotia of *C. cylindrospora* were recovered from soil that had been fallow for 7 years (Thies and Patton 1970). Pataky and Beute (1983) found that survival of microsclerotia of *C. ilicicola* in debris on the soil surface during the winter was less than that of samples buried 25 cm below the surface. Graham and Griffin (1988) placed soil naturally infested with microsclerotia of *C. ilicicola* at depths of 5, 13, or 25 cm in late fall; germination of microsclerotia declined rapidly at all soil depths during the winter; leveled off during April, May, and June; and declined gradually from July to November.

Henricot and Culham (2002) reported survival of *C. pseudonaviculata* for 5 years in infested litter and suggested that survival was due to mycelium because they did not observe microsclerotia. However, Weeda and Dart (2012) were able to detect microsclerotia in infested boxwood tissue. Ganci (2014) conducted field experiments over a period of 2 years in commercial boxwood production fields in western North Carolina and concluded that *C. pseudonaviculata* could survive from season to season in diseased leaves on the

surface or subsurface (5 cm) of the soil and soilless potting mix. Results from these field experiments indicated that the fungus survived better in diseased leaves that were buried rather than on the surface of the soil or soilless potting mix. There was also a strong correlation between the percentage of leaves with microsclerotia and the ability of the microsclerotia to form spores.

Laboratory conditions. Evaluations of the survival and infectivity of conidia and microsclerotia were performed by Dart et al. (2015). Using boxwood leaf discs to bait the pathogen from infested soil, they demonstrated survival of microsclerotia at 40 weeks postinoculation and conidial survival out to 3 weeks, as well as infectivity at levels as low as approximately 25 microsclerotia/g of soil. They estimated that one boxwood leaf could contain upward of 3,600 microsclerotia. Shishkoff and Camp (2016) evaluated survival of the pathogen at psychrophilic and mesophilic temperatures: specifically, -10, 0, 10, 20, and 30°C. Both lab-grown (colonized cellophane) and naturally occurring (within infected plant tissue) microsclerotia were utilized. Samples were incubated in sand at two moisture levels: 36% (field capacity) and 5% (vol/vol). Samples were taken monthly and evaluated for sporulation on nutrient media. Sporulation was observed only in the first few samples at the more extreme temperatures (up to 5 months at 30°C and 7 months at -10°C for leaf tissue; up to 2 months at 30°C and 7 months at -10°C for culture-derived microsclerotia). However, microsclerotia in other treatments survived and sporulated for at least 30 months, suggesting that the pathogen might be able to sporulate after extended periods in infested plant material at less extreme temperatures. Yang and Hong (2018b) examined survival of microsclerotia of different ages at different temperatures. Younger microsclerotia were less sensitive to extreme temperatures of -10 and 40°C than older ones. For instance, 21-day-old microsclerotia survived at almost twice the rate of 70-day-old ones after a 24-h exposure to -10°C. Likewise, the 21-day-old microsclerotia survived at much greater rates than 70-day-old ones after a 24-h exposure to 40°C. The authors suggested that heat treatments such as solarization and composting were promising measures to eradicate *C. pseudonaviculata* microsclerotia and restore infested sites.

In compost or after treatment with compost tea. Harvey et al. (2019a) reported that *C. pseudonaviculata* microsclerotia survived 72 h of exposure to 40°C in an incubator without compost, whereas exposure to the same temperature and time but with compost added to a bioreactor system decreased survival to 24 h. Harvey et al. (2019b) did further work to demonstrate that, for both *C. pseudonaviculata* and *C. henricotiae* grown on ammonia-amended agar in Petri plates, radial growth of the pathogens was reduced. In studies with *C. pseudonaviculata*, gaseous ammonia reduced survival of microsclerotia. This suggested that ammonia released in the composting process, as well as heat, was reducing microsclerotia survival. Samavat et al. (2020) tested the inhibitory effects of humic and fulvic acids extracted from compost on growth and infectivity of *C. pseudonaviculata* and found reduced growth and spore germination.

Survival under conditions used for heat treatment in propagation. Miller et al. (2018) found that 90% of *C. henricotiae* and *C. pseudonaviculata* conidia in water suspensions (10,000 conidia/ml) were killed by heat treating for 26.8 min at 45°C and 15.3 min at 47.5°C. Culture-produced microsclerotia of *C. henricotiae* and *C. pseudonaviculata* in a water suspension required greater exposure to heated water than conidia, with the latter being inactivated after 20 min at 45 and 47.5°C and 5 to 10 min at 50 and 52.5°C. Treatment of microsclerotia at 45°C for 60 min was not effective. Microsclerotia of *C. henricotiae* were significantly more resistant to heat treatment than those of *C. pseudonaviculata* at 47.5 and 50.0°C, whereas microsclerotia of both species were rapidly killed at 55°C. Experiments conducted on diseased, detached boxwood leaves of susceptible cultivar 'Justin Brouwers' at 47.5, 50, or 52.5°C showed significantly reduced production of conidia from tissue infected with *C. henricotiae* or *C. pseudonaviculata* after 25 to 30 min of exposure to 47.5°C water or 12 min of exposure to 50°C water. After 8 min of exposure to 52.5°C water, little or no sporulation was observed for either fungal species (Shishkoff et al. 2021). These authors investigated

the possibility of using such hot-water treatments to insure clean propagation material. Shishkoff et al. (2021) studied how heat treatments would affect rooting of boxwood cuttings, finding that 47.5°C for 30 min appeared to be a cardinal (maximum) temperature for rooting most cultivars, minimizing leaf damage associated with the application of heat to boxwood cuttings and suppressing sporulation of *C. henricotiae* and *C. pseudonaviculata*.

Fumigation and soil solarization. Studies to determine the efficacy of soil fumigants such as dazomet and metam sodium against *Calonectria* spp. have been performed (Crous 2002), and Aiello et al. (2018) investigated the effects of lower doses of dazomet and metam sodium on *Calonectria* spp. when applied under low-permeability films in greenhouse and field trials. However, nonfungicide methods of reducing pathogen levels in soil are more ecologically sound. With soil solarization, a film of plastic covers an area of soil in order to use solar radiation to heat it, suppressing pathogens without affecting beneficial soil microbes (Gamliel and Katan 2012). Solarization has been used to eradicate resting propagules of other *Calonectria* spp. (Mohan 2007; Vitale et al. 2013a). Mohan (2007) solarized forest soil with and without the addition of biological control agents and found solarization to reduce pathogen populations, including *C. quinquesepitata*. Vitale et al. (2013a) used isolates of *C. pauciramosa* and *C. polizii* in solarization tests and found no viable *Calonectria* inoculum after 12 days in an ethylene-tetrafluoroethylene greenhouse and at 15-cm depth from solarized plots inside an ethylene-vinyl-acetate greenhouse. These results suggest that solarization is a promising method to remediate boxwood blight-infested nursery sites and landscapes.

Disease management. Once *Calonectria* spp. are well established in nurseries, their control is very difficult because of polycyclic disease cycles, broad host ranges, durable infections over multiple seasons, and long-term persistence of microsclerotia in soil and plant debris (Crous 2002; Daughtrey 2019; Richardson et al. 2020; Vitale et al. 2013b). Consequently, disease management should include tactics involving pathogen eradication, reduction of primary inoculum, and decreasing disease development rate. Currently, worldwide *Calonectria* disease control is addressed mainly by combining scheduled fungicide spray programs and appropriate cultural practices. Unfortunately, application of fungicides is not always cost effective for an ornamental crop, or applicable in all nursery, gardening, and urban landscape contexts; moreover, fungicides present numerous hazards for the environment and human health and, in the case of materials with single-site mode of action, increase the risk of developing fungicide-resistant pathogen strains (Daughtrey 2019; Vitale et al. 2013b).

Because of these considerations, integrated disease management of *Calonectria* infections in nursery, gardening, and urban landscapes should be addressed with consideration of environmentally sustainable global policies as recommended by the Strategy on Sustainable Use of Pesticides in the European Union and, more recently, by the Green New Deal worldwide (European Commission 2009; European Parliament 2020).

Chemical control. Several fungicides (Table 2) have been tested and used to effectively manage *Calonectria* infections on many hosts of different botanical families, including Buxaceae (Aiello et al. 2013; Cinquerrui et al. 2017; Crous 2002; Henricot et al. 2008; LaMondia 2014, 2015; Palmer and Shishkoff 2014). Performance and benefits of both protectant and systemic fungicides always depend on mode of action, application timing and method, infection site (phyllosphere or rhizosphere), disease pressure, phytotoxic effect, and fungicide sensitivity within the *Calonectria* genus or species (Aiello et al. 2013; Crous 2002; Gehesquière et al. 2016; Ivors et al. 2013; Palmer and Shishkoff 2014; Polizzi and Azzaro 1996; Vitale et al. 2013b).

An important concern with chemical control in nurseries is the risk of resistance developing within *Calonectria* populations that leads to in-field breakdown of the efficacy of fungicides, as observed for methyl benzimidazole carbamates and other fungicides (Alfenas et al. 1988; Guarnaccia et al. 2012, 2014; Palmer and Shishkoff 2014; Polizzi and Vitale 2001; Vitale et al. 2009b). Extensive monitoring is needed to detect shifts in fungicide sensitivity, because fungicide-resistant strains can appear quickly following repeated use of single-site antifungal compounds. Resistance has been reported for

prochloraz used on *C. pauciramosa* populations (Guarnaccia et al. 2014; Vitale et al. 2009b). Researchers have underlined the advantages of alternating or tank mixing fungicides that have a high resistance risk with materials having different modes of action (different FRAC groups [https://www.frac.info]) or with inherently low resistance risk (Daughtrey 2019; Palmer and Shishkoff 2014; Vitale et al. 2013b).

A third concern with chemical controls is related to the ability of most *Calonectria* spp. to infect their hosts starting from low to medium temperatures (e.g., *C. pseudonaviculata* and other *Calonectria* spp. at 5°C), typical of the mild climatic conditions typically found in the Mediterranean basin. Pathogens active over a wide range of temperatures will require treatment over a long part of the growing season in temperate climates, thus making the number of applications so numerous that they are too expensive, too laborious, and environmentally unfriendly, especially in public gardens and landscaping.

Use of disease-free planting material and cultural practices.

Because dispersal of *Calonectria* spp. happens by infected propagation material, plant debris, or contaminated soil media or gardening equipment, reliable agronomic measures should be adopted to exclude fungal pathogens in greenhouses, nurseries, and public gardens before planting and at each stage of the crop cycle. Healthy planting material, selective shoot harvesting by technicians, use of inoculum-free growing pots or trays, and mulching at the base of plants can decrease primary infections on the root collar and root of young plants and also reduce reinfection from dropped leaves. In addition, pruning of infected portions back to healthy tissue, elimination of diseased plants and debris, removal of infested potting media, and favoring drip irrigation instead of overhead irrigation systems are very useful to minimize the development rate of *Calonectria* spp. in public gardens and nurseries (Daughtrey 2019; Henricot et al. 2008; Likins et al. 2019; Lovell-Smith 2017; Vitale et al. 2013b).

Unfortunately, other potential human-based activities can contribute strongly to a greater dispersal of *Calonectria* spp. For example, pruning practices that shed water and improve air circulation could suppress disease development but are very often in conflict with well-established ornamental purposes (LeBlanc et al. 2018; Lovell-Smith 2017). Additional examples of common practices that promote disease include employing leaf blowers in gardens and reusing clippers or other tools from nurseries or other landscapes without prior sanitation (Bush et al. 2016; Daughtrey 2019; Vitale et al. 2013b). Distribution of boxwood wreaths during winter holidays was also found to be responsible for spreading *C. pseudonaviculata* interstate within the United States (Daughtrey 2019). Preferably, new plants should be propagated on site from healthy mother plants and, when it is not possible, quarantine practices should be followed on imported *Buxus* and other ornamental plants to minimize risk of introducing exotic *Calonectria* spp. (Lovell-Smith 2017).

Host resistance. Whole-plant screening of susceptibility and resistance of boxwood and other ornamental germplasm to *Calonectria* diseases can provide both alternative choices for nurserymen and a crucial foundation for breeding programs supporting the nursery trade and green industry worldwide (Daughtrey 2019; LaMondia and Shishkoff 2017; Vitale et al. 2013b).

High susceptibility of *Buxus sempervirens* and *B. sempervirens* ‘Suffruticosa’ to *C. pseudonaviculata* and *C. henricotiae* (Henricot et al. 2008; LaMondia and Shishkoff 2017; Shishkoff et al. 2015) contrasts with the less prized Balearic boxwood, which was more tolerant to boxwood blight (Henricot et al. 2008). Although some trade European boxwood hybrids were less susceptible than English boxwood, Japanese (*B. macrophylla* var. *japonica*) and Korean (*B. sinica* var. *insularis*) boxwood exhibited a stronger disease resistance, on average (Ganci 2014; Ganci et al. 2013; LaMondia and Shishkoff 2017; Shishkoff et al. 2015). Use of ‘Korean’, ‘Winter Gem’ and ‘Nana’ accessions should be promoted for large-scale planting for their reduced susceptibility to boxwood blight, whereas ‘Common’, ‘True Dwarf’, and well-known Justin Brouwers *B. sempervirens* accessions should be discouraged because they are extremely susceptible (Avenot et al. 2017; LaMondia 2015; LaMondia and Maurer 2016). *Pachysandra axillaris* and *P. terminalis* show

Table 2. Chemical active ingredients used to control *Calonectria* infections under containerized or field conditions on boxwood and other ornamental plants^a

Active ingredient	<i>Calonectria</i> spp. (host)	Efficacy ^b	Application mode	Reference
Cu oxychloride ^{phy}	<i>C. pauciramosa</i> ^N (<i>Myrtus communis</i>)	3	Foliar spray	Polizzi and Azzaro 1996
Benomyl; chlorothalonil; dithianon; prochloraz; ziram	—	1	Foliar spray	—
Cyproconazole; propamocarb plus fosetyl-Al; K posphite; thiophanate-methyl	<i>C. cylindrospora</i> and <i>C. pauciramosa</i> ^N , (<i>C. pseudomexicana</i> , <i>C. pauciramosa</i> , <i>C. mexicana</i>) ^C (<i>Callistemon</i> spp., <i>Feijoa sellowiana</i>)	1 to 3	Foliar spray (on bottlebrush), soil drench (on <i>Feijoa</i>)	Aiello et al. 2013
Cu hydroxide; fosetyl-Al; prochloraz; prochloraz plus ciproconazole; azoxystrobin; tebuconazole	—	3	—	—
Trifloxystrobin	—	2 to 3	—	—
Boscalid plus pyraclostrobin; fludioxonil plus cyprodinil	<i>C. cylindrospora</i> , <i>C. pauciramosa</i> , <i>C. mexicana</i> , <i>C. pseudomexicana</i> ^N , (<i>Callistemon</i> spp., <i>Metrosideros excelsa</i> , <i>Dodonaea viscosa</i>)	3	Foliar spray	Cinquerrui et al. 2017
Etridiazole	—	2	—	—
Fludioxonil; fluopyram	—	3	—	—
Fosetyl-Al; propamocarb plus fosetyl-Al	—	2 to 3	—	—
Benomyl; chlorothalonil	<i>C. cylindrospora</i> (<i>Eucalyptus</i> seedlings) ^{C&N}	3	Foliar spray (benomyl also as soil drench)	Barnard 1984
Cu hydroxide	—	2	—	—
Bitertanol; mancozeb; metiram; triadimefon	—	1	—	—
Chlorothalonil; fludioxonil; fludioxonil plus cyprodinil; chlorothalonil plus thiophanate methyl; thiophanate methyl; polyoxin Zn salt; tebuconazole; trifloxystrobin; fluoxastrobin plus chlorothalonil; metconazole; pyraclostrobin; boscalid plus pyraclostrobin; propiconazole plus chlorothalonil; azoxystrobin	<i>C. pseudonaviculata</i> (<i>Buxus</i> spp.) ^C	3	Foliar spray	Ivors et al. 2013
Triflumizole; triticonazole; propiconazole	—	1 to 2	—	—
Fluoxastrobin; iprodione	—	2	—	—
Boscalid plus pyraclostrobin	<i>C. pseudonaviculata</i> (<i>Buxus</i> spp.) ^{C&N}	2	Foliar spray	LaMondia 2015
Chlorothalonil; etridiazole; etridiazole plus pyraclostrobin; fludioxonil plus cyprodinil; kresoxim-methyl; propiconazole; propiconazole plus pyraclostrobin; myclobutanil; myclobutanil plus pyraclostrobin	—	3	—	—
Azoxystrobin; kresoxim-methyl	<i>C. pseudonaviculata</i> (<i>Buxus</i> spp.) ^C	1	Foliar spray	Henricot and Wedgwood 2013
Penconazole; epoxiconazole plus pyraclostrobin	—	1 to 2	—	—
Carbendazim; benomyl	—	2 to 3	—	—
Epoxiconazole plus kresoxim-methyl plus pyraclostrobin	—	3	—	—
Chlorothalonil; prochloraz; boscalid plus pyraclostrobin	—	3**	—	—
Boscalid plus pyraclostrobin; fosetyl-Al	Disease complex (<i>C. pauciramosa</i> , <i>C. pseudomexicana</i> , <i>Fusarium oxysporum</i> , <i>Neocosmospora solani</i> , and <i>Rhizoctonia</i> AG-R) (<i>Polygala myrtifolia</i>) ^{C&N}	3	Soil drench	Aiello et al. 2020b
Propamocarb plus fosetyl-Al	—	1 to 2	—	—

^a Superscript abbreviations: C = containerized grown experiments, N = in-field experiments, and phy = phytotoxicity effects.

^b Scale of 1 = ineffective, 2 = moderate efficacy, and 3 = effective; asterisks (**) indicate in combination within a spray program of fungicide.

a high tolerance to boxwood blight and are preferred to more susceptible *P. procumbens* (LaMondia 2017). It is also better to choose more resistant *Sarcococca ruscifolia* and *S. ruscifolia* var. *chinensis* 'Dragon Gate' (highly tolerant) instead of *S. confusa* (LaMondia 2017; Ryan et al. 2018).

A range of susceptibility to *C. spathiphylli* also exists for *Spathiphyllum* spp. The lesser-prized *Spathiphyllum floribundum* and *S. canifolium* accessions showed a high level of resistance to *Calonectria* infections whereas no trade cultivar showed meaningful resistance (Henny and Chase 1986; Norman et al. 1999; Schoulties and El-Gholl 1983).

Further surveys showed that *Polygala myrtifolia* var. *myrtifolia* (largely used in Italy) was more susceptible than *P. myrtifolia* var. *grandiflora* to collar and root rot caused by *C. pauciramosa*, and a trade hybrid of *P. myrtifolia* × *oppositifolia* 'Bibi Pink' should be promoted because it was highly disease resistant (Vitale et al. 2013b). Relatively low susceptibility to *C. pauciramosa* was also observed on *M. communis* var. *lusitanica* compared with *M. communis* var. *baetica* (Vitale et al. 2003).

Sanitation. Sanitizers that have been studied worldwide to eradicate different *Calonectria* inocula include hydrogen dioxide, ethanol, quaternary ammonium compounds, acetic acid, and sodium hypochlorite, with some encouraging results (Bika et al. 2021; Douglas 2013; Shishkoff and Camp 2016). Use of flaming to eliminate infected plants or debris in an infested area, and of hot water to sanitize cuttings and soil solarization to suppress microsclerotia in soil, gave variable

performance depending upon exposure time, *Calonectria* heat sensitivity, application mode, and timing, as well as other factors (Crous 2002; Dart et al. 2012; Miller et al. 2018; Shishkoff et al. 2021; Vitale et al. 2013a). Similarly, soil application of fumigants such as dazomet and metam-sodium applied at low rates provided variable results depending on targeted species and the permeability of the mulching film (Aiello et al. 2018). In summary, although sanitization, fumigation, and heat-based treatments have been successfully employed in some contexts, their application is strongly limited to narrow operative conditions. For this reason, they cannot be suggested on a large scale or thought of as measures to be applied alone.

Biological management. Application of several *Trichoderma* fungal species and some antagonistic bacteria, recovered from natural sources or from recycled organic media, irrigation water, or suppressive soils, gave variable performance in reducing *Calonectria* infections (Fig. 2E). However, these findings were often limited to controlled experiments in laboratories or under other unique conditions; consequently, it is not possible to draw generalized conclusions. Indeed, the success of biological control agents (BCAs) depended strictly on the targeted *Calonectria* spp., application method and timing, and many other factors (Aiello et al. 2020b; Daughtrey and Benson 2005; Dumas et al. 1996; Harman 2000; Kong and Hong 2017; Polizzi and Vitale 2002; Vitale et al. 2012; Yang and Hong 2018a; Yang et al. 1995). Although numerous *Trichoderma* spp., including the widely studied *Trichoderma harzianum* strain T22, are currently registered as commercial bioformulations in

Table 3. Biocontrol agents worldwide employed against *Calonectria* infections under containerized or field conditions on boxwood and other ornamental plants^a

Active ingredient	<i>Calonectria</i> spp. (host)	Efficacy ^b	Application mode	Reference
<i>Trichoderma harzianum</i> T22	<i>C. pauciramosa</i> (Feijoa sellowiana, Polygala myrtifolia) ^{C&N}	3	Soil drench	Polizzi and Vitale 2002; Vitale et al. 2012
<i>T. koningiopsis</i> Mb2	<i>C. pseudonaviculata</i> (Buxus spp.) ^{C&N}	3	Foliar spray	Kong and Hong 2017
<i>Pseudomonas protegens</i>	<i>C. pseudonaviculata</i> (Buxus spp.) ^C	3	Foliar spray	Yang and Hong 2018a
<i>Burkholderia cepacia</i>	<i>C. pseudonaviculata</i> (Buxus spp.) ^C	3	Foliar spray	Kong and Hong 2020
<i>Pseudomonas lactis</i>	<i>C. pseudonaviculata</i> (Buxus spp.) ^N	3	Foliar spray	Kong 2019
<i>Bacillus amyloliquefaciens</i> subsp. <i>plantarum</i> D747	<i>C. cylindrospora</i> , <i>C. pauciramosa</i> , <i>C. mexicana</i> , <i>C. pseudomexicana</i> , (<i>Callistemon</i> spp., <i>Metrosideros excelsa</i> , <i>Dodonaea viscosa</i>) ^{C&N}	2	Foliar spray	Cinquerrui et al. 2017
<i>B. subtilis</i> QST713	—	2	—	—
<i>Streptomyces griseoviridis</i> K61	—	1 to 2	—	—
<i>T. asperellum</i> TV1	—	1 to 2	—	—
<i>T. atroviride</i> T11 plus <i>T. asperellum</i> T25	—	1 to 2	—	—
<i>T. harzianum</i> ICC012 plus <i>T. viride</i> ICC080	—	1 to 2	—	—
<i>T. harzianum</i> T22	—	1 to 2	—	—
<i>B. amyloliquefaciens</i> subsp. <i>plantarum</i> D747	Disease complex (<i>C. pauciramosa</i> , <i>C. pseudomexicana</i> , <i>Fusarium oxysporum</i> , <i>Neocosmospora solani</i> , and <i>Rhizoctonia</i> AG-R) (<i>Polygala myrtifolia</i>) ^{C&N}	1 to 2	Soil drench	Aiello et al. 2020b
<i>B. amyloliquefaciens</i> (<i>B. subtilis</i>) QST713	—	1 to 2	—	—
<i>Streptomyces</i> K61 (<i>S. griseoviridis</i>)	—	1 to 2	—	—
<i>T. gamsii</i> (<i>T. viride</i>) ICC080 plus <i>T. asperellum</i> (<i>T. harzianum</i>) ICC012, T25 and TV1	—	1 to 2	—	—
<i>T. harzianum</i> strains T-22 and 908	—	1 to 2	—	—
<i>T. atroviride</i> (<i>T. harzianum</i>) T11 and IMI206040 plus <i>T. asperellum</i> (<i>T. harzianum</i>) ICC012, T25 and TV1	—	1 to 2	—	—
<i>Pseudomonas chlororaphis</i> strain MA312	—	1 to 2	—	—
<i>T. asperellum</i> (<i>T. harzianum</i>) ICC012, T25 and TV1	—	1 to 2	—	—

^a Superscript abbreviations: C = containerized-grown experiments, N = in-field experiments, and phy = phytotoxicity effects.

^b Scale of 1 = ineffective, 2 = moderate efficacy, and 3 = effective.

Europe and the United States, there remains a significant gap in effectiveness between synthetic chemical fungicides and BCAs (Cinquerrui et al. 2017; Daughtrey and Benson 2005; Yang and Hong 2017). A list of BCAs is provided in Table 3. Consequently, the use of BCAs should be thought of as one component of an integrated pest management (IPM) strategy in combination with other measures, including fungicide application.

Outlook

The large number of described species observed in the genus *Calonectria* and emergence of new pathogen species such as the causal agents of boxwood blight suggest that *Calonectria* diseases will continue to be a challenge for ornamental horticulture industries in Europe and the United States. Research continues to address important questions about the biology and management of *Calonectria* spp. as pathogens of ornamental plants. However, many aspects of these pathosystems still remain poorly understood.

Resistant commercial cultivars are needed for long-term sustainable management of *Calonectria* diseases on ornamental plants. There are high levels of disease susceptibility among popular commercial cultivars of ornamental plants, including those in the genera *Buxus*, *Pachysandra*, and *Spathiphyllum*. In addition to traditional breeding, transfer of genetic material that confers disease resistance using genetic engineering can help overcome challenges associated with the time needed to breed long-lived perennial ornamental plants (Chandler and Sanchez 2012; Hammond et al. 2006). Further evaluation and integration of disease resistant cultivars with integrated chemical, biological, and cultural controls will provide additional options for management of *Calonectria* diseases on ornamental plants in nursery production systems and the landscape.

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