Offered Review

CALONECTRIA DISEASES ON ORNAMENTAL PLANTS IN EUROPE AND THE MEDITERRANEAN BASIN: AN OVERVIEW

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SUMMARY

Species of *Calonectria* and their cylindrocladium-like asexual morphs are important plant pathogens of agronomic and forestry crops, especially in the tropical and subtropical regions of the world. *Calonectria* species have been associated with a wide range of disease symptoms on a large number of plant hosts. On horticultural crops, most records of *Calonectria* species come from the Northern Hemisphere, where they occur mainly in gardens and ornamental nurseries. In Europe and the Mediterranean basin, several species are widespread in nurseries and cause extensive damage to ornamental plants. In the past, identification of species was based on phenotypic characters and sexual compatibility using standardised media. More recently, morphological characteristics, phylogenetic studies (DNA sequence data of the β-tubulin, histone H3 and translation elongation factor-1α gene regions) and mating studies have revealed the presence of several cryptic species complexes that were formerly treated as single *Calonectria* species. These studies resulted in the introduction of several new species. Other studies aimed at understanding environmental sustainability focused attention on soil solarisation and biological control as means for controlling these pathogens. The potential use of biological control agents (BCAs) and chemicals for controlling Calonectria-induced diseases has recently been addressed. In this review we discuss the *Calonectria* species detected in Europe and the Mediterranean basin, and the disease management strategies. In view of the mandatory implementation of integrated pest management (IPM) for all European countries by 2014, this paper provides basic information as a platform for the adaptation of more sustainable integrated measures to control Calonectria diseases in European nurseries.

Key words: *Calonectria*, *Cylindrocladium*, epidemiology, identification, integrated control strategies, ornamental nursery, phylogeny, sexual compatibility, sustainable plant production, taxonomy.

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INTRODUCTION

Cut-flowers and ornamental plants are important economic commodities in European countries. Europe accounts for *ca.* 10% of the area given over in the world to cut-flowers and ornamental plant production. In the European Union increasing levels of production result in one of the world's highest densities of cut-flower and ornamental plant production per hectare i.e. 44% of global cut-flower and ornamental plant production. The total nursery production area was estimated to be approximately 71,000 ha in 2010 with a production value of about € 19.7 billion, concentrated in the Netherlands (32.8%), Italy (13.4%), Germany (11.9%), France (11.9%), Spain (10.6%) and UK (5.5%) (http://ec.europa.eu/agriculture/flowers/index_en.htm).

Based on literature reports and a 20-year period of field observations, *Calonectria* spp. are considered key pathogens of many nursery ornamental plants as listed in Table 1.

Calonectria (*Ca.*) is placed in the Nectriaceae, one of three families of Hypocreales (Rogerson, 1970; Rossman, 1983, 1996; Rossman *et al.*, 1999; Hirooka *et al.*, 2012). This genus is distinguished from the other hypocrealean genera based on its cylindrocladium-like asexual morphs that are commonly encountered in nature (Crous, 2002).

The genus *Calonectria* was erected in 1867 by the Italian cryptogamic botanist Giuseppe De Notaris (1805-1877) based on *Ca. daldiniana* De Not., collected on dead leaves of *Magnolia grandiflora* in Locarno (Switzerland). Over a century later, Rossman (1979) reduced *Ca. daldiniana* to synonymy under *Ca. pyrochroa* (Desm.) Sacc., then establishing (Rossman, 1983) that *Ca. pyrochroa* could be linked to the asexual morph *Cy. ilicicola* (Hawley) Boedijn et Reitsma based on isolations obtained from *Pittosporum undulatum* collected in Madeira (insular Portugal). However, Lechat *et al.* (2010), showed that this asexual morph is linked to *Ca. lauri* (Vanderw.) Lechat et Crous. Therefore no asexual morph is presently linked to *Ca. pyrochroa*.

The asexual genus *Cylindrocladium* was first described by Morgan (1892) based on *Cy. scoparium* Morgan, which was collected as saprobe from a dead pod of honey locust

Table 1. *Calonectria* species and symptoms detected on ornamental plants in Europe and Mediterranean basin.

Family	Host plant	Symptoms	Calonectria species	Country	Reference
Anacardiaceae	*Pistacia lentiscus*	CRR, DE, LS, SL	*Ca. morganii*, *Ca. pauciramosa*	Italy	Vitale and Polizzi, 2007, 2008
Aquifoliaceae	*Ilex aquifolium*	LS	*Ca. lauri*	France, The Netherlands	Lechat *et al.,* 2010
Araceae	*Spathiphyllum* sp.	CRR, PL	*Ca. spathiphylli*	Italy	Carrai and Garibaldi, 1990
Araliaceae	*Hedera helix*	LS	*Ca. hederae*	UK, France	Both and Murray, 1960; Peerally, 1974
Arecaceae	*Brahea armata, B. edulis, Chamaerops humilis*	LS	*Ca. pauciramosa*	Italy	Polizzi *et al.*, 2007a
Buxaceae	*Buxus sempervirens, B. microphylla, B. sinica*	DE, LS, SL	*Ca. pseudonaviculata*	UK	Henricot *et al.*, 2000; Henricot and Culham, 2002; Henricot *et al.*, 2008
	B. sempervirens	CRR	*Ca. lauri*	Belgium	Lechat *et al.,* 2010
Ericaceae	*Arbutus unedo*	CRR, LS, SL	*Ca. pauciramosa*	Italy	Polizzi and Crous, 1999; Vitale *et al.*, 2009a
	A. unedo	LS	*Ca. polizzii*	Italy	Lombard *et al.,* 2010b
	Azalea hybrids	CRR	*Ca. morganii*	Belgium	de Prest and Poppe, 1988
	Calluna vulgaris, Erica spp.	CRR	*Ca. morganii*	the UK	Litterick and McQuilken, 1998
	Rhododendron hybrids	CRR	*Ca. morganii*	Germany, The Netherlands	Heimann, 1976 Rattink, 1973
Fabaceae	*Acacia retinodes*	LS, SL	*Ca. pauciramosa*	Italy	Polizzi and Crous, 1999
Lauraceae	*Laurus nobilis*	CRR	*Ca. ilicicola*	Italy	Polizzi *et al.*, 2012
	L. nobilis	W	*Ca. lauri*	UK	Brayford and Chapman, 1987; Crous *et al.,* 2006
Myrtaceae	*Callistemon* spp.	LS	*Ca. polizzii*	Italy, Tunisia	Lombard *et al.*, 2010b, 2011
	Callistemon spp.	LS	*Ca. mexicana*, *Ca. pseudomexicana*, *Ca. tunisiana*	Tunisia	Lombard *et al.*, 2011
	Eucalyptus spp.	LS	*Ca. pauciramosa*	Italy	Polizzi, 1996; Polizzi and Crous, 1999
	Eugenia myrtifolia	CRR	*Ca. pauciramosa*	Italy	Polizzi *et al.*, 2009c
	Feijoa sellowiana	CRR, LS	*Ca. pauciramosa*	Italy	Polizzi and Crous, 1999; Vitale *et al.*, 2008
	Melaleuca acuminata	DE, LS, SL	*Ca. morganii*	Italy	Polizzi *et al.*, 2009b
	Melaleuca spp.	CRR, DE, LS, SL	*Ca. pauciramosa*	Italy	Polizzi and Crous, 1999; Polizzi *et al.* 2009c
	Metrosideros spp.	DE, LS	*Ca. pauciramosa, Ca. polizzii*	Italy, Tunisia	Polizzi and Crous, 1999; Lombard *et al.*, 2011
	Myrtus communis	CRR, DE, LS, SL	*Ca. pauciramosa*	Italy, Portugal	Polizzi and Azzaro, 1996; Henricot and Beales, 2003
	M. communis	CRR, DE, LS, SL	*Ca. mexicana, Ca. polizzii, Ca. pseudomexicana, Ca. tunisiana*	Tunisia	Lombard *et al.*, 2011
Pteridaceae	*Adiantum* sp	LS, PL	*Ca. rumohrae*	The Netherlands	Crous *et al.*, 1999
Polygalaceae	*Polygala myrtifolia*	CRR	*Ca. pauciramosa*	Italy, Spain	Polizzi and Crous, 1999; Pérez Sierra *et al.*, 2006
Rhamnaceae	*Ceanothus thyrsiflorus*	CRR	*Ca. pauciramosa*	Italy	Polizzi *et al.*, 2006a
	C. thyrsiflorus	SL	*Ca. pauciramosa*	UK	Lane *et al.*, 2006
Rosaceae	*Rosa* hybrids	CRR	*Ca. morganii*	UK	Storey, 1964
Sapindaceae	*Dodonaea viscosa*	LS	*Ca. pauciramosa*	Italy	Polizzi and Catara, 2001
	D. viscosa	LS	*Ca. mexicana, Ca. polizzii, Ca. pseudomexicana, Ca. tunisiana*	Tunisia	Lombard *et al.*, 2011

CRR = crown and root rot; DE = defoliation; LS = leaf spot; PL = petiole lesion; SL = stem lesion; W = wilting.

(*Gleditsia triacanthos*) in Ohio (USA). Representative isolates of *Calonectria* were initially considered to be saprobic (Graves, 1915). The first record of these fungi as serious pathogens of greenhouse-grown roses was by Massey (1917). Extensive reports exist on the pathogenicity of several other *Calonectria* species documenting symptoms such as damping off, root rot, crown rot, crown and stem canker, fruit rot, tuber rot, and leaf spot. The host range includes about 100 families and approximately 335 plant species (Crous, 2002; Lombard *et al*., 2010a).

The aim of this review is to present an overview of the genus *Calonectria* and their cylindrocladium-like morphs with emphasis on species detected on ornamental plants in Europe and the Mediterranean basin, and their disease management in nurseries.

HISTORY AND IMPORTANCE OF CALONECTRIA DISEASES IN EUROPE AND THE MEDITERRANEAN BASIN

Species of *Calonectria* were little kown in Europe and the Mediterranean basin until the 1960's, when Booth and Murray (1960) described a leaf spot of *Hedera helix* caused by *Ca. hederae* G. Arnaud ex C. Booth. et D. Murray. Later, Storey (1964) detected damping-off of rose cuttings caused by *Ca. morganii* Crous, Alfenas et M.J. Wingf. (as *Cy. scoparium*), a pathogen from North America (Crous and Wingfield, 1994), which was apparently brought to Europe with infected herbs imported from the USA (Overmeyer *et al*., 1996). In the following years, Rattink (1973) and Heimann (1976) reported the spread of *Ca. morganii* in other European countries (Germany and the Netherlands), causing blight and wilt of rhododendron and azalea.

Carrai and Garibaldi (1990) were first to identify *Ca. spathiphylli* Schoult., El-Gholl et Alfieri in Italy, following isolation from *Spathiphyllum* cv. Mauna Loa with symptoms of root and petiole rot. This pathogen is responsible for a devastating disease of *Spathiphyllum* cultivars in Europe. It was first introduced from Central or South America to North America (Chase and Poole, 1987), from where it moved to Europe (Crous, 2002). Since then, several species of *Calonectria* have been recorded in Europe or in the Mediterranean basin (Table 1).

The first report of a new disease of milkwort (*Polygala myrtifolia*) as well as the first detection of the causal agent, *Ca. pauciramosa* C.L. Schoch et Crous (= *Cy. pauciramosum* C.L. Schoch et Crous) in Italy and Europe, was published by Polizzi and Crous (1999). Initially, this fungus had been recovered from myrtle plants and identified as *Ca. morganii* (Polizzi and Azzaro, 1996). *Ca. pauciramosa* has a centre of origin in Central and South America and is well established in Australia, Europe, South Africa and the USA. Variability in the DNA sequence data from a set of *Ca. pauciramosa* isolates from Australia, California, Italy, South Africa and South America illustrated the possibility that more than one introduction of this species could have occurred into Italy from South Africa or other countries (Schoch *et al.,* 2001). In Europe, diseases caused by *Ca. pauciramosa* are a serious threat to nursery production in Portugal, Spain, and the UK (Henricot and Beales, 2003; Lane *et al*., 2006; Pérez Sierra *et al*., 2006, 2007). Since the early 1990s, diseases associated with *Ca. pauciramosa* have regularly been observed in most nursery areas of southern Italy, where this pathogen is well established in a broad and ever-expanding range of pot-grown hosts (Polizzi and Catara, 2001; Polizzi *et al*., 2006a; Vitale et *al*., 2008; Polizzi *et al*., 2009a, 2010). *Ca. pauciramosa* is a prevalent pathogen in commercial nurseries of southern Italy, and the cause of considerable losses to ornamental plants. Based on DNA sequence data, the previous report of *Ca. morganii* (as *Cy. scoparium*) from Europe may have been incorrect, as it possibly represented *Ca. pauciramosa* (Polizzi and Crous, 1999; Schoch *et al.,* 1999).

The first confirmation of *Ca. morganii* (as *Cy. scoparium*) in Europe was published by Polizzi *et al.* (2006b), who linked this pathogen to several new diseases (leaf spot, blight, and crown rot) of mastic trees (*Pistacia lentiscus*). Soon afterwards, Vitale and Polizzi (2008) provided the first evidence of the co-existence of *Ca. pauciramosa* and *Ca. morganii* in the same host. Subsequently, *Ca. morganii* became established in Sicilian ornamental nurseries (southern Italy) and its presence was documented on new plant hosts or cultivars (Polizzi *et al*., 2007b, 2009b).

Since 1994, a new blight disease was observed on *Buxus sempervirens* in Hampshire (UK) by Henricot *et al.* (2000) who, based on DNA sequence and morphological data, suggested that a new species could be the cause of the disease. The causal agent was described as *Ca. pseudonaviculata* L. Lombard, M.J. Wingf. et Crous (= *Cy. pseudonaviculatum* Crous, J.Z. Groenew. et C.F. Hill) by Crous *et al.* (2002) and was later reported from New Zealand (Crous *et al*., 2004). However, in December 2002 Henricot and Culham (2002) described this species as *Cy. buxicola* Henricot and, recently, a proposal to conserve the name *Cy. buxicola* against *Ca. pseudonaviculata* has been filed by Henricot *et al.* (2012). The origin of this new fungal species remains unknown, but it was hypothesized that it was first introduced into Europe, then to New Zealand (EPPO, 2004). Cylindrocladium boxwood blight is one of the main diseases of *Buxus* spp. To date, the disease has been reported on *B. microphylla*, *B. sempervirens,* and *B. sinica* (Henricot *et al*., 2008) and more recently on *B. colchica* (Gorgiladze *et al*., 2011), and also on genera such as *Sarcococca* and *Pachysandra* (Henricot *et al.,* 2008; LaMondia *et al.,* 2012). In Europe, the pathogen is widespread in many countries including Austria, Belgium, Croatia, Czech Republic, France, Germany, Italy, the Netherlands, Slovenia, Spain, and Switzerland (Crepel and Inghelbrecht, 2003; Brand, 2005; CABI, 2007; Henricot *et al*., 2008; Saracchi *et al*., 2008; Benko Beloglavec *et al*., 2009; Varela

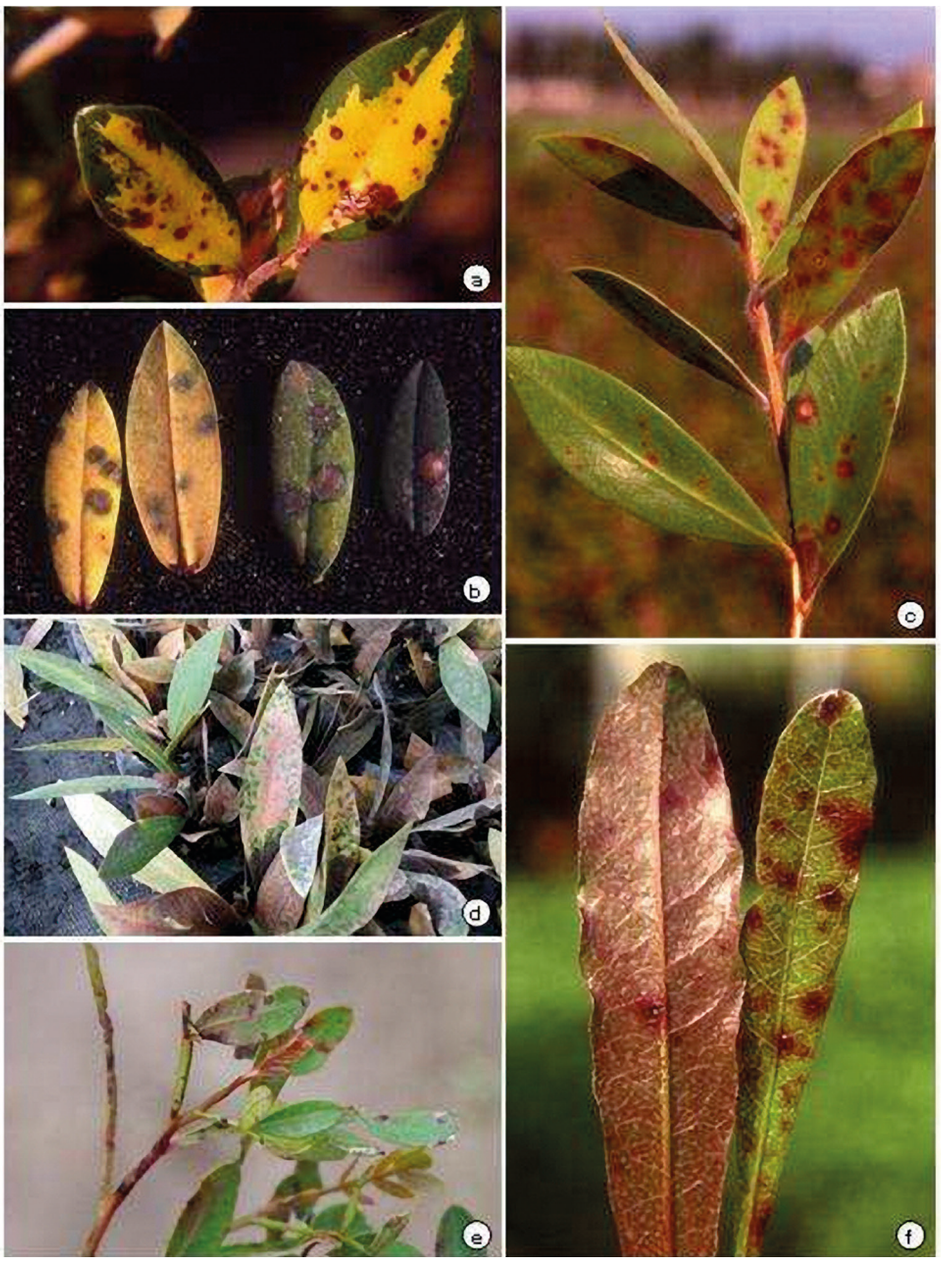

Fig. 1. Disease symptoms caused by *Calonectria morganii, Ca. pauciramosa* and *Ca. polizzii* on ornamental hosts: (a) leaf spots on *Metrosideros excelsa*; (b) leaf spots on *Melaleuca hypericifolia*; (c, d) leaf spots on *Callistemon citrinus*; (e) leaf spots and stem lesions on *Pistacia lentiscus* caused by coexisting infections due to *Ca. morganii* and *Ca. pauciramosa*; (f) leaf spots on *Dodonaea viscosa*.

et al., 2009; Cech *et al*., 2010; Šafránková *et al*., 2012). *Ca. pseudonaviculata* (syn. *Cy. buxicola*) was included on the EPPO alert list (EPPO, 2004), but has subsequently been removed (EPPO, 2008).

Calonectria ilicicola Boedijn et Reitsma (= *Cy. parasiticum* Crous, M.J. Wingf. Et Alfenas) induces peg, pod, and root necrosis of peanuts, but also leaf spot, damping-off, blight, crown and root rot of several plant hosts (Crous, 2002). In Europe, *Ca. ilicicola* was first reported on *Nerium oleander* in the UK (Crous, 2002), but this report could not be substantiated. The first report of the occurrence of a disease caused by *Ca. ilicicola,* supported by molecular data, was published by Polizzi *et al.* (2012), who demonstrated *Ca. ilicicola* to be the causal agent of a new crown and root rot disease on potted *Laurus nobilis* plants. Formerly, Brayford and Chapman (1987) had reported a wilting disease of *L. nobilis* in nurseries on the Isles of Scilly (UK). The causal agent was identified as *Cy. ilicicola* (now known as *Ca. lauri*), but incorrectly linked to the sexual morph *Ca. ilicicola*. Later, based on molecular comparison of the ex-type strain, Crous *et al.* (1993) showed *Ca. ilicicola* to be linked to the asexual morph *Cy. parasiticum*. *Calonectria lauri* has since been isolated from infected leaves of *Ilex aquifolium* in France and the Netherlands, as well

as from roots of *B. sempervirens* in Belgium (Lechat *et al*., 2010). New species of *Calonectria* related to *Ca. scoparia* (= *Cy. candelabrum*) have been described based on multigene phylogenetic analyses, morphological characters, and mating compatibility (Lombard *et al*., 2010b). Noteworthy is the species *Ca. polizzii* L. Lombard, Crous et M.J. Wingf., which was detected on *Arbutus unedo* and *Callistemon citrinus* plants showing leaf spot symptoms collected in 1997 in Sicily, although its pathogenicity was not confirmed at that time (Lombard *et al*., 2010b). Pathogenicity to several ornamental plants was established later and the fungus was also found to occur in Tunisia (Lombard *et al*., 2011) and sporadically in Italy (Aiello *et al*., 2013). Recent DNA analyses revealed that *C. polizzii* is widespread throughout Italy, where it occurs on several new plant hosts (Vitale *et al*., 2013).

During a survey conducted in an ornamental nursery in Tunisia, two previously undescribed *Calonectria* spp., *Ca. pseudomexicana* L. Lombard, G. Polizzi, et Crous and *Ca. tunisiana* L. Lombard, G. Polizzi, et Crous, were recognised (Lombard *et al*., 2011) and determined to be closely related to *Ca. mexicana*. In the same survey, *Ca. mexicana* was found for the first time in the African continent and Mediterranean basin. These two species, together with *Ca. polizzii,* cause crown and root rot and leaf spot on seedlings of *Callistemon* spp., *Dodonaea viscosa*, *Metrosideros* spp. and *Myrtus communis*.

A list of *Calonectria* species, their main ornamental host plants, symptoms, geographic locations and references in Europe and the Mediterranean basin is provided in Table 1. The host plants reported are common species produced in nurseries.

SYMPTOMS

Calonectria species can cause devastating diseases, especially on young plants grown in greenhouses, shade houses and in the open field. The symptoms may include leaf spot, stem lesion, stem or crown canker, root rot, crown rot and stem rot (Fig. 1 and 3). As a consequence of crown, root and stem rots, plants also display symptoms of damping-off, wilting, stunting, chlorosis, or shedding of the leaves (Fig. 2 and 3). On ornamental species such as *P. myrtifolia*, *A. unedo* and *Feijoa sellowiana* crown rot infections can progress slowly over time (G. Polizzi, unpublished information). These plants may be asymptomatic for some years prior to collapse. Thus, *Calonectria* crown and root infections, which start early during plant growth, can result in severe economic losses. Stem lesions, stem cankers or severe leaf spots usually lead to shoot blight. During the propagation of cuttings, the infection usually starts at the lower ends of the stems shortly followed by profuse sporulation. On seedlings, symptoms consist of a water-soaked constriction at the soil level, leading to collapse within 1-2 weeks from emergence. Leaf spots caused by *Calonectria* spp. are characterised by minute brown or purple spots. They first appear as water-soaked lesions, turning light to dark brown, surrounded by a red, dark brown or purple border and a chlorotic zone. Young, non-lignified, terminal shoots often exhibit dieback symptoms or lesions similar to those on the leaves. Under warm and wet conditions, leaf spots may coalesce and cause blighting. On *Melaleuca acuminata, M. hypericifolia*, *Myrtus communis* and *Pistacia lentiscus,* Calonectria diseases can lead to total plant defoliation. For some heterothallic species and most homothallic species, the sexual stage can be detected on symptomatic tissues (Fig. 3) (Vitale and Polizzi, 2007; Polizzi *et al*., 2012).

DIAGNOSIS

Morphological identification. The monograph of *Cylindrocladium* by Crous and Wingfield (1994) emphasized the importance of asexual state characteristics in the taxonomy of *Calonectria*. Identification of species was previously based on morphological characteristics and sexual compatibility using standardised media (Boedijn and Reitsma, 1950; Peerally, 1991; Crous *et al*., 1992). Carnation leaf agar (CLA) has widely been used to study *Calonectria* spp. in culture (Fisher *et al.,* 1982; Crous, 2002). More recently, synthetic nutrient-poor agar (SNA; Nirenburg, 1981; Lombard *et al.,* 2009, 2010b) and minimal salt agar (MSA; Guerber and Correll, 2001; Halleen *et al*., 2006; Lombard *et al*., 2010b) with sterile toothpicks have been used to induce the morphological characters necessary for diagnosis. The latter technique successfully induced the sexual state in culture (Lombard *et al*., 2010b).

Morphological characteristics of the asexual state needed for species identifications include vesicle shape, stipe extension length and macroconidial septation and dimensions (Boesewinkel, 1982; Peerally, 1991; Crous and Wingfield, 1994; Crous, 2002). The sexual *Calonectria* morph provides less informative morphological information for identification. However, ascospore septation and dimensions, ascospore number within the asci and perithecial colour are relevant characters.

Recently, synoptic and dichotomous keys to species of *Calonectria* have been provided by Lombard *et al.* (2010c). In closely related species, a high degree of plasticity is found in some characters, which makes identification problematic (Schoch *et al*., 1999) and therefore sequence data is needed to confirm morphological diagnoses.

Lombard *et al.* (2010b) found that morphologically, *Ca. polizzii* can be distinguished from *Ca. pauciramosa* by its smaller 1-septate macroconidia (Lombard *et al*., 2010b). Using a larger set of cultures, Guarnaccia (2012) observed no differences in macroconidia dimensions between *Ca. polizzii* and *Ca. pauciramosa*. As a further example, *Ca. pseudomexicana* is morphologically similar to *Ca. mexicana,* but can be distinguished by having four or less

conidiophore branches (Lombard *et al.,* 2011) while *Ca. mexicana* has five (Schoch *et al.,* 1999). *Calonectria tunisiana* is similar to *Ca. mexicana* and *Ca. pseudomexicana*, but can be distinguished from both taxa by its shorter stipe extensions and fewer fertile branches (Lombard *et al*., 2011).

Optimal growth temperatures are mostly determined on 2% malt extract agar (MEA) at 5-35°C in 5-7°C intervals, in the dark. Colony colours are determined after 7 days on MEA at 25°C in the dark. Based on cardinal growth temperatures, *Calonectria* species have been classified as low-temperature (min. below 10°C, max. not above 30°C), moderate-temperature (between 10 and 30°C), eurithermal (min. below 10°C, max. above 30°C), and high-temperature (min. 10°C or above, max. above 30°C) (Crous, 2002). The majority of *Calonectria* spp. are eurithermal (*Ca. morganii*, *Ca. pauciramosa*, *Ca. polizzii*, etc.) or high-temperature species (*Ca. mexicana*, *Ca. spathiphylli*, *Ca. ilicicola*, etc.), whereas *Ca. pseudonaviculata* grows well both at low (min. above 5°C) and high temperature (max. below 35°C) (Crous *et al*., 2002).

Mating compatibility and strategies. Several species of *Calonectria* reported from Europe have a homothallic mating system i.e. they produce perithecia containing viable ascospores from a single isolate. For example, a single-spored isolate of *Ca. ilicicola* is able to produce perithecia in culture (Alfieri *et al*., 1982) and its sexual morph is often observed on infected plant tissue in nature (Lechat *et al*., 2011). For some species of *Calonectria* such as *Ca. pseudonaviculata*, no sexual morph has been observed *in vitro* or *in vivo* although phylogenetic inference place these species in the genus *Calonectria* (Lombard *et al.,* 2010c).

Other species of *Calonectria* including *Ca. morganii*, *Ca. pauciramosa,* and *Ca. spathiphylli* are self-sterile hermaphrodites and therefore heterothallic. Female structures consist of protoperithecia, which can be spermatised by conidia or hyphae from an opposite mating type. Normally, protoperithecia are formed within 2-3 weeks producing perithecia with viable ascospores within 4-8 weeks. Perithecia of heterothallic species are not often encountered in nature.

Both homo- and heterothallic mating systems have been identified in the same species complexes (Alfieri *et al*., 1982; Schubert *et al*., 1989; Crous and Wingfield, 1994; Crous *et al*., 2004). The *Ca. scoparia* species complex was initially regarded as having a biallelic, heterothallic mating system (Schoch *et al*., 1999, 2001). However, sexual compatibility tests between various isolates representing this species complex resulted in the identification of a homothallic species, *Ca. zuluensis* L. Lombard, Crous et M.J. Wingf. (Lombard e*t al.,* 2010b).

Multigene phylogeny and cryptic species. Phylogenetic inference using multigene sequence data has significantly changed the taxonomy of *Calonectria* by identifying species complexes. The first major phylogenetic study of *Calonectria* by Schoch *et al.* (2000) using β-tubulin (BTUB) sequence data only laid the foundation for this taxonomic approach. Subsequently multigene DNA sequence data relative to the nuclear ribosomal internal transcribed spacer (ITS), BTUB, Histone H3 (HIS3) and translation elongation factor 1-α (TEF-1α) were used in taxonomic studies of the genus *Calonectria*. Lombard *et al.* (2010c) provided the first comprehensive multigene data set of *Calonectria* employing seven gene regions [28S large subunit (LSU), actin (ACT), BTUB, calmodulin (CAL), HIS3, ITS and TEF-1α], and was able to identify 11 species complexes. Seven of these complexes contain species reported from Europe (Crous, 2002; Lombard *et al*., 2010b, 2010c), highlighting the importance of DNA sequence data for correct species identification.

Although past studies have shown that the ITS gene region provides limited information to separate *Calonectria* spp. (Schoch *et al*., 1999, 2001; Crous, 2002; Henricot and Culham 2002; Crous *et al*., 2004, 2006), it is still of vital importance as a diagnostic tool. This gene region has been formally accepted as the universal DNA barcode marker for *Fungi* (Schoch *et al*., 2012). Combination of ITS with BTUB and TEF-1α sequence data provides sufficient resolution for the identification of cryptic species within *Calonectria* species complexes.

BIOLOGY AND EPIDEMIOLOGY

Calonectria disease epidemics are classified as polycyclic. Thus, the major disease management strategies include strategies for reduction of both initial inoculum and infection rates. Primary inoculum consists of chlamydospores forming microsclerotia in soil and plant debris, that are able to overwinter for extended periods (Crous, 2002). Although low temperatures and severe drought conditions reduce the number of viable microsclerotia (Thies and Patton, 1970; Sung *et al*., 1980), they can survive for up to 15 years when buried in the soil (Thies and Patton, 1970; Sobers and Littrell, 1974; Phipps and Beute, 1977, 1979; Crous, 2002). In ornamental nurseries, inoculum spreads mostly through the movement of water, and infection occurs when water splashes conidia originating from microsclerotia or infected host tissue, onto potting mixes and plants. Disease severity is most prevalent under warm, wet and humid conditions. Spread via aerial dissemination (conidia and ascospores) has also been recorded (Crous, 2002). The environmental conditions necessary for shoot multiplication and cutting propagation and rooting in greenhouses, provide ideal conditions for infection by *Calonectria* spp. Furthermore, mist and overhead irrigation systems, as well as the use of plastic film employed for mulching in ornamental nurseries, increase the diffusion and severity of Calonectria infections. The recent trend of 'sustainable nursery production', i.e. obtaining substrates

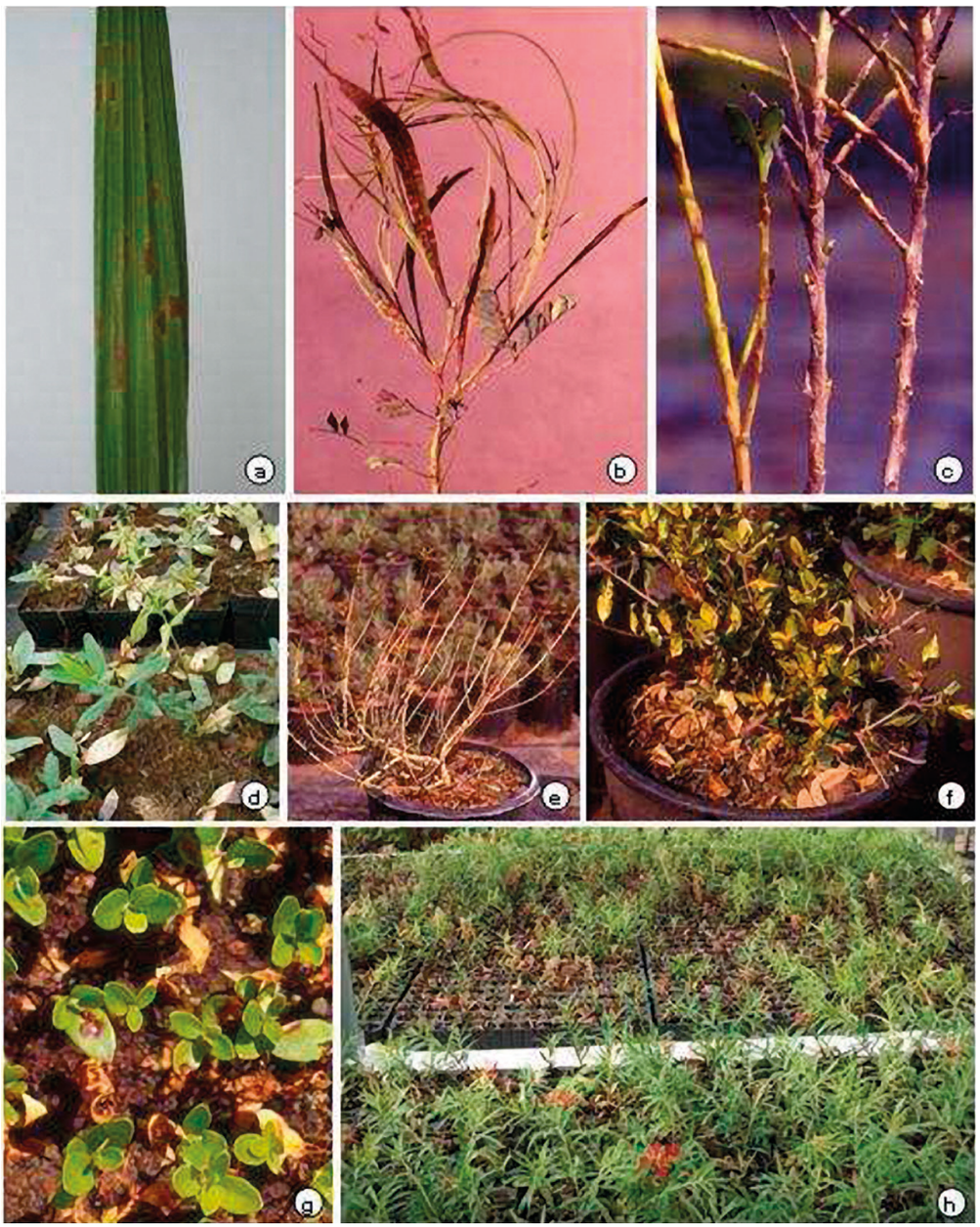

Fig. 2. Disease symptoms caused by *Calonectria morganii, Ca. pauciramosa* and *Ca. polizzii* on ornamental hosts: (a) leaf spots on *Chamaerops humilis*; (b) leaf spots and stem lesions on *Acacia retinodes*; (c) stem lesions on *Myrtus communis*; (d) damping-off of *Polygala myrtifolia*; (e) total defoliation of *M. communis*; (f) leaf spots and defoliation on *Metrosideros excelsa*; (g) leaf spots and damping of on *Feijoa sellowiana* seedlings; (h) damping-off of *Callistemon citrinus* cuttings.

for pot-grown plants from recycled and composted organic materials (Chong, 2005; Walters, 2009), could also increase infection risks by these pathogens in ornamental nurseries (Noble and Roberts, 2004).

DISEASE MANAGEMENT

Management of Calonectria diseases in the nursery is very complex and depends on an integrated control strategy. Since only preventative measures are effective for Calonectria disease control, chemical applications should always be adopted in association with good nursery practices. These practices include reduction of primary inoculum, use of resistant and disease-free plant species or cultivars for propagation, removal of infected plants, and utilisation of uncontaminated potting medium (Crous, 2002). The use of other sustainable strategies, such as soil solarisation or biological control agents, can also improve disease control (Vitale *et al.,* 2012a, 2013). With the mandatory implementation of an integrated pest management (IPM) system for European countries by 2014, a more sustainable integrated system is required to prevent Calonectria infections in nurseries (Anonymous, 2011).

Cultural management. Spread of Calonectria diseases between nurseries occurs via movement of infected plants, infested plant debris, soil or equipment. Every precaution should be taken to exclude Calonectria diseases from the nursery during the growing stage i.e. seeding, rooting, and pot-transplanting. The use of disease-free plant material, soil and equipment is the first step to avoid introduction of fungal pathogens into nurseries during plant propagation. To control Calonectria diseases during the rooting stage of cuttings, efforts must be made to reduce primary inoculum through the use of healthy shoots and adopting management practices such as selective and continuous shoot harvesting, and the use of inoculum-free trays (Silveira, 1996). Nurserymen must systematically inspect plants and propagative materials to prevent pathogen introduction. A drip irrigation system

Fig. 3. Disease symptoms and signs associated with *Calonectria* species on ornamental hosts: (a) death of *Callistemon* cuttings during the first stage of propagation due to *Ca. morganii*; (b) wilting of *Polygala myrtifolia* plants caused by mixed infection of *Ca. pauciramosa* and *Ca. polizzii*; (c) crown rot and root rot of *Arbutus unedo* associated to *Ca. pauciramosa*; (d) crown and root rot of *Laurus nobilis* caused by *Ca. ilicicola*; (e) mycelium of *Ca. morganii* on *Callistemon* cutting; (f) crown rot and mycelium of *Ca. ilicicola* on *L. nobilis*; (g) crown rot and perithecia of *Ca. ilicicola* on *L. nobilis*; (h) perithecia of *Ca. pauciramosa* on crown and stem of *Pistacia lentiscus* seedlings.

that does not create excess moisture or humidity is recommended. Overhead irrigation generates optimal conditions for disease development and should be avoided. Once Calonectria diseases are established in production nurseries, regular use of fungicides is required (Crous, 2002). In Italy, Calonectria diseases are controlled by use of fungicides in association with good nursery practices, which include immediate removal of diseased plants, use of new and uninfected potting medium, and minimal watering.

Since chemical control is expensive, development and use of resistant cultivars is the most desirable solution to managing Calonectria diseases. Of the species and cultivars of *Spathiphyllum* that were tested for susceptibility to *Ca. spathiphylli*, only *S. floribundum* and *S. cannifolium* exhibited a high degree of resistance against infections (Schoulties and El-Gholl, 1983; Henny and Chase, 1986). Unfortunately, none of the commercial *Spathiphyllum* cultivars tested were immune to *Ca. spathiphylli* and the resistant species have limited ornamental value (Norman *et al*., 1999). In experimental trials, Henricot *et al.* (2008) showed that none of the 10 boxwood species and cultivars tested were resistant to Calonectria infections. *Buxus balearica* and *Sarcococca* sp. were the most resistant to the pathogens tested. In southern Italy field observations in commercial nurseries showed that *P. myrtifolia* var. *myrtifolia* was more susceptible than *P. myrtifolia* var. *grandiflora* to crown and root rot caused by *Ca. pauciramosa*. In addition, the hybrid *P. myrtifolia* x *oppositifolia* 'Bibi Pink' was found highly resistant to infections (D. Aiello and G. Polizzi, unpublished information). In a chemical trial conducted against foliar infection by *Ca. pauciramosa* on the botanical varieties '*baetica*' and '*lusitanica*' of *Myrtus communis,* a lower susceptibility of *M. communis* var. '*lusitanica*' was observed (Vitale *et al*., 2003).

Fungicide resistance and chemical management. Several authors have reported the effectiveness of benzimidazoles (MBCs) against *Calonectria* species (Barnard, 1984; Chase, 1987; Nan *et al*., 1992; Kucharek and Atkins, 1993). However, the continuous use of these fungicides in nurseries may result in the selection of resistant strains with a consequent decrease in the effectiveness of the fungicide (French and Menge, 1978; Alfenas *et al*., 1988). The constant use of benomyl induced the development of resistant strains of *Ca. morganii* at concentrations close to 1000 µg/ml (Alfenas *et al*., 1988). An experiment was carried out in a greenhouse containing cuttings of rhododendron to assess the presence of sensitive and resistant strains to carbendazim in a population of *Ca. morganii*. In conditions of low pathogen pressure, a treatment with prochloraz in the soil showed good control of sensitive and resistant fungal strains, whereas none of the tested compounds was effective in the presence of high inoculum levels (de Prest and Poppe, 1988). Resistance to benzimidazole has also been found in an Italian *Ca. morganii* population and the benomyl-resistant isolates were also resistant to carbendazim (Vitale *et al.,* 2009b).

In vitro trials have shown that the mycelial growth of six strains of *Ca. pauciramosa* was completely inhibited at concentrations of 1 µg/ml of active ingredient (a.i.) carbendazim, while 500 µg a.i./ml did not completely inhibit four other strains (Polizzi, 2000). Resistance to benomyl was observed in 58% of 200 strains of *Ca. pauciramosa* collected from different nurseries in southern Italy (Polizzi and Vitale, 2001). Many resistant strains grew at concentrations higher than 500 µg a.i./ml, while no growth was observed for sensitive strains at a concentration of 1 µg a.i./ml. Benomyl-resistant isolates showed cross-resistance to carbendazim. In addition, the highly resistant strains showed a slow-growing phenotype when compared to the sensitive fast-growing isolates (Polizzi and Vitale, 2001). Subsequently, some of these isolates were identified as *Ca. polizzii* (Vitale *et al*., 2013). More recently, MBCs-resistant isolates of *Ca. mexicana, Ca. polizzii, Ca. pseudomexicana* and *Ca. tunisiana* with MIC values >100 µg a.i./ml were found in Tunisia (Guarnaccia *et al*., 2012).

Considering the risks arising from the continuous use of benzimidazoles, an anti-resistance strategy should be implemented to limit their use, especially where the number of resistant isolates is high. Alternatively MBCs can be used in mixture with or in succession to other fungicides that have a different mode of action or low risk of resistance development.

In general, Calonectria diseases are difficult to control with fungicides and trials revealed that their efficacy against these pathogens varied depending on application procedures, the type of infection (leaf spot or crown and root rot), and the inoculum levels of the pathogens. At high inoculum levels, no fungicides provided complete protection from infection, especially when it occurred in the crown and root regions.

In the past, several fungicides have been proposed to control Calonectria diseases. They included chlorothalonil, copper compounds, MBCs and prochloraz (Crous, 2002). Repeated copper compounds application was found to induce phytotoxicity to some ornamental species (Barnard, 1984; Vitale *et al*., 2003). In addition, chlorothalonil or prochloraz show variable control of natural infections in nurseries (Polizzi and Azzaro, 1996; Crous, 2002; Polizzi and Vitale, 2002). A satisfactory activity against *Calonectria* spp. was disclosed by fludioxonil (Haralson *et al*., 2007), fosetyl-Al, prochloraz + cyproconazole, trifloxystrobin, azoxystrobin and K phosphite, whose efficay, however, varied with the type of infection (Aiello *et al*., 2013).

Soil fumigation with methyl bromide, chloropicrin, metham sodium and dazomet has been effective in reducing soil populations of some *Calonectria* spp. (Crous, 2002). We are working on the effects of different rates of metham sodium, dazomet and the new fumigant, dimethyl disulfide, on soil survival of microsclerotia of several *Calonectria* spp. These fumigants could replace methyl bromide

for the disinfection of soil and substrates in nurseries.

Biological control. According to European legislation on the "Sustainable Use of Pesticides", research should focus on efforts to implement non-chemical alternatives such as biological control of *Calonectria* spp. in nurseries. Little information exists on the use of biological control agents (BCAs) for an effective reduction of Calonectria infections in nurseries. Currently, bioformulates containing different *Trichoderma* spp. (*T. asperellum* TV1, *T. harzianum* strains Rifai T22 and ICC 012, *T. viride* ICC 080), applied exclusively as soil treatments, are commercially available for ornamental plants. Although some of these biological formulations are effective against specific pathogens (Hjeljord and Tronsmo, 1998), their efficacy against Calonectria infections is variable (Harman, 2000; Polizzi and Vitale 2002; Daughtrey and Benson, 2005; Vitale *et al.,* 2012). Calonectria infections on *Spathiphyllum* spp. were effectively managed using *T. harzianum* Rifai T22, although weekly applications were required (Harman, 2000). The antimicrobial activity was primarily assessed under laboratory or controlled conditions (Dumas *et al.,* 1996; Ngueko and Xu-Tong, 2002). Results were correlated with *Calonectria* species and specific isolate, application modes and timings (Yang *et al.,* 1995; Harman, 2000; Polizzi and Vitale 2002; Vitale *et al.,* 2012). *Trichoderma harzianum* strain T22 is the most widely studied among *Trichoderma* isolates (Daughtrey and Benson, 2005). Recently, *T. harzianum* strain T22 controlled isolates of *C. pauciramosa* on feijoa, although its efficacy depended on the virulence of the specific isolate of the pathogen (Vitale *et al.,* 2012). Other effective biological control applications against *Calonectria* spp., such as *Ca. morganii* on *Eucalyptus grandis* and *Ca. spathiphylli* on banana, involved the use of *Bacillus* isolates (Bettiol *et al.,* 1988; Santos *et al.,* 1993; de Wit *et al.,* 2009). In addition to these BCAs, pre-inoculation of plants with mycorrhizal fungi resulted in a significant decrease of the detrimental effects caused by Calonectria infections, in the case of *Ca. canadensis, Ca. morganii* and *Ca. spathiphylli* (Natarajan and Govindasamy, 1990; Morin *et al.,* 1999; Declerck *et al.,* 2002). Encouraging results were also obtained using silicon (Si) and alkaloids from *Catharanthus roseus* against *Ca. spathyphilli* and *Ca. morganii*, thus opening the way to non-chemical fungicide alternatives (Vermeire *et al.,* 2011; Vardhan *et al.,* 2012). However, their effectiveness is generally less than fungicide treatments (Aiello *et al.,* 2012). As mentioned, these biological and/or alternatives control measures could be used in combination with fungicides within IPM programmes.

Soil solarisation and Calonectria thermal sensitivity. *Calonectria* spp. are able to survive as primary inoculum in soil and potting media as resting structures, i.e. microsclerotia (Crous, 2002). The substrates infested by these fungi act as inocula, introducing these plant pathogens to susceptible ornamental hosts. *Calonectria* species could be eradicated from soil or soil-less media through chemical fumigation, heat treatment using steam, composting or with environmentally friendly approaches such as solarisation (Crous, 2002; Polizzi *et al.,* 2003; Walters, 2009). Little information is available on the effects of soil temperature and moisture on microsclerotia of *Calonectria* species (Phipps and Beute, 1977; Pataky and Beute, 1983; Crous, 2002; Kuruppu *et al.,* 2004). Recently, Vitale *et al.* (2013) demonstrated that solarisation performed in closed greenhouses during summer is able to suppress *Ca. pauciramosa* and *Ca. polizzii* microsclerotia, buried at 30 cm depth, within 6-9 days. This study showed that maintaining temperature above 35°C for an adequate duration can significantly reduce microsclerotia survival and that heat sensitivity may vary among *Ca. pauciramosa* and *Ca. polizzii* isolates.

CONCLUDING REMARKS

The ornamental industry is a dynamic economic sector of European agriculture, for whose improvement process and product innovations are crucial. Propagation and cultivation of foreign plants determine the movement of plant stocks and accompanying soil between nurseries around the world. With these stocks, *Calonectria* species have been introduced into Europe and today represent a great threat to the ornamental nursery industry of several European countries.

Diagnosis of Calonectria diseases and identification of their causal agents is required for implementing appropriate disease management strategies. Management of Calonectria diseases in ornamental crops can be extremely difficult and only preventative measures are effective. In addition, crop production practices in nurseries such as overhead irrigation, high humidity, prolonged leaf wetness, and poor light penetration, encourage the development of diseases due to Calonectria infections.

Various fungicides and different methods of application have been proposed to control *Calonectria* spp. in nurseries but they are expensive and are unable to control properly the development of epidemic Calonectria infections. Following the enforcement of the European Union directive according to which all EU countries will convert to the use of an IPM system in agricultural production by 2014, the use of chemicals will be reduced/adjusted as necessary and growers will be encouraged to use need-based pesticide doses and/or alternative control methods. To manage cutting and seedling attacks by *Calonectria* spp. during the rooting stage, chemical control should be adopted in association with good nursery practices, including reduction of primary inoculum, removal of infected plants, and utilisation of uncontaminated potting medium. The use of other control strategies such as solarisation or biological control agents according to European legislation on the sustainable use of pesticides, could further improve disease control.

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