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Short communication

Root and collar rot of milkwort caused by *Cylindrocladium pauciramosum*, a new record for Europe

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Abstract

A new disease of milkwort (*Polygala myrtifolia*) was observed on several commercial nurseries in southern Italy. Diseased plants showed wilting, stunting, chlorosis or loss of foliage, and rotting of the basal stem as well as the crown and roots. A *Cylindrocladium* species was consistently found associated with crown, basal stem, and root lesions. The etiology of this disease was proved on milkwort, by fulfilling Koch's postulates. Two hundred *Cylindrocladium* isolates were collected from the most important Sicilian and Calabrian ornamental production areas from different host plants. Isolates were identified as *Cylindrocladium pauciramosum* (teleomorph *Calonectria pauciramosa*) on the basis of their obpyriform to broadly ellipsoidal terminal vesicles, conidiophore branching pattern, conidium morphology, as well as mating type studies with tester strains of *C. pauciramosum* for selected isolates. This is the first record of this pathogen from Europe and it is the first report of *C. pauciramosum* on milkwort.

Milkwort (*Polygala myrtifolia* L.) is an attractive ornamental perennial shrub with rich purple flowers, of commercial importance in southern Italy. Cultivation is concentrated in warm regions (Calabria, Sardinia and Sicily), or more temperate areas as a potted plant. A severe disease of milkwort was observed in different nurseries of eastern Sicily in 1993 and has appeared each subsequent year. Diseased plants showed wilting, stunting, chlorosis, or loss of foliage. Extensive necrotic areas were observed on the crowns and roots. Sometimes the necrotic areas in the crowns developed into basal stem cankers. Girdling appeared related to the rapid collapse of the aerial portions of the plant. A *Cylindrocladium* Morgan species was consistently isolated from diseased milkwort tissues.

Cylindrocladium spp. are reported worldwide causing crown and root rot, stem canker, leaf spot, seedling and shoot blight as well as post-harvest fruit decay of numerous hosts, especially under humid conditions (Cordell et al., 1971; Sobers and Alfieri, 1972; Bertus, 1976; Alfenas et al., 1979; Mims et al., 1981; Mohanam and Sharma, 1985; Boesewinkel, 1986; Sepiah, 1990; Crous et al., 1991; El-Gholl et al., 1993). Species of *Cylindrocladium* (*Cy*.) are identified primarily on the basis of conidium, vesicle, conidiophore and culture characteristics (Peerally, 1991; Crous et al., 1992) as well as their *Calonectria* (*Ca*.) teleomorphs (El-Gholl et al., 1986; Peerally, 1991; Crous and Wingfield, 1994). In closely related species, a high degree of plasticity is found in some of these characters, which makes identification difficult (Schoch et al., 1999). A species complex that is beset with taxonomic problems includes *Cy. scoparium* Morgan (teleomorph *Ca. morganii* Crous, Alfenas and M.J. Wingf.) and *Cy. candelabrum* Viégas (teleomorph *Ca. scoparia* Peerally). *Cy. scoparium* was circumscribed as having ellipsoidal to pyriform or clavate vesicles (widest above the middle) (Figure 1), while those of *Cy. candelabrum* were

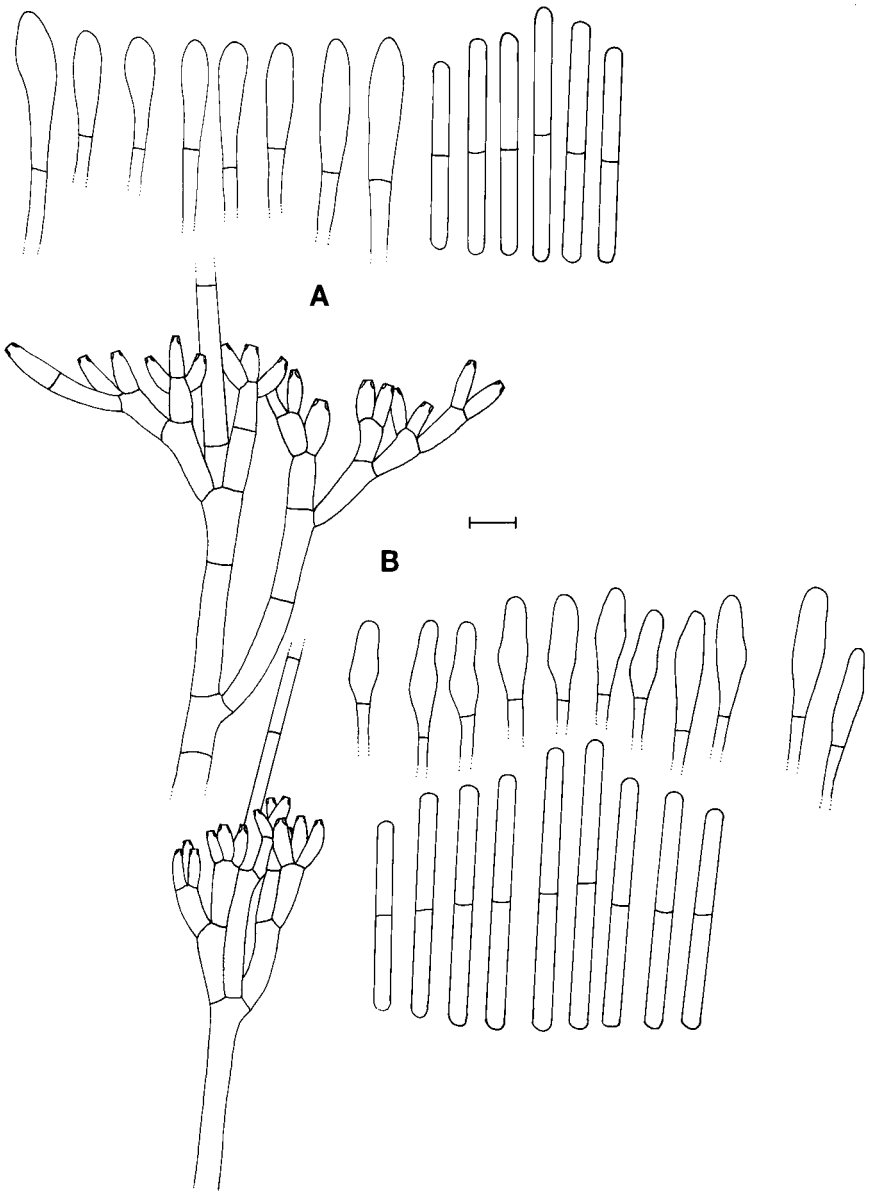

Figure 1. Conidiophores, conidia and vesicles of *Cy. scoparium* (A), and *Cy. pauciramosum* (B). Bar = 10 μm.

ellipsoidal to obpyriform (widest below the middle) (Crous et al., 1993). Recently results of mating studies have shown four distinct mating populations to exist in the *Cy. candelabrum* species complex (Schoch et al., 1999). Morphologically the isolates from Milkwort closely resembled others in the *Cy. candelabrum* complex. The aim of the present study was to correctly identify the *Cylindrocladium* species involved, and also determine its host range in Italy.

Plants with leaf spots, stem lesions, cankers, shoot blight, crown and root rot symptoms were collected during 1996 and 1997 from different hosts grown in nurseries in areas of Sicily and Calabria (Table 1). Infected leaf, crown and root tissues collected from different nurseries were surface-sterilized for 1 min in 2% sodium hypochlorite, 30 s in 70% ethanol, and plated on potato-dextrose agar (PDA; Oxoid) and corn meal agar (CMA; Oxoid). Conidia and conidiophores

Table 1. Host range and symptoms of 200 *Cylindrocladium pauciramosum* isolates collected in commercial nurseries in Sicilian and Calabrian ornamental production areas

Isolate number[1]	Location	Province[2]	Host	Symptoms[3]
1^B, 2^B, 190	Praiola	Catania (S)	*Polygala myrtifolia*	C.R.; R.R.; S.C.
3^A	Lamezia 1	Catanzaro (C)	*P. myrtifolia*	C.R.; R.R.
4, 5^B, 6^A-8	Milazzo	Messina (S)	*Callistemon citrinus*	L.S.
9^A	Carruba	Catania (S)	*Metrosideros robustus*	L.S.
10^B-16, 19, 20^B	Carruba	Catania (S)	*C.* 'Mauve Mist'	L.S.
21^A, 22	Praiola	Catania (S)	*M. robustus*	L.S.
23–27	Grotte	Catania (S)	*Myrtus communis*	L.S.
-31^B, 32^B, 37, 38	Carruba	Catania (S)	*C.* 'Mauve Mist'	L.S.
40^A-46^B-50^B, 51	Carruba	Catania (S)	*Eucalyptus viminalis*	L.S.
28, 29, 36, 43-47	Carruba	Catania (S)	*C. citrinus*	L.S.
17, 18, 30, 33, 39, 41, 42, 48-50, 52, 53^B-60^B	Carruba	Catania (S)	*M. communis*	L.S.
34, 35	Carruba	Catania (S)	*M. robustus*	L.S.
61, 62^B-67^B-69^B-74^B	Milazzo	Messina (S)	*C. citrinus*	L.S.
75^B-77^B-79^B-81^B, 129-148	Barcellona 1	Messina (S)	*C. viminalis*	L.S.
149^B-155^B-171	Barcellona 2	Messina (S)	*C. viminalis*	L.S.
82, 83, 84^A-87^A-92^A-96^A-98^B, 99	San Marco 1	Messina (S)	*Acacia retinodes*	L.S.; S.L.
100-104^A-115^A, 116	San Marco 2	Messina (S)	*A. retinodes*	L.S.; S.L.
117-124^B-126^A, 172	Carruba	Catania (S)	*Arbutus unedo*	L.S.
127^B, 128^B	Barcellona 1	Messina (S)	*C. citrinus*	L.S.
173	Lamezia 2	Catanzaro (C)	*C. citrinus* 'Splendens'	L.S.
174, 175^B, 176, 188, 189	Grotte	Catania (S)	*M. robustus*	L.S.
177^B, 178^B, 179^B	Carruba	Catania (S)	*P. myrtifolia*	C.R.; R.R.
180-182	Praiola	Catania (S)	*C. citrinus* 'Splendens'	L.S.
183^B-185	Grotte	Catania (S)	*E. rostrata*	L.S.
186^A, 187	Praiola	Catania (S)	*Melaleuca hypericifolia*	L.S.
191^B, 192^B, 193^B-195^B, 196	Lamezia 1	Catanzaro (C)	*P. myrtifolia*	C.R.; R.R.
197-199^B, 200	Praiola	Catania (S)	*M. communis*	L.S.

[1]Isolates grouped according to location and host. Mating type of 48 selected isolates indicated individually. Isolates producing perithecia with viable progeny when paired with A = STE-U 1670 (MAT1-1), or B = STE-U 971 (MAT 1-2), the two mating type testers of *Cy. pauciramosum*.
[2]S = Sicily, C = Calabria.
[3]C.R. = crown rot; L.S. = leaf spot; R.R. = root rot; S.C. = stem canker; S.L. = stem lesion.

were also collected with a sterile needle from sporulating colonies on diseased tissue, suspended in sterile water, and streaked over the agar surface. Plates were incubated at room temperature (approximately 20 °C) under fluorescent cool-white lights on a 12-h light/dark regime.

A *Cylindrocladium* sp. was consistently isolated from crown and root rot, leaf spot, stem lesions and cankers of several hosts in addition to *Polygala myrtifolia*, namely *Acacia retinodes* Schltdl., *Arbutus unedo* L. and various other Myrtaceae (Table 1).

Four-month-old seedlings of *P. myrtifolia* were used in pathogenicity tests. Inocula were prepared by harvesting conidia from sporulating 14-day-old colonies derived from single conidia growing on PDA. Plates were flooded with distilled, sterile water, and conidial suspensions quantified and adapted with a haemocytometer to 1×10^4 conidia/ml. Fifty millilitres of inoculum were mixed into the top 3 cm of soil in each pot. Control plants were treated with the same amount of autoclaved inoculum. Twenty replicates were used for both treatments, and the experiment was repeated once. All plants were maintained at moisture saturation for 24 h before being transferred to a greenhouse where the temperature varied from 18–32 °C during the experiment. Results were recorded

2 months after the experiments were initiated. Root rot and stem tissue discolouration was visible on all inoculated *P. myrtifolia* plants, while the controls remained healthy. The *Cylindrocladium* species was also successfully re-isolated from the lesions produced, confirming it to be the causal organism of the disease.

Two hundred single-conidial isolates (Table 1) collected from *P. myrtifolia* and other hosts were subcultured on carnation leaf agar (CLA; Fisher et al., 1982), incubated at 25 °C under near-ultraviolet light and examined after 7 days (Crous et al., 1992). Only material occurring on the carnation leaves was examined. *Cylindrocladium* isolates were identified using the keys of Crous and Wingfield (1994) and Schoch et al. (1999). Conidia were observed to be in the range of 30–60 × 3.5–5.0 µm, while conidiophores mostly had only two or three series of branches, and stipes terminated in obpyriform to broadly ellipsoidal vesicles, closely resembling *Cy. pauciramosum* C.L. Schoch and Crous (teleomorph *Ca. pauciramosa* C.L. Schoch and Crous) and *Cy. insulare* C.L. Schoch and Crous (teleomorph *Ca. insularis* C.L. Schoch and Crous).

Fifty selected single-conidial *Cylindrocladium* isolates (Table 1) originating from various geographic locations and collected from different hosts were mated with tester strains of both species. Testers used for *Cy. pauciramosum* were STE-U 1670 (MAT1-1) (Brazil, *Eucalyptus* sp.) and STE-U 971 (MAT1-2) (South Africa, soil), and for *Cy. insulare* were STE-U 766 (MAT1-1) (Madagascar, soil) and STE-U 768 (MAT1-2) (Madagascar, soil). After matings were conducted on CLA as described by Crous et al. (1993), plates were stacked, sealed in plastic bags, and incubated in the laboratory at 22 °C. Results from matings were determined after one month. All isolates except two (108 and 158) produced perithecia with viable progeny when mated with testers of *Cy. pauciramosum* (Table 1), determining this to be the species present in Italy. The two isolates that remained infertile indicate that other factors possibly also play a role in sexual compatibility, as these isolates also resembled *Cy. pauciramosum* in general morphology (Figure 1).

Defoliation of myrtle (*Myrtus communis*) and other hosts by a species of *Cylindrocladium* was first observed in Italy during 1993 (Polizzi, 1996; Polizzi and Azzaro, 1996). The disease was originally identified as *Cy. scoparium* Morgan, a pathogen known primarily from North America (Crous and Wingfield, 1994), but which has apparently also spread to Europe with infected herbs imported from the U.S.A. (Overmeyer et al., 1996). To date, we have been unable to obtain any authentic isolates of *Cy. scoparium* from Europe. This is the first report of *Cy. pauciramosum* from Italy and Europe, as it has previously only been reported from Australia, Brazil, Colombia, Mexico and South Africa (Schoch et al., 1999). Other species in this complex include *Cy. candelabrum*, which has been confirmed from Brazil and Venezuela, *Cy. insulare* from Brazil, Indonesia, Madagascar, Malaysia, Mauritius, Mexico and the U.S.A. (Hawaii), and *Cy. mexicanum* C.L. Schoch and Crous from Mexico (Schoch et al., 1999). Although disease symptoms attributed to *Cy. pauciramosum* have only been observed in Italy since 1993, no additional evidence is available at present to support the fact that it is a recent introduction to this country. However, it is possible that previous *Cylindrocladium* reports from Europe have been incorrectly identified, and could have been representative of *Cy. pauciramosum*.

In the field the disease is favoured by high temperature and humidity conditions. In fungicide trials conducted on infected myrtle plants, good control was obtained using foliar sprays of copper oxychloride ($0.9\,g\,l^{-1}$), while benomyl ($0.5\,g\,l^{-1}$), chlorothalonil ($1.02\,ml^{-1}$), dithianon ($0.75\,g\,l^{-1}$), prochloraz ($0.5\,g\,l^{-1}$) and ziram ($1.35\,g\,l^{-1}$) proved to be ineffective (Polizzi and Azzaro, 1996). Furthermore, the disease also appears more severe in areas where strict nursery sanitation, e.g. removal of abscised leaves and dead plants, is not carried out.

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