

Research Papers

## Virulence and cross-infection potential of *Ilyonectria* spp. to grapevine

ANA CABRAL<sup>A</sup>, CECÍLIA REGO<sup>A</sup>, PEDRO W. CROUS<sup>B,C,D</sup> and HELENA OLIVEIRA<sup>A</sup>

<sup>a</sup> CEER-Biosystems Engineering, Instituto Superior de Agronomia, Technical University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal

<sup>b</sup> CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, The Netherlands

<sup>c</sup> Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

<sup>d</sup> Wageningen University and Research Centre (WUR), Laboratory of Phytopathology, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

**Summary.** Black foot is an important disease of grapevines, affecting vines in nurseries as well as in young plantations. In recent years the disease has increased in incidence and severity throughout the world. Black foot is associated with at least two *Campylocarpon* and 12 *Ilyonectria* species, most of which have only recently been described. The recognition of previously unknown species, together with published reports of variability in virulence between and within species identified as *I. macrodidyma* and *I. lirioidendri*, underlined the need to compare the virulence of isolates from these complexes. A further objective of this work was to determine the cross-infection potential of isolates of these species from other hosts to grapevine. Results from this study revealed recently described species such as *I. lusitanica*, *I. estremocensis* and *I. europaea* to be more virulent to grapevine than the species previously accepted as the main causal agents of black foot, such as *I. lirioidendri* and *I. macrodidyma*. Furthermore, these results also provided support for isolates obtained from non-grapevine hosts to be as virulent to grapevines as isolates obtained from grapevine, underlying the cross-infection potential of these pathogens.

**Key words:** black foot disease; *Cylindrocarpon* root rot; pathogenicity; *Vitis vinifera*.

### Introduction

Black foot is an important disease of grapevines in most countries throughout the world. In recent years the disease has been reported with an increased incidence and severity, affecting both nurseries and young plantations, causing typical darkening of the basal end of rootstock plants (Halleen *et al.*, 2004; Oliveira *et al.*, 2004). Declining plants are frequently found in infected vineyards, showing slow growth, reduced vigour, retarded sprouting, shortened internodes, sparse and chlorotic foliage (Rego *et al.*, 2000; Halleen *et al.*, 2006a), resulting frequently in plant

death and forcing growers to replant considerable areas.

Black foot is caused by several *Cylindrocarpon*-like species residing in the genera *Campylocarpon* and *Ilyonectria*. Two species of *Campylocarpon* have been reported, namely *Campyl. fasciculare* Schroers, Halleen & Crous and *Campyl. pseudofasciculare* Halleen, Schroers & Crous (Halleen *et al.*, 2004), although these have thus far only been reported from South Africa (Halleen *et al.*, 2004) and Uruguay (Abreo *et al.*, 2010). The genus *Ilyonectria* was recently established within what was formerly regarded as *Neonectria* s. lat., accommodating well-known pathogens such as *Ilyonectria lirioidendri* (Halleen, Rego & Crous)

Corresponding author: Cecília Rego

Fax: +351 21 365 3100

E-mail: crego@isa.utl.pt

P. Chaverri & C. Salgado and *I. macrodidyma* (Halleen, Schroers & Crous) P. Chaverri & C. Salgado (Chaverri *et al.*, 2011). In fact, *I. liriodendri* and *I. macrodidyma* are the species most commonly reported from affected grapevines (Petit and Gubler, 2005; Halleen *et al.*, 2006b; Alaniz *et al.*, 2007). Recent studies have shown, however, that many of these records actually represent some newly described species (Cabral *et al.*, 2012a, 2012b). These include *I. alcacerensis* A. Cabral, Oliveira & Crous, *I. estremocensis* A. Cabral, Nascimento & Crous, *I. novozelandica* A. Cabral & Crous and *I. torresensis* A. Cabral, Rego & Crous which were described from within the *I. macrodidyma* species complex (Cabral *et al.*, 2012b), and *I. europaea* A. Cabral, Rego & Crous, *I. lusitanica* A. Cabral, Rego & Crous, *I. pseudodestructans* A. Cabral, Rego & Crous, *I. robusta* (A.A. Hildebr.) A. Cabral & Crous and *I. vitis* A. Cabral, Rego & Crous, which emerged from the *I. radicola* (Gerlach & L. Nilsson) P. Chaverri & C. Salgado species complex (Cabral *et al.*, 2012a). *Ilyonectria torresensis* was found to be associated with *Vitis vinifera*, *Abies nordmanniana*, *Fragaria* sp. and *Quercus* sp. in countries throughout the world. In contrast, *I. alcacerensis* has thus far only been reported from *V. vinifera* in the Iberian Peninsula. *Ilyonectria novozelandica* was associated with *V. vinifera* in New Zealand, South Africa and the USA, but also reported on *Festuca duriuscula* in Portugal. *Ilyonectria estremocensis* was isolated from *V. vinifera* in Portugal and *Picea glauca* in Canada (Cabral *et al.*, 2012b). *Ilyonectria europaea*, *I. pseudodestructans* and *I. robusta* were found on *V. vinifera* in Portugal and on other host plants in different parts of the world, while *I. lusitanica* and *I. vitis* were thus far exclusively reported from grapevines (Cabral *et al.*, 2012a). Besides these, "*Cylindrocarpon*" *pauciseptatum* Schroers & Crous was associated with diseased roots of *Vitis* spp. in New Zealand and Slovenia (Schroers *et al.*, 2008), in Uruguay (Abreo *et al.*, 2010), in Portugal (Cabral *et al.*, 2012a) and in Spain (Martín *et al.*, 2011).

*Ilyonectria macrodidyma* was reported as more virulent to grapevines than *I. liriodendri*, although variation in virulence among groups of *I. macrodidyma* was also found (Alaniz *et al.*, 2009). However, no other comparative virulence studies have been reported among the pathogens causing black foot disease of grapevine. This is becoming particularly relevant, as at least 12 species are currently recognised to be associated with this disease. Moreover, most of these species are not exclusive to grapevine, and infect

several other hosts, underlining the cross-infection potential of isolates from other hosts to grapevines. Therefore, the objective of this work was to compare the virulence of isolates from different species associated with black foot disease of grapevines, as well as to test the pathogenicity of isolates from other hosts to grapevine.

## Materials and methods

A total of 60 isolates were analysed, 36 of which are from grapevines (Table 1). The other hosts include *Olea europaea* (five isolates) and *Quercus* spp. (five isolates), among others. Species covered in this study include "*C.*" *pauciseptatum* (three isolates from grapevine and one from *Olea europaea*), *I. alcacerensis* (two isolates from grapevine), *I. estremocensis* (four isolates from grapevine), *I. europaea* (two isolates from grapevine, one from *Actinidia chinensis* and one from *Aesculus hippocastanum*), *I. liriodendri* (four isolates from grapevine, one from *Liriodendron tulipifera*, one from *Malus domestica*, and one from *Quercus suber*), *I. lusitanica* (one isolate from grapevine), *I. macrodidyma* (three isolates from grapevine and two from *Olea europaea*), *I. novozelandica* (five isolates from grapevine and one from *Festuca duriuscula*), *I. pseudodestructans* (two isolates from grapevine and two from *Quercus* sp.), *I. robusta* (three isolates from grapevine, two from *Quercus* spp., two from *Panax quinquefolium*, one from *Prunus cerasus*, one from *Tilia petiolaris*, one from *Thymus* sp. and one from an aquarium with *Anodonta*), *I. vitis* (one isolate from grapevine), *I. torresensis* (four isolates from grapevine, one from *Fragaria x ananassa* and one from *Olea europaea*), an *I. estremocensis*-like undescribed species, here referred as *Ilyonectria* sp2 (L. Mostert, personal communication; two isolates from grapevine, one from *Pinus laricio* and one from an unknown host) and an *I. venezuelensis*-like undescribed species, here referred as *Ilyonectria* sp1 (Cabral *et al.*, unpublished data).

Cuttings of the susceptible rootstock 1103P (Alaniz *et al.*, 2010) were rooted for 1.5 to 2 months at 20°C in a rooting bench containing perlite and sand. Irrigation was carried out by overhead nebulisation for 5 s every 10 min.

After the rooting period, plants were removed from the bench and the roots were slightly pruned. The wounded cuttings were dip-inoculated by immersing the roots and the basal end of the cuttings in a 10<sup>6</sup> mL<sup>-1</sup> conidial suspension (for each isolate listed

Table 1. List of isolates studied with collection details.

| Strain code <sup>a</sup>              | Collected/isolated by year    | Host/substrate  | Location                                   |
|---------------------------------------|-------------------------------|---|--|
| <i>"Cylindrocarpum" pauciseptatum</i> |                               |   |  |
| OL-CM3                                | C. Rego, 2008                 | <i>Olea europaea</i>  | Portugal, Campo Maior                      |
| Cy196                                 | N. Cruz, 2005                 | <i>Vitis vinifera</i> , basal end of a 4-year-old plant; scion Alvarinho; rootstock 196-17  | Portugal, Melgaço / Monção                 |
| Cy217                                 | A. Cabral, 2007               | <i>Vitis vinifera</i> , asymptomatic; scion Gouveio   | Portugal, Torres Vedras                    |
| Cy238                                 | C. Rego, 2008                 | <i>Vitis vinifera</i> , basal end of a 2-year-old plant; scion Petit Verdot; rootstock 110R   | Portugal, Vidigueira                       |
| <i>Ilyonectria vitis</i>              |                               |   |  |
| CBS 129082; Cy233                     | C. Rego, 2008                 | <i>Vitis vinifera</i> , basal end of a 2-year-old plant; scion Touriga Nacional; rootstock 110R   | Portugal, Vidigueira                       |
| <i>Ilyonectria altacerensis</i>       |                               |   |  |
| Cy134; IAFM Cy20-1                    | J. Armengol                   | <i>Vitis vinifera</i>   | Spain, Ciudad Real, Villarubia de los Ojos |
| CBS 129087; Cy159                     | A. Cabral & H. Oliveira, 2004 | <i>Vitis vinifera</i> , basal end of a 3-year-old plant with root discoloration and decline symptoms; scion Sangiovese; rootstock 1103P | Portugal, Alcácer do Sal, Torrão           |
| <i>Ilyonectria</i> sp2                |                               |   |  |
| CBS 173.37; IMI090176                 | T.R. Peace, 1937              | <i>Pinus laricio</i> , associated with dieback  | UK, England, Devon, Haldon                 |
| Cy108                                 | C. Rego, 1999                 | <i>Vitis vinifera</i> , basal end of a 4-year-old plant showing decline symptoms; scion Aragonez; rootstock SO4                         | Portugal, Nelas                            |
| Cy200                                 | N. Cruz, 2005                 | <i>Vitis vinifera</i> , basal end of a 16-year-old plant; scion Alvarinho; rootstock 196-17   | Portugal, Melgaço                          |
| CBS 159.34; IMI113891                 | H.W. Wollenweber, 1934        | -   | Germany                                    |
| <i>Ilyonectria estremocensis</i>      |                               |   |  |
| Cy135                                 | C. Rego & T. Nascimento, 2003 | <i>Vitis vinifera</i> , basal end of a 1.5-year-old plant showing decline symptoms; scion Aragonez; rootstock 3309C                     | Portugal, Estremoz                         |
| CBS 129085; Cy145                     | C. Rego & T. Nascimento, 2003 | <i>Vitis vinifera</i> , basal end of a 1.5-year-old plant showing decline symptoms; scion Aragonez; rootstock 3309C                     | Portugal, Estremoz                         |
| Cy152                                 | C. Rego & T. Nascimento, 2003 | <i>Vitis vinifera</i> , asymptomatic 1.5-year-old plant; scion Aragonez; rootstock 3309C  | Portugal, Estremoz                         |
| Cy243                                 | C. Rego, 2008                 | <i>Vitis vinifera</i> , basal end of a 2-year-old plant; scion Touriga Nacional; rootstock 110R   | Portugal, Vidigueira                       |
| <i>Ilyonectria macrodidyma</i>        |                               |   |  |
| OL_CM4                                | C. Rego, 2008                 | <i>Olea europaea</i>  | Portugal, Campo Maior                      |
| OL_CM6                                | C. Rego, 2008                 | <i>Olea europaea</i>  | Portugal, Campo Maior                      |
| Cy128                                 | W.D. Gubler                   | <i>Vitis vinifera</i>   | USA, California                            |
| Cy175                                 | C. Rego, 2004                 | <i>Vitis vinifera</i> , basal discoloration; scion Touriga Nacional; rootstock 1103P  | Portugal, Torre de Moncorvo                |
| Cy250                                 | C. Rego, 2008                 | <i>Vitis vinifera</i> , basal end of a 2-year-old plant; scion Chardonnay rootstock 110R  | Portugal, Vidigueira                       |

(Continued)

Table 1. *Continues.*

| Strain code <sup>a</sup>          | Collected/isolated by year       | Host/substrate  | Location   |
|-----------------------------------|----------------------------------|---|--|
| CBS 112615                        | F. Halleen, 2000                 | <i>Vitis vinifera</i> , roots, asymptomatic nursery plant; scion Sultana; rootstock 143-B Mgt     | South Africa, Western Cape, Malmesbury, Jakkalsfontein |
| <i>Ilyonectria novaezelandica</i> |                                  |   |  |
| Cy230                             | F. Caetano, 2005                 | <i>Festuca duriuscula</i>   | Portugal, Lisbon                                       |
| CBS 113552                        | R. Bonfiglioli, 2003             | <i>Vitis</i> sp. decline of nursery plants dead rootstocks  | New Zealand, Candy P New Ground                        |
| Cy129                             | W.D. Gubler                      | <i>Vitis vinifera</i>   | USA, California  |
| Cy130                             | W.D. Gubler                      | <i>Vitis vinifera</i>   | USA, California  |
| CBS 112593                        | F. Halleen, 2000                 | <i>Vitis vinifera</i> , roots of asymptomatic nursery plant; scion Pinotage; rootstock 101-14 Mgt | South Africa, Western Cape, Wellington, Voorgroenberg  |
| <i>Ilyonectria torresensis</i>    |                                  |   |  |
| Cy222                             | L. Leandro                       | <i>Fragaria x ananassa</i>  | USA, North Carolina, Asheville                         |
| HC7                               | C. Rego, 2007                    | <i>Olea europaea</i>  | Portugal, Avis   |
| Cy118                             | W.D. Gubler                      | <i>Vitis vinifera</i>   | USA, California  |
| Cy214                             | A. Cabral, 2007                  | <i>Vitis vinifera</i> , asymptomatic; scion Grenache  | Portugal, Torres Vedras                                |
| CBS 129086; Cy218                 | A. Cabral, 2007                  | <i>Vitis vinifera</i> , asymptomatic; scion Chenin  | Portugal, Torres Vedras                                |
| Cy260                             | C. Rego, 2008                    | <i>Vitis vinifera</i> , basal end of 2-year-old plant; scion Cabernet Sauvignon; rootstock 110R   | Portugal, Vidigueira                                   |
| <i>Ilyonectria europaea</i>       |                                  |   |  |
| Cy131                             | P. Lecomte & S. Chamont, 2000    | <i>Actinidia chinensis</i> 'Hayward', internal lesion of stem                                     | France, St. Chicq-du-Gaue                              |
| CBS 537.92                        | V. Demoulin, 1992                | <i>Aesculus hippocastanum</i> , wood  | Belgium, Liège   |
| CBS 129078; Cy241                 | C. Rego, 2008                    | <i>Vitis vinifera</i> , basal end of a 2-year-old plant; scion Petit Verdot; rootstock 110R       | Portugal, Vidigueira                                   |
| Cy155                             | C. Rego & H. Oliveira, 2004      | <i>Vitis vinifera</i> , 2-year-old, with decline symptoms, scion Alfrocheiro; rootstock SO4       | Portugal, Alter do Chão                                |
| <i>Ilyonectria liriodendri</i>    |                                  |   |  |
| CBS 110.81; IMI 303645            | J.D. MacDonald & E. Butler, 1978 | <i>Liriodendron tulipifera</i> , root   | USA, California, Yolo Co., Davis                       |
| Cy164                             | C. Rego, 1997                    | <i>Malus domestica</i> ; cultivar Lysgolden; rootstock MMI106                                     | Portugal, Porto de Mós, Valbom                         |
| Cy232                             | L. Inácio & J. Henriques, 2007   | <i>Quercus suber</i> , stem   | Portugal, Macedo de Cavaleiros                         |
| Cy5                               | C. Rego, 1992                    | <i>Vitis vinifera</i> , 4-year-old, with decline symptoms; scion Boal Branco; rootstock 99R       | Portugal, Torres Vedras, Dois Portos                   |

(Continued)

Table 1. *Continues.*

| Strain code <sup>a</sup>             | Collected/isolated by year    | Host/substrate  | Location                    |
|--------------------------------------|-------------------------------|---|-----------------------------|
| CBS 117526; Cy68                     | C. Rego, 1999                 | <i>Vitis vinifera</i> , asymptomatic rootstocks; rootstock 99 R, clone 179F                         | Portugal, Ribatejo e Oeste  |
| Cy190                                | N. Cruz, 2005                 | <i>Vitis vinifera</i> , basal end of a 6-year-old plant; scion Alvarinho; rootstock 196-17          | Portugal, Monção, Cortes    |
| Cy253                                | C. Rego, 2008                 | <i>Vitis vinifera</i> , basal end of a 2-year-old plant; scion Petit Verdote; rootstock 110R        | Portugal, Vidigueira        |
| <i>Ilyonectria lusitanica</i>        |                               |   |                             |
| CBS 129080; Cy197                    | N. Cruz, 2005                 | <i>Vitis vinifera</i> , below grafting zone, 6-year-old; scion Alvarinho; rootstock 196-17          | Portugal, Melgaço, Alvaredo |
| <i>Ilyonectria</i> sp1               |                               |   |                             |
| OL2                                  | C. Rego & H. Oliveira, 2007   | <i>Olea europaea</i>  | Portugal, Évora             |
| <i>Ilyonectria pseudodestructans</i> |                               |   |                             |
| CBS 117824                           | E. Halmshlager, 1993          | <i>Quercus</i> sp., root  | Austria, Patzmannsdorf      |
| CBS 117812                           | E. Halmshlager, 1993          | <i>Quercus</i> sp., root  | Austria, Patzmannsdorf      |
| CBS 129081; Cy20                     | C. Rego, 1996                 | <i>Vitis vinifera</i> , 4-year-old, with decline symptoms, scion Malvasia Fina; rootstock 1103P     | Portugal, Gouveia, São Paio |
| Cy22                                 | C. Rego, 1996                 | <i>Vitis vinifera</i> , 5-year-old, with decline symptoms, scion Aragonez; rootstock 99R            | Portugal, Viseu, Silgueiros |
| <i>Ilyonectria robusta</i>           |                               |   |                             |
| CD1666                               | R. D. Reeleder, 1998          | <i>Panax quinquefolium</i>  | Canada, Nova Scotia         |
| CBS 308.35                           | A.A. Hildebrand               | <i>Panax quinquefolium</i>  | Canada, Ontario             |
| CPC 13532; DAOM 139398               | -                             | <i>Prunus cerasus</i> cultivar Montmorency  | Canada, Ontario             |
| CBS 117813                           | E. Halmshlager, 1993          | <i>Quercus robur</i> , root   | Austria, Niederweiden       |
| CBS 117815                           | E. Halmshlager, 1993          | <i>Quercus</i> sp., root  | Austria, Patzmannsdorf      |
| Cy231                                | F. Caetano, 2005              | <i>Thymus</i> sp.   | Portugal, Lisbon            |
| CBS 605.92                           | R. Schröer, 1992              | <i>Tilia petiolaris</i> , root  | Germany, Hamburg            |
| Cy23                                 | C. Rego, 1997                 | <i>Vitis</i> sp. rootstock 99R clone 179F in nursery  | Portugal, Ribatejo e Oeste  |
| Cy158                                | C. Rego & T. Nascimento, 2004 | <i>Vitis vinifera</i> , 1-year-old, died before sprouting; scion Alicante Bouschet; rootstock 1103P | Portugal, Lamego, Cambres   |
| CBS 129084; Cy192                    | N. Cruz, 2005                 | <i>Vitis vinifera</i> , basal end of 25-year-old plant; scion Alicante; rootstock 196-17            | Portugal, Monção            |
| CBS 773.83                           | J. Hemelraad                  | Water, in aquarium with <i>Anodonta</i>   | Netherlands, Utrecht        |

<sup>a</sup> CBS: CBS-KNAW Fungal Biodiversity Centre (Centraalbureau voor Schimmelcultures), Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; Cy:OL and HC: Cylindrocarpon collection housed at Laboratório de Patologia Vegetal "Veríssimo de Almeida" - ISA, Lisbon, Portugal; DAOM: Agriculture and Agri-Food Canada National Mycological Herbarium, Canada; IAFM: Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Spain; IMI: International Mycological Institute, CABI-Bioscience, Egham, Basingstoke, U.K.

in Table 1), for 60 min. Conidia were harvested by flooding 14 d old potato-dextrose agar (PDA, Difco, USA) cultures with sterile distilled water, and dislodged with a sterile glass rod. The spores and mycelium were then filtered through a double layer of cheesecloth, and the conidial concentration estimated using a haemocytometer, which was then adjusted with sterile distilled water. After inoculation, the rooted cuttings were planted in 1 L bags containing a mixture of soil, peat and sand (2:1:1, v/v/v), and maintained in a greenhouse at 24±5°C (day) and 18°C (night) with approximately a 12 h photoperiod. For negative control plants, sterile distilled water was used instead of conidial suspension.

The plants were grown on the greenhouse for 4.5 months and, following this period, results were evaluated for each isolate (10–12 plants per isolate, including the control), and compared to the control. The parameters analysed were focused on the loss of root (number and root dry weight, and the length of the longest root) and shoot (number of shoot nodes and the length and shoot dry weight; usually a single shoot was formed) biomass and on the intensity of wood colonisation by the pathogens (percentage of reisolation). For the latter, 10 pieces of wood from the basal end of each rootstock plant (at least 2 cm above the bottom) were excised, disinfected (for 1 min in a NaClO solution with 0.35% w/w as active chlorine), rinsed with distilled water and placed in Petri dishes containing PDA amended with 250 mg L<sup>-1</sup> chloramphenicol (BioChemica, AppiChem, Germany). The dishes were incubated at 20°C for up to 2 weeks and observed for the presence of *Ilyonectria* colonies, which was confirmed through morphological appearance of colonies and conidial characteristics. The percentage of reisolation was calculated as the proportion of wood pieces from which *Ilyonectria* colonies were recovered, versus the total number of pieces of wood for each plant.

All data were subjected to a one-way ANOVA and means compared using the Tukey's test at a 5% significance level (STATISTICA 8.0). Before analysis, arcsine-square root transformation was performed for data expressed as percentage.

To confirm results from this experiment, data from a subsequent, smaller experiment were used for comparison under the same conditions as stated above. Isolates tested were from the following species: *I. estremocensis* (isolates Cy135, Cy144, Cy145, Cy152 and Cy153 from grapevine), *I. europaea* (iso-

late Cy131 from *Actinidia chinensis*), *I. liriodendri* (isolates Cy5, Cy68 and Cy76 from grapevine, Cy164 from *Malus domestica* and Cy232 from *Quercus suber*), *I. novozelandica* (isolate Cy230 from *Festuca* sp.), *I. pseudodestructans* (isolates Cy20 and Cy22 from grapevine, and CBS 117812 from *Quercus* sp.), *I. robusta* (CBS 117818 from *Quercus* sp. and Cy231 from *Thymus* sp.), *I. torresensis* (isolates OL1 from *Olea europaea*, Cy96 from *Quercus* sp. and Cy221 and Cy222 from *Fragaria x ananassa*) and *Ilyonectria* sp1 (isolate OL2 from *Olea europaea*).

## Results

At the end of the first experiment, root rot symptoms were visible in inoculated plants, in contrast to the uninoculated control plants. Symptoms included root lesions, vascular discolouration, and necrosis in the basal plant tissues, although the quantification of these lesions and discolouration was not possible. Symptoms related to reduced vigour were more readily quantifiable. In general, inoculated plants had shorter shoots with less nodes, as well as less and shorter roots, although significant differences were found among isolates (Table 2).

The percentage of reisolation ranged from a minimum of 18.6% for isolate OL2 (*Ilyonectria* sp1, from *Olea europaea*) to a maximum of 96.5% for isolate CBS 537.92 (*I. europaea*, from *Aesculus hippocastanum*). Control plants had 0% reisolation, differing significantly from all tested isolates except OL2 and Cy230. This trait had the fewest homogeneous groups among all the traits studied.

The average number of roots in the control plants was 36.3, which did not differ significantly from the maximum value recorded from inoculated plants (35.8 for isolate CBS 112615; *I. macrodidyma* from grapevine). The minimum value for NR was 19.2 for isolate CBS 117526 (*I. liriodendri*, from grapevine), which represents a 47% reduction in the number of roots.

The root dry weight ranged from a maximum of 4.50 g for isolate OL2 (which did not differ significantly from the control plants; 4.08 g) to a minimum of 0.49 for Cy243 (*I. estremocensis*, from grapevine; 88% reduction from control).

The length of the longest root for the control plants was 49.6 cm, with all inoculated plants showing a significant reduction from that value, ranging from a minimum reduction of 23% for isolate OL2 to

**Table 2.** Comparison (average and homogeneous groups) among *Ilyonectria* spp. isolates for their effect on the frequency of reisolation (%RI), on the number of roots (NR), length of the longest root (LR, cm) root dry weight (RDW, g), number of shoot nodes (NSN), shoot length (SL, cm) and shoot dry weight (SDW, g) of grapevine rootstock 1103P.

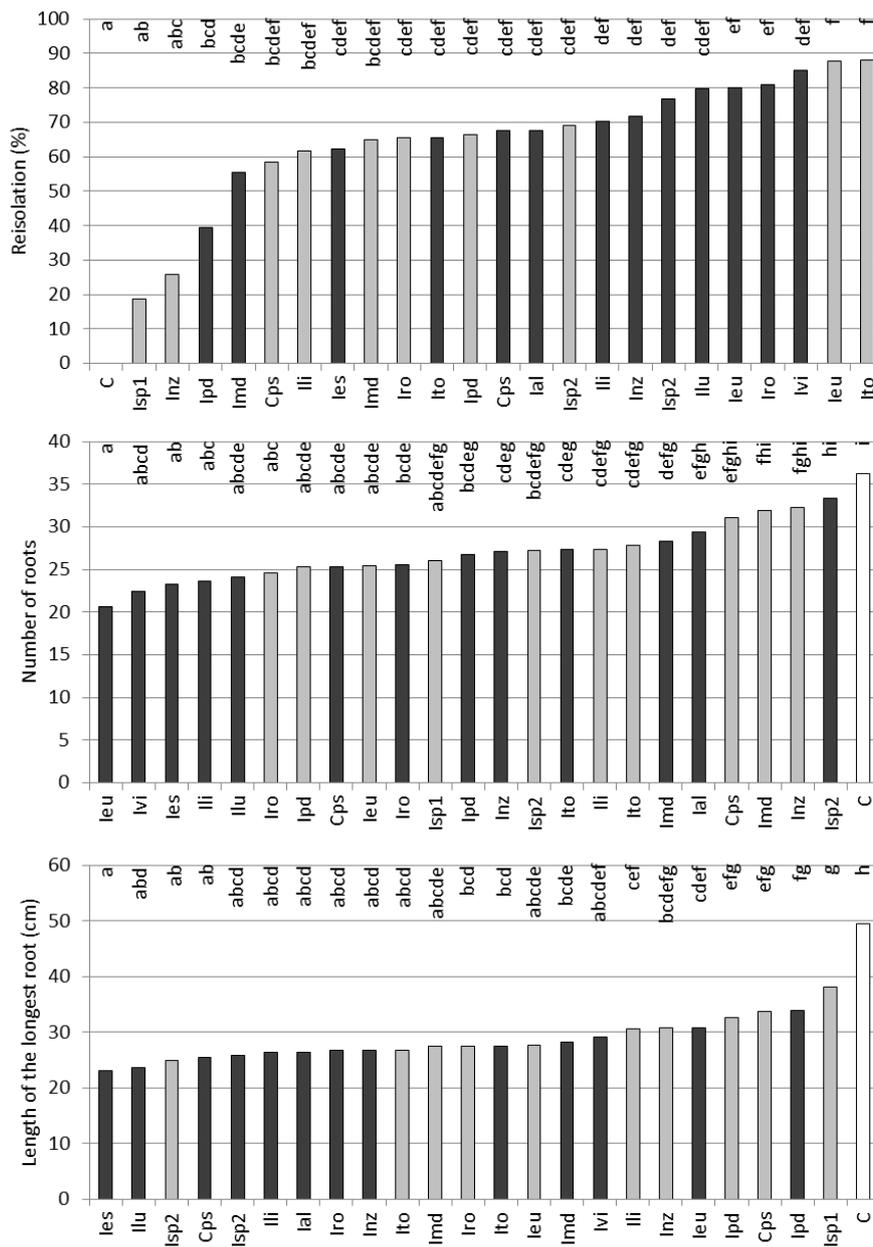
| Species  | Strain code | Host/substrate             | %RI <sup>a</sup> | NR          | LR         | RDW        | NSN      | SL         | SDW        |
|--|-------------|----------------------------|------------------|-------------|------------|------------|----------|------------|------------|
| Control  |             |                            | 0 a              | 36.3 t      | 49.6 s     | 4.08 tuv   | 15.9 g   | 52.2 o     | 0.95 g-k   |
| " <i>Cylindrocarpum</i> " <i>pauciseptatum</i> | OL-CM3      | <i>Olea europaea</i>       | 56 b-g           | 31.3 m-t    | 33.8 o-r   | 4.25 uv    | 12.5 c-f | 35.8 j-n   | 0.67 b-j   |
|  | Cy196       | <i>Vitis vinifera</i>      | 90 efg           | 23.7 a-k    | 22.4 abc   | 1.63 b-h   | 10.5 a-f | 33.0 f,h-m | 0.53 a-e   |
|  | Cy217       | <i>Vitis vinifera</i>      | 52 b-g           | 29.6 i-s    | 30.1 d-p   | 3.82 r-v   | 11.4 a-f | 30.1 b-l   | 0.72 b-j   |
|  | Cy238       | <i>Vitis vinifera</i>      | 56 b-g           | 22.8 a-i    | 23.7 a-f   | 0.99 abc   | 10.8 a-f | 22.4 abc   | 0.51 a-d   |
| <i>Ilyonectria vitis</i>                       | Cy233       | <i>Vitis vinifera</i>      | 85 d-g           | 22.4 a-h    | 29.1 c-p   | 2.98 k-s   | 13.2 efg | 36.1 j-n   | 0.76 b-k   |
| <i>Ilyonectria alacerensis</i>                 | Cy134       | <i>Vitis vinifera</i>      | 51 b-g           | 33.2 q-t    | 27.7 b-o   | 2.10 d-n   | 10.3 a-f | 30.3 c-l   | 0.74 b-j   |
|  | CBS 129087  | <i>Vitis vinifera</i>      | 83 d-g           | 25.2 a-m    | 25.0 b-i   | 2.00 c-l   | 11.6 a-f | 27.2 b-i   | 0.68 b-j   |
| <i>Ilyonectria</i> sp2                         | CBS 173.37  | <i>Pinus laricio</i>       | 68 b-g           | 25.0 a-n    | 24.9 b-j   | 1.92 b-l   | 10.0 a-f | 31.8 d-m   | 0.53 a-e   |
|  | Cy108       | <i>Vitis vinifera</i>      | 76 c-g           | 33.8 rst    | 26.0 b-j,l | 2.06 d-l,n | 10.7 a-f | 40.0 mn    | 0.93 g-k   |
|  | Cy200       | <i>Vitis vinifera</i>      | 78 c-g           | 32.9 p-t    | 25.6 b-j   | 2.71 i-q   | 12.8 efg | 43.0 n     | 0.99 i,k   |
|  | CBS 159.34  | Unknown                    | 70 b-g           | 29.0 g-r    | 25.0 b-i   | 1.98 c-l   | 10.0 a-f | 31.5 d-l   | 0.57 a-f   |
| <i>Ilyonectria estremocensis</i>               | Cy135       | <i>Vitis vinifera</i>      | 63 b-g           | 25.7 a-n    | 23.2 a-d   | 1.57 b-g   | 11.2 a-f | 34.8 h-n   | 0.67 b-j   |
|  | CBS 129085  | <i>Vitis vinifera</i>      | 71 b-g           | 27.1 d-q    | 26.0 b-l,n | 1.68 b-j   | 10.3 a-f | 31.7 e-l   | 0.71 b-j   |
|  | Cy152       | <i>Vitis vinifera</i>      | 63 b-g           | 20.3 abc,e  | 25.4 b-j   | 1.49 a-f   | 9.8 a-f  | 30.0 b-l   | 0.61 b-g   |
|  | Cy243       | <i>Vitis vinifera</i>      | 53 b-g           | 20.3 abc,e  | 16.8 a     | 0.49 a     | 8.9 abc  | 18.1 a     | 0.22 a     |
| <i>Ilyonectria macrodidyma</i>                 | OL_CM4      | <i>Olea europaea</i>       | 57 b-g           | 30.3 k-t    | 24.8 b-h   | 1.40 a-e   | 10.5 a-f | 35.3 i-n   | 0.82 d-k   |
|  | OL_CM6      | <i>Olea europaea</i>       | 74 b-g           | 33.8 rst    | 30.3 e-p   | 3.36 q-u   | 10.2 a-f | 35.5 i-n   | 0.92 f-k   |
|  | Cy128       | <i>Vitis vinifera</i>      | 61 b-g           | 20.9 abc,ef | 22.0 abc   | 1.63 b-h   | 9.8 a-f  | 28.8 b-k   | 0.56 a-e   |
|  | Cy175       | <i>Vitis vinifera</i>      | 70 b-g           | 26.4 c-o    | 28.2 b-o   | 2.20 d-n   | 11.0 a-f | 34.2 h-m   | 0.79 c-k   |
|  | Cy250       | <i>Vitis vinifera</i>      | 33 bcd           | 30.0 j-s    | 29.8 d-p   | 2.14 d-n   | 10.2 a-f | 23.3 a-d   | 0.50 a-d   |
| <i>Ilyonectria novaezelandica</i>              | CBS 112615  | <i>Vitis vinifera</i>      | 58 b-g           | 35.8 st     | 32.7 k,m-r | 1.98 c-l   | 10.0 a-f | 29.0 b-k   | 0.77 c-k   |
|  | Cy230       | <i>Festuca duriuscula</i>  | 26 abc           | 32.3 o-t    | 30.7 e-p   | 2.39 e-q   | 10.6 a-f | 31.4 d-l   | 0.84 d-k   |
|  | CBS 113552  | <i>Vitis</i> sp.           | 71 b-g           | 24.4 a-l    | 27.7 b-o   | 2.11 d-n   | 10.4 a-f | 35.2 i-n   | 1.08 k     |
|  | Cy129       | <i>Vitis vinifera</i>      | 75 c-g           | 25.2 a-m    | 26.2 b-l   | 1.97 c-k   | 12.1 b-f | 35.6 i-n   | 0.70 b-j   |
|  | Cy130       | <i>Vitis vinifera</i>      | 72 b-g           | 27.0 d,g-p  | 24.3 b-e   | 1.75 b-h   | 11.7 b-f | 33.1 h-m   | 0.65 b-h   |
| <i>Ilyonectria torresensis</i>                 | CBS 112593  | <i>Vitis vinifera</i>      | 69 b-g           | 32.1 n-t    | 29.9 d-p   | 2.63 h-q   | 12.4 c-f | 35.5 i-n   | 0.97 h-k   |
|  | Cy222       | <i>Fragaria x ananassa</i> | 92 efg           | 27.5 d,g-r  | 28.6 b-o   | 2.28 d-p   | 11.6 a-f | 37.7 lmn   | 0.95 g-k   |
|  | HC7         | <i>Olea europaea</i>       | 84 d-g           | 28.3 g-r    | 25.2 b-j   | 2.63 h-q   | 11.5 a-f | 35.7 j-n   | 0.77 b-k   |
|  | Cy118       | <i>Vitis vinifera</i>      | 70 b-g           | 25.0 a-l    | 25.4 b-j   | 1.71 b-i   | 10.9 a-f | 36.3 j-n   | 0.63 b-h,j |
|  | Cy214       | <i>Vitis vinifera</i>      | 76 b-g           | 27.4 d,f,r  | 27.7 b-o   | 3.08 m-t   | 9.8 a-f  | 27.9 b-k   | 0.78 b-k   |
|  | CBS 129086  | <i>Vitis vinifera</i>      | 73 b-g           | 25.3 a-m    | 28.8 c-o   | 3.25 o-u   | 8.1 a    | 23.4 a-e   | 0.70 b-j   |

(Continued)

Table 2. *Continues.*

| Species                              | Strain code | Host/substrate                 | %RI <sup>a</sup> | NR         | LR         | RDW        | NSN      | SL         | SDW      |
|--------------------------------------|-------------|--------------------------------|------------------|------------|------------|------------|----------|------------|----------|
| <i>Ilyonectria europaea</i>          | Cy260       | <i>Vitis vinifera</i>          | 37 b-e           | 30.7 l-t   | 28.0 b-o   | 3.03 k-t   | 11.0 a-f | 28.5 b-k   | 0.71 b-j |
|                                      | Cy131       | <i>Actinidia chinensis</i>     | 73 b-g           | 22.6 a-h   | 31.0 e-r   | 2.19 d-o   | 10.8 a-f | 32.3 f-m   | 0.71 b-j |
|                                      | CBS 537.92  | <i>Aesculus hippocastanum</i>  | 96 g             | 28.1 g-r   | 24.8 b-h   | 2.73 i-q   | 9.6 a-f  | 26.6 a-h   | 0.61 b-g |
| <i>Ilyonectria liriodendri</i>       | CBS 129078  | <i>Vitis vinifera</i>          | 76 c-g           | 21.3 a-f   | 30.6 f-p   | 2.03 d-l   | 11.1 a-f | 28.0 b-j   | 0.71 b-j |
|                                      | Cy155       | <i>Vitis vinifera</i>          | 85 d-g           | 19.8 abc   | 31.0 e-r   | 2.21 d-o   | 11.1 a-f | 26.6 a-h   | 0.62 b-h |
|                                      | CBS 110.81  | <i>Liriodendron tulipifera</i> | 75 b-g           | 27.5 d-r   | 23.8 a-h   | 0.77 ab    | 10.0 a-f | 36.7 j-n   | 0.73 b-k |
|                                      | Cy164       | <i>Malus domestica</i>         | 51 b-g           | 29.0 h-r   | 33.0 m,o-r | 2.90 k-r   | 13.0 fg  | 37.1 lmn   | 0.63 b-g |
|                                      | Cy232       | <i>Quercus suber</i>           | 64 b-g           | 25.2 a-n   | 33.3 m-r   | 2.77 j-r   | 11.3 a-f | 32.7 f,h-m | 0.74 b-k |
|                                      | Cy5         | <i>Vitis vinifera</i>          | 51 b-g           | 23.6 a-j   | 33.0 k-r   | 3.10 m,o-t | 11.6 a-f | 33.0 f,h-m | 0.77 b-k |
|                                      | CBS 117526  | <i>Vitis vinifera</i>          | 88 efg           | 19.2 a     | 38.0 qr    | 1.99 c-l   | 11.3 a-f | 31.8 e-m   | 0.66 b-j |
| <i>Ilyonectria lusitanica</i>        | Cy190       | <i>Vitis vinifera</i>          | 75 b-g           | 25.4 a-m   | 24.9 b-h   | 1.65 b-h   | 10.7 a-f | 32.2 f,h-m | 0.76 b-k |
|                                      | Cy253       | <i>Vitis vinifera</i>          | 67 b-g           | 26.0 a-o   | 32.4 j-r   | 3.97 s-v   | 11.0 a-f | 26.1 a-h   | 0.61 b-h |
|                                      | Cy197       | <i>Vitis vinifera</i>          | 80 c-g           | 24.1 a-l   | 23.7 a-f   | 1.28 a-d   | 8.9 a-d  | 24.3 a-g   | 0.41 ab  |
|                                      | OL2         | <i>Olea europaea</i>           | 19 ab            | 26.1 b-o   | 38.2 r     | 4.50 v     | 8.8 ab   | 21.8 ab    | 0.45 abc |
|                                      | CBS 117824  | <i>Quercus</i> sp.             | 83 d-g           | 20.0 ab    | 31.2 h-r   | 1.34 a-d   | 9.8 a-f  | 30.8 c-l   | 0.88 e-k |
| <i>Ilyonectria pseudodestructans</i> | CBS 117812  | <i>Quercus</i> sp.             | 48 b-f           | 30.6 l-t   | 34.1 o-r   | 3.06 m,o-s | 11.5 a-f | 30.5 c-l   | 0.75 b-k |
|                                      | CBS 129081  | <i>Vitis vinifera</i>          | 53 b-g           | 28.2 g-r   | 36.2 pqr   | 3.35 p-u   | 11.3 a-f | 33.8 h-m   | 0.97 g-k |
|                                      | Cy22        | <i>Vitis vinifera</i>          | 32 bcd           | 26.3 b-o   | 31.8 i-r   | 1.49 a-f   | 10.3 a-f | 32.5 f,h-m | 0.67 b-j |
|                                      | CD1666      | <i>Panax quinquefolium</i>     | 42 b-e           | 21.3 a-f   | 26.7 b-n   | 1.53 b-f   | 9.7 a-e  | 23.8 a-e-g | 0.54 a-d |
|                                      | CBS 308.35  | <i>Panax quinquefolium</i>     | 74 b-g           | 25.7 a-o   | 24.0 a-g   | 1.45 a-f   | 11.1 a-f | 34.5 h-m   | 0.62 b-g |
|                                      | CPC 13532   | <i>Prunus cerasus</i>          | 77 b-g           | 22.2 a-g   | 32.8 k-r   | 3.44 q-v   | 12.5 c-g | 34.9 h-n   | 0.99 ijk |
|                                      | CBS 117813  | <i>Quercus robur</i>           | 72 b-g           | 23.1 a-i   | 30.8 e-q   | 2.60 g-q   | 11.4 a-f | 34.9 h-n   | 0.74 b-k |
|                                      | CBS 117815  | <i>Quercus</i> sp.             | 75 b-g           | 27.5 d,g-r | 21.4 ab    | 2.47 f-q   | 12.6 d-g | 36.5 k-n   | 0.64 b-j |
|                                      | Cy231       | <i>Thymus</i> sp.              | 96 fg            | 26.6 b-q   | 27.0 b-o   | 3.40 p-v   | 11.6 a-f | 35.7 h-n   | 0.60 a-j |
|                                      | CBS 605.92  | <i>Tilia petiolaris</i>        | 50 b-g           | 24.3 a-l   | 28.0 b-o   | 1.49 a-f   | 10.2 a-f | 31.5 e-l   | 0.91 f-k |
| <i>Ilyonectria pseudodestructans</i> | Cy23        | <i>Vitis vinifera</i>          | 92 efg           | 20.0 a-f   | 20.7 ab    | 1.52 a-h   | 10.1 a-f | 32.1 e-m   | 0.65 b-j |
|                                      | Cy158       | <i>Vitis vinifera</i>          | 80 c-g           | 25.9 a-o   | 26.2 b-n   | 2.01 c-l,n | 11.3 a-f | 34.8 h-n   | 0.71 b-j |
|                                      | CBS 129084  | <i>Vitis vinifera</i>          | 71 b-g           | 28.8 g-r   | 31.0 g-q   | 1.96 c-l   | 11.5 a-f | 34.7 h-m   | 0.78 b-k |
|                                      | CBS 773.83  | water                          | 40 b-e           | 28.0 g-r   | 28.6 b-o   | 3.00 l-s   | 10.9 a-f | 31.8 e-m   | 0.73 b-j |

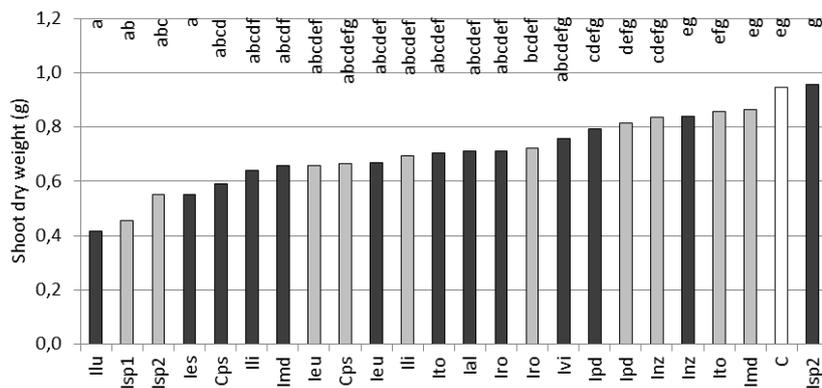
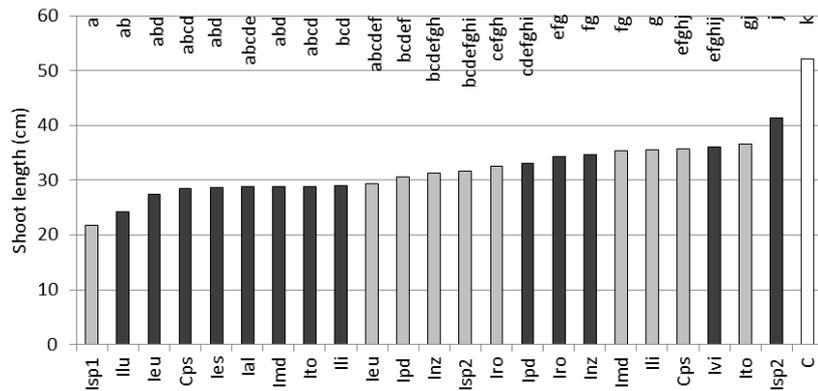
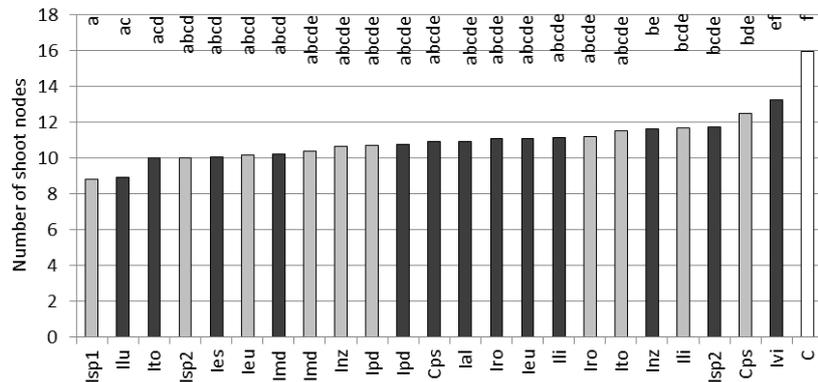
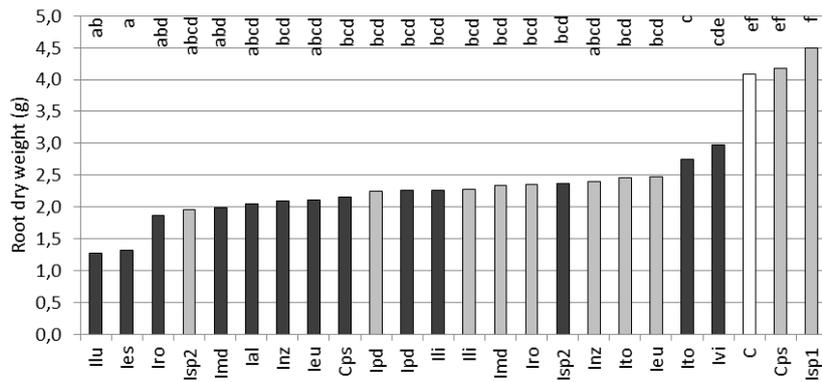
<sup>a</sup> In each column, values followed by the same letter do not differ statistically according to Tukey's test ( $P=0.05$ )



(Continued)

**Figure 1.** Comparison among *Ilyonectria* spp. isolates from grapevines (black bars) and from other hosts (grey bars) for their effect on grapevine rootstock 1103P in: frequency of reisolation; number of roots; length of the longest root; root dry weight; number of shoot nodes; shoot length; shoot dry weight. Bars affected by the same letter do not differ statistically according to Tukey's test ( $P=0.05$ ). C, Control (white bars); Cps, "*C.*" *pauciseptatum* (average of three isolates from grapevine and one isolate from another host); Ial, *I. alcacerensis* (two isolates from grapevines); Ies, *I. estremocensis* (four isolates from grapevines); Ieu, *I. europaea* (two isolates from grapevines and two from other hosts); Ili, *I. liriodendri* (four isolates from grapevines and three from other hosts); Ilu, *I. lusitanica* (one isolate from grapevines); Imd, *I. macrodidyma* (four isolates from grapevines and two from other hosts); Inz, *I. novozelandica* (four isolates from grapevines and one from another host); Iro, *I. robusta* (three isolates from grapevines and eight from other hosts); Isp1, *Ilyonectria* sp1 (one isolate from olive); Ipd, *I. pseudodestructans* (two isolates from grapevines and two from other hosts); Isp2, *Ilyonectria* sp2 (two isolates from grapevines and two from other hosts); Ito, *I. torresensis* (four isolates from grapevines and two from other hosts); Ivi, *I. vitis* (one isolate).

(Continues)



a maximum of 66% (16.8 cm) for isolate Cy243.

The average number of shoot nodes in the control plants was 15.9, ranging for the inoculated plants from 13.2 nodes for isolate Cy233 (*I. vitis*, from grapevine), which did not differ significantly from the control, to 8.1 nodes for isolate CBS 129086 (*I. torresensis*, from grapevine), which represents a 49% reduction.

The average shoot length was 52.2 cm in the control plants, ranging from 43.0 cm for isolate Cy200 (*Ilyonectria* sp2, from grapevine; 43.0 cm, 18% reduction) to 18.1 cm for isolate Cy243 (18.1 cm, 65% reduction).

The shoot dry weight ranged from a maximum of 1.08 g for isolate CBS 113552 (*I. novozelandica*, from grapevine), which did not differ significantly from the control (0.95 g), to a minimum of 0.22 g (Cy243), which represents a 80% reduction.

Considering the isolates obtained from grapevine separate from the isolates from other hosts, significant differences were observed among species and to the control (Figure 1). The percentage of reisolation ranged between 39.4% for *I. pseudodestructans* and 85.0% for *I. vitis* for grapevine isolates, all of them differing significantly from the control. Results for isolates from other hosts ranged between 88.1% for *I. torresensis* and 18.6% for *Ilyonectria* sp1 (which did not differ from the control, along with *I. novozelandica*; the latter was the single species with significant differences among isolates from grapevine and other hosts).

For grapevine isolates, the number of roots ranged between a maximum of 33.3 for *Ilyonectria* sp2 (the single species that did not differ significantly from the control; 36.3) and a minimum of 20.7 for *I. europaea*, representing a 43% reduction in the number of roots. Among the isolates from other hosts, "*C.*" *pauciseptatum*, *I. macrodidyma* and *I. novozelandica* did not differ statistically from the control (non-grapevine isolates from "*C.*" *pauciseptatum* and *I. novozelandica* differed significantly from grapevine isolates), while inoculations with *I. robusta* resulted in the lowest number of roots (a 32% reduction).

The length of the longest root was significantly lower for all samples when compared to the control, ranging between a maximum of 33.8 cm for *I. pseudodestructans* (a 32% reduction from the control) and a minimum of 22.9 cm for *I. estremocensis* (54% reduction) for grapevine isolates, and between 38.2 cm for *Ilyonectria* sp1 and 24.9 cm for *Ilyonectria* sp2 for isolates from other hosts. Significant differences

were recorded, however, for "*C.*" *pauciseptatum* inoculations between grapevine (25.4 cm) and non-grapevine isolates (33.7 cm).

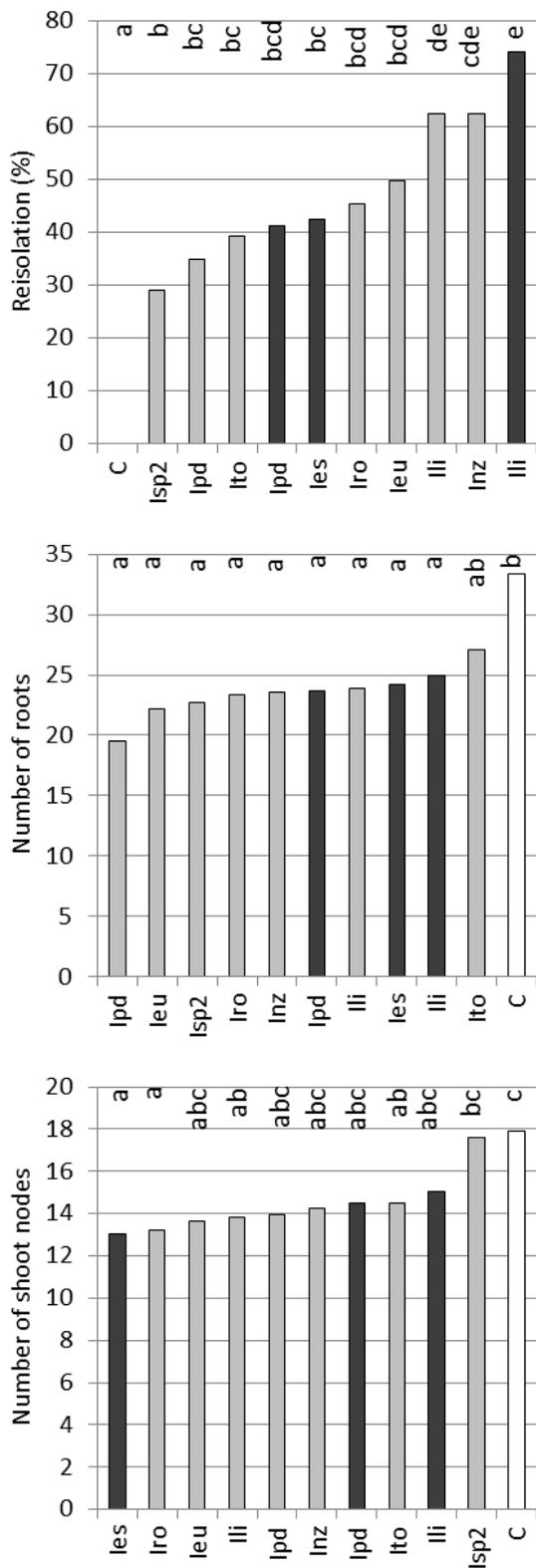
For grapevine isolates, the root dry weight of inoculated plants ranged between a maximum of 2.98 g for *I. vitis* (the single species that does not differ significantly from the control; 4.08 g) and a minimum of 1.28 g for *I. lusitanica* (a 69% reduction from the control). Among non-grapevine isolates, "*C.*" *pauciseptatum* and *Ilyonectria* sp1 did not differ statistically from the control (and "*C.*" *pauciseptatum* non-grapevine isolates differed significantly from grapevine isolates), while inoculations with *Ilyonectria* sp2 resulted in a root dry weight of 1.95 g (a 52% reduction).

Similarly, the number of shoot nodes ranged between a maximum of 13.2 for plants inoculated with *I. vitis* (the single species that did not differ significantly from the control; 15.9) and a minimum of 8.9 for plants inoculated with *I. lusitanica* (a 44% reduction from the control) for grapevine isolates, and between 12.5 for "*C.*" *pauciseptatum* and 8.8 for *Ilyonectria* sp1 among isolates from other hosts. For each species, no significant differences were found between grapevine and non-grapevine isolates.

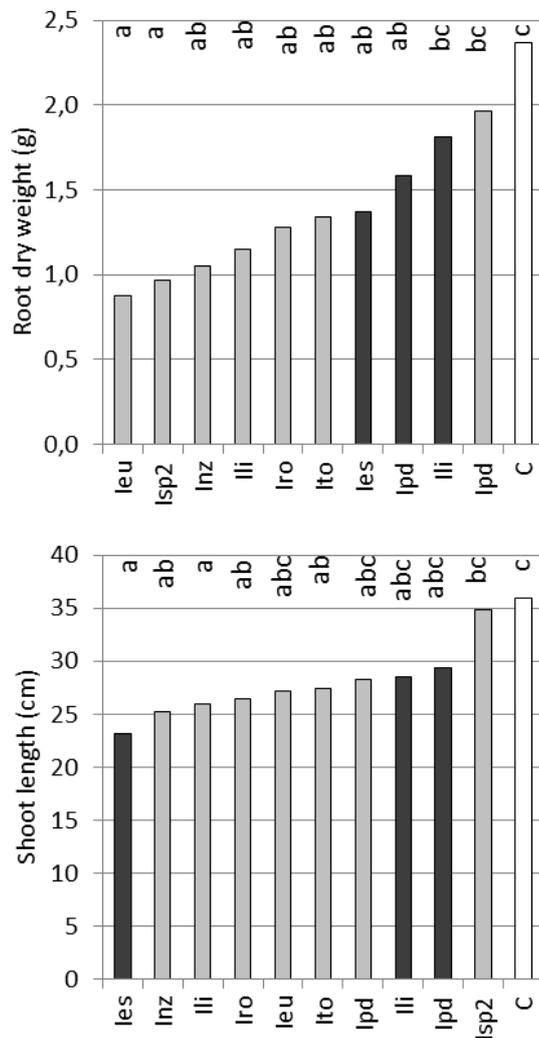
Shoot length was significantly shorter than that of the control for all samples, ranging between a maximum of 41.4 cm for *Ilyonectria* sp2 (a 21% reduction from the control) and a minimum of 24.3 for *I. lusitanica* (53% reduction) for grapevine isolates, and between 36.7 cm for *I. torresensis* and 21.8 cm for *Ilyonectria* sp1 for isolates from other hosts. Non-grapevine isolates had significantly higher values than grapevine isolates for several species, such as "*C.*" *pauciseptatum*, *I. liriodendri*, *I. macrodidyma* and *I. torresensis*, while the opposite was recorded for *Ilyonectria* sp2.

The shoot dry weight ranged between a maximum of 0.96 g for *Ilyonectria* sp2 and a minimum of 0.41 g for *I. lusitanica* (a 57% reduction from the control) for grapevine isolates ("*C.*" *pauciseptatum*, *I. estremocensis*, *I. liriodendri*, *I. lusitanica* and *I. macrodidyma* were significantly lower than the control) and of 0.45 g for *Ilyonectria* sp1 for non-grapevine isolates. Differences between grapevine and non-grapevine isolates were only recorded for *Ilyonectria* sp2 (0.96 g and 0.55 g, respectively).

Inoculated plants in the second experiment also revealed typical black foot symptoms, with significant reductions in root and shoot biomass as com-



**Figure 2.** Comparison among *Ilyonectria* spp. isolates from grapevines (black bars) and from other hosts (grey bars) for their effect on grapevine rootstock 1103P in: frequency of reisolation; number of roots; root dry weight; number of shoot nodes; shoot length. Bars affected by the same letter do not differ statistically according to Tukey's test ( $P=0.05$ ). C, Control (white bars); Ies, *I. estremocensis* (five isolates from grapevines); Ieu, *I. europaea* (one isolate from another host); Ili, *I. liriodendri* (three isolates from grapevines and two from other hosts); Inz, *I. novozelandica* (one isolate from another host); Ipd, *I. pseudodestructans* (two isolates from grapevine); Iro, *I. robusta* (three isolates from other hosts); Isp1, *Ilyonectria* sp1 (one isolate from olive); Ipd, *I. pseudodestructans* (two isolates from grapevines and two from other hosts); Isp2, *Ilyonectria* sp2 (one isolate from another host); Ito, *I. torresensis* (four isolates from other hosts).



pared to the control plants (Figure 2). Considering the species for which grapevine isolates were analysed, *I. estremocensis* was slightly more virulent than *I. liriodendri* and *I. pseudodestructans*, particularly in parameters concerning the aerial plant part, although the frequency of reisolation was significantly lower than that of *I. liriodendri*. Furthermore, results confirmed most non-grapevine isolates to be as virulent as grapevine isolates.

## Discussion

Black foot disease symptoms recorded at the end of the experiments were associated with a reduction in plant growth and vigour, less shoot internodes and roots, and shorter and thinner shoots. These are illustrated by a reduction in the number of roots (up to 47%), shoot nodes (up to 49%), shoot length (up to 65%), length of the longest root (up to 66%), shoot dry weight (up to 80%), and root dry weight (up to 88%).

Frequency of reisolation was the least informative character, only separating the control plants and the isolates OL2 (*Ilyonectria* sp1) from *Olea europaea* and Cy230 (*I. novozelandica*) from *Festuca duriuscula*, from the remaining isolates. Traits related to the roots were slightly more informative than those related to the aerial plant parts, thus corroborating results from Alaniz *et al.* (2010).

In general, grapevine isolates from the species *I. lusitanica*, *I. estremocensis*, *I. europaea* and "*C.*" *pauciseptatum* were the most virulent, while those from species such as *I. novozelandica*, *I. pseudodestructans*, *I. vitis* and *Ilyonectria* sp2 were the least virulent, with intermediate results for *I. robusta*, *I. liriodendri*, *I. macrodidyma*, *I. torresensis* and *I. alcacerensis*. For some species however, differences were recorded between characters related to the roots and to the aerial plant parts. Symptoms related to inoculations by *I. lusitanica*, *I. estremocensis* and "*C.*" *pauciseptatum* isolates were equally prominent based on root and aerial part parameters. In contrast, symptoms caused by *I. europaea*, *I. novozelandica* and *I. robusta* isolates were more prominent on roots than on aerial parts, while symptoms of *I. torresensis* and *I. macrodidyma* were more noticeable on aerial plant parts. However, the effect of these pathogens in the aerial parts should be interpreted while taking into consideration that only ungrafted rootstocks were studied here. Experiments using grafted plants would be necessary to

reach conclusions on the effect of these pathogens on the aerial parts of grapevine plants. In spite of this, the results obtained here reveal that different *Ilyonectria* species and "*C.*" *pauciseptatum* induce diverse levels of severity on the aerial plant parts. This observation may be relevant in infected fields of rootstock mother-plants, because, most likely, the canes will be shorter, thinner and of poorer quality, thus compromising the later success of cuttings made from such vines.

A comparison among all isolates revealed isolates Cy243 (*I. estremocensis*), Cy197 (*I. lusitanica*), Cy23 (*I. robusta*), Cy238 ("*C.*" *pauciseptatum*) and Cy128 (*I. macrodidyma*), all from grapevines, to be the most virulent, while the least virulent were isolates OL-CM3 ("*C.*" *pauciseptatum*) from *Olea europaea*, Cy200 (*Ilyonectria* sp2) from grapevine, CBS 129081 (*I. pseudodestructans*) from grapevine, CBS 112593 (*I. novozelandica*) and Cy164 (*I. liriodendri*) from *Malus domestica*. Virulence to the roots varied among isolates, which in turn exhibit different effects on the aerial parts. Isolates Cy23 (*I. robusta*), Cy128 (*I. macrodidyma*), Cy152 (*I. estremocensis*), Cy196 ("*C.*" *pauciseptatum*), CBS 110.81 (*I. liriodendri*, from *Liriodendron tulipifera*) or CBS 117824 (*I. pseudodestructans*, from *Quercus* sp.) showed high virulence in roots, but limited effects on the aerial parts. On the contrary, isolates CBS 129086 (*I. torresensis*), Cy250 (*I. macrodidyma*), CBS 537.92 (*I. europaea*, from *Aesculus hippocastanum*), CBS 159.34, and particularly isolate OL2 (*Ilyonectria* sp1, from *Olea europaea*) had low reisolation frequency and caused little effect on roots, but a very prominent effect on the above ground parts of inoculated plants. When isolate OL2 was inoculated on olive plants, it was found to be highly virulent (Cabral *et al.*, unpubl. data), inducing not only aerial symptoms but also root and crown necroses. This indicates that *Ilyonectria* sp1 may be more host-specialized than the other species studied here, suggesting that although there are taxa with wide host ranges, host specialisation also occurs in some species of *Ilyonectria*. However, the unexpected pattern of symptoms produced by OL2 or other isolates, suggests that further work is required to fully elucidate the grapevine-*Ilyonectria* pathosystem. To date little information exists on the mechanisms of host infection and root colonization, as well as the concomitant mechanisms of host-defense response. In apple trees for example, it was hypothesized that the most virulent "*Cylindrocarpum*" isolates do not proliferate extensively within the host

tissue, but rather cause damage to the host by the secretion of cell wall degrading enzymes or toxins (Tewoldemedhin *et al.*, 2011).

For each fungal species, comparisons between grapevine and non-grapevine isolates could not suggest specific trends, with the notable exception of isolates from *Olea europaea* (and to some extent from *Festuca duriuscula*), which were always less virulent than grapevine isolates from the same species ("*C.*" *pauciseptatum*, *I. macrodidyma* and *I. torresensis*). However, frequency of re-isolation did not differ significantly to that of other isolates, suggesting that these isolates are fully capable of infecting and colonizing the inoculated plants. The capacity of isolates from hosts such as *Actinidia chinensis*, *Fragaria x ananassa*, *Malus domestica* and *Quercus* spp. to be as virulent as the grapevine isolates, including isolates from some of the most virulent species, such as *I. europaea*, raises the importance of the cross-infection potential of isolates from other hosts to grapevine, particularly for plants that are likely to precede grapevine in cultivation, either in a vineyard or nursery. In fact, a recent study addressing apple replant disease (Tewoldemedhin *et al.*, 2011) revealed the involvement of species also pathogenic to grapevine in the present study, such as "*C.*" *pauciseptatum*, *I. macrodidyma* and *I. lirioidendri*, supporting their polyphagous nature.

Furthermore, many isolates of the *I. macrodidyma* species complex were obtained from roots of several monocotyledons and dicotyledons weed families sampled in Spanish vineyards. When inoculated on grapevines, these isolates were able to induce typical black foot disease symptoms (Agustí-Brisach *et al.*, 2011). In addition to the hosts referred to above, therefore, weeds may represent an important inoculum source of *I. macrodidyma* s. lat. in vineyards.

Besides the importance of cross-infection potential as well as indications of host specificity, the present study also revealed that grapevine isolates from newly described species such as *I. lusitanica*, *I. estremocensis* and *I. europaea* are more virulent to grapevine than the species previously accepted to represent the main causal agents of black foot, such as *I. lirioidendri* and *I. macrodidyma*.

## Acknowledgments

We thank M.Sc. Teresa Nascimento, M.Sc. Ana Teresa Vaz, Mrs. Amélia Marques and Mrs. Olga Natividade for technical assistance. This article is part

of a Ph.D. dissertation (Instituto Superior de Agronomia, Technical University of Lisbon, Portugal). This work was partially supported by Fundação para a Ciência e a Tecnologia, Portugal (grant number SFRH/BD/24790/2005; project PTDC/AGR-AAM/099324/2008).

## Literature cited

- Abreo E., S. Martínez L., Bettucci and S. Lupo, 2010. A Morphological and molecular characterization of *Campylo-carpon* and *Cylindrocarpon* spp. associated with black foot disease of grapevines in Uruguay. *Australasian Plant Pathology* 39, 446–452.
- Agustí-Brisach C., D. Gramaje, M. León, J. García-Jiménez and J. Armengol, 2011. Evaluation of vineyard weeds as potential hosts of black-foot and Petri disease pathogens. *Plant Disease* 95, 803–810.
- Alaniz S., J. Armengol, M. León, J. García-Jiménez and P. Abad-Campos, 2009. Analysis of genetic and virulence diversity of *Cylindrocarpon lirioidendri* and *C. macrodidymum* associated with black foot disease of grapevine. *Mycological Research* 113, 16–23.
- Alaniz S., J. García-Jiménez, P. Abad-Campos and J. Armengol, 2010. Susceptibility of grapevine rootstocks to *Cylindrocarpon lirioidendri* and *C. macrodidymum*. *Scientia Horticulturae* 125, 305–308.
- Alaniz, S., M. León, A. Vicent, J. García-Jiménez, P. Abad-Campos, and J. Armengol, 2007. Characterization of *Cylindrocarpon* species associated with black foot disease of grapevine in Spain. *Plant Disease* 91, 1187–1193.
- Cabral A., J.Z. Groenewald, C. Rego, H. Oliveira and P.W. Crous, 2012a. *Cylindrocarpon* root rot: multi-gene analysis reveals novel species within the *Ilyonectria radialis* species complex. *Mycological Progress* 11, 655–688.
- Cabral A., C. Rego, T. Nascimento, H. Oliveira, J.Z. Groenewald and P.W. Crous, 2012b. Multi-gene analysis and morphology reveal novel *Ilyonectria* species associated with black foot disease of grapevines (*Vitis* spp.). *Fungal Biology* 116, 62–80.
- Chaverri, P., C. Salgado, Y. Hirooka, A.Y. Rossman, and S.J. Samuels, 2011. Delimitation of *Neonectria* and *Cylindrocarpon* (Nectriaceae, Hypocreales, Ascomycota) and related genera with *Cylindrocarpon*-like anamorphs. *Studies in Mycology* 68, 57–78.
- Halleen F., P.H. Fourie and P.W. Crous, 2006a. A review of black-foot disease of grapevines. *Phytopathologia Mediterranea* 45, S55–S67.
- Halleen F., H.-J. Schroers, J.Z. Groenewald, C. Rego, H. Oliveira and P.W. Crous, 2006b. *Neonectria lirioidendri* sp. nov., the main causal agent of black foot disease of grapevines. *Studies in Mycology* 55, 227–234.
- Halleen F., H.-J. Schroers, J.Z. Groenewald and P.W. Crous, 2004. Novel species of *Cylindrocarpon* (*Neonectria*) and *Campylo-carpon* gen. nov. associated with black foot disease of grapevines (*Vitis* spp.). *Studies in Mycology* 50, 431–455.
- Martín M.T., L. Martín, M.J. Cuesta and P. García-Benavides,

2011. First report of *Cylindrocarpon pauciseptatum* associated with grapevine decline from Castilla y León, Spain. *Plant Disease* 95, 361.
- Oliveira H., C. Rego and T. Nascimento, 2004. Decline of young grapevine caused by fungi. *Acta Horticulturae* 652, 295–304.
- Petit E. and W.D. Gubler, 2005. Characterization of *Cylindrocarpon* species, the cause of black foot disease of grapevine in California. *Plant Disease* 89, 1051–1059.
- Rego C., H. Oliveira, A. Carvalho and A. Phillips, 2000. Involvement of *Phaeoacremonium* spp. and *Cylindrocarpon destructans* with grapevine decline in Portugal. *Phytopathologia Mediterranea* 39, 76–79.
- Schroers H.-J. M., Žerjav, A. Munda, F. Halleen and P.W. Crous, 2008. *Cylindrocarpon pauciseptatum* sp. nov., with notes on *Cylindrocarpon* species with wide, predominantly 3-spore macroconidia. *Mycological Research* 112, 82–92.
- Tewoldemedhin Y.T., M. Mazzola, M. Mostert and A. McLeod, 2011. *Cylindrocarpon* species associated with apple tree roots in South Africa and their quantification using real-time PCR. *European Journal of Plant Pathology* 129, 637–651.

*Accepted for publication: January 20, 2012*