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Black foot of grapevine: sensitivity of *Cylindrocarpon destructans* to fungicides

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Summary. Black foot disease of grapevine is caused by *Cylindrocarpon* spp., with *C. destructans* being the main pathogen isolated from vine cuttings and young vineyards in Portugal. Few recommendations for black foot disease control are presently available, and they are not easy to implement within commercial nurseries. In this study, 14 fungicides were evaluated for their effect on the mycelial growth and conidium germination of four field isolates of *C. destructans*. Mycelial growth of the pathogen was inhibited by DMI fungicides, prochloraz (EC_{50} values <0.09 mg l^{-1}) and to a lesser extent by difenoconazole (EC_{50} values <2.25 mg l^{-1}), by the benzimidazole fungicide benomyl (EC_{50} values <0.35 mg l^{-1}), and by the mixtures cyprodinil + fludioxonil and carbendazim + flusilazole, which gave EC_{50} values <0.75 mg l^{-1}. Among these, only cyprodinil + fludioxonil (EC_{50} values <0.15 mg l^{-1}), the strobilurin fungicides, azoxystrobin and trifloxystrobin (EC_{50} values <2.27 mg l^{-1}) and the phenylsulphamide fungicide tolylfluanid (EC_{50} < 0.54 mg l^{-1}) were effective in reducing conidium germination. Results from *in vivo* studies, carried out on potted grapevine plants (cultivar Castelão) showed that benomyl, tebuconazole and the mixtures carbendazim + flusilazole and cyprodinil + fludioxonil significantly (α=0.05) improved plant growth (plant height and number of roots) and decreased disease incidence compared with non-treated plants.

Key words: *Cylindrocarpon*, black foot disease, chemical control, *Vitis vinifera*.

Introduction

Cylindrocarpon species, in particular *Cylindrocarpon destructans* (Zins.) Scholten, teleomorph *Neonectria radicicola* (Gerlach & Nilsson) Mantiri & Samuels, are responsible by black foot disease of grapevine (Halleen *et al*., 2004). These fungi together with *Phaeomoniella chlamydospora*, the main causal agent of Petri disease, are responsible for decline and dieback of young vineyards (Rego *et al*., 2000). Over the last years, the incidence and severity of both diseases have increased all over the world and are of major concern to grapevine nurserymen and grape producers. Infected grapevine propagating material, mainly rootstock cuttings, is believed to be the main means of spread of *Pa. chlamydospora* (Mugnai *et al*., 1999; Fourie and Halleen, 2002; 2004a, 2004b; Whiteman *et al*., 2004), although the pathogen can also be present in the soil (Rooney *et al*., 2001; Whiteman *et al*., 2004). *C. destructans* could also be present in rootstock cuttings (Rego *et al*., 2001a), but it is isolated at much higher frequencies from both rooted cuttings and graftlings in nurseries (Rego *et al*., 2000; Fourie and Halleen, 2001). Vineyards settled with such infected materi-

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al soon show a high percentage of declining plants with slow growth, reduced vigour, retarded sprouting, shortened internodes, sparse and chlorotic foliage. Internally, plants exhibit black discolouration and brown to dark streaks in the wood, mainly at the basal end of the rootstock. *C. destructans* has been the most isolated fungus from these plants in Portugal; *Pa. chlamydospora* was also frequently obtained (Rego *et al*., 2000; Oliveira *et al*., 2004). Infected plants frequently die and growers are consequently forced to replant considerable vineyard areas, resulting in serious losses and compromising the sustainability of vineyards.

Despite the actuality and importance of these diseases, a comprehensive understanding of the bioecology of *Pa. chlamydospora* and *Cylindrocarpon* spp. infecting grapevine is still lacking. This knowledge is essential to develop rational control methods to prevent and manage the decline and dieback of young grapevines. In recent years, promising results have been achieved with respect to Petri disease management (Crous *et al*., 2001; Fourie *et al*., 2001; Jaspers, 2001; Laukart *et al*., 2001; Rooney *et al*., 2001; Fourie and Halleen, 2004a, 2004b) but no comparable data are available for black foot disease control. Fourie *et al*. (2001) showed the attributes of *Trichoderma* treatments (dips, soil amendments and drenches) against several fungi in grapevine nurseries, including *Cylindrocarpon* spp. Also, Gubler *et al*. (2004) reported unpublished data concerning the excellent control of black foot by using the mychorrizal fungus *Glomus intraradices*. Hot water treatment (HWT) of dormant nursery vines has also been recommended in some countries to control *Cylindrocarpon* spp. and other fungi like *Phaeomoniella* and *Phaeoacremonium* spp., but this treatment has a short-term effect and grapevine cuttings might be re-infected after the rooting process in open field (Crous *et al*., 2001; Halleen *et al*., 2003, 2005; Fourie and Halleen, 2004b). Therefore, Crous *et al*. (2001) suggested its combined use with a biological control agent.

Biological and physical treatments to control black foot disease are very important but it is also advisable to have effective fungicides that might be used as alternative or combined with other control methods at different stages of the grapevine propagation process. Practices in the grapevine nursery industry are responsible for a large number of wounds on propagating material, during preparation of cuttings and graftlings. These wounds are potential infection sites for *Cylindrocarpon* spp., as well as other soilborne pathogens. Also, a rooting process for these graftlings is actually imposed in open field for one season. Therefore, even if cuttings are taken from healthy rootstocks mother plants, they could be infected during the rooting process (Rego *et al*., 2001b; Halleen *et al*., 2003).

The aim of this study was to evaluate the effect of selected fungicides on grapevines growing in *Cylindrocarpon* infested soil, as might be expected during the rooting process in the nursery. To achieve this objective the fungicides were first tested for their effects on mycelial growth and spore germination of *C. destructans*. Preliminary results of this study were published by Rego *et al*. (2004).

Materials and methods

Cylindrocarpon isolates and cultural conditions

Four *C. destructans* isolates were used in this study. They were selected from the collection at the Laboratório de Patologia Vegetal 'Veríssimo de Almeida' (LPVVA), Lisbon, Portugal, as follows: Cy1, as *C. destructans* typical strain (IMI 357400, Rego 1994); Cy68 (CBS 117526), Cy21 and Cy32, as representing variation within *C. destructans* collection (Rego *et al*., 2001b). They were collected in Portugal from either young grapevines showing black foot disease symptoms (Cy1 from cv. Seara Nova grafted on 99R and Cy21 from cv. Malvasia Fina grafted on 1103P) or 99R rooted rootstocks (Cy32, Cy68). The isolates were maintained on slants of 'Spezieller Nährstoffarmer Agar' containing 0.1% yeast extract (SNAY) at 5ºC (Brayford, 1992), and routinely subcultured on Petri dishes containing potato dextrose agar (PDA, Difco Laboratories, Detroit, MI, USA) at 24ºC, in darkness. For liquid cultures, 500 ml-Erlenmeyer flasks containing 250 ml of Czapek-Dox liquid medium (Modified) (Oxoid, LTD) were inoculated with 3 mm diameter agar plugs cut from the edge of colonies grown under the conditions referred to above. Flasks were placed in a reciprocal shaker (90 strokes min^{-1}) for 12 days at room temperature in the dark.

Fungicides

Commercial formulations of 14 fungicides were tested in all experiments (Table 1). They were either registered for use on grapevine or were known

Table 1. Details of the 14 fungicides studied.

Chemical group	Fungicide name	Trade name	Company	Concentration a. i.	Field rate
Amide	Carpropamid	KTU 3616300SC	Bayer	300 g l^{-1}	1.00 ml l^{-1}
Anilopyrimidine	Cyprodinil	Chorus 50%	Novartis	50%	0.30 g l^{-1}
	Pyrimethanil	Scala	Agrevo	400 g l^{-1}	2.50 ml l^{-1}
Benzimidazole	Benomyl	Benlate	Rhône-Poulenc	50%	0.45 g l^{-1}
	Thiabendazole	Tecto 500SC	Novartis	500 g l^{-1}	2.10 ml l^{-1}
DMI (imidazole)	Prochloraz	Sportak 45 HF	Syngenta	450 g l^{-1}	1.00 ml l^{-1}
DMI (triazole)	Tebuconazole	Folicur WG25	Bayer	25%	0.40 g l^{-1}
	Difenoconazole	Score 250 EC	Syngenta	250 g l^{-1}	0.40 ml l^{-1}
Phosphonate	Fosetyl-Al	Alliette	Aventis	80%	2.50 g l^{-1}
Strobilurin	Azoxystrobin	Quadris	Syngenta	250 g l^{-1}	1.00 ml l^{-1}
	Trifloxystrobin	Flint 50WG	Bayer	50%	0.15 g l^{-1}
Phenylsulfamide	Tolylfluanid	Euparene M	Bayer	50%	2.00 g l^{-1}
Mixture	Cyprodinil + Fludioxonil	Switch 62.5 WG	Novartis	37.5% cyprodinil + 25% fludioxonil	1.00 g l^{-1}
Mixture	Carbendazim + Flusilazole	Escudo	DuPont	10 g l^{-1} carbendazim + 5 g l^{-1} flusilazole	2.50 ml l^{-1}

to be effective against fungi related to *C. destructans*. Stock fungicide suspensions (10 mg a.i. l^{-1}) were obtained by suspending the commercial products directly in sterile distilled water (SDW). For each fungicide, a series of six decreasing concentrations 5, 1, 0.5, 0.1, 0.05 and 0.01 mg a.i. l^{-1} was assayed, and for each concentration six replicates were included. All experiments were repeated once within 15 days.

Mycelial growth assay

Appropriate volumes of each fungicide were added to molten PDA at 50°C to obtain the test concentrations. Aliquots of 15 ml of the fungicide-amended PDA were poured immediately into 9-cm-diameter Petri dishes. After cooling, one mycelial plug (3 mm diameter) cut from the growing edge of *C. destructans* cultures was transferred to the centre of each plate. In control plates SDW was used instead of a fungicide dilution. The dishes were incubated for 12 days in the dark at 24°C, whereafter the colony diameters were measured. The inhibition of mycelial growth was determined as a percentage, relating to the mycelial growth in presence and in the absence of fungicide.

Conidial germination assay

Conidia were harvested from 12-day-old colonies growing on PDA. Sterile distilled water (10 ml) was added to each plate, and the surface was gently scraped with a sterile loop to release the spores. The resulting suspensions of conidia were transferred to sterile glass culture tubes, filtered through two layers of sterile cheesecloth to remove any mycelial fragment, and diluted with SDW to give the desired concentration of 10^5 conidia ml^{-1}. Experiments to evaluate conidial germination were done according to Olaya and Koller (1999). Each conidial suspension (150-μl aliquots) was dispensed into Eppendorf tubes (1.5 ml) containing 150-μl aliquots of each fungicide dilution, prepared as mentioned above, or SDW (150 μl) as control. Four 30 μl droplets of these suspensions were subsequently placed in the base of polystyrene Petri dishes and covered with a cover glass (2.2×2.2 cm). After five hours of incubation at 24°C in the dark, conidium germination was determined by microscopic examination (160× magnification) of 200 conidia (50 per droplet) for each combination of fungicide concentration and isolate. A conidium was rated as germinated if germ tube(s) were at least

the length of the conidium. All assays were conducted twice within 15 days. The inhibition of spore germination was determined as a percentage of germination in the absence of fungicide.

***In vivo* assay**

Studies were conducted in a greenhouse at 24 ± 5ºC day/18ºC night with 12 h of daylight. Inoculum was prepared from 12-day-old cultures of each *C. destructans* isolate grown in Czapek-Dox liquid medium. The conidial suspensions were filtered through a double layer of cheesecloth, quantified with a hemacytometer and adjusted to a final concentration of approximately 10^8 conidia ml^{-1}. Each conidial suspension was subsequently added (1:10, v:v) to an autoclaved mixture of soil, peat and sand (2:1:1, v:v) and mixed thoroughly by hand. Substrate infested with each isolate was placed separately in 1-liter plastic pots. Control plants were similarly treated, but SDW was used instead of inoculum.

Aqueous suspensions of fungicides were prepared according to the recommended field rates on the label for other grapevine diseases or similar fungi (Table 1). Roots of 10 grapevine cuttings of grapevine cv. Castelão had their extremities pruned and the basal ends of cuttings (5–10 cm) were dipped for 50 min in each fungicide suspension. Control plants were similarly treated, but SDW was used instead of a fungicide. Treated and untreated grapevine plants were potted (one plant per pot) in the infested soil mixture and placed at random in the greenhouse. Plants were checked for other diseases or pests and watered weekly. After three months, all plants were uprooted and evaluated for the total number of roots and plant height according to the methods described by Adalat *et al*. (2000). Isolations were made from necrotic tissues located within 5 cm of the basal end of rootstocks (Rego *et al*., 2000) and the identity of *C. destructans* isolates was confirmed on the basis of morphological characters. The incidence of *Cylindrocarpon*-black foot disease was determined as the mean percentage of grapevine plants that were infected by each isolate of *C. destructans*.

Data analysis

For each fungicide and isolate, percentages of mycelial growth and conidial germination inhibitions were converted to probits and plotted against $\log_{10}$ values of the fungicide concentration. Probit regression analysis was used to calculate the effective concentration values that inhibited mycelial growth and spore germination by 50% (EC_{50} values) and their 95% confidence limits values, using STATISTICA 6.0 (StatSoft Inc., Tulsa, OK, USA).

For the *in vivo* assay, plant height, total number of roots and *C. destructans* incidence data were subjected to analysis of variance (ANOVA) and treatment means compared by using Tukey's test at a 5% significance level (STATISTICA 6.0). Percentages were transformed to arcsine-square root values before analyses.

Results

In probit regression analyses, only coefficients of determination (r^2) higher than 0.80 and confidence limits (CL) of EC_{50} lower than 0.05 were used. Significant isolate × treatment interactions were observed in assays ($P<0.05$; ANOVA not shown) and data for each isolate were therefore treated separately.

Of the 14 fungicides tested mycelial growth of *C. destructans* (isolates Cy1, Cy21, Cy32 and Cy68) was significantly reduced only by the demethylation inhibitor (DMI) fungicides, prochloraz and difenoconazole, by the benzimidazole fungicide benomyl, and by the mixtures carbendazim + flusilazole and cyprodinil + fludioxonil (Table 2). Prochloraz was the most effective fungicide (EC_{50} values ≤0.09 mg l^{-1}), followed by carbendazim + flusilazole (EC_{50} values ≤0.23 mg l^{-1}), benomyl (EC_{50} values ≤0.35 mg l^{-1}) and cyprodinil + fludioxonil (EC_{50} values ≤0.75 mg l^{-1}). Within isolates, variation of sensitivity was mainly observed with difenoconazole, with isolates Cy68 and Cy21 being less sensitive than Cy1 and Cy32. From the DMI fungicide, only tebuconazole was considered ineffective, because of its inability to inhibit mycelial growth of isolate Cy68. However, the remaining isolates showed sensitivity to this fungicide, with EC_{50} values less than the highest concentration tested (5 mg l^{-1}).

Among the fungicides effectively reducing mycelial growth of *C. destructans*, only the mixture cyprodinil + fludioxonil was able to inhibit conidium germination of the fungus, with EC_{50} values less than 0.15 mg l^{-1} (Table 2). The strobilurin fungicides, azoxystrobin and trifloxystrobin (EC_{50} values ≤2.27 mg l^{-1}) and the phenylsulfamide tolylflu-

Table 2. Effective concentration (EC_{50}) values (mg a.i. l^{-1}) of 14 fungicides against mycelial growth and conidial germination of *Cylindrocarpon destructans*, isolates Cy1, Cy21, Cy32 and Cy68.

Fungicide name	Mycelial growth (EC_{50})				Spore germination (EC_{50})			
	Cy1	Cy21	Cy32	Cy68	Cy1	Cy21	Cy32	Cy68
Tolylfluanid	>5[a]	>5	>5	>5	0.31[a]	0.42	0.40	0.54
Cyprodinil	>5	>5	>5	>5	>5	>5	>5	>5
Difenoconazole	0.40	1.51	0.49	2.25	>5	>5	>5	>5
Thiabendazole	>5	>5	>5	>5	>5	>5	>5	>5
Pyrimethanil	>5	>5	>5	>5	>5	>5	>5	>5
Trifloxystrobin	>5	>5	>5	>5	1.38	0.93	1.13	0.38
Carpropamid	>5	>5	>5	>5	>5	>5	>5	>5
Fosetyl-Al	>5	>5	>5	>5	>5			
Azoxystrobin	>5	>5	>5	>5	0.87	1.41	0.33	2.27
Prochloraz	0.05	0.07	0.08	0.09	>5	>5	>5	>5
Cyprodinil + fludioxonil	0.59	0.41	0.36	0.75	0.08	0.14	0.15	0.15
Tebuconazole	2.59	3.91	3.14	>5	>5	>5	>5	>5
Carbendazim + flusilazole	0.23	0.23	0.23	0.19	>5	>5	>5	>5
Benomyl	0.31	0.22	0.30	0.35	>5	>5	>5	>5

[a] Values of >5 were given when mycelial growth or spore germination was not inhibited by 50% at 5 mg a.i. l^{-1}.

anid (EC_{50} values ≤0.54 mg l^{-1}) also inhibited conidium germination of *C. destructans*, although they were ineffective in inhibiting mycelial growth. The mixture cyprodinil + fludioxonil was the most effective in reducing spore germination, as revealed by lowest EC_{50} values (0.08–0.15 mg l^{-1}).

In the *in vivo* assay, potted grapevine plants grown in soil mixture previously infested with each *C. destructans* isolate (Cy1, Cy21, Cy32 and Cy68) consistently became diseased when water was used as treatment (100% disease incidence; Table 3). Symptoms developed within three months after inoculation and included poor growth, shortened internodes, sparse foliage and low number of roots. When plants were cut for isolations, typical internal black foot necroses were visible.

The response of grapevine plants to the fungicides was dependent on the *C. destructans* isolate with Cy1 being the least effective regarding the plant height parameter. The mixture carbendazim + flusilazole significantly improved the height of grapevine plants grown in soil mixture infested with isolate Cy1. For plants grown in soil infested with isolates Cy21 or Cy32, significant differences (α=0.05) in plant height were found between those treated with carbendazim + flusilazole, cyprodinil + fludioxonil, tebuconazole or benomyl fungicides and the untreated controls. Similar results were obtained with isolate Cy68, except for the cyprodinil + fludioxonil mixture (Table 3).

When the total number of roots was analysed, only benomyl was able to improve this parameter for plants grown in soil infested with Cy1 isolate. In contrast, concerning Cy68 isolate, plants treated with cyprodinil + fludioxonil, carbendazim + flusilazole, tebuconazole, benomyl and prochloraz fungicides had significantly more roots than controls (α=0.05). A comparable result was obtained for isolate Cy32, except for prochloraz effect. For plants grown in soil infested with isolate Cy21, only the two fungicide mixtures and benomyl were able to significantly improve the total number of roots (Table 3).

From internal black foot necroses, *C. destructans* was reisolated from all untreated plants grown in soil infested with each fungal isolate (Table 3). Significantly lower incidences (α=0.05) were recorded for some of the fungicide treatments. For all isolates, a reduced *Cylindrocarpon* incidence was observed in the internal black foot necroses when the fungicides tebuconazole, benomyl and the mixtures cyprodinil + fludioxonil and carbendazim + flusilazole were used. Although significant differences were not observed between these fungicides and some of the others, they were always significantly different from the controls. Unexpectedly, disease incidence on plants grown in Cy1 in-

Table 3. Effects of pre-planting fungicide treatments on plant height, total number of roots and *Cylindrocarpon* incidence of grapevine plants cv. Castelão grown in soil mixture infested by four isolates of *Cylindrocarpon destructans* (Cy1, Cy21, Cy32 and Cy68).

Treatment	Plant height (cm)				Total number of roots				*Cylindrocarpon* incidence (%)			
	Cy1	Cy21	Cy32	Cy68	Cy1	Cy21	Cy32	Cy68	Cy1	Cy21	Cy32	Cy68
Water control	16.2 a[a]	24.2 a	14.8 a	15.0 a	25.2 ab	25.9 ab	16.6 a	14.4 a	100 b	100 c	100 b	100 d
Carpropamid	17.6 a	22.6 a	17.3 a	24.2 a	23.9 ab	23.6 ab	19.8 a	20.9 a	60 ab	70 bc	70 ab	100 d
Cyprodinil	16.1 a	19.1 a	19.5 a	19.6 a	16.5 a	18.2 a	17.1 a	22.2 a	40 ab	60 abc	60 ab	70 abcd
Pyrimethanil	17.8 a	22.9 a	16.4 a	16.9 a	24.0 abc	23.6 ab	21.1 a	21.4 a	30 a	50 abc	60 ab	80 bcd
Benomyl	32.4 a	61.8 c	76.2 d	53.5 c	44.5 c	48.1 c	90.1 c	64.6 b	0 a	10 ab	20 a	40 abc
Thiabendazole	21.5 a	13.4 a	18.4 a	17.1 a	22.5 ab	20.6 ab	22.7 a	18.2 a	20 a	40 abc	70 ab	80 bcd
Prochloraz	31.0 a	34.8 ab	25.4 ab	33.8 abc	32.4 abc	29.7 abc	22.3 a	51.7 b	30 a	30 ab	40 ab	80 bcd
Tebuconazole	28.0 a	57.5 c	58.9 c	47.6 bc	36.1 abc	40.0 bc	65.9 b	63.0 bc	10 a	30 ab	10 a	30 ab
Difenoconazole	19.0 a	18.2 a	16.3 a	19.1 a	24.9 abc	20.1 a	19.0 a	18.6 a	60 ab	60 abc	60 ab	100 d
Fosetyl-Al	19.0 a	23.1 a	26.5 ab	23.2 a	24.9 abc	21.7 ab	24.9 a	20.9 a	30 a	60 abc	70 ab	100 d
Azoxystrobin	35.4 ab	28.9 ab	25.0 ab	20.6 a	29.6 abc	25.9 ab	27.7 a	22.5 a	20 a	40 abc	60 ab	100 d
Trifloxystrobin	20.0 a	17.4 a	18.5 a	18.1 a	22.3 ab	21.8 ab	23.4 a	22.8 a	40 ab	50 abc	50 ab	90 cd
Tolylfluanid	19.4 a	17.0 a	17.2 a	18.0 a	20.7 ab	19.6 a	22.6 a	18.4 a	40 ab	40 abc	50 ab	90 cd
Cyprodinil + fludioxonil	26.2 a	48.4 bc	44.5 bc	31.7 ab	37.4 bc	48.5 c	54.8 b	57.4 b	20 a	10 ab	20 a	20 a
Carbendazim + flusilazole	53.9 b	62.5 c	68.9 d	56.2 c	36.0 abc	48.1 c	72.9 bc	80.0 c	0 a	12 a	10 a	20 a

[a] In each column data followed by the same letter did not differ significantly (α=0.05) according to Tukey's test; each value is the mean of 10 repetions of the plant data.

fested substrate was also significantly reduced by pyrimethanil, fosetyl-Al and thiabendazole, which had not shown *in vitro* efficacy. In addition, azoxystrobin and prochloraz, which revealed *in vitro* efficacy in inhibiting spore germination and mycelial growth of *C. destructans*, respectively, were only able to significantly reduce disease incidence for plants grown in soil infested with isolates Cy1 and Cy21.

Discussion

In the last couple of years, special focus has been placed on the control of Petri disease by using chemical, biological and physical approaches (Crous *et al*., 2001; Fourie *et al*., 2001; Jaspers, 2001; Rooney and Gubler, 2001; Fourie and Halleen, 2004b). Earlier studies on the use of fungicides to control black foot disease were reported by Dumot *et al*. (1999). However, these results were inconclusive, because *C. destructans* was not reisolated from control plants (artificially infested and untreated plants).

Results from the present study lead us to conclude that some fungicides identified as being effective against *C. destructans* have potential to be used in commercial nurseries. The mixture cyprodinil + fludioxonil was able to significantly reduce mycelial growth and spore germination (*in vitro*), as well as the incidence of *C. destructans in vivo*. This mixture is registered for use in integrated pest management (IPM) of grapes in Portugal to control *Botrytis cinerea*, and is also reported to be effective in inhibiting mycelial growth and spore germination of *Pa. chlamydospora* (Jaspers, 2001). Besides this, the fungicides benomyl, tebuconazole and carbendazim + flusilazole also proved to be effective against *C. destructans* in the present experimental conditions. Benomyl and tebuconazole were also noted for by their efficacy against *Pa. chlamydospora* (Groenewald *et al*., 2000; Jaspers, 2001). However, benomyl was withdrawn from the market, according to Annex I to Council Directive 91/414/EEC.

The effectiveness of fungicides *in planta* was always better related with results obtained for mycelial growth reduction of *C. destructans* than with those achieved for inhibition of spore germination. Only difenoconazole, and to a certain extent prochloraz, broke this trend. In addition

to cyprodinil + fludioxonil, the mixture carbendazim + flusilazole was one of the most effective fungicides against *C. destructans* in *in vivo* assays. In different countries, including Portugal, it is registered for use as a wound protectant in grapevine for preventing infections by *Eutypa lata* and esca-associated fungi (Larignon and Molot, 2004). However, its use for drenching propagating materials has not been studied or reported. Protection of the large wounds produced during the different nursery stages is of the utmost importance, in order to avoid *Pa. chlamydospora* and *C. destructans* infections. For the latter pathogen, this protection could be very effective in preventing infections from soilborne inoculum, as suggested by the present study. Even in the presence of a high inoculum concentration in soil, as in this case, some fungicides were able to protect grapevine plants from infection, and wood colonisation (data not shown). Moreover, although some variable results were obtained, these fungicides and mixtures markedly improved the plant height and the total number of roots of the treated plants, when compared with the other treatments and the controls.

In conclusion, results from these *in vitro* and *in vivo* assays indicate that some fungicides may inhibit infection and colonisation of *C. destructans* within grapevine propagation material, and improve plant growth and root development. It is possible that regular dipping of rootstock cuttings and graftlings in effective fungicides, in particular before the rooting process, may prevent infection during this stage, when the inoculum pressure is higher. For its feasibility and longer-term effect, this practice compares favourably with HWT. Results obtained in the present study are very promising but need to be evaluated further in nursery conditions.

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