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Pathogenicity and molecular detection of Uruguayan isolates of *Greeneria uvicola* and *Cadophora luteo-olivacea* associated with grapevine trunk diseases

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Summary. Species from different fungal genera have been indicated as responsible for the development of trunk diseases of grapevines. *Greeneria uvicola* is responsible for the bitter rot of *Vitis vinifera* grape bunches near harvest, and can also attack other *Vitis* species. In Uruguay, *G. uvicola* was isolated from dead arm-affected grapevines and as an endophyte from healthy canes. *Cadophora luteo-olivacea* is a phialophora-like ascomycete with a wide distribution that was isolated from asymptomatic wood tissues in *Vitis* and Petri disease-affected nursery plants in Uruguay. Pathogenicity of isolates of both species was evaluated on *Vitis vinifera* cv. Tannat and Cabernet Sauvignon, and rootstocks SO4 and 3309C. Specific primers were developed for the ITS rDNA region for both species. Number of plants showing discoloration, length of discoloration, number of re-isolations and amplifications confirmed the pathogenicity of *G. uvicola* isolates. Pathogenicity of the isolate of *C. luteo-olivacea* obtained from symptomatic tissues is discussed. Specific primers can be used to detect the presence of these fungi in asymptomatic tissues.

Key words: ITS, specific primers, dead arm, Petri disease.

Introduction

Trunk diseases of grapevines have recently been revisited with regards to their increased incidence in the last decade, ancient denominations still in use, overlapping symptoms, and diversity of fungi associated with them (Graniti *et al.*, 2000; Surico, 2009). Whereas some fungal species, notably *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*, have been proven to be pathogenic and the ultimate cause of Petri disease and esca, species of fungi associated with dead spurs, dead arm and dieback have been more elusive. Species from different fungal genera have been indicated as responsible for the development of these latter symptoms which acquire different denominations to account for the fungus responsible for the disease: *Phomopsis* dead arm (Chamberlain *et al.*, 1964), *Eutypa* dieback (Magarey and Carter, 1986) and black dead arm or Bot canker caused by Botryosphaeriaceae species (van Niekerk *et al.*, 2006; Urbez-Torres and Gubler, 2009). In addition, other species belonging to the genera *Eutypella* and *Greeneria* have been isolated from plants showing typical necrosis of Eutypa dieback and Bot canker (Castillo-Pando *et al*. 2001; Trouillas and Gubler, 2004; Abreo *et al.*, 2008).

Greeneria uvicola is an ascomycete responsible for the bitter rot of *Vitis vinifera* grape bunches near harvest, and this fungus can also attack other *Vitis* species. This rot has been observed in the USA, Brazil, and other countries (Farr *et al.*, 2001; Pfenning *et al.*, 2006; Steel *et al.*, 2007; Longland and Sutton, 2008). This species was also isolated from dead arms of grapevines in Australia but its participation in the develop-

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ment of symptoms was not established (Castillo-Pando *et al.*, 2001). In Uruguay, *G. uvicola* was isolated from dead arm-affected grapevines and its pathogenicity was demonstrated in detached 1-year-old canes *in vitro* (Navarrete *et al.,* 2009).

Cadophora luteo-olivacea is a phialophora-like ascomycete with a wide distribution. Usually isolated from soils, it has been isolated also from asymptomatic wood tissues in *Vitis* (Halleen *et al*., 2007; Casieri *et al*., 2009), and from decayed wood in extreme environments where it can produce soft rot (Blanchette *et al*., 2004). It has been shown to produce stem lesions on grapevine (Halleen *et al*., 2007), and black streaking of xylem tissues in young vines in Spain and California (Rooney-Latham, 2005; Gramaje *et al*., 2010), and has also been isolated from affected tissues of plants suffering from Petri and esca diseases in Uruguay (Abreo *et al.,* 2008).

The presence of this range of fungi and the damage associated with them have led to suggest that pruning wounds should be protected, and that more sensitive techniques are needed for their detection (Halleen *et al.,* 2007). Molecular tools such as specific primers can be useful for the early detection and identification of pathogenic fungi that are responsible for similar symptoms, as it has been done with *Eutypa lata* and *Eutypella vitis* (Catal *et al*., 2007). In turn, early and proper identification can lead to better plan management and consequently to get a reduction in the incidence of grapevine trunk diseases.

The objective of the present study was to further evaluate the pathogenicity of Uruguayan isolates of *G. uvicola* obtained from symptomatic dead arm tissues and healthy canes, and of one isolate of *C. luteo-olivacea* obtained from black streaked xylem tissues of plants showing symptoms of Petri disease. In addition, specific primers for both species were designed and evaluated on inoculated plants to allow for early and sensitive detection of these fungi.

Materials and methods

Isolates

Cadophora luteo-olivacea FI2131 was selected from a series of morphologically similar isolates obtained from nursery plants showing external and internal symptoms of Petri disease, and for this reason it was selected for further pathogenicity testing. Identification was based on macro- and micro-morphological characteristics, such as conidia and conidiogenous cell shape and size, and was confirmed by PCR amplification of ITS rDNA (White *et al*., 1990) and subsequent sequencing (Macrogen, Seoul, Korea). The sequence was submitted to GenBank (GenBank accession No. HQ586008).

Greeneria uvicola FI12007 (GenBank accession No. HQ586009) was isolated from Bot canker-affected plants, and *G. uvicola* FI12008 (GenBank accession No. HQ586010) was isolated as an endophyte from healthy canes (Navarrete *et al.*, 2009). These isolates were selected for pathogenicity testing based on the different health status of the tissues from which they were isolated and on their different macromorphological characteristics. Three other isolates of *G. uvicola* obtained from Bot canker-affected grapevines, FI2132 (GenBank accession No. HQ610174), FI2133 (GenBank accession No. HQ610175) and FI2134 (GenBank accession No. HQ610176), were grown and their DNA purified, amplified and sequenced to be used for the design of specific primers.

All specimens remain at the fungal culture collection of the Laboratorio de Micología (Facultad de Ciencias-Facultad de Ingeniería, Montevideo, Uruguay).

Pathogenicity test

Pathogenicity of two isolates of *G. uvicola* and one isolate of *C. luteo-olivacea* was tested by inoculating mycelium-containing agar plugs in either the scion or rootstock of grafted nursery plants obtained from a local commercial nursery.

Two-year-old plants were potted, and moved to a distant greenhouse. Either scion or rootstock of each plant was wounded with a 5 mm diameter cork borer at the second internode starting from the graft union, inoculated with a mycelium-containing agar disc of 5 mm diameter and covered with parafilm. These discs were cut from the edge of colonies of the fungi actively growing on PDA at 25°C in the dark.

Inoculation took place in March (early autumn) after the plants had shown active growth.

An experimental design was set in which treatments consisted of inoculating the three selected

fungal isolates into five intact plants each of *Vitis vinifera* cv. Tannat and Cabernet Sauvignon and rootstocks SO4 (*V. berlandieri* × *V. riparia*) and 3309C (*V. riparia* × *V. rupestris*). In parallel, five control plants of each cultivar and rootstock were inoculated with sterile agar discs. Plants were kept in a greenhouse under natural photoperiod and temperature conditions (11.5–13 h of daylight, 12–25°C). Inoculated plants were evaluated after 3 months. Scions and rootstocks were cut and split lengthwise, the bark was removed and the size of internal lesions was measured. Split stems were surface-disinfected by immersion in ethanol 70% for 1 min, NaClO 4% for 2 min, washed in sterile distilled water and dried with sterile paper. Eight segments (3×2 mm) were cut from the lesions (two from the inoculation point, one from each lesion border and other four from elsewhere in the lesion) with a sterile blade. In the cases when stems did not show any lesion, two segments were taken from the inoculation point and six more were taken randomly from the rest of the internode. Segments were transferred to fresh PDA medium, incubated at 25°C and 12 h illumination to recover the inoculated fungi. The plates were checked on a daily basis during 3 weeks for new emerging colonies.

Statistical analysis

Lesion size data were subjected to Kolmogorov-Smirnov test (P=0.05) to verify significant differences between treatments as data did not follow a normal distribution. The differences between treatments in the number of plants showing lesions, positive re-isolations and amplifications were assessed by building contingency tables and then using Fisher Exact test (P=0.05) for sample size lower than 5 and Chi-Square (P=0.05) for greater sample size.

DNA extraction from fungal material

For DNA extraction, colonies of the fungi were grown on PDA for 10 or 15 days, depending on the isolate, until colony size reached a diameter of 20 mm. Mycelium was harvested with a sterile scalpel and DNA was extracted and purified according to the protocol of Lee and Taylor (1990).

Development of specific primers

Specific primers for *C. luteo-olivacea* and *G. uvicola* were designed for the ITS rDNA region using Primer3 (Rozen and Skaletsky, 2000).

For *C. luteo-olivacea*, primers were designed from a consensus sequence that included the Uruguayan isolate (GenBank accession No. HQ 586008) and sequences from GenBank (Harrington and McNew, 2003). For *G. uvicola*, primers were based solely on sequences of the Uruguayan isolates (GenBank accession No. HQ 586009, HQ 586010, HQ610174, HQ610175 and HQ610176).

NCBI BLAST was used to select only those primer pairs that did not match other fungal species or *Vitis* spp., or matched them with very low alignment score.

Specificity of primers

Specificity of the selected primers was further evaluated *in vitro* against genomic DNA from the target fungi and several fungi associated with *Vitis* spp. and/or trunk diseases: *Acremonium ochraceum*, *C. luteo-olivacea, C. melinii, Campylocarpon pseudofasciculare*, *Colletotrichum* sp., *Cylindrocarpon olidum, Diplodia seriata, Eutypella vitis, Fusarium oxysporum, Fusicoccum aesculi, G. uvicola, Inocutis jamaicensis, Neofusicoccum luteum, N. parvum, Paraconiothyrium brasiliense, Phaeomoniella chlamydospora, Phaeoacremonium aleophilum, Ph. australiense, Phialophora* sp., *Phomopsis viticola* and *Phomosis* sp.

PCR products were visualized by electrophoresis on 1% agarose gels stained with ethidium bromide. The size of the PCR products was determined against a 100 bp DNA ladder (Fermentas, Glen Burnie, MD, USA).

Sensitivity of primers

Sensitivity of the designed primers was determined using serial dilutions of pure fungal DNA (from 10 ng to 0.001 fg). PCR products were visualized as described above.

Direct amplification of fungal DNA from inoculated plants

Inoculated stems were cut longitudinally in two segments, one for fungal reisolations in pathogenicity trials, and one for total DNA extraction (2 cm up and down from inoculation sites). DNA extraction was carried out using the

AxyPrep Multisource Genomic DNA Miniprep Kit (Axygen, Union City, CA, USA) following manufacturer instructions.

The specific primers designed for *C. luteo-olivacea* (CLO1F/CLO1R) and *G. uvicola* (GU2F/GU2R) were used to amplify fungal DNA from inoculated plants either in a single step reaction for *C. luteo-olivacea* primers or in nested PCR after initial amplification with universal primers ITS4/ITS5 (White *et al*., 1990) for *G. uvicola* primers.

The cycling conditions for PCRs carried out with ITS4/ITS5 primer pair were as in White *et al*. (1990). Amplification conditions for CLO1F/CLO1R were as follow: an initial denaturation cycle at 96°C for 3 min, 35 cycles at 95°C for 30 s, 64°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 3 min. GU2F/GU2R cycling conditions were the following: an initial denaturation cycle at 96°C for 3 min, 25 cycles at 95°C for 30 s, 68°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 5 min. PCR reaction mixture was:

PCR buffer 10× (Invitrogen) 2.5 μL, dNTPs 2.5 mM 2.5 μL, MgCl 50 mM / 25 mM*0.75 μL, Left Primer 10 μM / 7.5 μM* 0.5 μL, Right Primer 10 μM / 7.5 μM* 0.5μL, Taq DNA polymerase (1.25 U, Invitrogen) 0.25 μL, H_2O 16.0 μL. (Concentrations marked with * were used in the PCR mix for GU2 primer pair).

PCR products were visualized as described above and both strands were sequenced (Macrogen, Seoul, Korea).

Results

Pathogenicity

When comparing *G. uvicola* inoculated plants of all cultivars against control plants, the number of plants showing discoloration, the size of lesions, and the number of plants showing positive re-isolation were significantly greater in treated plants than in control plants ($P<0.01$) (Figure 1, Table 1). There were no significant differences between both *G. uvicola* isolates regarding the number of plants showing discoloration ($P=0.14$), number of plants showing re-isolation ($P=1$) and lesion length ($P=0.7$) (Table 1).

When comparing at cultivar level against control, some differences between cultivars became evident.

The number of plants showing discoloration was significantly different for all combinations of fungal isolates and cultivars, except for FI12007 inoculated in cv. SO4 ($P=0.24$) and cv. Tannat ($P=0.22$), and FI12008 inoculated in cv. Cabernet Sauvignon ($P=0.08$), in which the treatments were not different from controls.

Mean lesion length varied considerably within cultivars but differed from control, except for cv. Tannat inoculated with *G. uvicola* FI12007 ($P=0.31$) and cv. Cabernet Sauvignon inoculated with *G. uvicola* FI12008 ($P=0.53$) (Table 2).

When comparing susceptibility of rootstocks against scions (evaluated as lesion length), rootstocks were more susceptible than scions to *G.*

Table 1. Mean pathogenicity parameters measured in a trial where *Greeneria uvicola* and *Cadophora luteo-olivacea* were inoculated onto grapevine cultivars (all cultivars combined).

Fungal species	Strain	NPD[ab]	DL[ac]	NPR[ad]
G. uvicola	FI12007	13 a	21.7 ± 32.5 a	6 a
	FI12008	17 a	18.4 ± 15.8 a	6 a
	Control	1 b	0.6 ± 2.6 b	0 b
C. luteo-olivacea	FI2131	2 a	4.3 ± 16.4 a	0 a
	Control	2 a	3.1 ± 9.8 a	0 a

[a] Within each column, values with different letters are significantly different ($P<0.05$) according to Kolmogorov-Smirnov test (DL) or Chi-Square test (NPD and NPR).
[b] NPD, number of plants showing discoloration.
[c] DL, discoloration length in mm (mean ± standard deviation).
[d] NPR, number of plants from which the inoculated fungus could be reisolated.

Table 2. Mean pathogenicity parameters measured in a trial where *Greeneria uvicola* and *Cadophora luteo-olivacea* were inoculated onto different grapevine rootstocks and scion cultivars.

Fungal species	Strain	SO4 [a]			3309C [a]			Tannat [a]			Cabernet Sauvignon [a]		
		NPD	DL	NPR	NPD	DL	NPR	NPD	DL	NPR	NPD	DL	NPR
G. uvicola	FI12007	3 ab	28.8±39.1 a	0 a	4 a	39.6±51.9 a	3 a	2 ab	7±9.7 ab	0 a	4 a	11.4±9 a	3 a
	FI12008	5 b	24.4±6.2 a	2 a	4 a	29.8±26.5 a	1 a	4 b	11.4±7.3 b	2 a	3 ab	8±7.6 ab	1 a
	Control	1 a	2.4±5.3 b	0 a	0 b	0 b	0 a	0 a	0 a	0 a	0 b	0 b	0 a
C. luteo-olivacea	FI12131	0 a	0 a	0 a	1 a	15±33.5 a	0 a	0 a	0 a	0 a	1 a	2.4±5.4 a	0 a
	Control	0 a	0 a	0 a	2 a	12.6±18.3 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a

[a,b,c, d] See Table 1.

uvicola FI12008 (P=0.031) but not to *G. uvicola* FI12007.

The number of plants showing positive re-isolation ranged between 0 and 3 depending on the cultivar. Differences with control plants were not significant for any cultivar, in spite of the fact that the fungus was never isolated from control plants (Table 2). It is noteworthy that the fungus was isolated from plants either with or without discoloration (data not shown).

Cadophora luteo-olivacea was able to cause discoloration in one inoculated plant of 3309C rootstock and one plant of cv. Cabernet Sauvignon, whereas plants of cv. Tannat and SO4 rootstock showed no discoloration (Table 2). Difference from control plants, two of which showed discoloration, was not significant (P=0.15). *Cadophora luteo-olivacea* was never isolated either from inoculated or control plants (Table 2).

Development of specific primers

The sequences of the primers designed in this study are shown in Table 3. Under the established PCR conditions, GU2F/GU2R and CLO1F/CLO1R primer pairs amplified target DNA producing a specific 400 bp band (Figure 2). Sequences of both PCR products matched the genomic region for which the respective primers had been designed. GU2F/GU2R also produced an unspecific 600 bp band which could be seen when nested PCR was carried out with pure *G. uvicola* genomic DNA (data not shown). CLO1F/CLO1R also produced unspecific products for *Pa. chlamydospora*, *Neofusicoccum luteum* and *N. parvum* but of different size (Figure 2). Sensitivity of primer pair GU2 was 1 ng, whereas sensitivity of CLO1 was 0.1 ng.

Direct amplification of fungal DNA from inoculated plants

GU2F/GU2R primer pair was used to amplify target DNA from inoculated plants in a nested PCR after an initial amplification with ITS4 and ITS5 primers (Figure 3). Identity of the 400 bp products always belonged to *G. uvicola* target sequence (BLAST search, data not shown). In some cases, a 600 bp band could also be seen. The num-

Table 3. Sequences of primers, melting temperatures (Tm), and size of amplicons used in this study.

Primer	Sequence	Tm	Amplicon size (bp)
CLO1F	TACTAGAGCAAAGGACAGGCAGC	64	403
CLO1R	GTTATAATGACGCTCGAACAGGC	64	
GU2F	GGTGGCCCTGTAAACTCTTGTT	68	403
GU2R	TGATCCGAGGTCAACTTTCAGA	68	

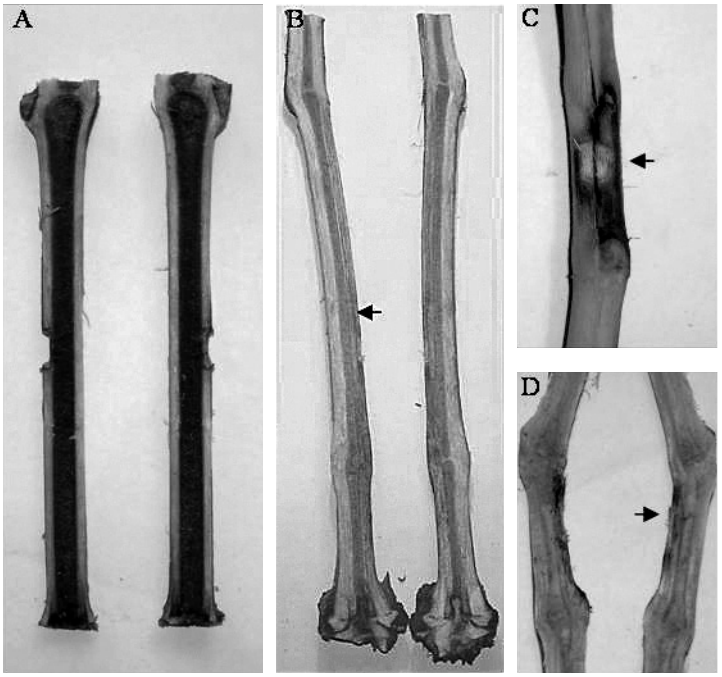

Figure 1. Lesions on stems/trunks of 2-year-old nursery plants 3 months after inoculation. A) Rootstock inoculated with sterile agar plug. B) Rootstock inoculated with *Greeneria uvicola*. C) Scion inoculated with *G. uvicola* (external lesions on xylematic tissues). D) The same scion as in C showing internal lesion in xylem.

ber of plants showing positive amplification varied widely depending on the cultivar. For each cultivar, it was always equal to, or higher than, the number of plants showing re-isolation (Table 4). When comparing *G. uvicola* inoculated plants of all cultivars against control plants, the number of plants showing positive amplifications was significantly greater in inoculated plants than controls, (P<0.01). There were no significant differences between both *G. uvicola* isolates regarding the number of plants showing positive amplification (P=0.1), even though cv. Tannat inoculated with FI12008 did produce a significantly greater number of amplifications than cv. Tannat plants inoculated with FI12007.

The CLO1F/ClO1R primer pair was used to amplify target DNA from inoculated plants in a single step (Figure 3). Identity of the 400 bp prod-

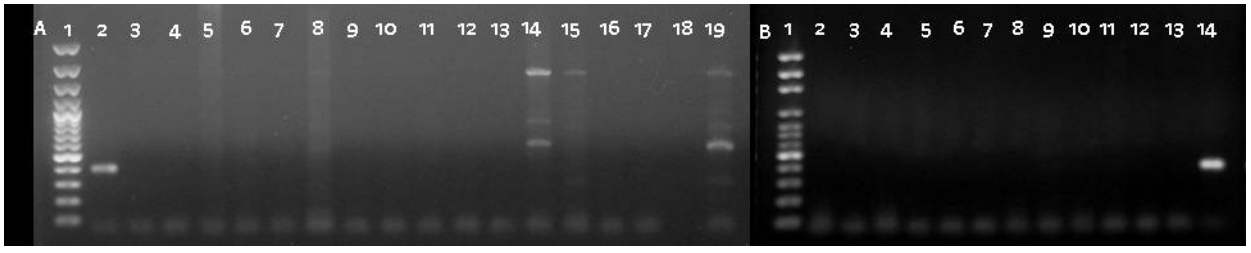

Figure 2. A) Specificity of CLO1F/CLO1R primer pair. Lanes 1–19, 100 bp DNA Marker, *C. luteo-olivacea, E. vitis, Phialophora* sp., *D. seriata, P. brasiliensis, F. aesculi, N. parvum, F. oxysporum, C. melinii, Ph. aleophilum, I. jamaicensis, C. olidum*, *Pa. chlamydospora, N. parvum, Phomopsis* sp., *G. uvicola, P. viticola, N. luteum*. B) Specificity of GU2F/GU2R primer pair. Lanes 1–14, 100 bp DNA Marker, *E. vitis, C. luteo-olivacea*, *Phomopsis* sp., *P. brasiliensis, F. aesculi, Ph. aleophilum, I. jamaicencis, C. pseudofasciculare, Cylindrocarpon* sp., *Pa. chlamydospora, F. oxysporum, N. luteum, G. uvicola.*

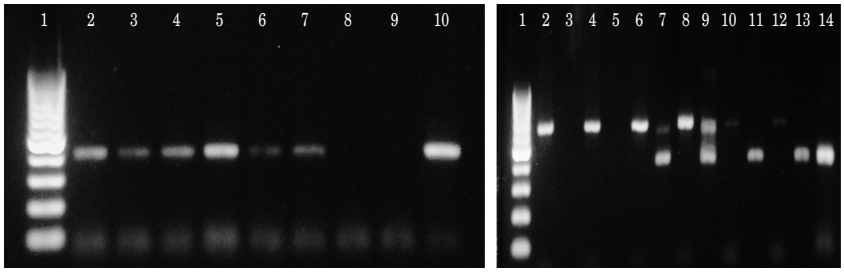

Figure 3. PCR amplification from inoculated plants. A) Amplification carried with CLO1F/CLO1R primers. Lane 1, 100 bp DNA Marker; lanes 2–7, plants inoculated with *C. luteo-olivacea*; lanes 8–9, plants inoculated with sterile agar discs; lane 10, *C. luteo-olivacea* genomic DNA. B) Amplification carried with GU2F/GU2R. Lane 1, 100 bp DNA Marker; lanes 2 and 4, PCR products obtained with ITS4/ITS5 from plants inoculated with sterile agar plugs; lanes 3 and 5, nested PCRs carried out with GU2F/GU2R; lanes 6, 8, 10, 12, PCR products obtained with ITS4/ITS5 from plants inoculated with *G. uvicola*; lanes 7, 9, 11, 13, nested PCR carried with GU2F/GU2R; lane 14, *G. uvicola* genomic DNA amplified with GU2F/GU2R.

ucts always belonged to *C. luteo-olivacea* target sequence (BLAST search, data not shown). For each cultivar, the number of plants showing positive amplification was always greater than the number of plants showing re-isolation, which were zero (Table 4). When comparing inoculated plants of all cultivars against the control plants, the former group was significantly different ($P<0.01$), despite one control plant of cv. Tannat and one control plant of 3309C which showed positive amplification for this fungus. Within cultivars, only cv. Tannat inoculated plants showed a significant difference with control plants (Table 4).

Discussion

In this study, the pathogenicity of isolates of *G. uvicola* in 2-year-old plants has been demonstrated, confirming the previous results obtained in detached 1-year-old canes (Navarrete *et al.*, 2009). Even though one of the isolates had been obtained as an endophyte from asymptomatic canes, and the other one from a plant showing dead arm symptoms, both isolates had similar virulence. This suggests that this fungus could be a latent pathogen, able to produce symptoms in stressed plants. The positive amplification of *G. uvicola* in control plants can be considered a further indication of this condition. However, the higher susceptibility of rootstocks, and the variability of the results across cultivars and fungal isolates possibly indicate different sensitivity of cultivars to both strains of the fungus. The observation of rootstocks being more sensitive also underlines the importance of the correct health management of rootstock mother plants in nurseries regarding the spread of this fungus.

In other countries, *G. uvicola* is known to

Table 4. Direct amplification from plant material inoculated with *Greeneria uvicola* and *Cadophora luteo-olivacea*.

Fungal species	Strain	SO4 [a]	3309C [a]	Tannat [a]	Cabernet Sauvignon [a]	All cultivars [a]
G . uvicola	FI12007	0 a	5 a	0 a	5 a	10 a
	FI12008	3 a	5 a	4 b	3 a	15 a
	Control	1 a	0 b	0 a	2 a	3 b
C. luteo-olivacea	FI2123	2 a	3 a	5 a	3 a	13 a
	Control	0 a	1 a	1 b	0 a	2 b

[a] Within each column, values with different letters are significantly different ($P<0.05$) according to Fisher Exact test and Chi-Square test.

cause bitter rot of grape berries (Farr *et al*., 2001; Pfenning *et al*., 2006; Steel *et al*., 2007; Longland and Sutton, 2008). However, there are no reports of this disease in Uruguay. Whereas it is possible that cultural and climatic conditions in Uruguay are not conducive to the development of bitter rot, the presence of the fungus in vineyards as an endophyte and associated to dead arms should be acknowledged, as infected trunks might act as sources of inoculum. Conversely, in countries where bitter rot of bunches is a frequent disease, it could lead to build up of inoculum that could later infect trunks, as has been suggested for Botryosphaeriaceae (van Niekerk *et al*., 2006).

The wood canker on grapevines attributed to *Eutypa lata* has been separated from cankers caused by Botryosphaeriaceae, because of the absence of the typical external symptoms due to the toxic fungal metabolites produced by *E. lata*, like eutypine, which give the characteristic stunted shoot development and small, cup-shaped leaves attributed to Eutypa dieback (Castillo-Pando *et al*., 2001). *Greeneria uvicola* might be considered an additional species to cause wood cankers with no specific foliar symptom. Specificity of GU2 primers was demonstrated. The second band (*c.* 600 bp) that could be seen in some of the nested PCRs could be due to a second, lower affinity binding site of the primers in the *G. uvicola* genome. When nested PCR was carried out with pure *G. uvicola* genomic DNA, which is more concentrated, this band also appeared. Although this unspecific band could be eliminated by reducing primer concentration in the reaction mix, this would also reduce the sensitivity of the assay. In assays performed on plant material, this second band would be expected in the cases when DNA of *G. uvicola* recovered from the plant is abundant, as would be the case under severe infections.

When some plants inoculated with sterile agar showed positive amplification, the identity of the amplicons always belonged to *G. uvicola*. The positive amplification of this fungus could be due to its presence as an endophyte.

Recently, Samuelian *et al.* (2011) also developed specific primers for the ITS region of *G. uvicola* and tested them *in vitro* against some grape bunch-associated fungi. It would be opportune to test the two sets of primers both on grape bunches and trunks.

Cadophora luteo-olivacea obtained from a plant with internal black streaking failed to produce discoloration of vessels, or any other disease symptom when inoculated into healthy plants. Likewise, this fungus could not be isolated from inoculated or control plants despite its presence being confirmed by amplification with specific primers, probably due to the size of segments.

The high number of stems showing positive amplification from tissues that were not visually affected indicates that this isolate of *C. luteo-olivacea* could be considered as non-pathogenic towards the evaluated cultivars. Alternatively, the fungus might need a longer incubation period to colonize the stems and cause lesions. Other isolates have been shown to colonize and cause discoloration and decline of rootstocks, and could cause disease symptoms in field trials (Halleen *et al*., 2007; Gramaje *et al*., 2010). Our divergent results could be due to differences among isolates in their ability to colonize plant tissues, as has been shown with isolates of other *Cadophora* spp. such as *Cadophora gregata* (Tabor *et al.,* 2007). Also the phenological and physiological stage of grapevine in autumn at the time of inoculation might not have initiated an infection, as has happened with Botryosphaeriaceous spp. when inoculated in seasons other than winter (Urbez-Torrez and Gubler, 2011). The fact that control plants showed positive amplification of *C. luteo-olivacea* could be the result of its presence as an endophyte (Casieri *et al.,* 2009). In either case, *C. luteo-olivacea* obtained either from an inoculated plant or as an endophyte, did not produce symptoms.

The sensitivity of GU2 and CLO1 primers was 1 ng and 0.1 ng respectively, similar or even greater than that achieved by other primers developed for grapevine trunk pathogens (Pollastro *et al*., 2001; Ridgway *et al*., 2002). The sensitivity could be further increased with the use of a different Taq polymerase (data not shown). In spite of this, both primers were useful to detect the presence of the target fungi in cases when their isolation was not possible.

In conclusion, *G. uvicola* is regarded as a grapevine pathogen that can cause lesions in wood and therefore could be associated with cankers on vines. The assayed isolate of *C. luteo-olivacea* obtained from diseased tissues failed to

cause necrotic symptoms in the inoculated tissues, and this isolate was therefore not considered pathogenic towards the evaluated cultivars under the experimental conditions applied in the present study. Specific primers used in inoculated plants showed greater sensitivity than isolation to detect these fungi, and the molecular method can be considered a useful tool for early diagnosis and pathogenicity studies.

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