BIODIVERSITY OF PATHOGENIC WOOD FUNGI ISOLATED FROM *XYLOTRECHUS ARVICOLA* (OLIVIER) GALLERIES IN VINE SHOOTS

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Abstract

Aim: Grapevine decline caused by wood fungi seriously threatens viticulture worldwide. In Spain, the polyphagous borer *Xylotrechus arvicola* (Coleoptera, Cerambycidae) is becoming a serious pest in different Qualified Designation of Origin (DOC) wine regions. The aim of the present work was to identify the fungal species growing in the galleries excavated by *X. arvicola* larvae inside the vine branches and investigate the possible relationship between wood pathogenic fungi and this borer.

Methods and results: Wood samples from branches of Tempranillo vines and *Prunus pisardi* trees affected by the borer *X. arvicola* were collected in La Rioja DOC vineyards (Northern Spain) and analyzed for the presence of fungi using both morphological techniques and genetic tools based on Iinternal Ttranscribed sSpacer 2 (ITS2) sequence. Among the 20 different fungal species/isolates identified, 7 belonged to fungi associated with grapevine decline (esca, Petri disease, and *Eutypa* dieback), 6 were plant pathogens, 6 were saprophytic and one was entomopathogenic (*Beauveria bassiana*).

Conclusion: The fact that 65 % of the fungi detected inside *X. arvicola* galleries belong to species recognized as plant pathogens suggests that this borer, in addition to causing a progressive decay of the branches, could facilitate the transmission of plant diseases, further threatening the vineyards by spreading grapevine decline pathogens.

Significance and impact of the study: The present work represents a first step in recognizing the association between the fungal pathogens associated with grapevine decline and the borer *X. arvicola*, suggesting a possible role of this insect as a vector in the transmission of these fungi.

Key words: grapevine decline, ITS2, *Xylotrechus arvicola*, La Rioja (Spain), wood fungi

Résumé

Objectif: Le dépérissement de la vigne causé par des champignons attaquant le bois menace sérieusement la viticulture mondiale. En Espagne, l'insecte envahissant *Xylotrechus arvicola* (Coleoptera, Cerambycidae) est en train de devenir un sérieux fléau dans plusieurs zones de production de vins d'origine et qualité garanties (DOC). Ce travail a permis d'identifier les espèces de champignons qui se développent dans les galeries creusées par les larves de *X. arvicola* à l'intérieur des sarments afin de déceler la possibilité d'une relation entre les champignons xylophages et cet insecte envahissant.

Méthodes et résultats: Des échantillons de sarments de Tempranillo et de *Prunus pisardi* affectés par l'insecte envahissant *X. arvicola* ont été collectés dans les vignobles DOC de La Rioja (Espagne du Nord) et analysés pour détecter la présence de champignons en utilisant à la fois des techniques morphologiques et des outils génétiques basés sur la séquence d'espaceurs transcrits internes ITS2. Parmi les 20 différentes espèces de champignons identifiées, 7 appartenaient à des champignons associés au dépérissement de la vigne (esca, maladie de Pétri, eutypiose), 6 étaient des agents pathogènes des plantes, 6 étaient saprophytes et 1 était entomopathogène (*Beauveria bassiana*).

Conclusion: Le fait que 65 % des champignons détectés à l'intérieur des galeries creusées par *X. arvicola* appartiennent à des espèces reconnues comme étant des pathogènes des plantes met en lumière que cet insecte envahissant, outre le fait qu'il cause une détérioration progressive des sarments, pourrait faciliter la transmission des maladies des plantes, ce qui menacerait encore plus la santé des vignobles avec la prolifération des agents pathogènes.

Signification et impact de l'étude: Ce travail est un premier pas vers la reconnaissance de l'action conjuguée des champignons pathogènes et de l'insecte envahissant *X. arvicola*, suggérant le rôle possible de cet insecte dans la transmission des champignons.

Mots clés: dépérissement de la vigne, ITS2, *Xylotrechus arvicola*, La Rioja (Espagne), champignons du bois

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INTRODUCTION

La Rioja is one of the most famous Qualified Designation of Origin (DOC) wine regions in the world. It covers an area of about 65,000 ha in Northern Spain, with Tempranillo accounting for almost 75 % of the planted area. This variety, which is considered the most representative red grapevine in Spain, is also widely grown in several other Spanish regions (Huetz de Lemps, 2009).

Vineyard infestations by Cerambycid borers were initially discovered in La Rioja in the 1970s, at pruning time, when exit holes made by the adults (about 4 mm in diameter) could be observed in the trunk and the thicker branches of the vines. Ocete and del Tío (1996) identified the pest as *Xylotrechus arvicola* (Olivier) (Coleoptera, Cerambycidae) (Figure 1) and observed that this infestation was spreading at an alarming rate in the region. At the same time, the pest began to be detected in several other wine producing regions in Spain, especially on some cultivars : Castilla-La Mancha (mainly on Cabernet sauvignon and Tempranillo), Castilla y León (on Tempranillo and Sauvignon blanc), and Navarra (on Garnacha Tinta, Tempranillo, Moscatel and Miguel del Arco cultivars) (Ocete *et al.*, 2002 and 2004).

The damage caused by *X. arvicola* is severe in terms of yield and wine quality. Large and numerous galleries are excavated by the insect larvae inside the wood, causing a drastic decrease in grape production, progressive decay and death of the affected branches, and eventually the premature death of the vine. Moreover, wine made with grapes from infested branches has significantly lower alcoholic percentage and higher organic acid concentration (Ocete *et al.*, 2008).

The use of chemical treatments as control method against *X. arvicola* is not feasible because of the endophytic development of the larvae, which lasts about two years, and because of the extended period of emergence of the adults, which lasts from June to August.

Grapevine trunk diseases, such as *Eutypa* dieback, esca and Petri disease, reduce vineyard longevity and productivity in almost every wine and grape production region worldwide. They have been related to a complex of pathogenic fungi from different Ascomycete taxa (Bertsch *et al.*, 2013 ; Edwards and Pascoe, 2004 ; Travadon *et al.*, 2012 ; Úrbez-Torres *et al.*, 2006 and 2009 ; van Nierkerk *et al.*, 2006) that produce spores which are disseminated from one vine to another by rain, wind and other factors. The appearance of symptoms and the development of these diseases are influenced by atmospheric conditions, mainly humidity and temperature. However, conditions that allow direct exposure of wood vessels, such as the wounds caused to the aerial or underground parts by pruning and other farming practices, hail or biotic agents, greatly favour the colonization by the spores of the pathogenic microorganisms (Eskalen *et al.*, 2007). Another possible source of infection is the manipulation of infected plants in nurseries (Fourie and Halleen, 2004 ; Aroca *et al.*, 2010).

The possibility that insect borers may facilitate the transmission of grapevine trunk diseases has never been investigated. In order to unravel possible synergic interactions between wood pathogenic fungi and xylophagous borers, the present study aimed at quantifying and examining the fungal biodiversity in the galleries excavated by *X. arvicola* larvae inside grapevine branches in La Rioja vineyards.

MATERIALS AND METHODS

1. Sample collection and study domain

The samples were collected from seven different plots in La Rioja DOC vineyards (Northern Spain). Twelve vines of *Vitis vinifera* L. cv. Tempranillo were initially selected along the major diagonals of each plot and all visible old exit holes of *X. arvicola* adults were marked. The following year, only four of the twelve vines from each plot were chosen, considering the ones that showed new *X. arvicola* exit holes and the most severe symptoms of vine decline. One wood sample per vine was taken from an affected branch that had



Figure 1 - Adult specimen of *Xylotrechus arvicola*.

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produced rachitic shoots the year before and each wood sample had a new exit hole and contained the pupation chamber of the borer. *Prunus pisardi* is a very common ornamental plant in the villages of this area, and plants older than 5 years show a particularly high degree of infestation by *X. arvicola* and symptoms very similar to the ones observed in grapevines. Using the same technique described for grapevines, one wood sample was taken from an eight-year-old specimen of *P. pisardi* showing the highest degree of infestation by the borer in the La Rioja examined area. Details of all samples are shown in Table 1.

The collected wood samples were superficially disinfected with methyl alcohol 99 % and flame. Splinters from the rot and necrotic lesions of the insect galleries were obtained by cross and longitudinal cuttings of the sampled wood, under sterile conditions. The splinters were then seeded in

Table 1 - Fungal species detected inside *X. arvicola* galleries in infested branches from grapevine (numbers 1-7) and *P. pisardi* (number 8) samples from different locations in La Rioja vineyards. Full name of the species is reported in Table 2.

Location and age of the sampled plants	No. of samples	Species identification and total no. of isolations (in parentheses)
1. Baños de Ebro 42º N 31´29´´N/ 2º 41´0.3´´W Age: 36 years	4	*Diplodia seriata* (6) *Phaeoacremonium aleophilum* (1) *Eutypa lata* (2) *Phaeomoniella chlamydospora* (1)
2. La Bastida 42º 34´20´´N/ 2º 48´39´´W Age: 22 years	4	*Diplodia seriata* (6) *Gibberella avenacea* (1) *Eutypa lata* (2) *Epicoccum nigrum* (1) *Phaeoacremonium tuscanicum* (3) *Phaeoacremonium aleophilum* (1)
3. Cubillas (Haro) 42º 35´38.8´´N/ 2º 52´19.7´´W Age: 37 years	4	*Diplodia seriata* (5) *Eutypa lata* (4) *Acremonium alternatum* (1) *Phaeoacremonium aleophilum* (2) *Cryptovalsa ampelina* (1)
4. Cuzcurrita 42º 33´32´´N/ 2º 58´0.0´´W Age: 33 years	4	*Phaeoacremonium aleophilum* (1) *Phomopsis viticola* (2) *Eutypa lata* (3) *Aspergillus tubingensis* (1) *Diplodia seriata* (2)
5. Gimileo 42º 32´42´´N/ 2º 49´22.7´´W Age: 43 years	4	*Phaeoacremonium angustius* (1) *Phaeomoniella chlamydospora* (1) *Diplodia seriata* (4)
6. Tirgo 42º 32´34´´N/ 2º 56´49´´W Age: 90 years	4	*Diplodia seriata* (2) *Eutypa lata* (2) *Gibberella avenacea* (1) *Fusarium acuminatum* (1) *Phomopsis viticola* (1) *Beauveria bassiana* (3) *Preussia africana* (2) *Phaeomoniella chlamydospora* (1)
7. Tondonia (Haro) 42º 35´15.7´´N/ 2º 49´47.6´´W Age: 45 years	4	*Diplodia seriata* (2) *Fomitiporia mediterranea* (2) *Eutypa lata* (1)
8. Briñas (*Prunus pisardi*) 42º 36´7.7´´N/ 2º 49´56.6´´W Age: 8 years	4	*Cytospora chrysosperma* (1) *Penicillium toxicarium* (1) *Collophora pallida* (1) *Eutypa lata* (2) *Phialocephala dimorphospora* (1)

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Table 2 - Detection frequency of fungal species isolated from *X. arvicola* galleries in infested branches from grapevines and *P. pisardi*.

Species	Total No. of isolations	% of isolations with the fungus	Nº of samples with the fungus	% of samples with the fungus
Diplodia seriata De Not.	27	35.52	17	53.1
Eutypa lata (Pers.) Tul. & C. Tul.	16	21.05	10	31.5
Phaeoacremonium aleophilum W. Gams, Crous, M.J. Wingf. & Mugnai	5	6.57	5	15.6
Phaeomoniella chlamydospora (W. Gams, Crous, M.J. Wingf. & Mugnai) Crous & W. Gams	3	3.95	3	9.4
Phomopsis viticola (Sacc.) Sacc.	3	3.95	3	9.4
Phaeoacremonium tuscanicum Essakhi, Mugnai, Surico & Crous	3	3.95	1	3.1
Beauveria bassiana (Bals.-Criv.) Vuill	3	3.95	1	3.1
Fomitiporia mediterranea M. Fisch.	2	2.63	2	6.3
Gibberella avenacea R.J. Cook	2	2.63	1	3.1
Phaeoacremonium angustius W. Gams, Crous & M.J. Wingf.	1	1.31	1	3.1
Epicoccum nigrum Link	1	1.31	1	3.1
Acremonium alternatum Link	1	1.31	1	3.1
Aspergillus tubingensis Mosseray	1	1.31	1	3.1
Preussia africana Arenal. Platas & Peláez	2	2.63	1	3.1
Cryptovalsa ampelina Abbado	1	1.31	1	3.1
Penicillium toxicarium I. Miyake	1	1.31	1	3.1
Collophora pallida Damm & Crous	1	1.31	1	3.1
Phialocephala dimorphospora W.B. Kendr.	1	1.31	1	3.1
Fusarium acuminatum Ellis & Everh.	1	1.31	1	3.1
Cytospora chrysosperma (Pers) Fr.	1	1.31	1	3.1
Total	76	100	32	

9-cm plates containing malt extract agar (MEA, Oxoid) supplemented with 400 mg/L of streptomycin sulphate and 120 mg/L of penicillin and incubated at 25ºC. Fungi from the splinters grown in MEA were isolated in potato dextrose agar (PDA, Sigma) plates and incubated at 25ºC in darkness. Colonies from potential phytopathogenic fungi were selected and sown twice until pure samples were obtained. Sequencing was performed on all isolated fungi, except those considered as exclusively saprophyte. Additional molecular analyses were carried out through internal transcribed spacer 2 (ITS2) sequencing. The extraction of genomic DNA from the different isolated fungi was performed using the REDExtract-N-AmpTM Plant PCR Kit (Sigma).

2. PCR amplification

Primers ITS4 and ITS5 described by White *et al.* (1990) were synthesized by Boehringer Mannheim (Mannheim, Germany) and used to amplify a rDNA fragment including ITS1 and ITS2 and the 5.8S rDNA gene. PCR amplifications were performed in a total volume of 20 ml by mixing 40 ng of template DNA with 0.2 mM of each primer, 200 mM of each deoxynucleoside triphosphate, and 2.5 U of *Taq* DNA polymerase in GeneAmp 103 PCR Buffer II (100 mM Tris-HCl, pH 8.3 ; 500 mM KCl) (Perkin-Elmer). These reactions were subjected to an initial denaturation of 2 min at 95 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 54 °C, and 1 min at 72 °C, with a final extension of 10 min at 72 °C in an Applied Biosystems 2720 thermal cycler. Aliquots (1 µl) were analyzed by electrophoresis in 1 % (wt/vol) agarose gel in 1x TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA [pH 8]), stained with ethidium bromide, and photographed over a transilluminator. The molecular size marker was fX174-*Hae*III (Promega, Madison, Wis.).

3. Sequencing of amplification products

Sequencing of the rDNA region, including the spacers ITS1 and ITS2 and the 5.8S rDNA gene, was carried out by automated DNA sequencing with fluorescent terminators using ABI 377 Prism Sequencer (Applied Biosystems, Foster City, Calif.) at the Department of Microbiology and

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Genetics, University of Salamanca, Spain. Prior to sequencing, PCR products were purified with Centricon-100 concentrators (Amicon, Madison, Wis. USA) according to the manufacturer's specifications. Each fungal isolate was sequenced in both directions using primers ITS1-ITS3 and ITS2-ITS4 with a T7 DNA Polymerase Sequencing Kit (Pharmacia, Uppsala, Sweden). Chromatograms were analyzed with the program Chromas and compared with those available in the databases of the National Center for Biotechnological Information (NCBI) and the European Bioinformatics Institute (EMBL-EBI), using the implementation of the Local Basic Alignment Search Tool (BLAST) (Altschul *et al.*, 1997).

RESULTS

A total of 32 wood samples taken from Tempranillo grapevine and *P. pisardi* branches affected by the xylophagous borer *X. arvicola* were analyzed, resulting in the identification of 20 different fungal species or isolates (Table 1). In all cases both morphological and molecular identification were performed. All the fungi determined by molecular analysis presented over 98 % homology to the related database. The incidence of all detected fungal species and the number of samples for each isolate are shown in Table 2.

Diplodia seriata was the most commonly detected species : it was the only one detected in all the sampling locations, found in more than half of the samples (53.1 %), and was isolated with the greatest frequency (35.52 %). *Eutypa lata* was detected in vine branches from six vineyard locations and was also isolated from the *P. pisardi* samples. Overall, it was detected in 31.5 % of the samples and corresponded to 21.05 % of the isolations. The fungus *Collophora pallida* was also isolated from the *P. pisardi* samples.

Phaeoacremonium aleophilum, representing 6.57 % of the isolations, was detected in 15.6 % of the samples from four locations ; *Phaeomoniella chlamydospora*, representing 3.95 % of the isolations, was detected in 9.4 % of the samples from three locations ; *Phaeoacremonium tuscanicum*, representing 3.95 % of the isolations, was detected in 3.1 % of the samples from one location ; *Fomitiporia mediterranea*, representing 2.63 % of the isolations, was detected in 6.3 % of the samples from one location ; and *Phomopsis viticola*, representing 3.95 % of the isolations, was detected in 9.4 % of the samples from two locations. The fungus *Beauveria bassiana*, which represented 3.95 % of the isolations, was detected in 3.1 % of the samples from only one location. Other fungal species were detected sporadically at the different locations.

DISCUSSION

The borer *X. arvicola* and the fungal pathogens associated to grapevine decline represent an increasing threat to Spanish viticulture, a situation that worsened after the banning of sodium arsenite spraying. The present work represents a first step in recognizing a possible association between microorganisms and the insect pest, with the identification of the fungi detected in the *X. arvicola* larval galleries in vine branches in La Rioja DOC vineyards, although further studies are needed to better ascertain the role of this borer.

D. seriata (Botryosphaeriaceae), the pathogen that was detected with the greatest frequency and in all sampling locations, is the agent of the *Botryosphaeria* dieback and the most frequently isolated species of this genus in esca-affected vines in Spanish vineyards (Luque *et al.*, 2009). Sometimes, as it occurred on the vines sampled in Haro (La Rioja), a longitudinal necrosis that arrives to the point of graft was observed, where narrow black streaks develop in the xylem of arms and trunks, as it was described by Lehoczky (1988). In Castilla y León DOC vineyards there is a high biodiversity of *Botryosphaeria*-like species involved in grapevine decline (Urbez-Torres *et al.*, 2006 ; van Nierkerk *et al.*, 2006) and they are associated with *Ph. chlamydospora* and *Phaeoacremonium* spp. (Martín and Cobos, 2007), as found here in the galleries of the *X. arvicola* borer pest.

Ph. chlamydospora, *Pm. aleophilum*, *Pm. tuscanicum* and *F. mediterranea* are fungal species typically associated with Petri and esca diseases. Our results show that at least one of these species was detected in at least one grapevine sample from each chosen La Rioja location. When considering these species altogether, it emerges that 34.4 % of the samples had fungi related to either one of these diseases and that overall these fungi represented 17.1 % of the fungal isolates. *Ph. chlamydospora* has been associated with typical Petri disease symptoms more often than species of *Phaeoacremonium* (Mugnai *et al.*, 1999 ; Edwards and Pascoe, 2004). Both *Ph. chlamydospora* and *Phaeoacremonium*, together with the wood decaying basidiomycete *F. mediterranea* and, to a lesser extent, *Stereum hirsutum*, are often

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associated with esca symptoms (Mugnai *et al.*, 1999 ; Fischer, 2002 ; Péros *et al.*, 2008). *Pm. tuscanicum* was identified for the first time in Spain and is also considered as a grapevine decline disease agent (Essakhi *et al.*, 2008). Studies carried out in California indicated that the teleomorph of *Pm. aleophilum*, *Togninia minima*, is also a causal agent of esca symptoms (Rooney-Latham *et al.*, 2005 ; Mostert *et al.*, 2006). *Pm. angustius* has also been associated with esca disease in Spain (Aroca and Raposo, 2009) and Portugal (Chicau *et al.*, 2000). Esca decline has been termed an 'elusive' disease due to the diversity and range of its symptoms, with several fungi probably acting in ecological succession (Mugnai *et al.*, 1999). Symptoms of esca, such as lighter and soft dead wood areas, were observed in those vines containing *F. mediterranea*, as previously reported in other Spanish DOC (Redondo *et al.,* 2001).

E. lata represented 21.05 % of the isolations and besides being detected in the grapevine samples from almost all locations, it was found in the *P. pisardi* samples. This fungus is a generalist ascomycete with worldwide distribution (Travadon *et al.*, 2012) that causes *Eutypa* dieback of grapevine. In the field, *Eutypa*-infected wood develops a rough, dark, black-brown area, clearly differentiated from the healthy wood, in the form of a hard and fragile wedge (Arias-Giralda, 1998). Besides grapevine, this fungus affects several botanical species (Farr *et al.*, 1995) such as *Fraxinus* spp., *Populus* spp., *Prunus* spp., *Quercus* spp. and *Ulmus* spp., which are usual hosts of *X. arvicola* in natural conditions (Ocete and del Tio, 1996). Moreover, the presence of *E. lata* has also been associated to *X. arvicola* infestation in vineyards from Castilla y León (Moreno, 2005).

P. viticola, isolated from 9.4 % of the samples, is known as the causal agent of the grapevine disease *Phomopsis* cane blight and leaf spot (Hewitt and Pearson, 1994), but in Spain it has also been detected in association with esca fungi, especially in cane and vine shoot samples (Sánchez-Torres *et al.*, 2008). On the other hand, the co-occurrence of *P. viticola* and *E. lata* has been hypothesized in explaining variation in the development of grapevine dieback (Péros *et al.*, 1999). The fungus *C. ampelina*, isolated only from one vine sample, has also been associated with grapevine decline in Spain and Chile (Martin *et al.*, 2009 ; Díaz *et al.*, 2011), although it does not appear to be a major pathogen because of its low virulence.

Among fungal agents of grapevine trunk disease, *Cylindrocarpon* species, causing Black Foot disease on the radicular area (Alaniz *et al.*, 2007), was never detected and this could be due to the type of sampling performed in this study, as the wood samples were collected from the aerial region of the vine stock.

Besides *E. lata*, the fungus *C. pallida* was also isolated in the wood samples from *P. pisardi* infested by *X. arvicola*. According to Damm *et al.* (2010), this ascomycete is the causal agent of a *Prunus* wood necrotic disease.

B. bassiana represented 3.95 % of the isolations and was found only in one of the sampled locations. This entomopathogenic species is used as a biological control agent against several pests (Ownley *et al.*, 2004) and has also been detected on larvae of *X. arvicola* in Castilla y León vineyards (García Calleja and Sánchez Urraca, 2002). The presence of this species in the vine samples is remarkable, in the view of using this fungus as a tool to decrease the infestation of the coleopteran borer.

The other species detected inside *X. arvicola* galleries in the vine branches and in the *P. pisardi* wood, which accounted for less than 3 % of the isolates each, were associated to other plant diseases such as *Gibberella avenacea* on cereals, *Preussia africana* on *Viburnum* spp. and *Cytospora chrysosperma* on *Populus* spp., some were saprophytic and occasional secondary colonizers of agricultural plants (*F. acuminatum*) or were saprophytic fungi like *Epicoccum nigrum*, *Acremonium alternatum*, *Aspergillus tubingensis*, *Penicillium toxicarium* and *Phialocephala dimorphospora*, a decomposer of rotten deciduous wood.

To summarize our findings, among the 20 fungal species detected inside *X. arvicola* galleries, 7 (*D. seriata*, *Ph. chlamydospora*, *Pm. aleophilum*, *Pm. tuscanicum*, *Pm. angustius*, *E. lata* and *F. mediterranea*), which were also the ones detected with the greatest frequency, are associated with grapevine decline diseases (esca, Petri disease, and *Eutypa* dieback), 2 are also grapevine pathogens (*P. viticola* and *C. ampelina*), 4 are related to other plant diseases, 6 are saprophytic and one is entomopathogenic. The detection of 13 species (65 % of the isolated fungi) recognized as plant pathogens inside the galleries of this polyphagous borer is a worrying evidence of a possible association between the fungi and the

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insect in the transmission of plant diseases. Such an hypothesis has already been investigated for declining oaks in Italy (Tiberi and Ragazzi, 1998): associations between xylophagous insects (Coleoptera, Scolytidae and Bostrichidae) and oak pathogenic fungi carried on their bodies were demonstrated and are considered to have an important role in triggering the oak decline.

Grapevine decline disease transmission starts on wounds made by pruning, frost or hail (Eskalen *et al.*, 2007). An un-investigated possibility is that *X. arvicola* could also play a role in facilitating the transmission of these diseases. The fungal biodiversity found inside *X. arvicola* galleries supports the hypothesis that this borer could act as a vehicle for fungal pathogens by mechanically transporting the spores on its body surface and "inoculating" the grapevines during egg laying, which occurs in cracks and pruning wounds between early June and early August (Moreno, 2005). Moreover, the exit holes performed by the newly emerged adults represent easy points of entrance for the spores accidentally transported by atmospheric agents.

CONCLUSION

Consequences of grapevine trunk diseases are serious worldwide. In Spain, the increasing infestation by the cerambycid borer *X. arvicola*, whose larvae excavate numerous galleries inside grapevine branches, causing their progressive decay and a decrease in grape production, represents a further threat in many DOC vineyards. By presenting the fungal biodiversity detected inside the galleries, the present work supports the idea that *X. arvicola* could dangerously facilitate the transmission of pathogenic wood fungi in vineyards by acting as a spore vehicle during egg laying as well as by increasing the spore entry points, through the exit holes of the newly emerged borers. In both cases, a high degree of infestation by these borers highly increases the probability of being infected by grapevine decline agents with deleterious consequences for the vineyard productivity. Further research on this subject is needed to clarify this association and to investigate sustainable and feasible methods to control *X. arvicola*. The potentiality of the entomopathogenic fungus *B. bassiana*, detected in one of the samples, as biological control agent against the borer should be investigated.

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