Action D1.
Analyses of data and samples, evaluation
and optimisation of techniques

Deliverable:

List of fungi associated to *Xylosandrus* spp.

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1	15/01/2021	Carmen Morales-Rodriguez (UNITUS)	Deliverable structure and introduction
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Summary

Insects, like many other organisms, live in association with many fungal symbionts, which can have a positive (i.e., mutualistic), negative (i.e., parasitic) or neutral (i.e., commensalistic) impact on their host's fitness. Symbiotic fungi associated with Ambrosia beetles contribute with insect damage to the impact to invaded environments. Also of providing food for the insect development stages, some of the Ambrosia beetle symbiotic fungi are severe pathogens of plant hosts. The most relevant example is *Xyleborus glabratus* carrying the fungus *Raffaela lauricola* cause of lethal vascular wilt of avocado, that is devastating the plantations in South-eastern United States. Thus, monitoring of alien fungi introduced through specific pathways (mostly trading of living plants), their identification and determination of pathogenicity behaviour is essential to design and apply prevention and mitigation quarantine measures. This deliverable describes the fungal community isolated from *X. compactus* and *X. crassiusculus*.

1. Bark beetle' and obligate symbiosis with fungi

Bark beetle is both a taxonomic and ecological designation. In the taxonomic sense, bark beetles are all species in the weevil subfamily Scolytinae, including species that do not consume bark. In the ecological sense, bark beetles are species of Scolytinae whose larvae and adults live in and consume phloem of trees and other woody plants. They are not obligatory associated with fungal symbionts, while ambrosia beetles are obligately associated with nutritional fungal symbionts. Obligate symbiosis with fungi is present in at least 11 independent scolytinae and platypodine groups. Ambrosia beetles are therefore not monophyletic, and the name is not a taxonomic designation. Ambrosia beetles are derived from bark beetles (Coleoptera: Curculionidae: Scolytinae). Bark beetles colonize and consume phloem, a tissue that is more nutrient-rich than wood. Bark beetles, like ambrosia beetles, are also often associated with fungal symbionts, usually ascomycotan and rarely basidiomycotan fungi, and the intensity of association is more variable, ranging from facultative to obligate (You L, 2015).

One of the most common symbioses in any forest ecosystem occurs between wood-boring insects and fungi.

This kind of symbiosis between Ambrosia beetles (Coleoptera: Curculionidae: Scolytinae and Platypodinae) and ambrosia fungi is ideal for studying many symbiosis-related questions. One reason is the diversity of ambrosia beetles which represent about 3000 species of wood-boring weevils that repeatedly evolved obligate symbioses with nutritional fungi possibly up to 16 times. Another reason is the easy manipulation of the symbiosis. Although the two partners require each other to complete their life cycle, they are perfectly separable in vitro. The beetles' transport-specific fungal symbionts from their natal galleries to newly established galleries in a storage organ termed a mycangium, but both can be kept in the laboratory on artificial media. Furthermore, their importance needs to be taken into consideration as in recent years, several ambrosia beetle-fungus symbioses have developed outbreaks causing significant economic and ecological damages. Unquestionably, it is important to understand the interactions between the beetle and the fungus has immediate scientific, economic and ecological implications (You Li1, 2018). Moreover, like many insects, ambrosia beetles may carry commensalist fungi on their body.

2. *Xylosandrus crassiusculus* (Motschulsky)

Xylosandrus crassiusculus (Asian ambrosia beetle or granulate ambrosia beetle) it is a highly polyphagous pest of woody plants of Asian origine and has been spread most probably with trade of plants and wood. In Africa it arrived hundreds of years ago while in the last few years it has been introduced to at least 14 African countries, 25 USA states, 3 countries of Central America, 2 South American countries, 6 countries of Oceania and 2 European countries (EPPO 2015, Fletchmann and Atkinson 2016), Italy and France, recently adding Spain (Gallego et al., 2017). Since the 1970s it has become a pest of fruit tree orchards and ornamental tree nurseries in the USA (EPPO Alert-list). It was first found in Europe in 2003, in cross-vane traps set up in Tuscany (Livorno, NW Italy) where no specific control measures were adopted (Pennacchio et al. 2003; EPPO 2015). Later, carob trees attacked by *X. crassiusculus* were found in in orchards in Central-North Italy and in gardens of nearby Liguria (Alassio and Pietraligure, NW Italy) in 2007 and 2008, and in NE Italy in Veneto (EPPO 2015) and Friuli Venezia Giulia (2015, personal observation of Massimo Faccoli). Maybe via Liguria, in 2014 the species arrived in SE France and in the Spanish Valencia Region.

Adults are small dark reddish brown scolytids (female: 2-3 mm long, males: 1.5 mm). Larvae are white, legless, C-shaped with a well-developed capsule, and cannot be easily distinguished from other scolytids. Populations essentially contain females (1:10 male-female ratio). Adult males do not fly and remain inside the galleries. Also *X. crassiusculus* is an inbreeding species (females mate with their brothers).

When females emerge, they leave infested plants and fly to new hosts. They start to bore a tunnel (round entrance hole of 2 mm diameter) with a brood chamber and one or more branches into the sapwood (and sometimes the heartwood). Eggs are laid in the brood chamber. Larvae have a length about 3.5 mm. and hatch and feed on the symbiotic fungus growing inside the galleries (Gardner, 1934, CABI Factsheet).

2.1. List of fungi isolated from *Xylosandrus crassiusculus*

The fungal isolation was done according to the protocol described on **SAMFIX Deliverable: *Fast routine protocol for detection of fungal symbiotic community associated with trapped Xylosandrus***. *Briefly,* the insects are crumbled in PBS and serial dilutions are plated on PDA. Different colonies are subcultured in new PDA plates. Morphotype designations are confirmed by ITS DNA sequencing and compared with sequences available at NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The fungi isolated from *X. crassiusculus* are on Table 1.

Table 1. List of fungi isolated from *Xylosandrus crassiusculus*

SPECIES (best hit)	PHYLUM	ORDER	BODY PART	FUNCTIONAL GUILD	OCCURANCE	REPORTED HOST/SUBSTRATE	REFERENCE
Penicillium expansum(P. spuinulosum)	A	Eurotiales	abdomen, head, external	Plant pathogen/Saprotrophs	Cosmopolitan	Numerous hosts.Fruit, decaying vegetation, seeds, etc. Causing Blue mold, fruit rot, postharvest decay.	https://nt.ars-grin.gov/fungaldatabases
Alternaria infectoria	A	Pleosporales	external	Plant pathogen	Widespread in temperate regions.	Substrate: Kernels, leaves. Disease: Black point disease.	https://nt.ars-grin.gov/fungaldatabases
Bipolaris sorokiniana	A	Pleosporales	abdomen	Plant pathogen	Cosmopolitan.	Leat spot, seedling blight, and root rot. Primarily on *Poaceae* but also numerous and diverse other hosts.	https://nt.ars-grin.gov/fungaldatabases
Clonostachys rosea	A	Hypocreales	mycangia	Plant pathogens/Saprotrophs	Cosmopolitan	Various plant parts both living and newly killed, associated with bark beetle galleries	Nygren et al., 2018
Cryphonectria parasitica	A	Diaporthales	mycangia	Plant pathogen	Asia, Europe, North America	*Castanea* spp., *Fagus sylvatica*, *Quercus* spp. (Fagaceae).	https://nt.ars-grin.gov/fungaldatabases

SPECIES (best hit)	PHYLUM	ORDER	BODY PART	FUNCTIONAL GUILD	OCCURANCE	REPORTED HOST/SUBSTRATE	REFERENCE
Didymella glomerata/ D._fabae|Aspergillus_niger	A	Pleosporales	external	Plant pathogens/Saprotrophs	Cosmopolitan	Various plant genera. Opportunistic pathogen. Found in association with blights, leaf spots, fruit rots.	https://nt.ars-grin.gov/fungaldatabases
Geosmithia pallida	A	Hypocreales	abdomen, mycangia, external	Plant pathogen	Widespread	Associated with bark beetle *Pseudopithyophthorus pubipennis*; Hosts: Quercus spp. (Fagaceae), Prunus spp., Malus (Rosaceae); Causing Foamy bark canker, dieback, death	Kolarik et al., 2017
Pestalotiopsis vismiae	A	Xylariales	mycangia	Plant pathogen	Asia (China, India), North America (USA).	Numerous hosts.Substrate: Trunk, bark, leaves, petioles, roots. Disease Note: Bark cracking, lesions; trunk disease.	https://nt.ars-grin.gov/fungaldatabases
Acremonium roseolum	A	Hypocreales	abdomen	Unassigned	Asia (Japan) , South America (Brasil)	Cryptomeria japonica and Manihot esculenta	https://nt.ars-grin.gov/fungaldatabases
Cladosporium sp.	A	Capnodiales	external	Saprotrophs	Cosmopolitan	Multiple genera in multiple families. Plant material and other organic substrates. Various spots and rots.	Bensch et al., 2012
Paraconiothyrium archidendri	A	Xylariales	abdomen, head, mycangia, external	Plant pathogen	Asia (Myanmar)	Leaf spot on *Pithecellobium bigeminum* (Fabaceae).	**Verkley et al., 2014**
Pleurostoma richardsiae	A	Calosphaeriales	external	Pathogen	Widespread	Wood streaking, canker; also a human pathogen. On Olea europaea (Oleaceae), Vitis vinifera (Vitaceae), Prunus dulcis (Rosaceae) and reports from diverse hosts.	https://nt.ars-grin.gov/fungaldatabases
Trichoderma harzianum	A	Hypocreales	external	Saprotrophs/Fungal antagonist	Cosmopolitan	Found on roots and other plant parts on numerous hosts;causing soft roots	Bissett et al., 2015; Han et al., 2017
Xenoacremonium falcatus	A	Hypocreales	external	Unassigned	Asia, Europe	Castanea sativa; other substrates	Aghyeva et al., 2017

SAMFIX Deliverable: List of fungi associated to *Xylosandrus* spp.

SPECIES (best hit)	PHYLUM	ORDER	BODY PART	FUNCTIONAL GUILD	OCCURANCE	REPORTED HOST/SUBSTRATE	REFERENCE
Fusarium solani	A	Hypocreales	abdomen, head, mycangia, external	Plant pathogen	Cosmopolitan	Broad host range; associated with ambrosia beetles	Sharma and Marques, 2018
Pithomyces chartarum	A	Pleosporales	head, external	Plant pathogens/Saprotrophs	Cosmopolitan	From *Pithomyces chartarum* leaves - Diverse plant families, but especially Poaceae.	https://nt.ars-grin.gov/fungaldatabases
Cladosporium cladosporioides	A	Capnodiales	abdomen, head, external	Saprotrophs/ hyperparasitic	Cosmopolitan	Multiple genera in multiple families; Leaves, seeds, inflorescences, often dead plant material; Causing leaf spot and blight, flower blight, scab, sooty mold.	Barkat et al., 2016
Talaromyces minioluteus	A	Eurotiales	mycangia, external	Plant pathogens/Saprotrophs	Cosmopolitan	Post harvest fruit rot.	**Palou et al., 2013**
Alternaria alternata	A	Pleosporales	abdomen, head, mycangia, external	Plant pathogens/Saprotrophs	Widespread	Wide-host range	Feng, Zheng, 2007
Trichoderma atroviride	A	Hypocreales	abdomen, external	Saprotrophs/Fungal antagonist	Widespread	On numerous hosts. Soil, wood, numerous other substrates.	https://nt.ars-grin.gov/fungaldatabases

3. Xylosandrus compactus (Eichhoff)

Xylosandrus compactus (black twig borer or shot-hole borer) is a highly polyphagous pest of woody plants that probably originates from Asia and has been introduced to other parts of the world, most probably with the trade of plants and wood. It is widely distributed in Africa, Asia and South America. It has been introduced in the Pacific Islands, New Zealand, Southeastern USA, and more recently in Europe in Italy and Southern France (EPPO Alert-list, Rabaglia et al., 2006, Wood, 1982; Chong et al., 2009). It was first found in Europe in 2011 (Garonna et al., 2012) in urban parks of the Campania region of Italy. Then, the species has been recorded in Italy's Campania, Tuscany and Liguria, and recently emerged in South-east France. The first report in Europe of X. compactus and associated ambrosia fungi in a natural environment has been recorded in September 2016, in the Italian National Park Circeo, Central Italy, in the Latium Region (Vannini et.al., 2017).

The adult females are dark brown to almost shiny black, 1.4-1.9 mm long and about two times longer than wide. The small, wingless males are reddish black and measuring 0.9–1.3 mm in length (Hara & Beardsley, 1979). Xylosandrus compactus is a species in which males are born from unfertilized eggs (0.3 -0.5 mm) and females from fertilized ones. After mating, which primarily occurs between siblings just after adult emergence, the male remains in the gallery while the female leaves the tunnel through the entry hole and colonizes branches of new hosts, boring an entry hole and a subsequent brood gallery (Hara & Beardsley, 1979; Greco & Wright, 2015). (CABI Factsheet).

3.1. List of fungi isolated from *Xylosandrus compactus*

Table 2 shows the fungal species isolated from the *X. compactus* specimens in the Circeo Park (Italy). The fungal isolation was done according to the protocol described on ***SAMFIX Deliverable:** Fast routine protocol for detection of fungal symbiotic community associated with trapped Xylosandrus*.

Table 2. List of fungi isolated from *Xylosandrus compactus*

SPECIES	PHYLUM	ORDER	BODY PART	FUNCTIONAL GUILD	OCCURANCE	REPORTED HOST/SUBSTRATE	REFERENCE
Ambrosiella xylebori	A	Microascales	abdomen, head, mycangia	Symbiont/plant pathogen	Cosmopolitan	Obligate, mutualistic symbionts of ambrosia beetles	Mayers et al., 205
Acremonium camptosporum	A	Hypocreales	abdomen, head, mycangia	Saprotrophs	Europe, Asia	dead plants or soil dwellers	Park, Thuong, Nguyen and Burm Lee, 2017
Acrodontium salmoneum	A	Pleosporales	mycangia	Animal pathogen	Widespread	Wide-host range	Steiman et al., 1995
Alternaria alternata	A	Pleosporales	abdomen, head, mycangia	Plant pathogens/Saprotrophs	Widespread	Wide-host range	Feng, Zheng, 2007
Aspergilllus spelaeus	A	Eurotiales	abdomen, mycangia	endophytes, food contaminants	Worldwide	soils and rhizospheres, indoor and cave environments	Hubka et al., 2017

SPECIES	PHYLUM	ORDER	BODY PART	FUNCTIONAL GUILD	OCCURANCE	REPORTED HOST/SUBSTRATE	REFERENCE
Aspergillus flavus	A	Eurotiales	abdomen	Plant pathogens/Saprotrophs	Cosmopolitan	Multitudinous substrates;secondary pathogen of some plants. Rots of fruit and food where it produces highly	Hubka et al., 2017
Aspergillus versicolor	A	Eurotiales	head	Plant pathogens/Saprotrophs	Cosmopolitan	Numerous biological substrates.	Behnke-Borowczyk et al., 2019
Beauveria bassiana	A	Hypocreales	abdomen	Animal pathogen	Cosmopolitan	Extremely wide host range; It can exist in diverse ecological environments including soil, plants and insects.	Imoulan et al., 2017
Beauveria pseudobassiana	A	Hypocreales	external	Animal pathogen	Cosmopolitan	Wide insect host range; can survive in diverse environments.	Imoulanet al., 2017
Cladosporium cladosporioides	A	Capnodiales	abdomen, head, mycangia	Saprotrophs/ hyperparasitic	Cosmopolitan	Multiple genera in multiple families; Leaves, seeds, inflorescences, often dead plant material; Causing leaf spot and blight, flower blight, scab, sooty mold.	Barkat et al., 2016
Cladosporium perangustum	A	Capnodiales	abdomen, head, mycangia	Saprotrophs	Widespread	Numerous hosts, associated with plants, fungi, food	Ogórek et al., 2012; Bensch et al., 2012
Cladosporium psychrotolerans	A	Capnodiales	head, abdomen, mycangia	Saprotrophs	Europe , North America , Dominican Republic.	Hypersaline water in the Mediterranean basin, indoor and outdoor environment, occasionally from plants	Bensch et al., 2012

SPECIES	PHYLUM	ORDER	BODY PART	FUNCTIONAL GUILD	OCCURANCE	REPORTED HOST/SUBSTRATE	REFERENCE
Cladosporium ramontenellum	A	Capnodiales	abdomen,head	Saprotrophs	South Africa, North America	Fruit and other plant material	K. Bensch et al., 2012
Cladosporium sphaerospermum.	A	Capnodiales	head, abdomen	Saprotrophs	Cosmopolitan	Wide-host range; decaying Citrus leaves and branches in Italy; soil; decaying stem	Dugan et al. 2008; Zalaret al.,2007
Cladosporium uwebrauniana	A	Capnodiales	head, mycangia	Unassigned	Europe	Indoor environment	Benschet al., 2012
Clonostachys byssicola	A	Hypocreales	abdomen, head, mycangia	Saprotrophs	Cosmopolitan	Fungi, plants	Alvindia and Hirooka, 2011
Clonostachys rosea	A	Hypocreales	abdomen, head, external	Plant pathogens/Saprotrophs	Cosmopolitan	Various plant parts both living and newly killed,associated with bark beetle galleries	Nygren et al., 2018
Clypeosphaeria phillyreae	A	Amphisphaeriales	abdomen	Unassigned	Europe	Phillyrea latifolia	Reblova, 2017
Cytospora acaciae	A	Diaporthales	abdomen, head	Plant pathogen	worldwide	Broad-host range; Ceratonia siliqua in Spain	N. Jiang et al., 2020
Fomes fomentarius	B	Polyporales	head	Saprotrophs	Temperate northern hemisphere	Broad host range; on dead or living hardwoods	Hashemi and Mohammadi, 2016.
Fusarium solani	A	Hypocreales	abdomen,head	Plant pathogen	Cosmopolitan	Broad host range; associated with ambrosia beetles	Sharma and Marques, 2018

SPECIES	PHYLUM	ORDER	BODY PART	FUNCTIONAL GUILD	OCCURANCE	REPORTED HOST/SUBSTRATE	REFERENCE
Fusarium sporothichioides	A	Hypocreales	head, mycangia, external	Plant pathogen	Temperate and tropical regions	Numerous hosts; found on roots, leaves, seeds, fruit causing root rot, leaf spot, dieback, etc	Arias et al., 2013; Taheri et al., 2017
Geosmithia flava	A	Hypocreales	head, abdomen	Plant pathogen	Europe, North America (USA: CA). Europe, Asia and Australia	Numerous hosts; Beetle galleries, other plant substrates. In association with bark beetles	Kolarik et al., 2007
Geosmithia pallida	A	Hypocreales	abdomen, head, mycangia	Plant pathogen	Widespread	Associated with bark beetle Pseudopithyophthorus pubipennis; Hosts: Quercus spp. (Fagaceae), Prunus spp., Malus (Rosa ceae); Causing Foamy bark canker, dieback, death	Kolarik et al., 2017
Geosmithia sp. 21 NL-2015	A	Hypocreales	abdomen,head	Unassigned	California	Wide range of host plants found in association with different bark and ambrosia beetles	Kolarik *et al*., 2017
Paraphoma fimeti	A	Pleosporales	mycangia	Saprotrophs	Cosmopolitan	Soil, dead plant tissues; Herbaceous and woody plants; roots of Juniperus comunis	Boeremaet al., 2004; De Gruyteret al., 2010;Moslemiet al., 2017
Penicillium citrinum	A	Eurotiales	head	Saprotrophs	Cosmopolitan	Soil, decaying vegetation, variety of organic substrates	Kozakiewwicz, 1992

SPECIES	PHYLUM	ORDER	BODY PART	FUNCTIONAL GUILD	OCCURANCE	REPORTED HOST/SUBSTRATE	REFERENCE
Penicillium coccotrypicola	A	Eurotiales	abdomen	Saprotrophs	Australia	Galleries of palm seed borer Coccotrypes carpophagus; Archontophoenix cunninghamiana (Arecaceae)	Crous et al., 2014
Penicillium glabrum	A	Eurotiales	external	Saprotrophs	Cosmopolitan	Numerous substrates; Numerous hosts; causing post harvest fruit rot	Duduk et al., 2017
Penicillium glabrum	A	Eurotiales	mycangia	Saprotrophs	Cosmopolitan	Numerous substrates; Numerous hosts; causing post harvest fruit rot	Duduk et al., 2017
Penicillium multicolor	A	Eurotiales	abdomen,head	Plant pathogens/Saprotrophs	Asia; Europe; USA	Leaves, seeds, coniferus and broadleaved sp.	Visagie et al., 2013
Penicillium pancosmium	A	Eurotiales	abdomen	Plant pathogens/Saprotrophs	Worldwide	on hardwood log, Isolated from soil, old Armillaria mellea on a hardwood log, Piptoporus (on Betula sp), nut of Juglans cinerea (butternut) and porcupine dung.	Houbraken et al.,2015
Peniophora meridionalis	B	Russulales	external	Plant pathogen	Europe	Wood; decidious species; Ceratonia siliqua, Erica sp. Eucalyptus sp., Q. Ilex, Q. Pyrenaica, Pistacia lentiscus, P. Halepensis, Arbutus unedo, Castanea sp., Viburnum tinus	https://www.gbif.org/species/2552347

SPECIES	PHYLUM	ORDER	BODY PART	FUNCTIONAL GUILD	OCCURANCE	REPORTED HOST/SUBSTRATE	REFERENCE
Pestalotiopsis biciliata	A	Xylariales	external	Plant pathogens/Saprotrophs	Cosmopolitan	Within woody species, both conifers and broadleaf trees are reported as hosts.causing a various range of symptoms including leaf spots, leaf blight, fruits rot as well as post-harvest diseases.	Morales-Rodríguez et al., 2018
Peziza ostracoderma	A	Pezizales	mycangia	Saprotrophs	North America and Europe	Peat mold	Lohr et al., 2017
Phanerochaete livescens	B	Polyporales	mycangia	Saprotrophs	Widespread	Alnus glutinosa, A. incana, A. hirsuta, Quercus sp., Fagus sylvatica, Populus tremula, Corylus avellana, Acer platanoides, Padus avium	Volubev et al., 2015
Sarocladium strictum	A	Hypocreales	external	Plant pathogen	Cosmopolitan	Broad host range, associated with ambrosia beetle Euwallacea fornicatus	Farr and Rossman, 2020; Li et al., 2016
Simplicillium lamellicola	A	Hypocreales	head	plant-parasitic, symbiotic, entomopathogenic	Widespread	broad spectrum of hosts and substrates, such as insects, plants, rusts, nematodes and mushrooms	De-Ping et al., 2019
Sistotrema brinkmannii	B	Cantharellales	abdomen	Saprotrophs	Widespread	Usually on wood, sometimes on plant debris and basidiomata	https://nt.ars-grin.gov/fungaldatabases

SPECIES	PHYLUM	ORDER	BODY PART	FUNCTIONAL GUILD	OCCURANCE	REPORTED HOST/SUBSTRATE	REFERENCE
Talaromyces amestolkiae	A	Eurotiales	head, abdomen, mycangium, ext	Animal pathogen	Cosmopolitan	Talaromyces contains species that are medically important. Emerging pathogen of agricultural crops.	Tsang et al., 2017; Yilmaz et al., 2014
Talaromyces purpurogenus	A	Eurotiales	head	Animal pathogen	Cosmopolitan	Talaromyces contains species that are medically important. Emerging pathogen of agricultural crops	Tsang et al., 2017; Yilmaz et al., 2014
Torrubiella alba	A	Hypocreales	head, mycangia	Animal pathogen	Cosmopolitan	Obligate symbiont with plants, animals and other fungal species	Johnson et al., 2008
Trichoderma hamatum	A	Hypocreales	head, abdomen mycangium, ext	Saprotrophs/Fungal antagonist	Cosmopolitan	Found on roots and other plant parts on numerous hosts;causing soft roots	Bissett et al., 2015; Han et al., 2017
Umbelopsis westeae	M	Mucorales	abdomen	Saprotroph	Australia	Different soil substrates	Wang et al., 2013
Ustilaginoidea virens	A	Incertae sedis	abdomen	Plant patogen	Worldwide in rice-growing regions	Substrate: Inflorescence/infructescense; Host: Oryza sativa, Zeamays, Brachiariabrizantha (Poaceae).	Kumari and Sharma, 2017; Fan et al., 2016
Xenoacremonium falcatus	A	Hypocreales	head	Unassigned	Asia, Europe	Castanea sativa; other substrates	Aghyeva et al., 2017

4. CONCLUSION

Fungi typically live in highly diverse communities composed of multiple ecological guilds. "Plant-pathogens" have been the most abundant fungal-guild present on the isolated fungi from *Xylosandrus crassiusculus* and *X. compactus*. Of particular interest is the finding of several plant pathogens associated to different parts of the insect and having as hosts the family of Fagaceae or other tree species. For example: *Cryphonectria parasitica*, the causal agent of chestnut blight, isolated form *X. crassiusculus* or *Pestalopsis biciliata*, the causal agent leaf blotch symptoms on Eucalyptus. The genus *Fusarium* and the species complex *Fusarium solani* (FSSC) were isolated from both *Xylosandurs* species. *Fusarium solani* it is a symbiotic fungus cultivated in tunnels of host plants by the female pest, which is attracted to volatiles from *F. solani* (Egonyu, 2017). Furthermore, members of genus *Fusarium* have been reported in association with other ambrosia beetles, and they are often reported as pathogenic to the host tree and other woody crops (i.e., avocado) in Sicily (Gugliuzzo *et al*., 2020). Species belonging to the genus *Fusarium* have diverse ecological functions as they can act as saprophytes, endophytes and animal and plant pathogens. It is important to mention that the genus *Fusarium* includes important plant pathogens that affect both forest and agricultural species by producing different types of wall-degrading enzymes (e.g., cellulases, glucanases and glucosidases) and mycotoxins such as beauvericin and fumonisins (Bezos, 2018; Sharma, 2018). *Geosmithia pallida* is a species native to Europe (Lynch *et al.,* 2014) and one of the most diffused fungal species in Mediterranean maquis, closely associated with alien species. *G. pallida* appear to be more a no specific commensal. It was reported from other plant-insect interactions, such as *Castanea sativa* and the Cynipidae wasp *Dryocosmus kuriphilus* (Morales-Rodríguez *et al*., 2019), *Carya illinoinensis* and *Quercus laurifolia* with *Pseudopityophthorus minutissimus* (Huang *et al*., 2019) or associated with *X. compactus* at the National Park of Circeo (Vannini *et al.,* 2017). But it can also behave as a plant pathogen, for instance, *G. pallida* have been reported in the literature as a causal agent of foamy bark canker in *Quercus agrifolia* in Californiain association with *Pseudopityophthorus pubipennis* (Lynch *et al.,* 2014). According to the literature, *G. pallida* was accidentally introduced from Europe, like an alien pathogen of live oaks in the United States (Lynch *et al.,* 2014).

The introduction of ambrosia beetle *Xylosandrus* and consequently fungal species, which represent a prevalent group of forest pathogens, as they are the major component of biodiversity in Europe and second-largest group of Eucaryotes right after insects. Many fungal species are considered as cryptogenic, which means they are most likely alien but with unknown origin, as they are poorly represented in alien species databases and unfortunately there is poor knowledge of their biogeography. For example, species as *Paraconiothyrium archidendri* reported on Asia, *Acremonium roseolum* on Asia and South America or *Geosmithia* sp. 21 on USA; wich roles should be more investigated.

Symbiosis plays a critical role when the insects attempt to invade a new habitat. Understanding the ecological factors that influence the adaptation of an organism in a new environment and the uptake of new microorganisms are the key to explain the mechanism of biological invasions. Right one of the most complex examples of symbiosis is the one between ambrosia beetles and ambrosia fungi. Nevertheless, should be considered that different kind of fungal species are associated to different species of ambrosia beetles and this is the reason why some of the fungal species associated to some beetles are not found in association with *X. compactus*/*crassiusculus* or vice versa, as there is the difference between fungal species that are associated to an exotic or native ambrosia beetle species. After the introduction of an exotic species such as *Xylosandus*, in a new environment, there is a series of biotic and abiotic forces that greatly influence the community of organisms in association with the insect. It is considered that forest habitat strongly influences the diversity of fungal species associated with the exotic ambrosia beetles. The absence of adaptation of exotic species could limit its establishment in a new environment. However, gaining microorganisms native to the invaded environment may support the exotic species to overcome these ecological barriers (Rassati et al., 2019).

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