Detection and control of forest invasive alien species in a dynamic world

September 25-28, 2019 Ljubljana Slovenia

Analysis of fungal community associated to the alien invasive ambrosia beetle *Xylosandrus compactus*: a specific contribute to the project LIFE17 NAT/IT/000609 SAMFIX: SAving Mediterranean Forests from Invasions of Xylosandrus beetles and associated pathogenic fungi.

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The project LIFE17 NAT/IT/000609 SAMFIX is co-financed by the European Union's LIFE Programme.

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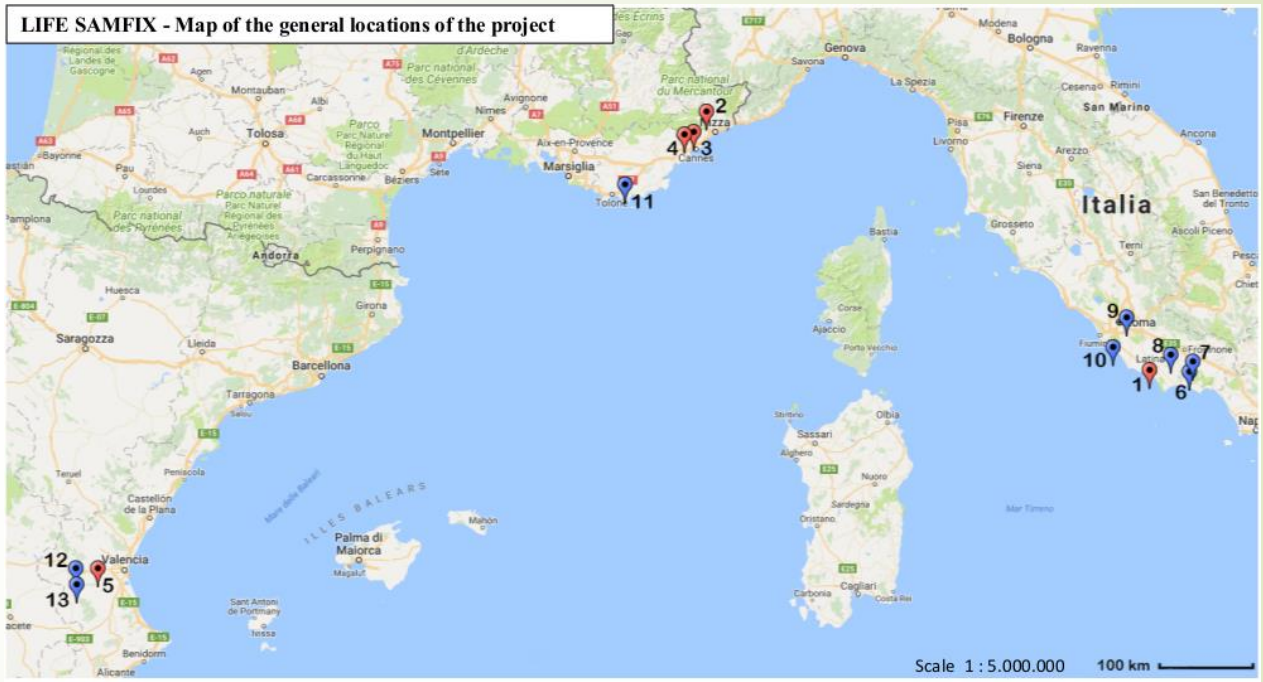
Saving Mediterranean Forests from Invasions of *Xylosandrus* beetles and associated pathogenic fungi

Objectives

SAMFIX aims to share and exploit these competences, finding and research efforts; to develop specific protocols and tools and trial them in a wide range of sites; to collect and assess herewith obtained data to better understand invasion pathways and routes, risks and efficacies, in order to achieve the following objectives:

- establish in and around 6 European detection sites in or close to natural parks effective protocols for prevention, early warning and rapid response, eradicating or containing current infestations and preventing future expansions.
- extend prevention and early warning protocols to 8 natural parks located in the surroundings of these sites to prevent expansions.
- disseminate knowledge on pathways and risks, and prevention, early detection and rapid response protocols amongst Mediterranean and EU bodies, networks and experts engaged in IAS policies and amongst natural parks managers to facilitate adoption of phytosanitary measures and replication of protocols to preserve Mediterranean forests and maquis landscapes.



Legend:

Core sites:
1. Circeo National Park IT6040017 - IT6040016 - IT6040014 - IT6040018
2. Corniches de la Riviera - FR9301568 / Saint-Jean-Cap-Ferrat / Mont Boron
3. Antibes (Bois de la Garoupe et Villa Thuret) - FR9301573
4. Ile Sainte Marguerite - FR9301573
5. El Tello and surroundings

Replication sites:
6. Natural Park of Riviera di Ulisse IT6040022 - IT6040023 - IT6040024
7. Natural Park of Monti Aurunci - IT6040043
8. Natural Park of Monti Ausoni - IT6040043
9. Natural Park of Castelli Romani IT6030018- IT6030017- IT6030039
10. Natural Reserve of Tor Caldara IT6030046
11. Port-Cros National Park - FR9301613
12. Sierras de Martés y el Ave - ES5233011
13. Muela de Cortes y el Caroche - ES5233040

Insect-fungus relationship

- Insect-fungus relationships described in the literature range from vague "associations" or "suspected" transmission, to facilitating entry via feeding wounds, to observation of the pathogen in the host insect, to fully established cases of experimental transmission (Mitchell et al. 2004; Brockerhoff et al., 2016).
- Nature of 'contractors' (alien vs native), and the host range of both insect and fungus, might determine the effectiveness of the interaction and the impact to the challenged hosts

Leach reviewed insect vectors of plant pathogens and established four rules to confirm that an insect was the vector of a given pathogen. He indicated that it was necessary to demonstrate:

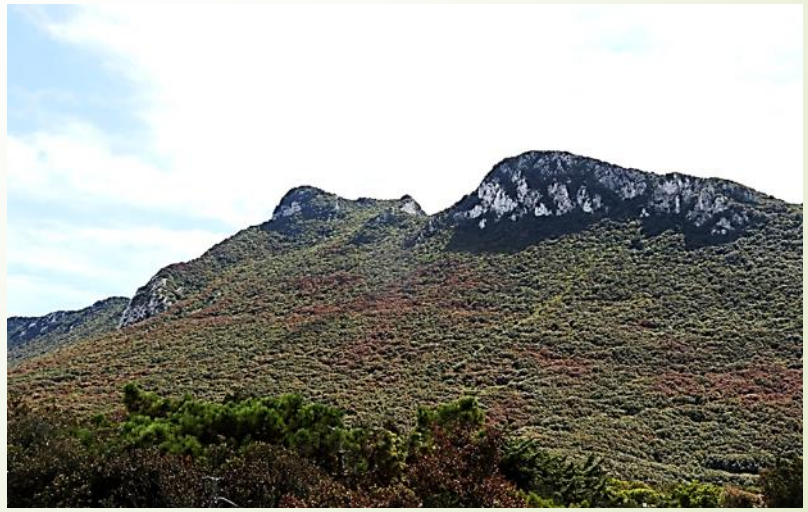
(i) a close association of the insect with diseased plants;
(ii) Regular visits of healthy plants by the insect;
(iii) an association of the pathogen with the insect; and
(iv) the development of the disease in healthy plants after interaction with pathogen-infested insects.

New invaders

- The Ambrosia beetle *Xylosandrus compactus*, a polyphagous alien pest from tropical areas in Asia and Africa. **Establishing symbiotic relationship with fungi, considered a true vector**

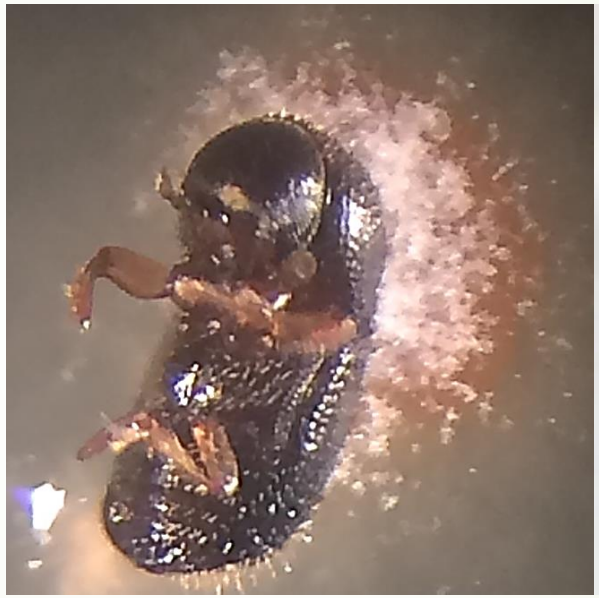
Xylosandrus compactus

- *X. compactus* is an Ambrosia beetle introduced in Europe probably during the first decade of this century probably from the tropical Asia, and actually widespread along the Mediterranean coast in Italy, France and Spain
- It is believed that *Xylosandrus* spp. were introduced in Europe with trade of living plants and that originally they moved from ornamentals in nurseries and parks to natural environments

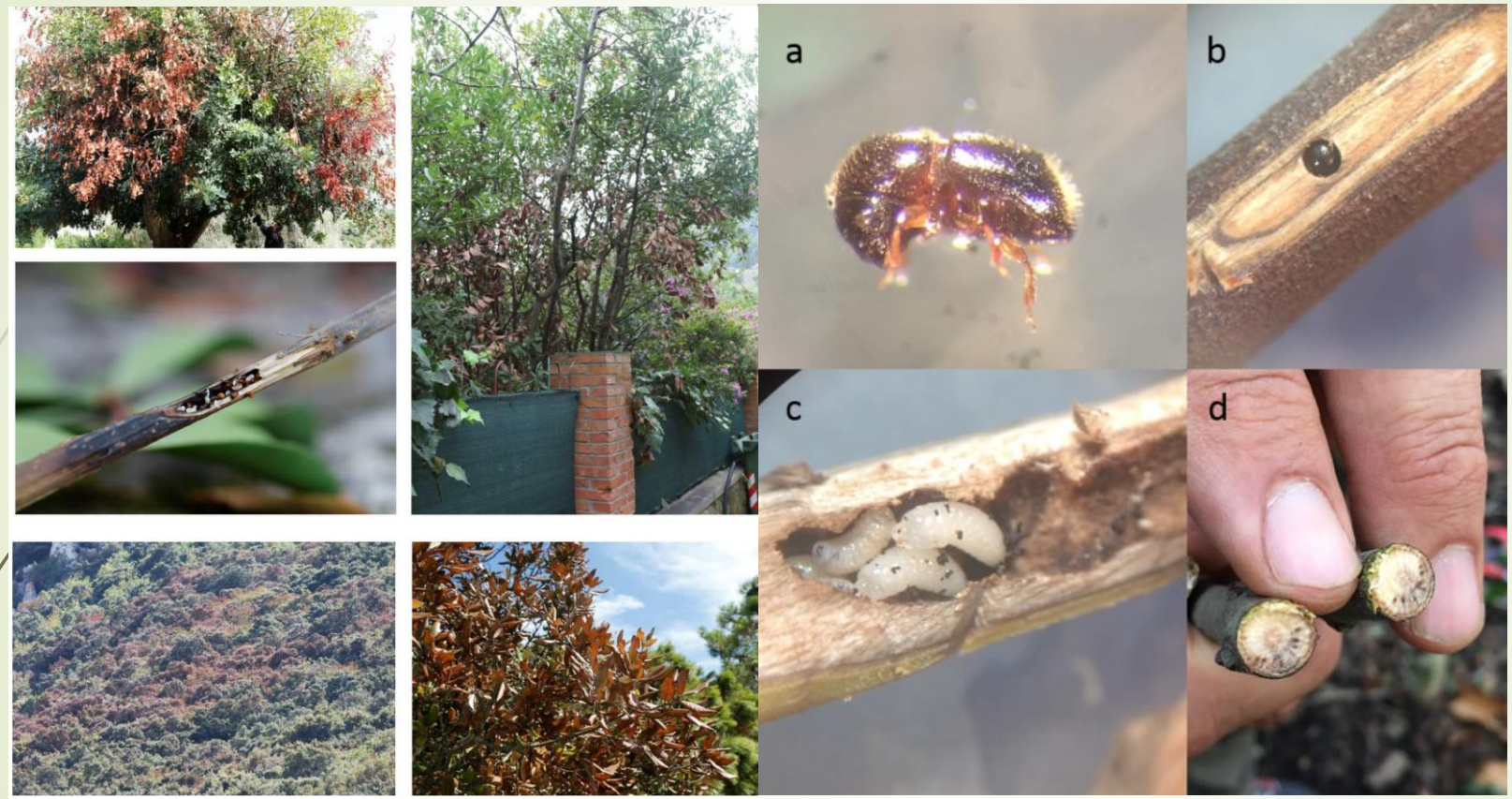


The symbiotic fungi

- The Ambrosia beetles are commonly associated with symbiotic fungi, some of which are harbored in a specialized structure named **mycangium**; others are associated with different parts of the insect body
- Some of these fungi, such as *Ambrosiella xylebori*, supplies the diet for the larvae into the galleries
- Others, are pathogenetic species that might contribute to symptoms developments on host plants



Xylosandrus compactus



- *X. compactus* digs galleries in young branches of trees **hosting symbiotic fungi**. Infested trees can show wilting, branch dieback, shoot breakage and general decline that is the result of the combined action of insect and pathogenic fungi

Xylosandrus compactus: a highly polyphagous species

- *Xylosandrus compactus* has 224 hosts outside Europe distributed in 62 families many of which also present in Europe
- In Italy only few of the hosts are known, *Laurus nobilis*, *Quercus ilex*, *Viburnum tinus*, *Ruscus aculeatus*, *Pistacia lentiscus*, *Ceratonia siliqua*

The highly polyphagous nature of the insect dramatically increase the risk of interaction with threatening fungi harbored by native and especially exotic hosts in nurseries and in the wild

Adoxaceae	Altingiaceae	Anacardiaceae	Annonaceae	Betulaceae
Bignoniaceae	Boxaceae	Cannabaceae	Cornaceae	Fabaceae
Fagaceae	Hydrangeaceae	Juglandaceae	Lamiaceae	Lauraceae
Magnoliaceae	Meliaceae	Mimosaceae	Moraceae	Myricaceae
Myrtaceae	Orchideaceae	Phyllanthaceae	Pinaceae	Platanaceae
Proteaceae	Rubiaceae	Salicaceae	Sapindaceae	Sterculiaceae
Symplocaceae	Thymeliaceae	Urticaceae	Verbenaceae	Vitaceae
Zingerberaceae				

Xyleborus glabratus and *Raffaelea lauricola:* a **Black swan** event in Tree pathology

- *Xyleborus glabratus and Raffaelea lauricola* are causing relevant damages in avocado plantations on the East coast of USA and in natural areas on *Persea borbonica* (redbay trees) and other Lauraceae
- Both the insect and the fungus were introduced in the USA in the early 2000 from the South-East Asia probably through trading untreated logs.
- In very short time they spread from Georgia to North Carolina, Texas and Florida causing the death of hundreds of millions of trees

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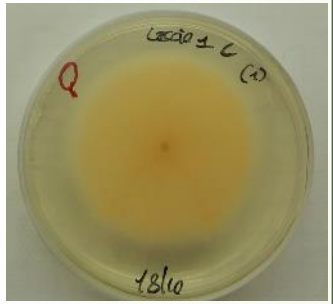
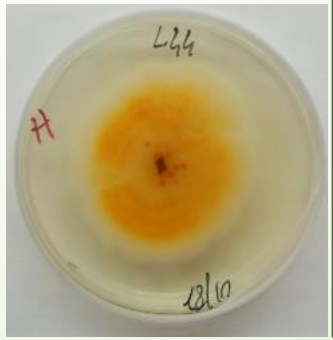
Back to *X. compactus* and associated fungi in Italy

- Up to 18 different fungal morphotypes were isolated in pure cutures
- Among them: *Ambrosiella xylebori* and *Fusarium solani* reported as the most frequent taxa associated to *Xylosandrus* spp. and other Ambrosia beetles
- Furthermore: *Fusarium proliferatum*, *Geosmithia pallida*, *Nectria haematococca*, *Epicoccum nigrum*
- *Clonostachys agrawalii* **an unespected asiatic species** associated with dead animals





Taxa
Alternaria sp.
Ambrosiella xylebori
Biscogniauxia mediterranea
Botryosphaeria stevensii
Clonostachys agrawalii
Cytospora sp.
Diaporthe sp.
Epicoccum nigrum
Fusarium proliferatum
Fusarium solani
Geosmithia pallida
Mucor racemosus
Nectria haematococca
Nigrospora sp
Penicillium sp.
Pestalotiopsis vismiae
Sarocladium strictum
Trichoderma sp.

The symbiotic fungi

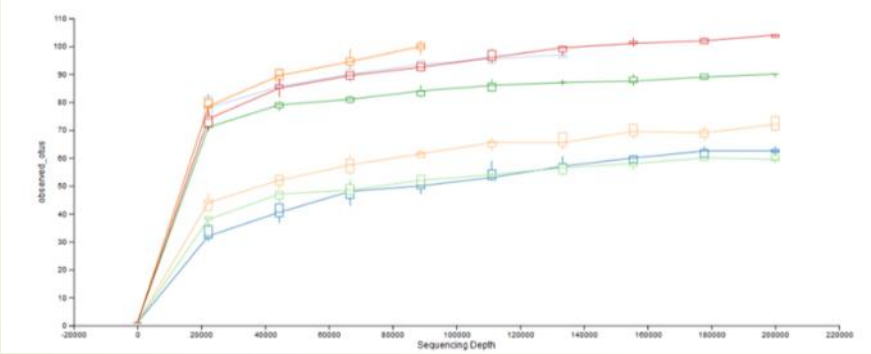
- *F. solani*, *F. proliferatum* and *N. haematococca* resulted the most aggressive causing necroses of several cm into the wood
- All the fungi colonized the tissues very slow
- The fungi were always re-isolated from the lesions

Microbial landscaping by HTS of fungal community associated to *Xylosandrus compactus* in the wild

- HTS was performed with the platform Illumina MiSeq.
- Three hosts: *Quercus ilex*, *Laurus nobilis* and *Ceratonia siiqua*
- Two dates: September 2016 and May 2017
- September 2016: 26 adults of *X. compactus* from *L. nobilis*, 48 from *Q. ilex*, and 50 *C. siliqua*
- May 2017: 15 adults of *X. compactus* from *L. nobilis*, 36 from *Q. ilex*, and 28 from *C. siliqua*.

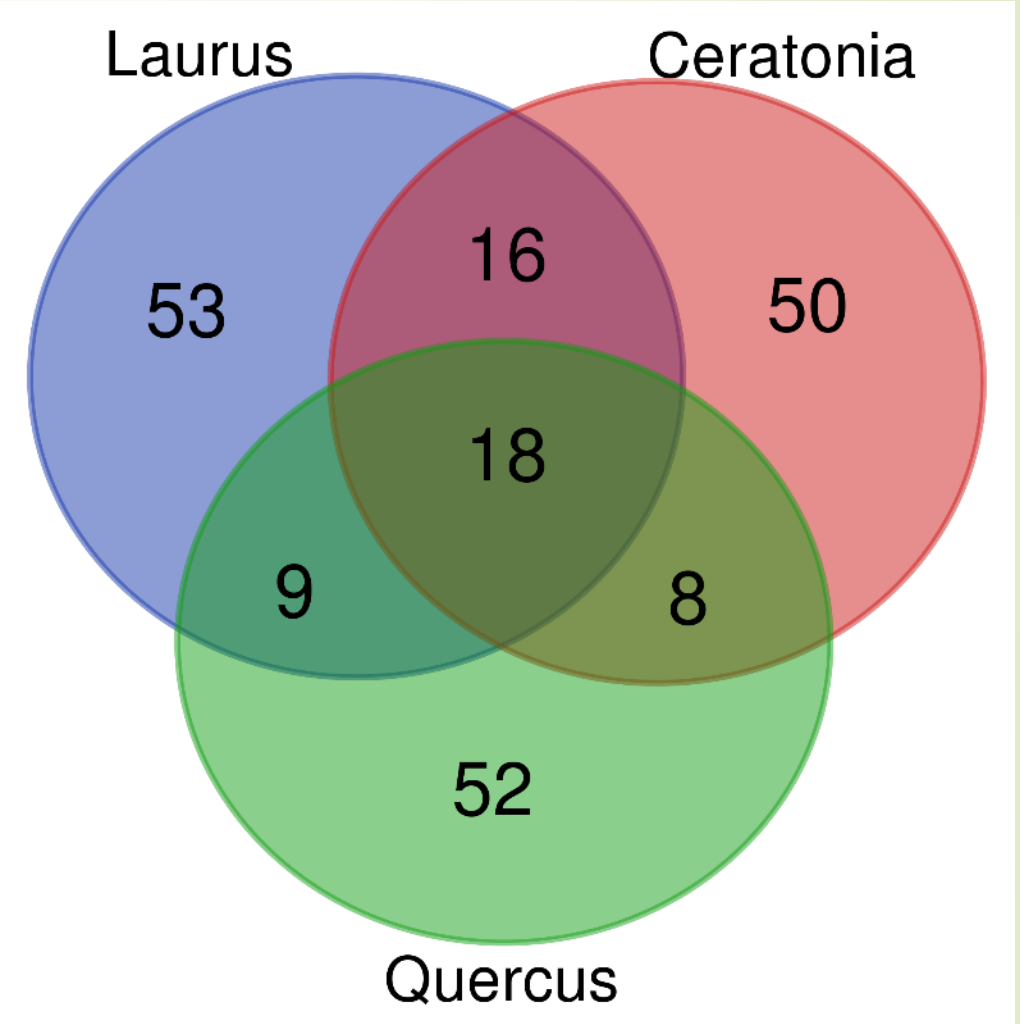
Illumina sequencing results

- A total of 1.513.183 reads were obtained from 7 samples
- Most of the samples biodiversity was explored



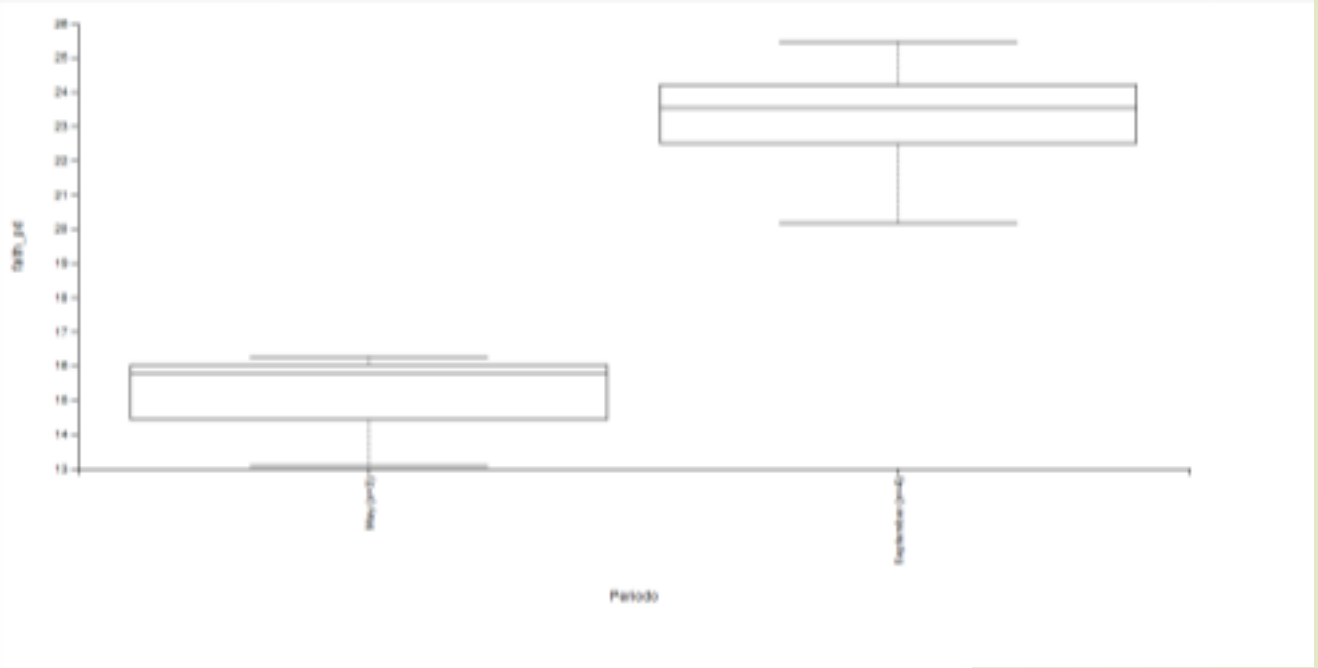
SampleID	Sequence Count
Laurus_Sep_2016	218.128
Laurus_May_2017	320.554
Quercus_Sep_2016	208.119
Quercus_May_2017	257.685
Ceratonia_Sep_2016	150.478
Ceratoniall_Sep_2016	108.930
Ceratonia_May_2017	249.289

- A total of 206 OTU's were identified
- Among the 18 OTU's (core biome) shared between the 3 hosts: *Fusarium solani*, *Fusarium sp.*, *Geosmithia pallida*, and *Ambrosiella xylebori*
- *F. solani* and *G. pallida* are the 2 most represented OTU's. These 2 taxa resulted pathogenetic to *Q. ilex* and *V. tinus* in 'in vivo' tests previously reported



Alpha diversity: Faith's Phylogenetic Diversity

Differences (P<0,05) were founded in the community richness of the fungal population between period of sampling



Kruskal-Wallis (all groups)

	Result
H	4.5
p-value	0.033894853524689295

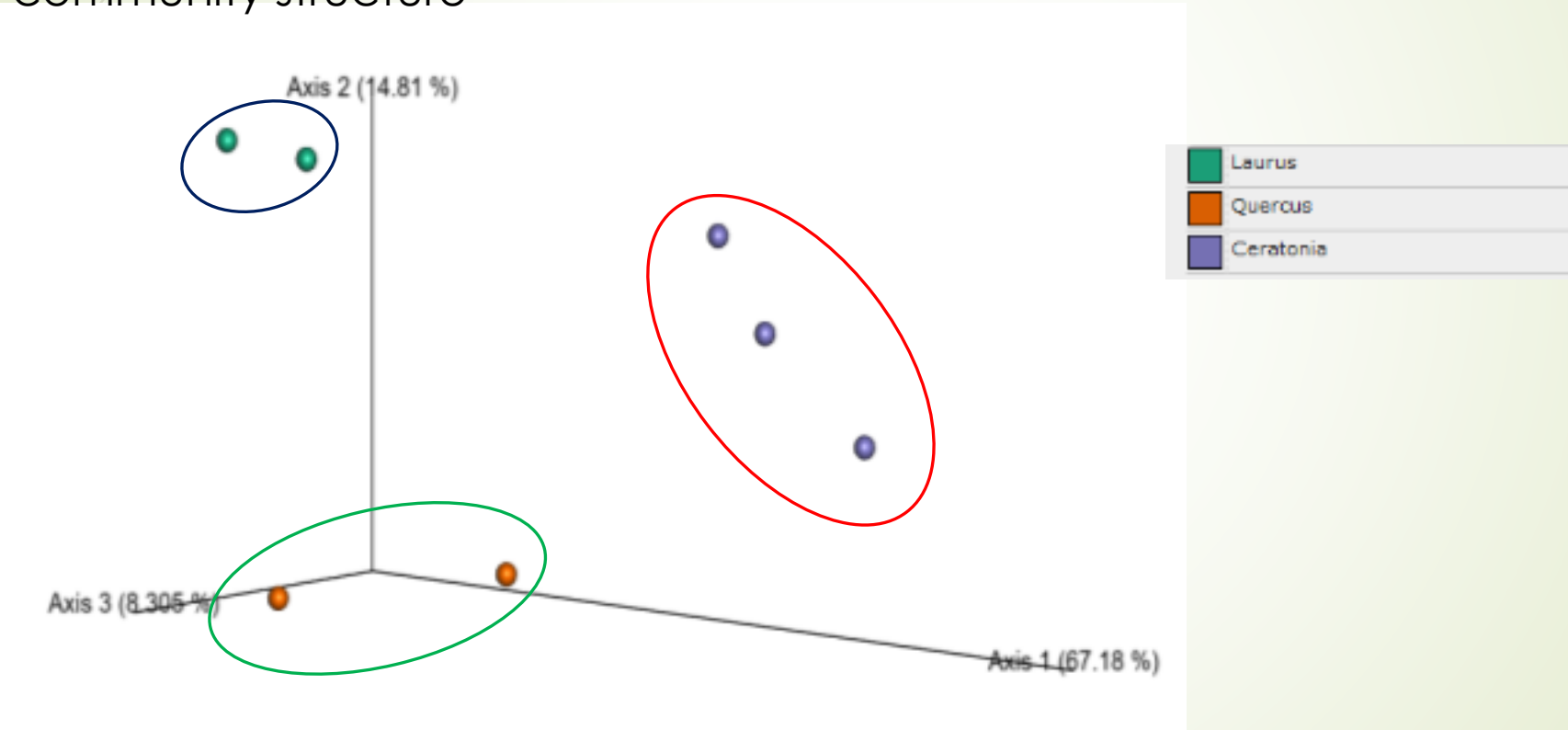
Kruskal-Wallis (pairwise)

Download CSV

		H	p-value	q-value
Group 1	Group 2			
May (n=3)	September (n=4)	4.5	0.033695	0.033695

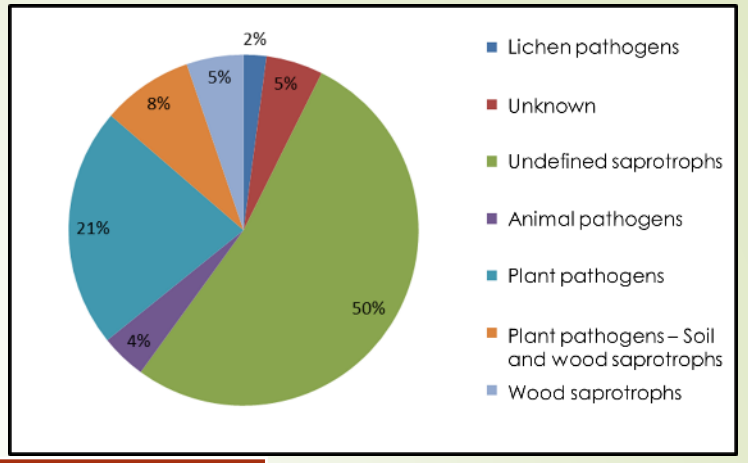
Beta diversity: weighted UniFrac distance

A quantitative measure of community dissimilarity that incorporates phylogenetic relationships between the features, useful for examining differences in abundance community structure



Principal coordinates analysis (PCoA) of weighted Unifrac distances

Functional groups: plant pathogens

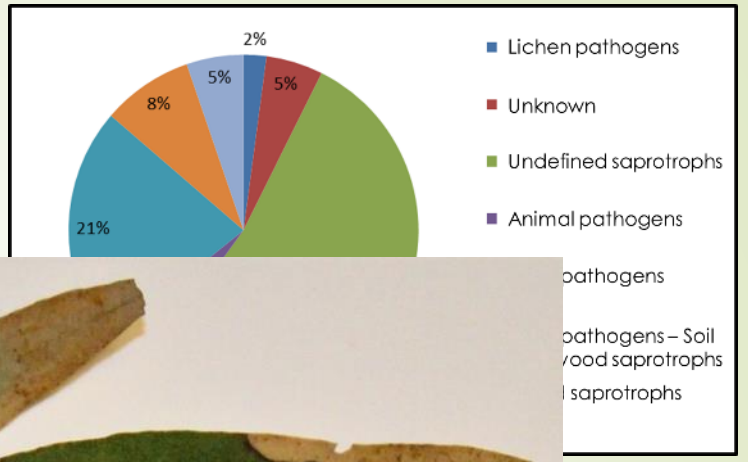


OTU	Species	Functional group
1	**Fusarium solani**	Plant pathogens – Soil and wood saprotrophs
2	Geosmithia pallida	Plant pathogens – Soil and wood saprotrophs
4	**Fusarium acuminatum**	Plant pathogens – Soil and wood saprotrophs
21	Clonostachys rosea	Plant pathogens
24	**Fusarium merismoides**	Plant pathogens – Soil and wood saprotrophs
35	Phaeoacremonium prunicola	Plant pathogens
41	Devriesia sardiniae	Plant pathogens
48	Ramularia eucalypti	Plant pathogens
51	Pestalotiopsis biciliata	Plant pathogens
70	Hortaea thailandica	Plant pathogens
77	Phaeoacremonium fraxinopennsylvanicum	Plant pathogens
86	Eutypa leptoplaca	Plant pathogens
110	Ramularia hydrangeae	Plant pathogens
113	Neofusicoccum luteum	Plant pathogens
214	Acrodontium crateriforme	Plant pathogens

Geosmithia pallida (G. Sm.) M. Kolarík, Kubátová & Paoutová is an emergent pathogen of oaks in the USA(Lynch et al., 2014) with affinities with a large number of xylophagous insects (Kolarik et al., 2004). This represents the first record of association, possibly as symbiont (core biome), with *Xylosandrus compactus*

Geosmithia pallida

Functional groups:



Functional groups: plant pathogens

- The genus Eucalyptus is an host of *Xylosandrus compactus*.
- The presence of the insects on *Eucalyptus* spp. in the Circeo area has not been verified yet
- The association of *X. compactus* with 2 pathogens of *Eucalyptus* spp., one of which of recent outbreak in the Circeo area, suggests an interaction between the insect and these specific hosts.
- Thus HTS analysis could also indicate new hosts of the insect based on identity of associated fungi

Alien taxa: core biome

Alien taxa

Adoxaceae	Altingiaceae	Anacardiaceae	Annonaceae	Betulaceae
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Zingerberaceae				

Final remarks

- *Xylosandrus* spp. represent a new risk for natural ecosystems in Europe
- The synergic activity of the insects and the associated pathogenic fungi might results in **unespected impact** on a wide range of host species
- The finding of new associations between the insects and pathogenic fungi (e.g. *G. pallida*) highlights the risk of insurgence of **novel interactions with alien invasive species in nurseries that might evolve in stable associations**
- Such event is favored by the **wide host range** of *X. compactus* spanning from exotic to native European species that could facilitate the host shift of associated fungi

Final remarks

- The detection of fungal species associated to the main hosts *of X. compactus* such as coffee (and others), and recorded from *X. compactus* native or invaded areas suggests a stable symbiosis between the beetle and the fungal taxa over the generations.
- Thus, evidence have been provided of the role of *Xylosandrus* compactus as a vector of alien fungal taxa during its colonization and invasion of new environments.
- This evidence reinforces the risk of a **black swan** event as the one caused by *Xyleborus glabratus* and *Raffaelea lauricola* in Eastern USA
- **Methodogically, we are shifting from the HTS Illumina MiSeq platform to the Nanopore MinION one that is faster, more flexible and potentially usable 'on site', dramtically reducing the time of analysis**