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SAving Mediterranean Forests from Invasions of Xylosandrus beetles and associated pathogenic fungi

CNiE 2021 TORINO

Bark and ambrosia beetles invasive of Mediterranean forest ecosystems

LIFE SAMFIX, co-funded by the European LIFE Programme Grant Agreement LIFE17 NAT/IT/000609

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UNIVERSITÀ DEGLI STUDI DELLA TUSCIA

Life

NATURA 2000

Xylosandrus invasive to Europe and their associated fungi: a symbiosis at high risk for natural environments

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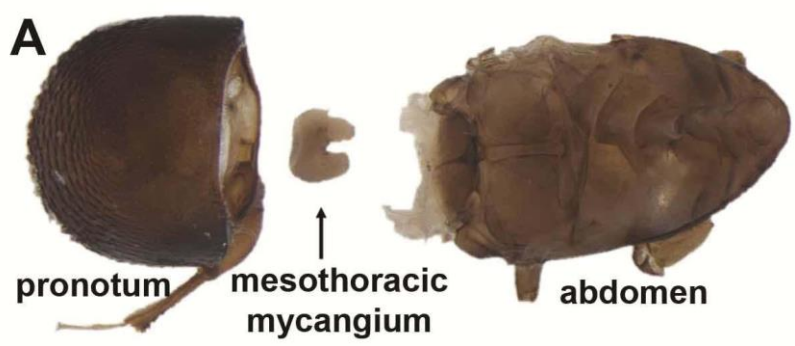
UNITUS , DIBAF

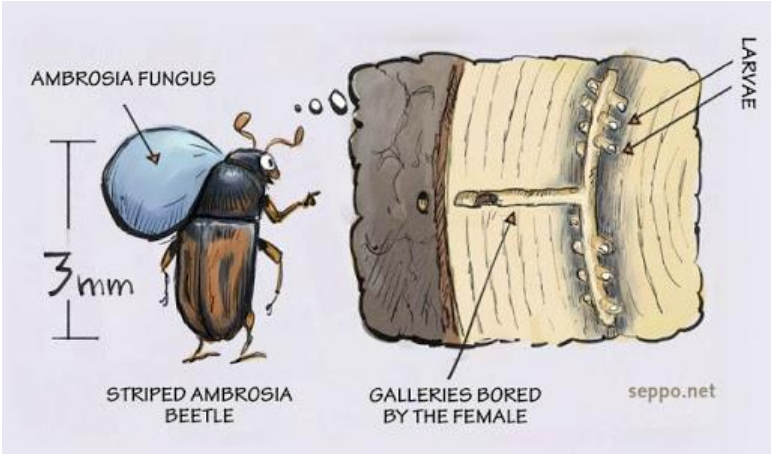
Bark and ambrosia beetles invasive of Mediterranean forest ecosystems

XXVI Congresso Nazionale Italiano di Entomologia
June 7-11, 2021

Ambrosia beetle

- Example of a mutualistic symbiosis
- Ambrosia beetles carry strictly symbiontic fungi in a specialized structure called mycangium





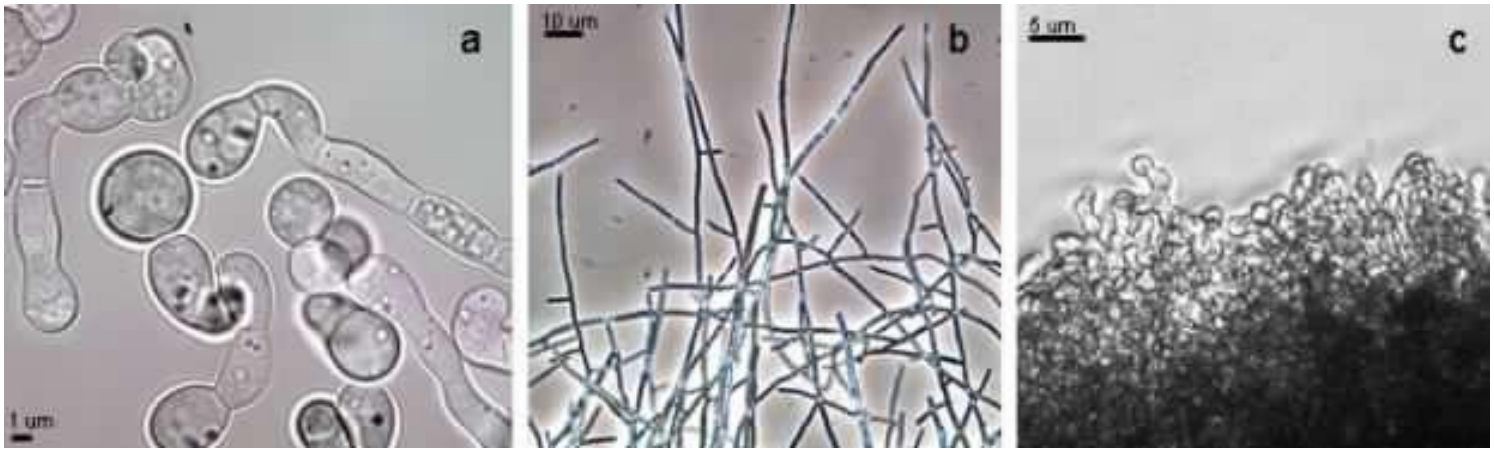
- The females dig galleries in the xylematic tissues typically very poor in nutrients
- Into the galleries the females ‘cultivate’ the symbiotic fungus that provides food for the larvae
- ***Fungus farming*** *insects*.

- Stable symbionts belongs mostly to the genera *Ambrosiella* (Ceratocystidiaceae), *Raffaelea* and *Dryadomyces* (Ophiostomataceae), *Ambrosiozyma* (yeast), and, occasionally, *Fusarium* (Nectriaceae).

Three main characteristics define an Ambrosia fungus:

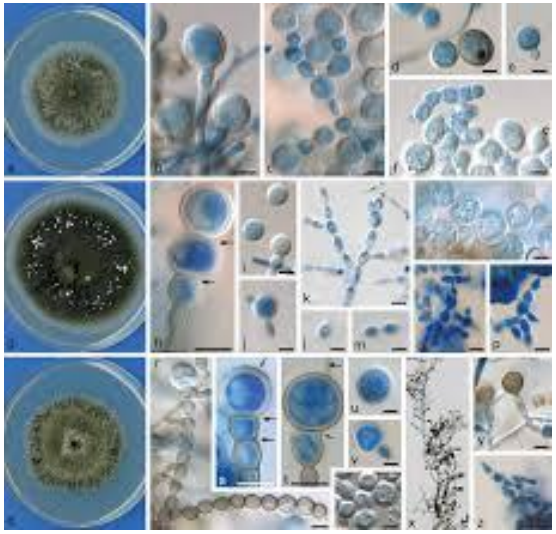
- Strict association with insect taxa that assures the horizontal transmission with mycangia between insect generations
- Polymorphism in vegetative structures that colonize wood and a yeast-like stage (or moniloid) in the mycangia
- Being a source of nutrition for the beetles

- An example of polymorphisms: *Ambrosiella hartigi* associated with *Xylosandrus germanus.*



- The basic compositional and dynamic patterns of the symbiotic consortium are unclear and contrasting evidences are provided with literature:
  - Clonal cultivation of a single species harboured in the mycangium
  - A biodiverse stable symbiotic fungal community harboured in mycangia and other part of the insect body
  - A variable fungal community intercheangeable with the invading environment

- It is not yet clear if it is the beetles that somehow choose the correct fungus among the many opportunistic fungi, if it is the other way around, or both.



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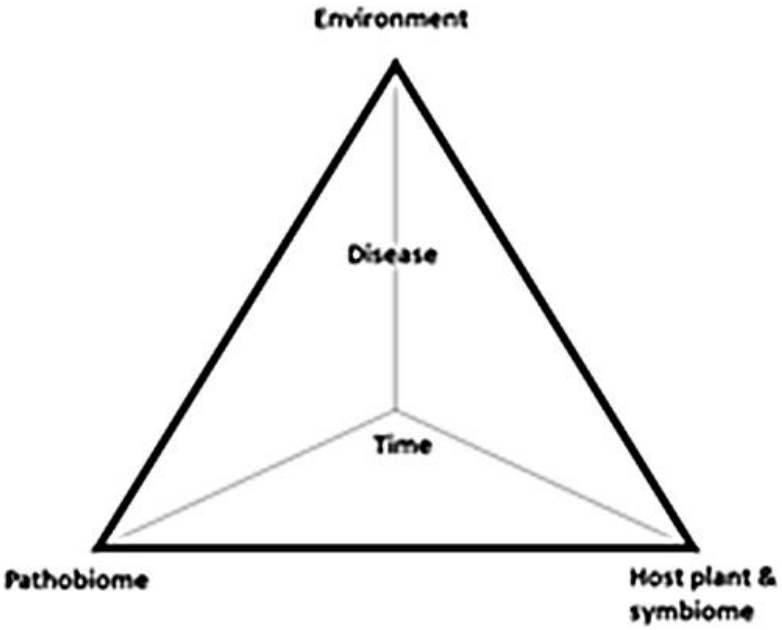
- What If an ambrosia fungus is also a plant pathogen?



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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
- *Raffaelea lauricola* is a permanent associate of *X. glabratus* that carry it into the mycangium
- 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

- *Raffaelea lauricola* was isolated from others Ambrosia beetle species

  - *Xyleborinus saxesenii*
  - ***Xylosandrus crassiusculus***
  - *Xyleborus ferrugineus*
  - *Xyleborus affinis*
  - *Xyleborus volvulus*
  - *Xyleborus bispinatus*
  - *Xyleborinus gracilis*

In summary the beetle is functional to facilitate different phases of the disease cycle of a pathogenic fungus

- Ability to act as a vector (*sensu stricto*, *sensu lato*)
- Ability to facilitate inoculation.
- Ability to facitate penetratio/colonization
- Ability to facilitate evasion

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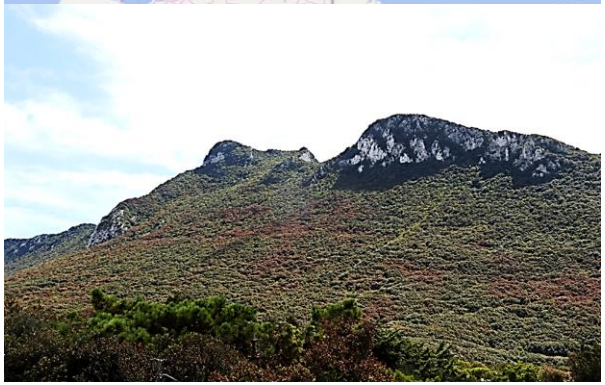
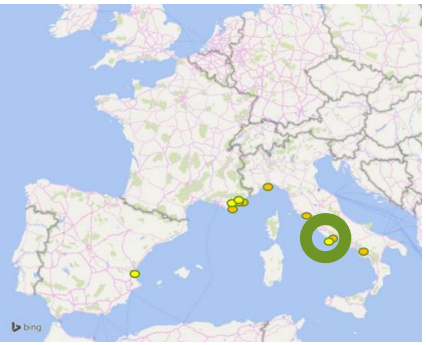
The fungal community associated to *Xylosandrus compactus*, *X. crassiusculus*, and *X. germanus* was studied different sites of the SAMFIX network

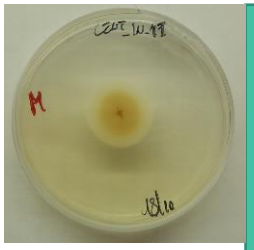
- Dissection of insects parts
- Isolation in pure culture
- Molecular barcoding
- HTS analysis

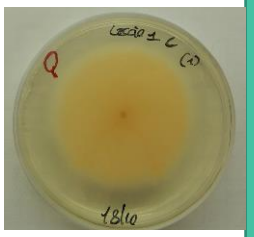


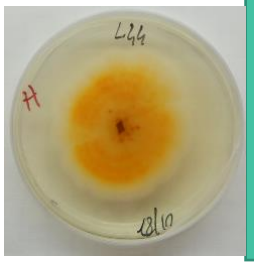
X. compactus fungal community isolated from insects in galleries (2016)



- 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







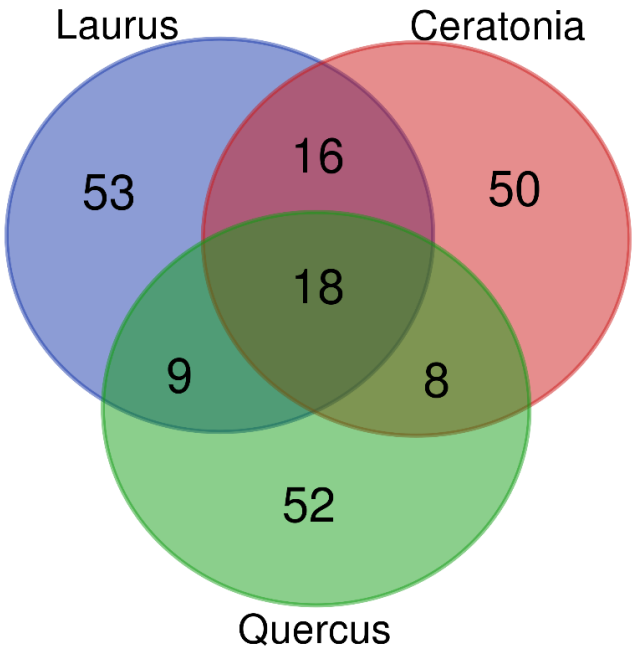
Taxa
Alternaria sp.
Ambrosiella xylebori
Biscogniauxia mediterranea
Botryosphaeria stevensii
Clonostachys agrawalii
Cytospora sp.
Diaporthe sp.
Epicoccum nigrum
Fusarium proliferatum
Fusarium solani
Geosmithia pallida
Mucor racemosus
Nectria haematococca
Nigrospora sp
Penicillium sp.
Pestalotiopsis vismiae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

- *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

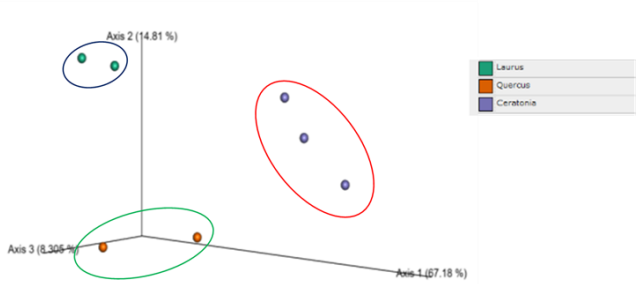
Microbial landscaping by HTS of fungal community associated to *Xylosandrus compactus* collected from galleries

- 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*.

- A total of 206 OTU's were identified

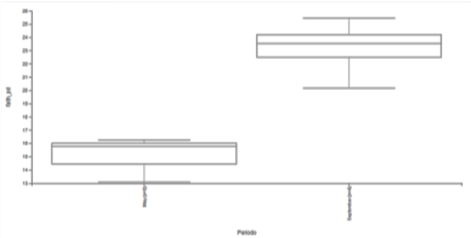


Beta diversity: weighted UniFrac distance



Alpha diversity: Faith's Phylogenetic Diversity

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



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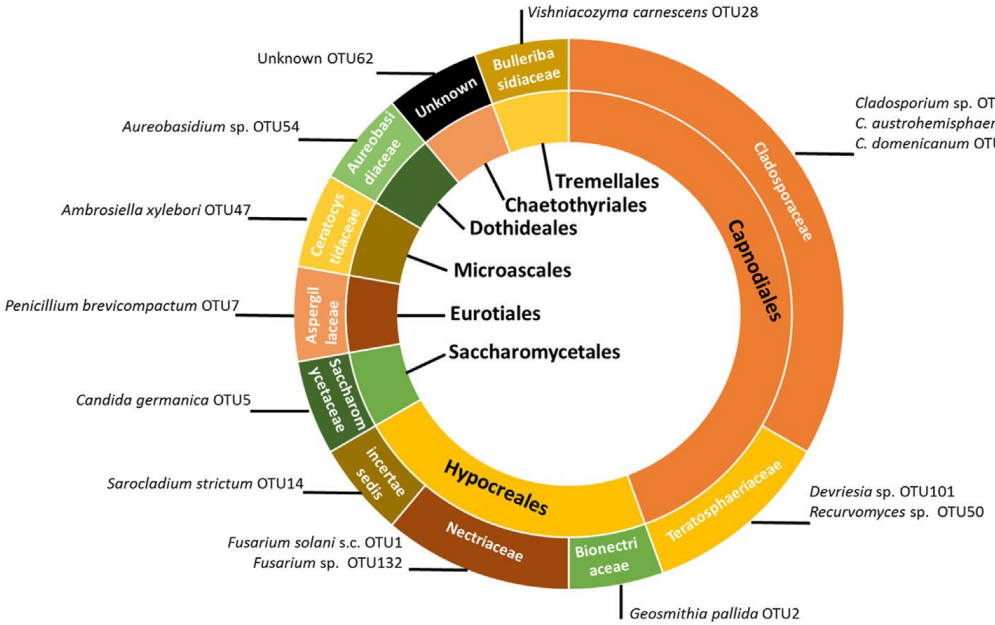
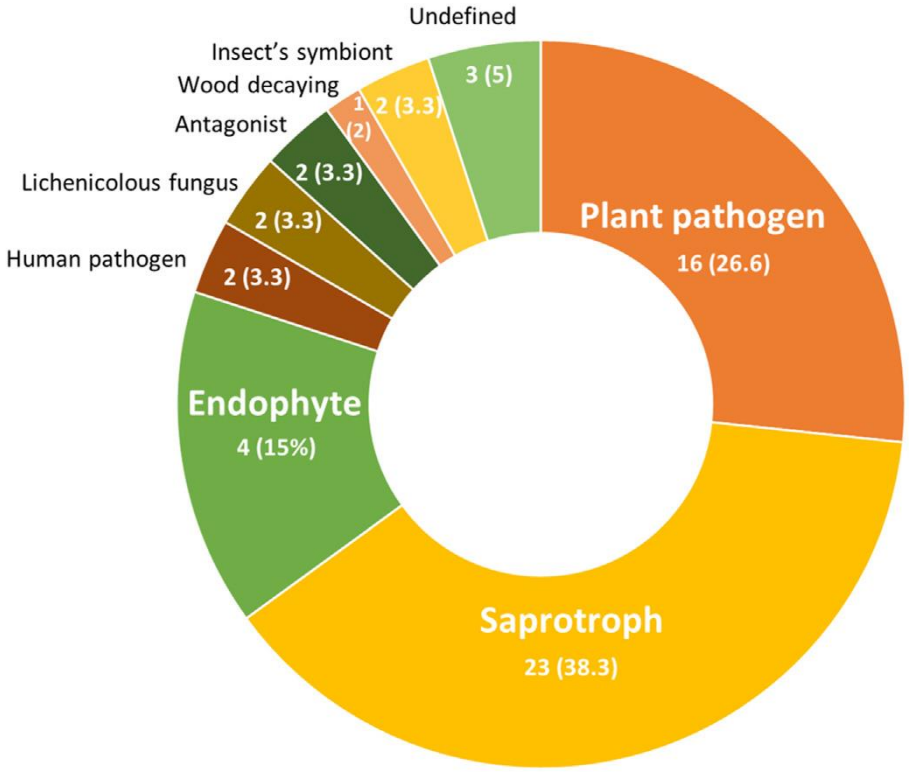
- Among the 18 OTU's (core biome) shared between the 3 hosts: *Fusarium solani*, *Fusarium sp.*, *Geosmithia pallida*, and *Ambrosiella xylebori*
- *F. solani* and *G. pallida* are the 2 most represented OTU's. These 2 taxa resulted pathogenetic to *Q. ilex* and *V. tinus* in 'in vivo' tests previously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



- Sixty-nine OTUs out of 206 were resolved at species level (including species complexes) identifying **60 different fungal species**
- Among these species, most were distributed worldwide or present in Europe.
- For six species this was the first record from Europe



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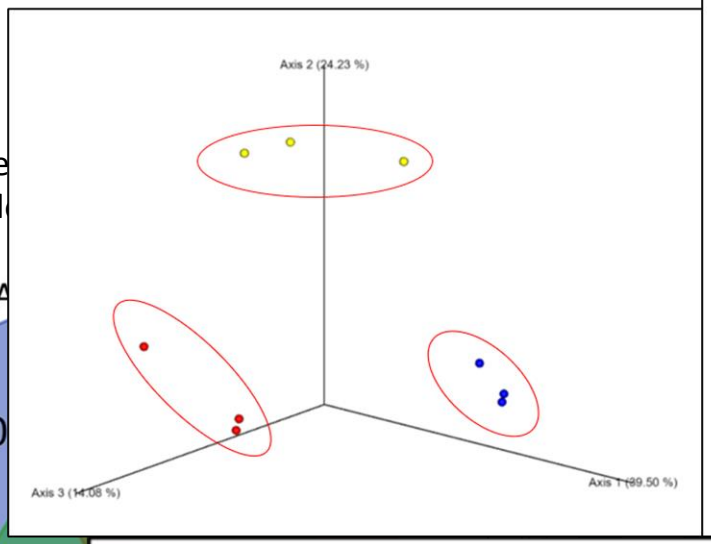
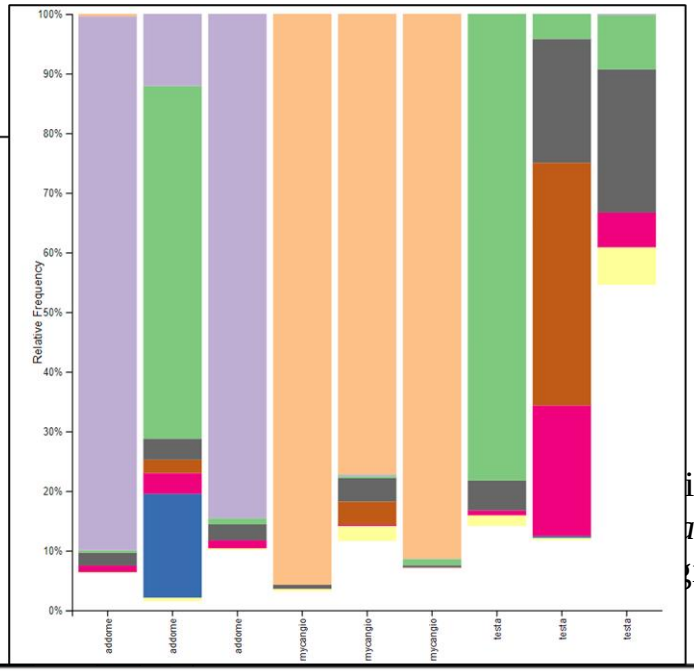
Xylosandrus germanus





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k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Microascales;f__Ceratocystidaceae;g__Ambrosiella;s__Ambrosiella grosmanniae
k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Sordariomycetes;f__Sordariomycetes;g__Phialemoniopsis;s__Phialemoniopsis sp.
k__Fungi;p__Ascomycota;c__Eurotiomycetes;o__Eurotiales;f__Aspergillaceae;g__Penicillium;s__Penicillium sp.
k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Capnodiales;f__Cladosporiaceae;g__Cladosporium;s__Cladosporium sp.
k__Fungi;p__Ascomycota;c__Eurotiomycetes;o__Pleosporales;f__Thyridariaceae;g__Pseudoneoconiothyrium;s__Pseudoneoconiothyrium euonymi
k__Fungi;p__Ascomycota;c__Dothideomycetes;o__Cladosporiales;f__Cladosporiaceae;g__Cladosporium;s__Cladosporium sp.
k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Nectriaceae;g__Fusarium;s__Fusarium solani
k__Fungi;p__Ascomycota;c__Sordariomycetes;o__Hypocreales;f__Bionectriaceae;g__Clonostachys;s__Clonostachys sp.

HEAD MYCANGIUM ABDOMEN

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Results related to *X. compactus* evidence the functionality of fungal community associated with Ambrosia beetle in providing information on the movement/host range of the insect population at local, regional and global scale

Example 1. local scale: *Pestalotiopsis biciliata*

Received: 1 October 2018 | Revised: 11 December 2018 | Accepted: 13 December 2018
DOI: 10.1111/efp.12492

ORIGINAL ARTICLE

WILEY Forest Pathology

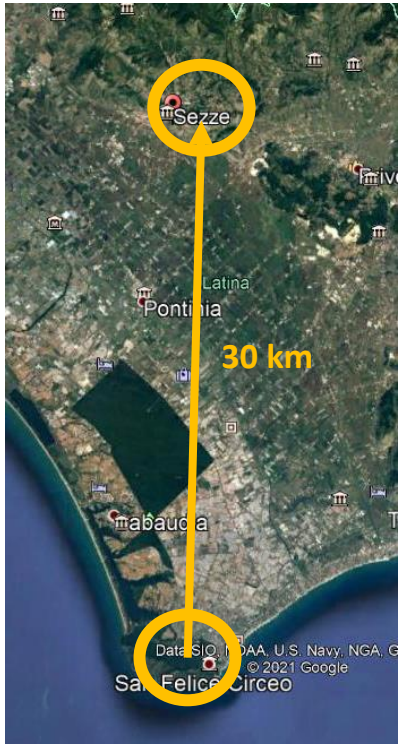
Pestalotiopsis biciliata, a new leaf pathogen of *Eucalyptus* spp. recorded in Italy

Carmen Morales-Rodríguez | Matteo Dalla Valle | MariaPia Aleandri | Andrea Vannini

Results related to *X. compactus* evidence the functionality of fungal community associated with Ambrosia beetle in providing information on the movement/host range of the insect population at local, regional and global scale

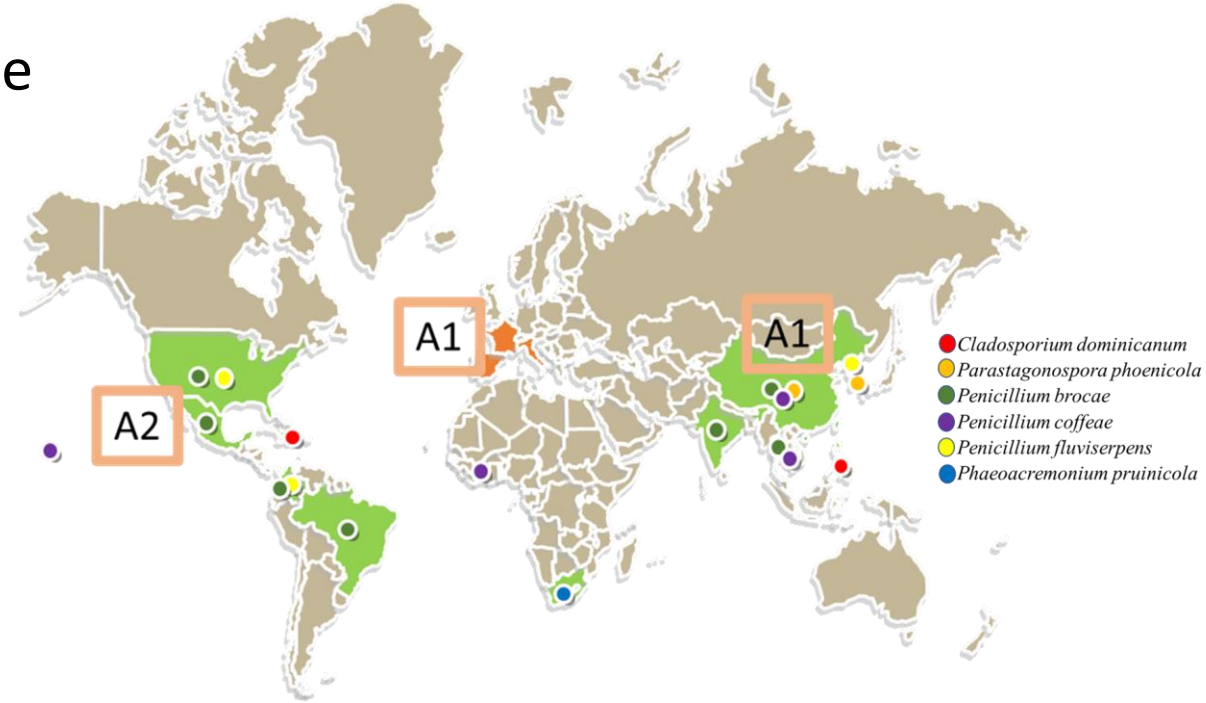
Example 2. medium scale: *Gnomoniopsis castanea* and *Cryphonectria parasitica* *(new to science)*

OTUs	SPECIES (obtained by HTS)	N° of reads/HEAD	N° of reads/ MYCANGIUM	N° of reads/ABDOMEN
11	***Gnomoniopsis castanea***	1519	487	15833
204	***Cryphonectria parasitica***	0	0	173

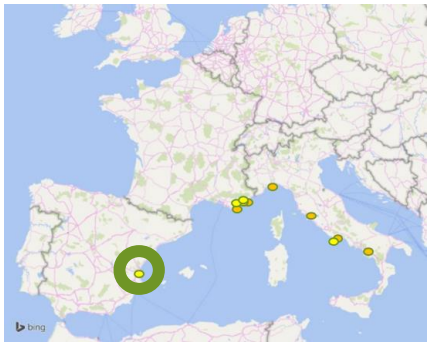


Results related to *X. compactus* evidence the functionality of fungal community associated with Ambrosia beetle in providing information on the movement/host range of the insect population at local, regional and global scale

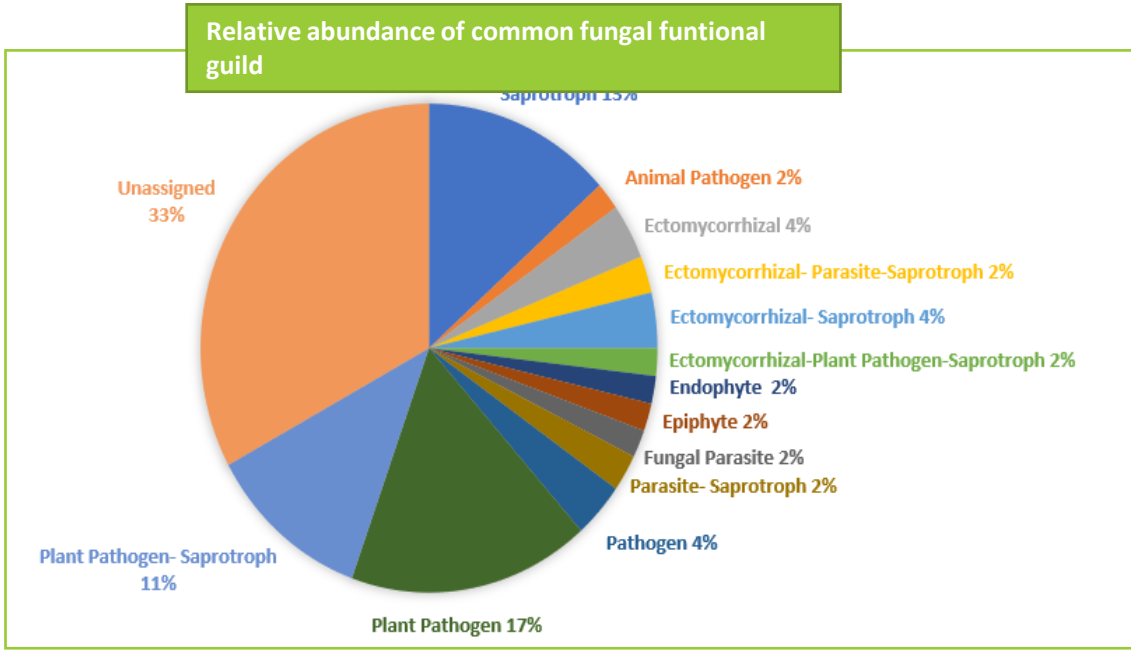
Example 3. large scale



Xylosandrus crassiusculus



- *Ambrosiella roeperi* as nutritional symbiont
- 32% plant pathogens



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Xylosandrus crassiusculus

Hosts (EPPO)

- *Acacia*
- *Ceratonia siliqua*
- *Diospyros kaki*
- *Eucalyptus*
- *Ficus carica*
- *Hibiscus*
- *Magnolia*
- *Malus domestica*
- *Populus*
- *Prunus avium*
- *Prunus domestica*
- *Prunus persica*
- *Quercus*
- *Salix*
- *Ulmus*

Vs.

OTU_24	*Alternaria alternata*	OTU_1	*Geosmithia sp_21*
OTU_49	*Aureobasidium pullulans*	OTU_8	*Gnomoniopsis smithogilvyi*
OTU_75	*Cercospora cf. citrulina*	OTU_155	*Ilyonectria liriodendri*
OTU_46	*Colletotrichum fioriniae*	OTU_228	*Paraconiothyrium brasiliense*
OTU_40	*Coniella quercicola*	OTU_35	*Penicillium glabrum*
OTU_305	*Cytospora acaciae/magnoliae*	OTU_245	*Plenodomus chrysanthemi/tracheiphilus*
OTU_84	*Cytospora rosae/cedri*	OTU_201	*Pseudocosmospora rogersonii*
OTU_91	*Dactylonectria novozelandica/macrodidyma*	OTU_145	*Ramularia eucalypti*
OTU_122	*Diaporthe amygdali*	OTU_183	*Ramularia pratensis/pistaceae*
OTU_143	*Didymella pedeiae*	OTU_347	*Diaporthe sp.*
OTU_7	*Didymella segeticola/bellidis*	OTU_77	*Sclerotinia pseudotuberosa*
OTU_50	*Diplodia gallae/corticola*	OTU_309	*Seiridium camelliae*
OTU_58	*Epicoccum nigrum*	OTU_256	*Sphaerulina menispermi*
OTU_119	*Epicoccum plurivorum*	OTU_98	*Stemphylium vesicarium*
OTU_205	*Fusarium acuminatum*	OTU_219	*Strelitziana mali*
OTU_130	*Fusarium equiseti*	OTU_69	*Taphrina carpini*
OTU_38	*Fusarium fujikuroi*	OTU_257	*Thelonectria veuillotiana*
OTU_62	*Fusarium proliferatum*	OTU_373	*Thysanorea rousseliana*
OTU_60	*Fusarium sambucinum*	OTU_356	*Vishniacozyma victoriae*
OTU_20	*Fusarium solani*	OTU_212	*Zasmidium fructicola*
OTU_67	*Geosmithia omnicola*	OTU_162	*Zymoseptoria verkleyi*

Xylosandrus crassiusculus

- *Diaporthe amygdali* (33 hosts)
  - *Malus* spp., *Prunus* spp.

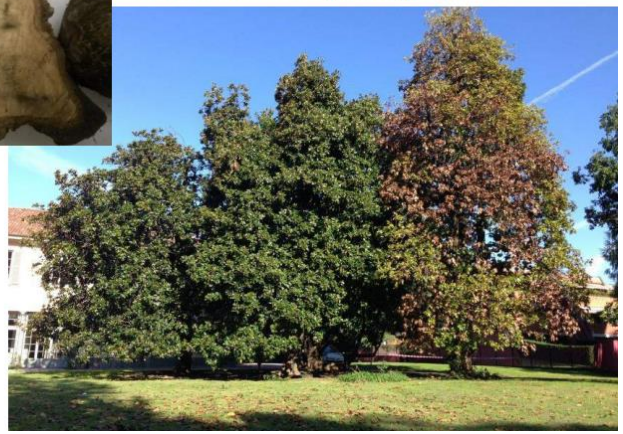
- *Diplodia gallae/corticola* (7/24 hosts)
  - *Quercus* spp., *Eucalyptus* spp.

https://doi.org/10.3390/agronomy10081062

Xylosandrus crassiusculus

- *Fusarium solani* (639 hosts)
- *Acacia*, *Eucalyptus*, *Hibiscus*, *Magnolia*, *Malus*, *Populus*, *Prunus*, *Quercus*, *Salix*, *Ulmus*

Fusarium solani: agente di tracheo-fusariosi della *Magnolia grandiflora*



Cancro alla corteccia del pioppo *Fusarium solani*

Populus x euramericana cv. A4A

https://www.regione.lombardia.it/wps/wcm/connect/8c1f56a8-0915-42b1-98f7-7e2413e23486/Malattie+fungine+delle+piante+ornamentali+e+forestali+%2815+aprile+2016%29.pdf?MOD=AJPERES&CACHEID=ROOTWORKSPACE-8c1f56a8-0915-42b1-98f7-7e2413e23486-lPFHiCH

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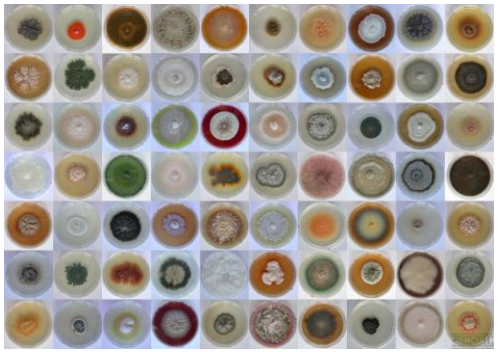
Xylosandrus crassiusculus

- The beetle is associated with fungal taxa commonly present in the invaded environment



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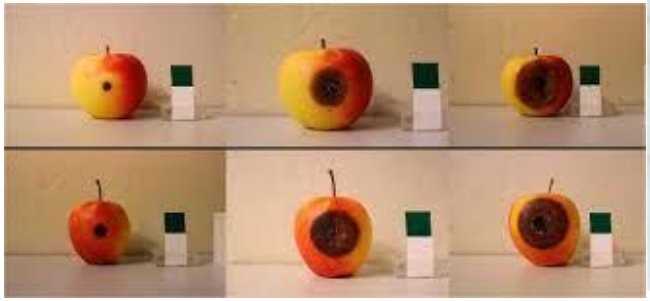
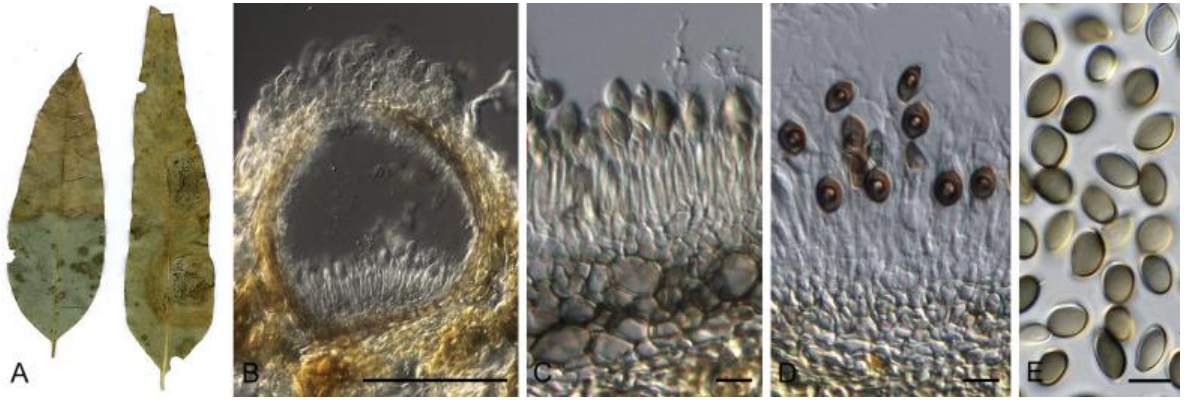
Xylosandrus crassiusculus

- *Colletotrichum fioriniae* (63 hosts)
- *Acacia*, *Magnolia*, *Malus*, *Prunus*.



Xylosandrus crassiusculus

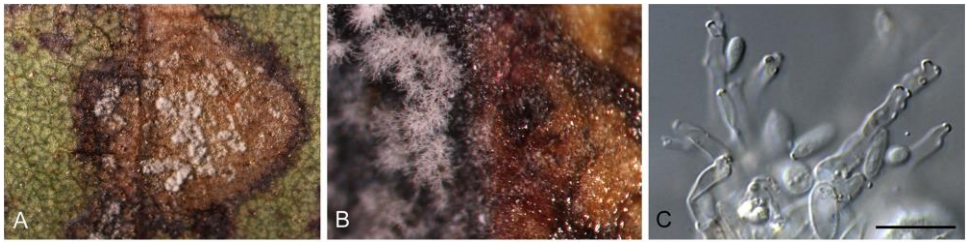
- *Coniella quercicola* (4 hospedadores)
- Eucalyptus, Quercus



https://doi.org/10.1016/j.simyco.2016.09.001

Xylosandrus crassiusculus

- *Ramularia eucalypti* (8 hospedadores)
- Malus, Eucaliptus



https://doi.org/10.1016/j.simyco.2019.08.001

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Xylosandrus crassiusculus

- The beetle is associated with fungal taxa commonly present in the invaded environment

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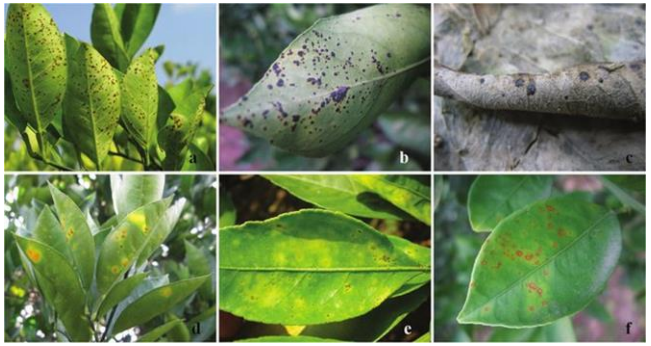
- The beetle is associated with fungal taxa present in Europe but not recorded in the invaded environment in Spain

Xylosandrus crassiusculus

- *Seiridium camelliae* (1 host)
  - **Distribution:** Asia (China).
  - **Substrate:** Living leaves.
  - **Disease Note:** Leaf blight.
  - **Host:** *Camellia reticulata* (Theaceae)

- *Zasmidium fructicola* (5 hosts)
  - **Distribution:** Asia (China).
  - **Substrate:** Fruit.
  - **Disease Note:** Fruit spot.
  - **Host:** *Citrus* spp. (Rutaceae).
  - Citrus grandis: China
  - Citrus paradisi China
  - Citrus reticulata China
  - Citrus sinensis China
  - Citrus unshiu China



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SAving Mediterranean Forests from Invasions of Xylosandrus beetles and associated pathogenic fungi

Xylosandrus crassiusculus

- The beetle is associated with fungal taxa commonly present in the invaded environment

+

- The beetle is associated with fungal taxa present in Europe but not recorded in the invaded environment in Spain

+

- The beetle is associated with fungal taxa not recorded in Europe

Bursaphelenchus xylophilus

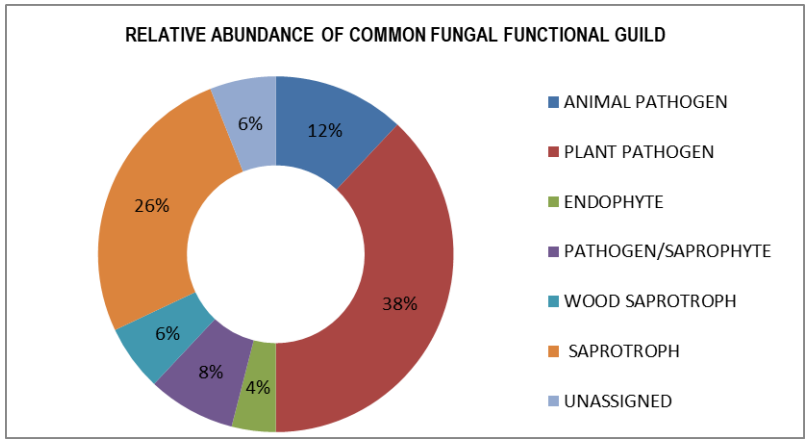
Xylella fastidiosa

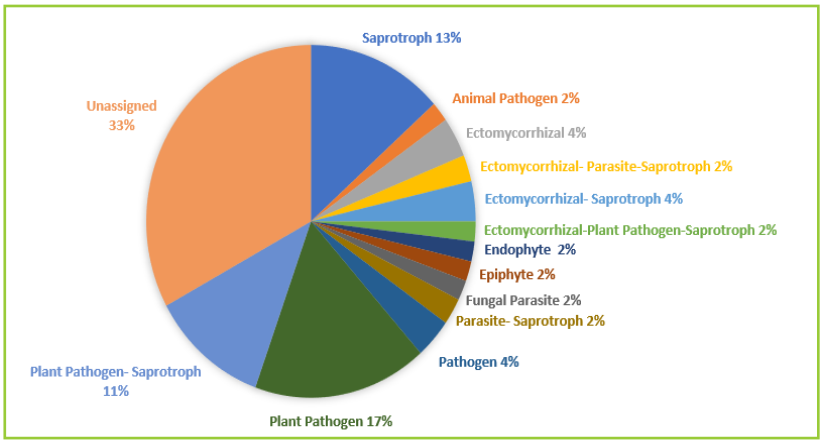
Hymenoscyphus fraxineus

Phytophthora infestans

Raffaelea lauricola

- A common feature of the invasive pathogens responsible for epidemics in forests is that most of them were first described (in Europe or elsewhere) after they had escaped from their geographic center of origin and started to cause damage in a susceptible plant community.
- In other words, they were unknown to science before the invasion.





- Described species but unknown life-style
- New species

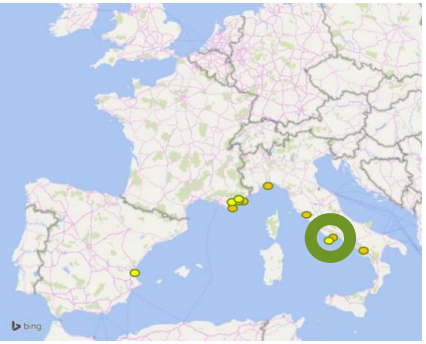
Nuevas enfermedades

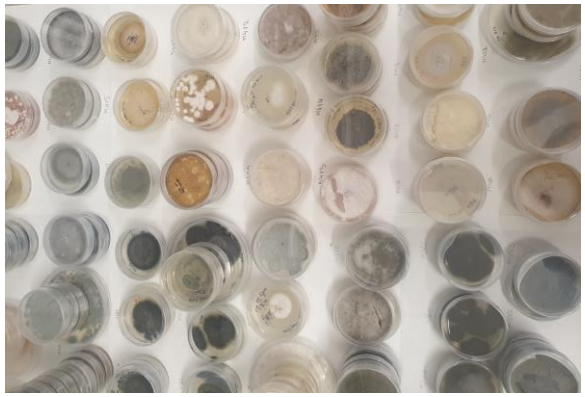
Monitoring nurseries

- About 57% of invasive fungi have been introduced into Europe through the plant trade
- Nurseries are one of the routes for the dispersal of pests and pathogens

Xylosandrus germanus

- Traps were placed nearby nurseries proximal to the Circeo National Park (Italy)
- Only *Xylosandrus germanus* specimens were trapped from which the isolation of associated fungi was carried out





Xylosandrus germanus

No-pathogenic fungal taxa alien to Europe

- *Cladosporium dominicanum*
  - Citrus sp.: Dominican Republic e Iran
  - Dracaena fragrans: Philippines
- *Cladosporium iranicum*
  - Citrus aurantium: Iran
  - Citrus sinensis Iran
- *Coniothyrium palmicola:*
  - Cocos nucifera: Southern Africa
  - Syagrus oleracea: Brazil

Xylosandrus germanus

No-pathogenic fungal taxa alien to Italy

- *Peniophora rufomarginata*
  - Quercus faginea: Portugal
  - Quercus ilex: Spain
  - Quercus robur, rotundifolia: Portugal
  - Salix aurita: Scotland
  - Tilia sp. : Ireland

Xylosandrus germanus

Pathogenic fungal taxa alien to Italy

- *Pestalotiopsis pini*
  - recorded in Portugal (2017) on *Pinus pinea*

- *Pseudosydowia eucalypti*
  - recorded in Africa (South Africa), Asia (Myanmar), Australia, Europe (Portugal) on *Eucalyptus* spp.
  - **Substrate:** Living leaves

Final remarks

- Along with their invasion patterns, the Ambrosia beetles modulate their associated fungal community that includes exotic species and newly aquired native/local species.
- This modulation in fungal community structure is likely influenced by the host community and the environmental conditions.
- The absence of adaptation of the exotic fungal species could limit the Ambrosia beetle establishment and survival in the newly invaded environment
- However, newly acquisition from the invaded environment can help alien species to overcome these ecological barriers.

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 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

SAMFIX
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THANKS

for your attention

Bark and ambrosia beetles invasive of Mediterranean forest ecosystems

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