COST Action FP1406
Action Title: Pine pitch canker - strategies for management of *Gibberella circinata* in greenhouses and forests (PINESTRENGTH)

Insects-fungi interactions, from symbiosis to occasional contamination: the case study of the Ambrosia beetle *Xylosandrus compactus* and the Chinese Gall Wasp *Dryocosmus kuriphilus*

Andrea Vannini and Carmen Morales-Rodriguez

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

Fusarium circinatum and insects

- In literature the interaction of *F. circinatum* with insects is widely reported and refers to bark and wood borer, shoot and foliage feeders, cone insects, predator insects.
- Most of the reported interactions are with native species in the area where the interaction take place, while interaction with no-native insects has been rarely reported (Brockerhoff et al., 2016).

forests MDPI

Article

Fungal Communities Associated with Bark Beetles in *Pinus radiata* Plantations in Northern Spain Affected by Pine Pitch Canker, with Special Focus on *Fusarium* Species

Diana Bezos [1,2,*,†], Pablo Martínez-Álvarez [1,2], Antonio V. Sanz-Ros [1,3], Jorge Martín-García [1,4], M. Mercedes Fernandez [1,5] and Julio J. Diez [1,2]

Biol Invasions (2016) 18:1177–1190
DOI 10.1007/s10530-016-1059-8
CrossMark

ORIGINAL PAPER

Role of insect vectors in epidemiology and invasion risk of *Fusarium circinatum*, and risk assessment of biological control of invasive *Pinus contorta*

Eckehard G. Brockerhoff · Margaret Dick · Rebecca Ganley · Alain Roques · Andrew J. Storer

Insect-fungus relationship

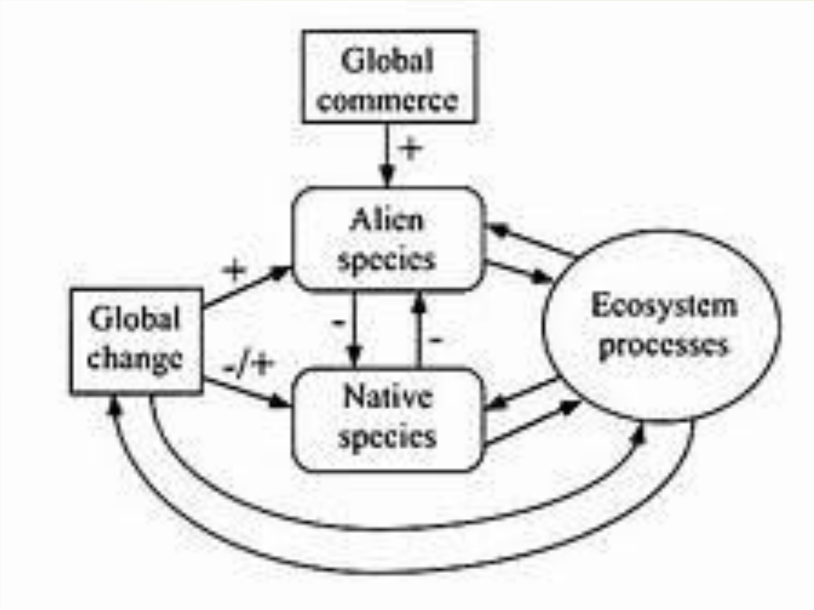
- Insect-fungus relationships described in the literature range from vague "associations" or "suspected" transmission, to facilitating entry via feeding wounds, to observation of the pathogen in the host insect, to fully established cases of experimental transmission (Mitchell et al. 2004; Brockerhoff 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.

Insect-fungus relationship in a biological invasion context: direct and indirect interactions

- Invasion by alien plant pests represents an important fraction of the global biological invasions having a tremendous **direct impact** to hosts species in the invaded environments.
- Differently, **indirect interactions** occur when one species **influences a second via its interactions with a third species** (Waser et al., 2015). In the context of alien invasive plant pests, these interactions **can modify third species behavioural traits for instance by interacting with patterns of dispersal or colonization** (Gandhi and Hermes, 2010).



New invaders, two opposite 'study cases'involving alien insects

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

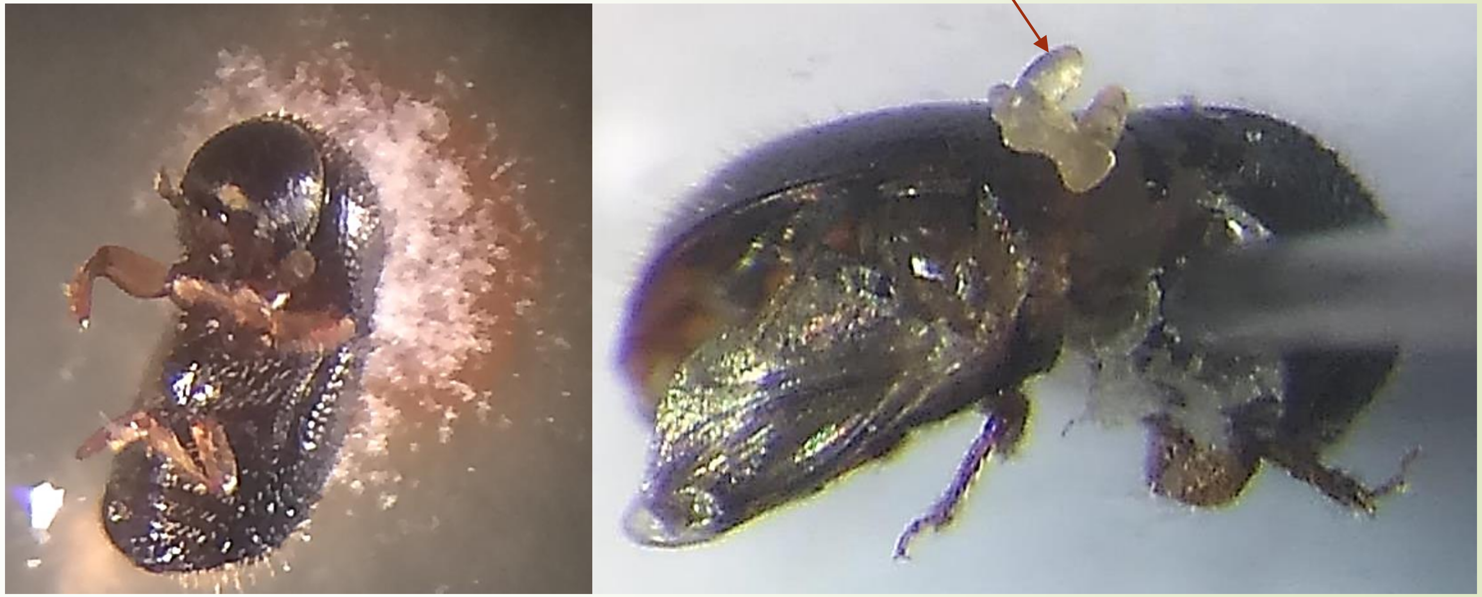
- The Chinese Gall Wasp *Dryocosmus kuriphilus*, a monophagous alien pest from Eastern Asia. **Apparently a pest occasionaly interacting with fungi as many gall-inducing insects**

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

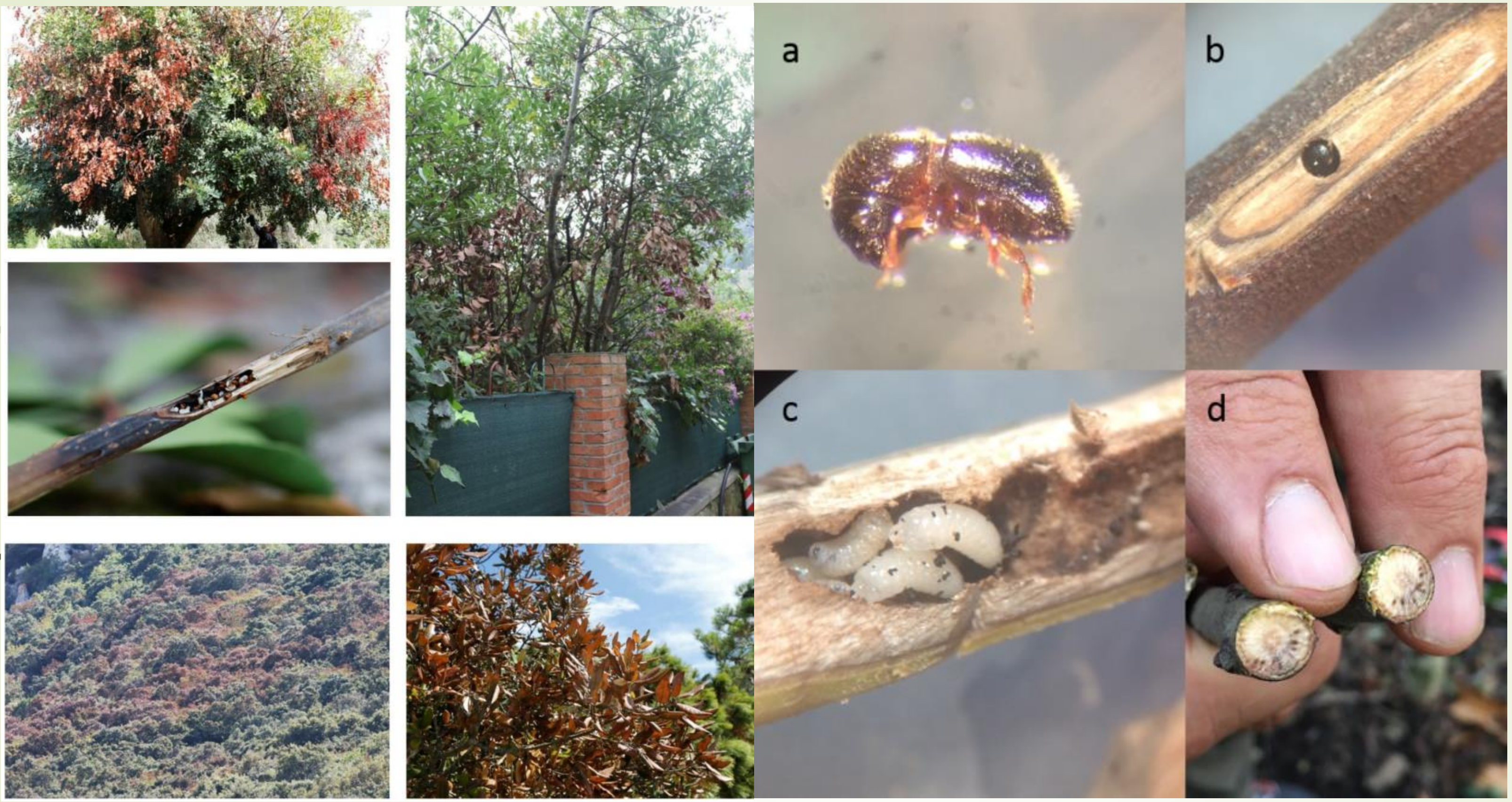
The symbiotic fungi

- 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



Mycangium

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

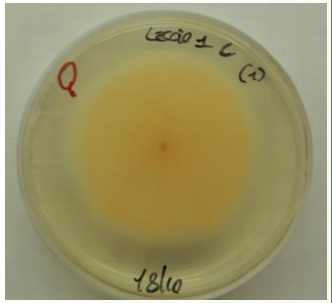
Adoxaceae	Altingiaceae	Anacardiaceae	Annonaceae	Betulaceae
Bignoniaceae	Boxaceae	Cannabaceae	Cornaceae	Fabaceae
Fagaceae	Hydrangeaceae	Juglandaceae	Lamiaceae	Lauraceae
Magnoliaceae	Meliaceae	Mimosaceae	Moraceae	Myricaceae
Myrtaceae	Orchideaceae	Phyllanthaceae	Pinaceae	Platanaceae
Proteaceae	Rubiaceae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

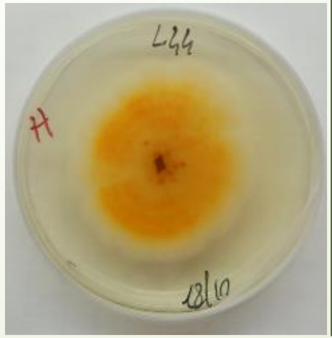
Xyleborus glabratus and *Raffaelea lauricola:* a **Black swan** event in Tree pathology

- *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 Geargia to North Carolina, Texas and Florida causing the death of hundreds of millions of trees

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

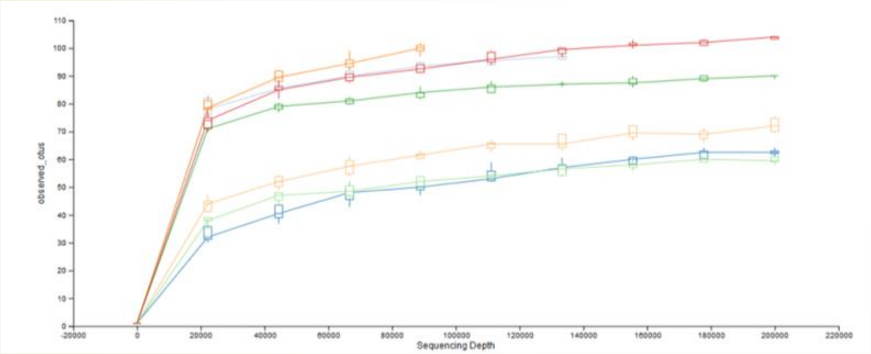
- *F. solani*, *F. proliferatum* and *N. haematococca* resulted the most aggressive causing necroses of several cm into the wood
- All the fungi colonized the tissues very slow
- The fungi were always re-isolated from the lesions

Analysis 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*.

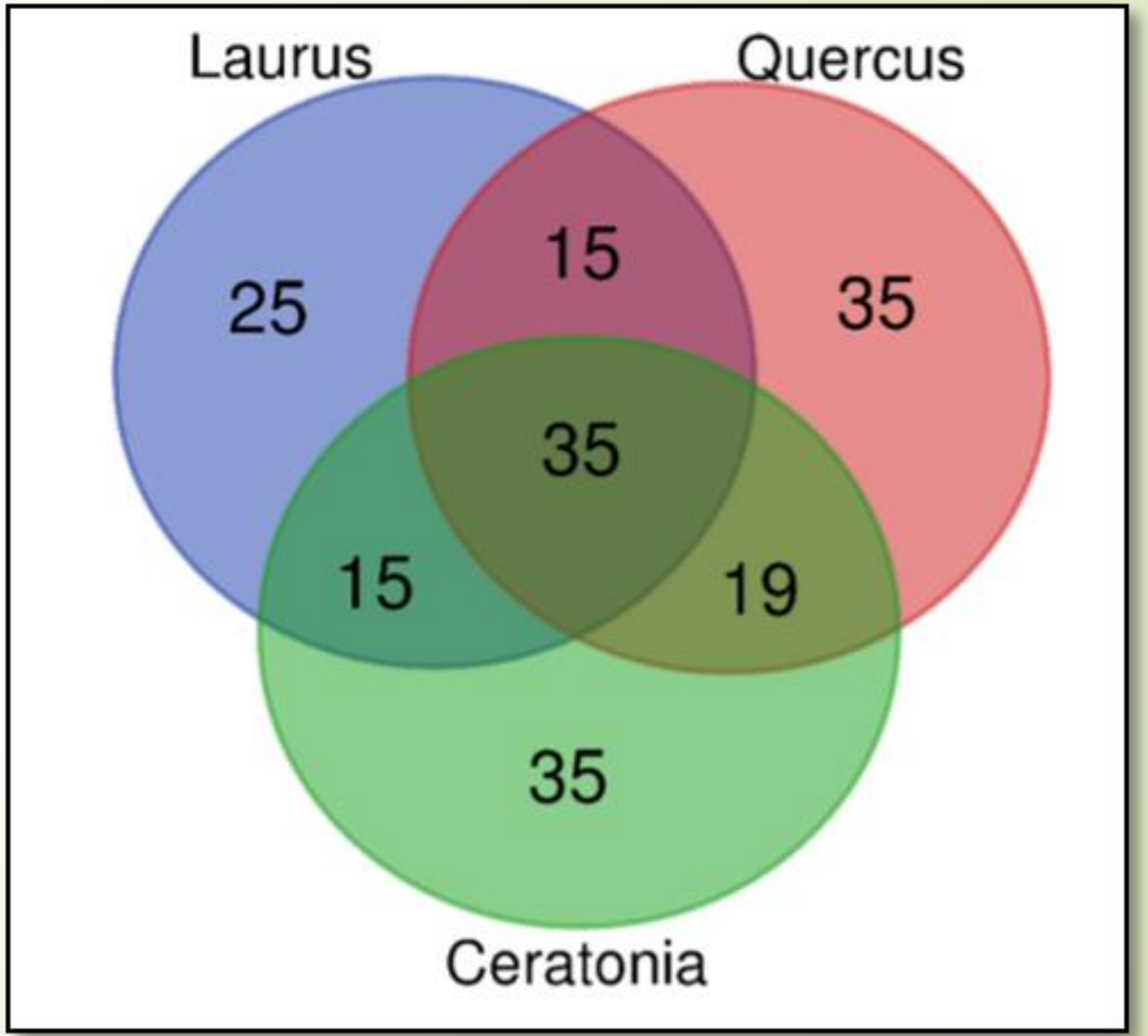
Illumina sequencing results

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



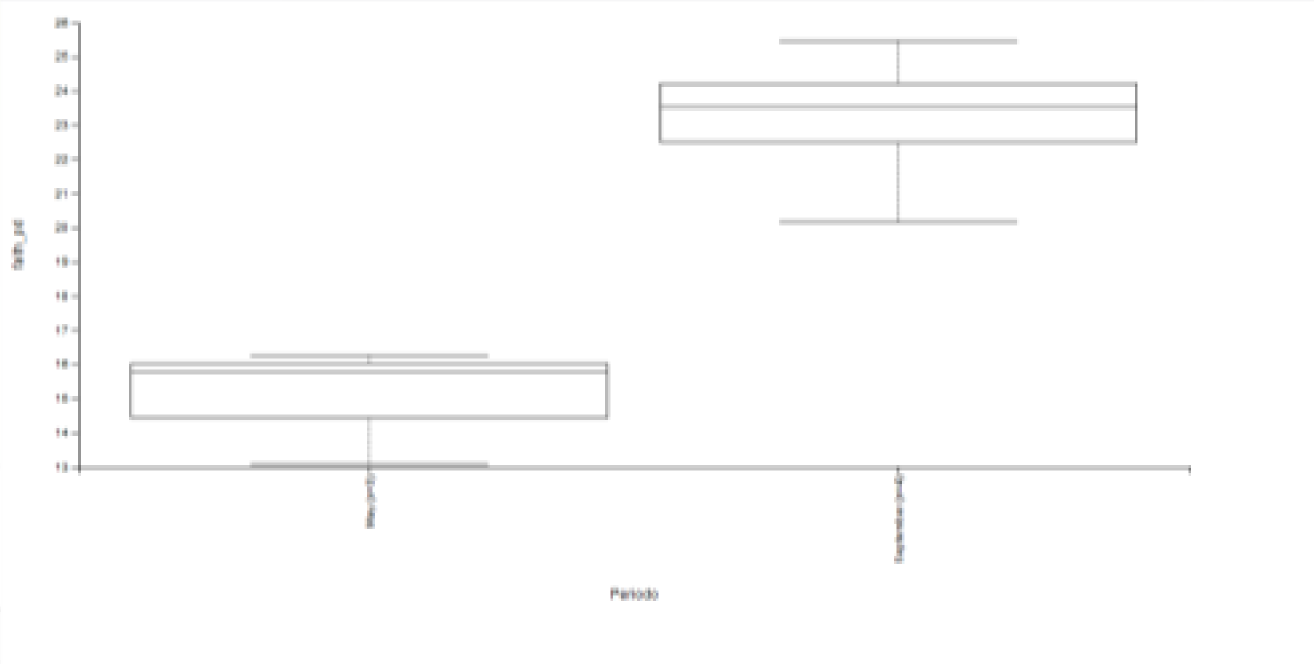
SampleID	Sequence Count
Laurus_Sep_2016	218.128
Laurus_May_2017	320.554
Quercus_Sep_2016	208.119
Quercus_May_2017	257.685
Ceratonia_Sep_2016	150.478
CeratoniaII_Sep_2016	108.930
Ceratonia_May_2017	249.289

- A total of 179 OTU's were identified
- Among the 35 OTU's (core biome) shared between the 3 hosts: *Fusarium solani*, *Fusarium oxysporum*, *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

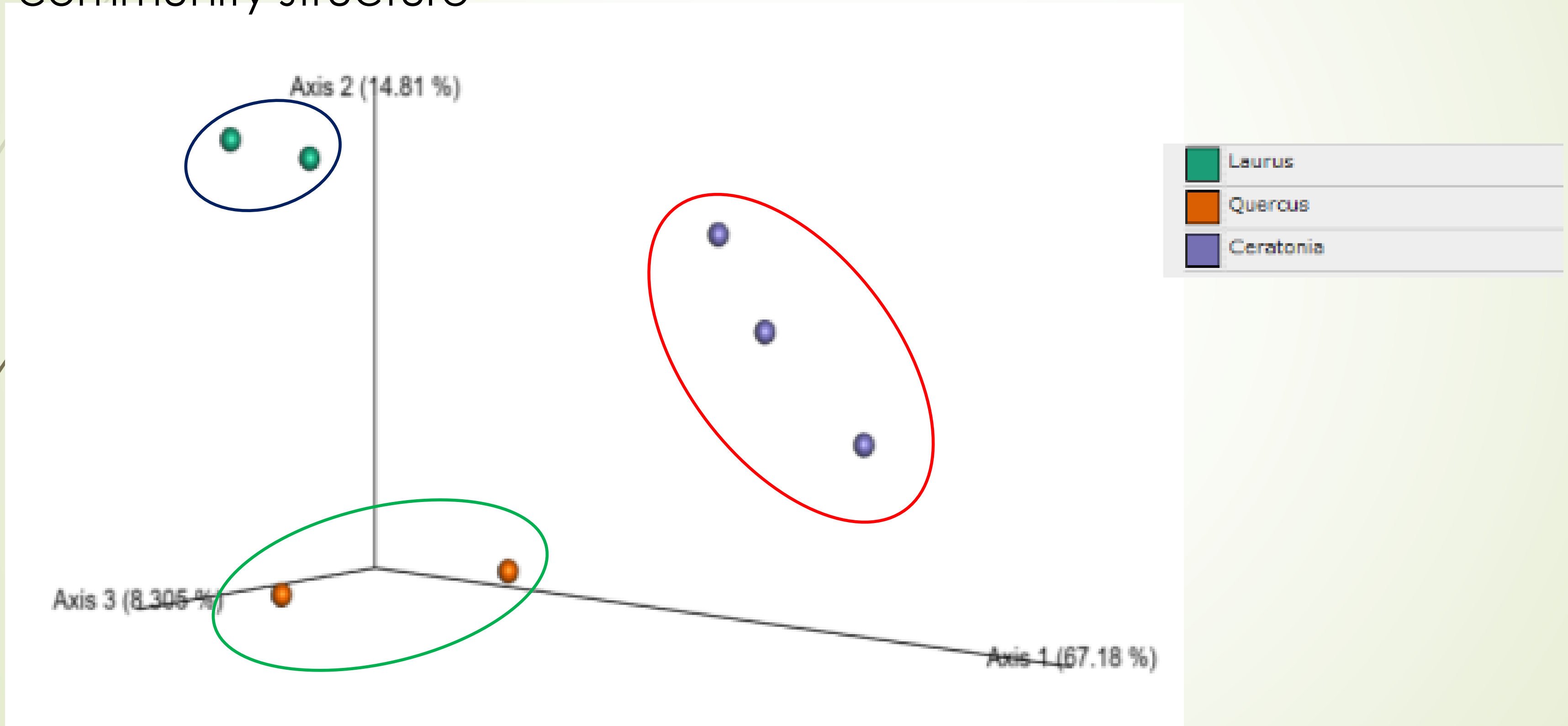
Kruskal-Wallis (pairwise)

Download CSV

		H	p-value	q-value
Group 1	**Group 2**			
May (n=3)	**September (n=4)**	4.5	0.033895	0.033895

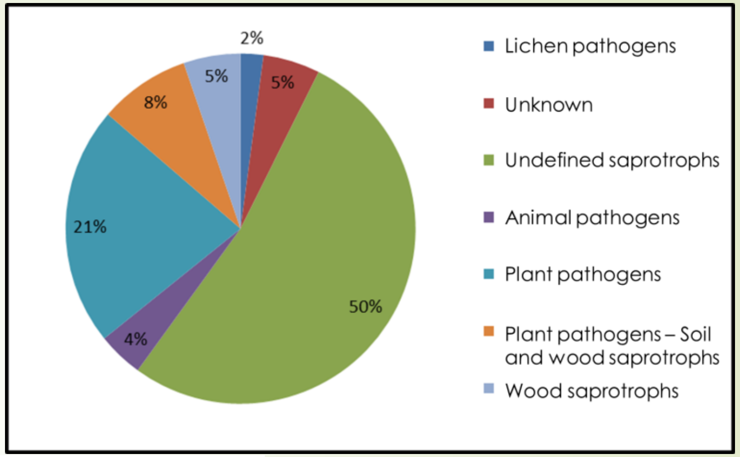
Beta diversity: weighted UniFrac distance

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

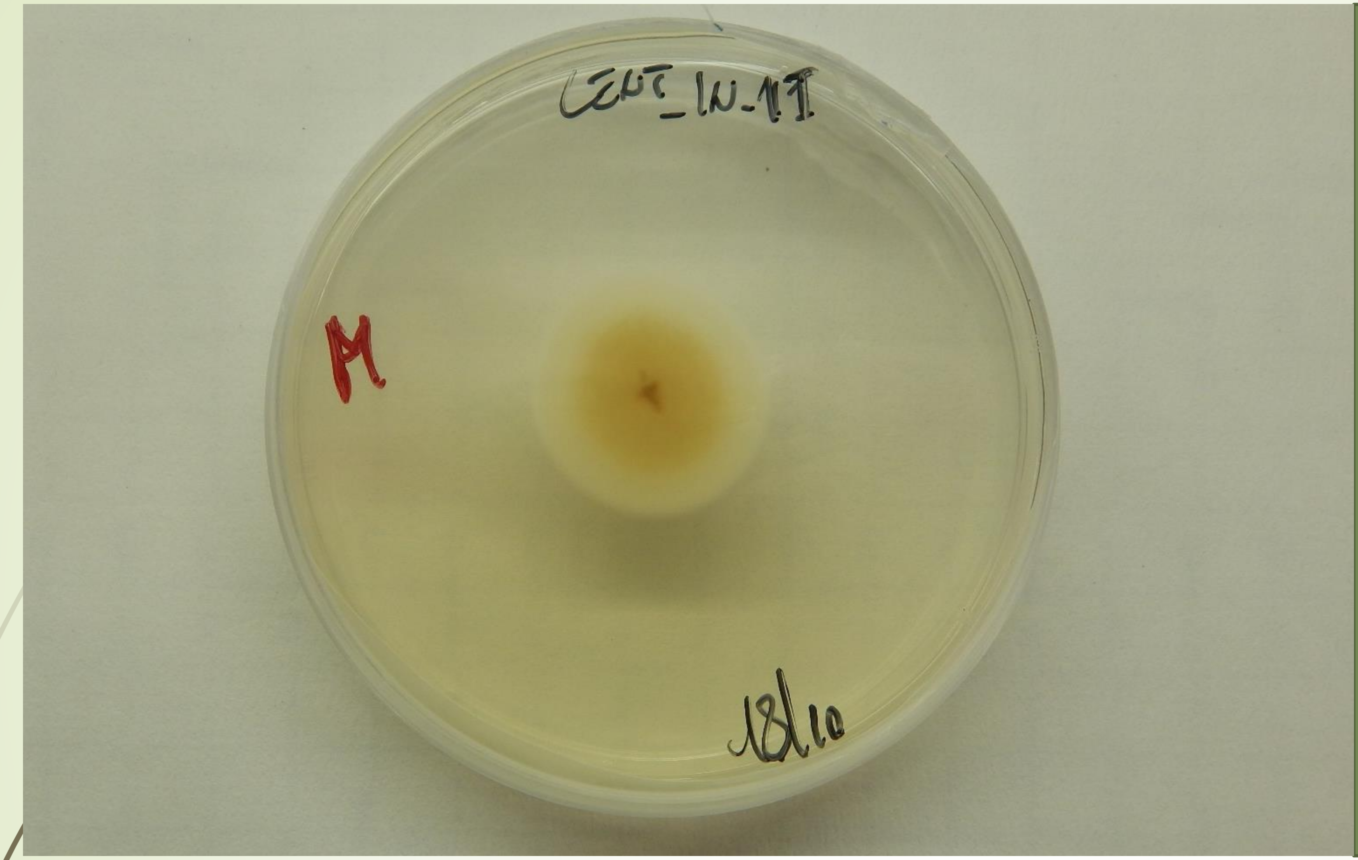


Principal coordinates analysis (PCoA) of weighted Unifrac distances

Functional groups: plant pathogens



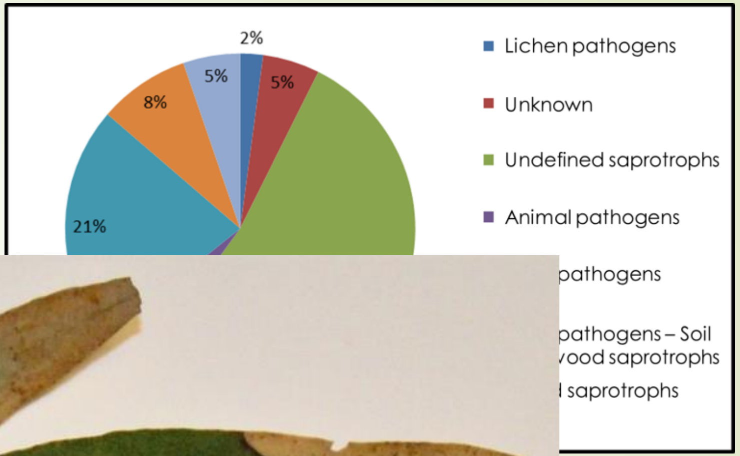
OTU	Species	Functional group
1	**Fusarium solani**	Plant pathogens – Soil and wood saprotrophs
2	Geosmithia pallida	Plant pathogens – Soil and wood saprotrophs
4	**Fusarium acuminatum**	Plant pathogens – Soil and wood saprotrophs
21	Clonostachys rosea	Plant pathogens
24	**Fusarium merismoides**	Plant 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

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
- A theoretical risk exist that *X. compactus* could interact in the future with *F. circinatum*, in consideration of the high affinity with *Fusarium* spp. and having *Pinus* spp. among the susceptible hosts

Chinese gall wasp: *Dryocosmus kuriphilus*

- Invasion started in early 2000 in Italy
- The insect is actually widespread in Europe in all chestnut areas
- It is a monophagous species on chestnut (*Castanea* spp. and hybrids)

Dryocosmus kuriphilus and *Gnomoniopsis castanea* (GC): indirect interaction

- After gall wasp infestation, a dramatic increase in fruits rot has been recorded in post-harvest conditions
- *Gnomoniopsis castanea* (syn *Gnomoniopsis smithogilvy*) **(or the reverse ☺)** is recognized as the causal agent of brown rot of chestnut kernels in Europe and Australasia (Smith & Ogilvy 2008; Visentin et al. 2012; Shuttleworth et al. 2013; Dennert et al. 2015).
- Most recently *G. castanea* was recorded in Michigan associated to brown-rot of kernels (Fulbright, in Press)

Dryocosmus kuriphilus and *Gnomoniopsis castanea* (GC): indirect interaction

- Brown rot is not new to chestnut as disease. It has been recorded for many years in several chestnut areas worldwide and associated to different synonyms (*Phoma endogena*, *Phomopsis endogena*, *Phomopsis castanea*, *Gnomonia pascoe*).
- Before Chinese Gall Wasp Invasion, it represented a minor problem responsible, together with the other agent of fruit damage, it was responsible of the loss of 2-5% of the production
- After Chinese Gall Wasp invasion, it become a primary problem being able to cause damage up to 50% and 60-70% of the production in pre- and post-harvest yields (Maresi et al. 2013).

GC and the Chinese Gall Wasp


FUNGAL BIOLOGY 121 (2017) 44–52

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Does *Gnomoniopsis castanea* contribute to the natural biological control of chestnut gall wasp?

Andrea VANNINI[a], AnnaMaria VETTRAINO[a,*], Diana MARTIGNONI[a], Carmen MORALES-RODRIGUEZ[c], Mario CONTARINI[b], Romina CACCIA[b], Bruno PAPARATTI[b], Stefano SPERANZA[b]

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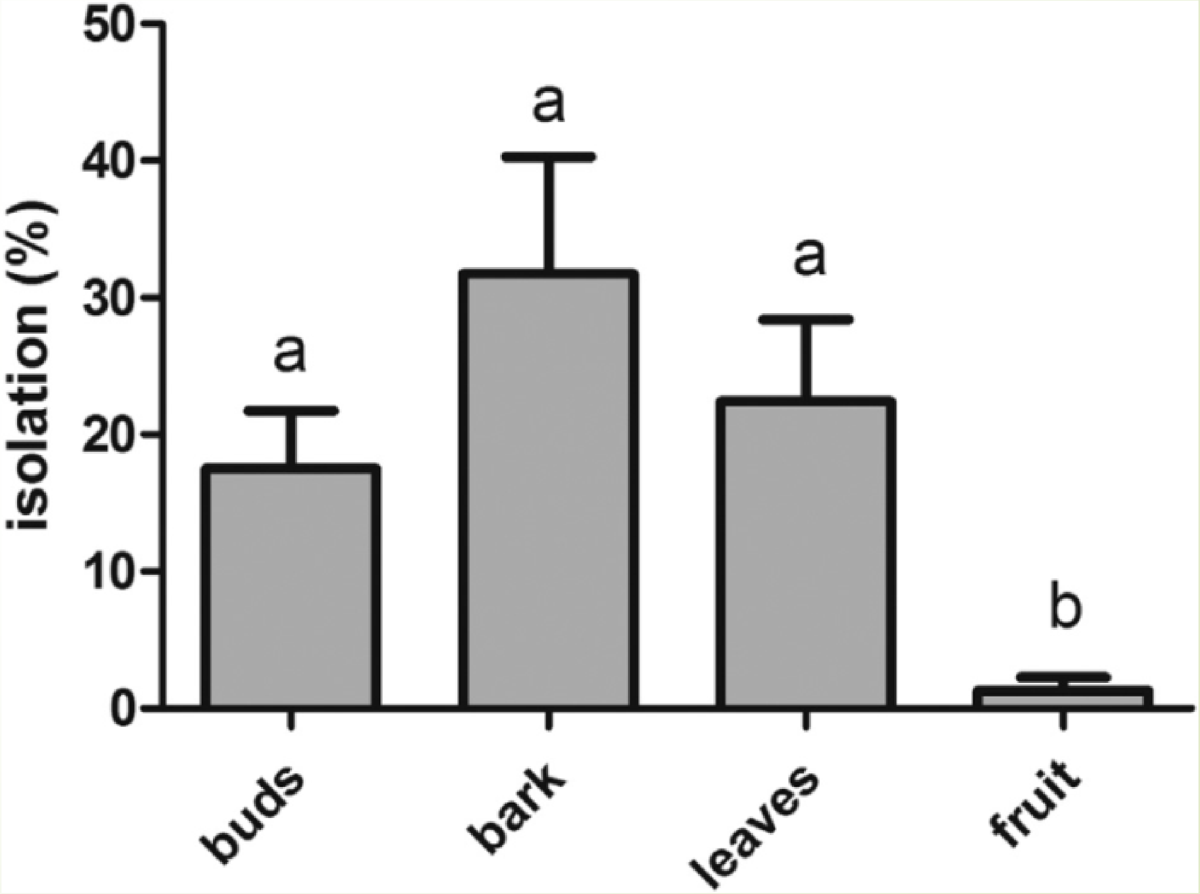
- GC is and endophyte in chestnut tissue & organs such as buds, bark, leaves and fruits. The graphic shows the percentage of isolation from tissues sampled in October 2010



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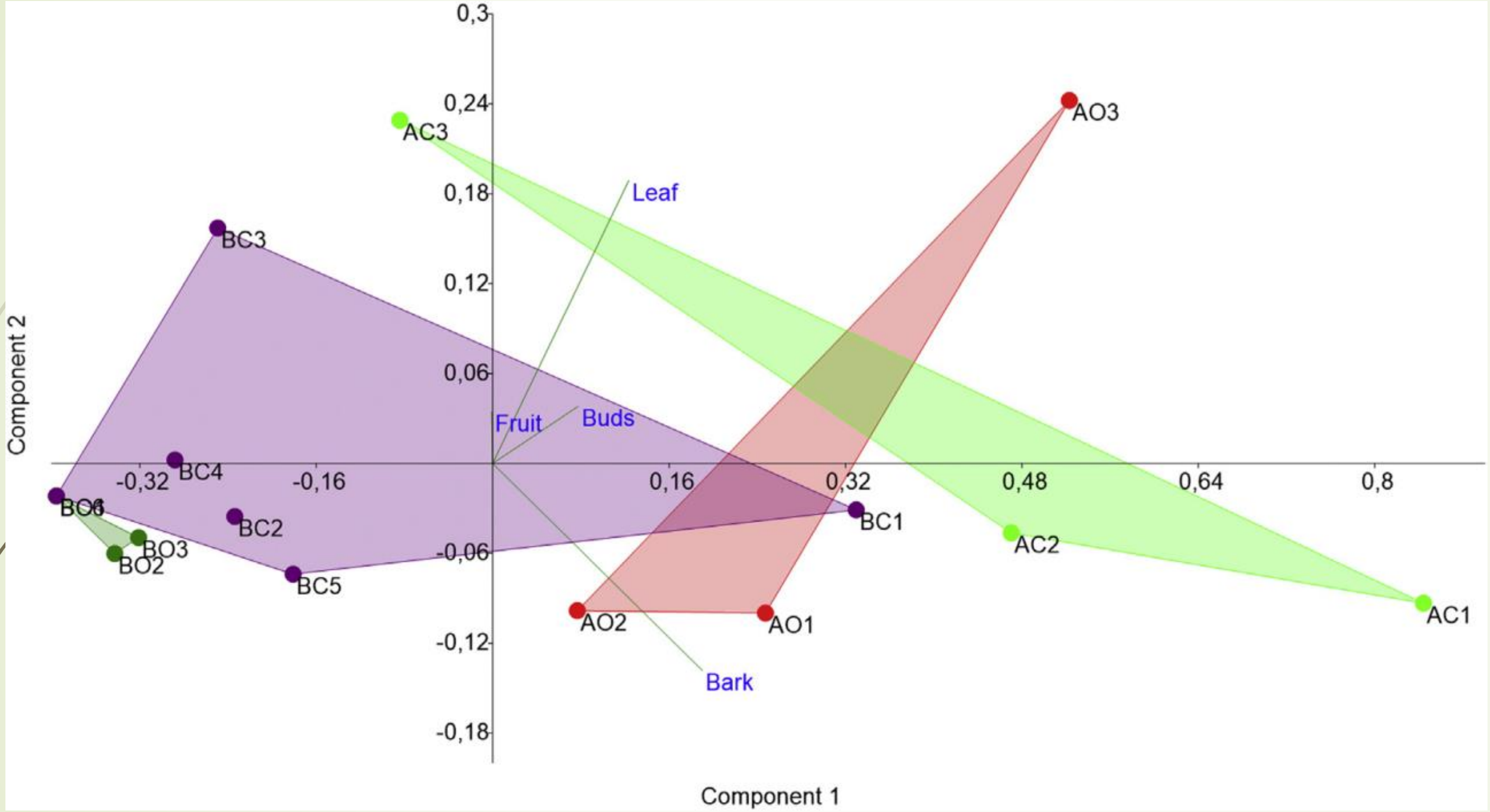
- Infact, GC found galls an optimal substrate to obtain nutrients supporting its growth.

GC and the Chinese Gall Wasp

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GC was more abundant in orchards with a longer history of Chinese Gall Wasp infestation

Table 1 – Description of the sweet chestnut sites investigated in the present study.

Site	Geographic coordinates	Forest types	Gall wasp first record in the area
A	42°17′31.4″N 12°09′01.9″E	Orchard & coppice	2006
B	42°18′38.3″N 12°13′29.5″E	Orchard & coppice	2010
C	42°17′42.0″N 12°08′23.3″E	Young plantation	2006

GC and the Chinese Gall Wasp

- Comparison of independent fits of the 2011 (○) and 2012 (●) with Extra sum-of-square F test revealed that one curve was representative of the two datasets for both incidence and severity.
- Patterns of gall necrosis development is independent from seasonal meteorological events, supporting the colonizations of galls from endophytic mycelium
- 427 versus 197 mm of rain in the period March and July 2011 and 2012, respectively

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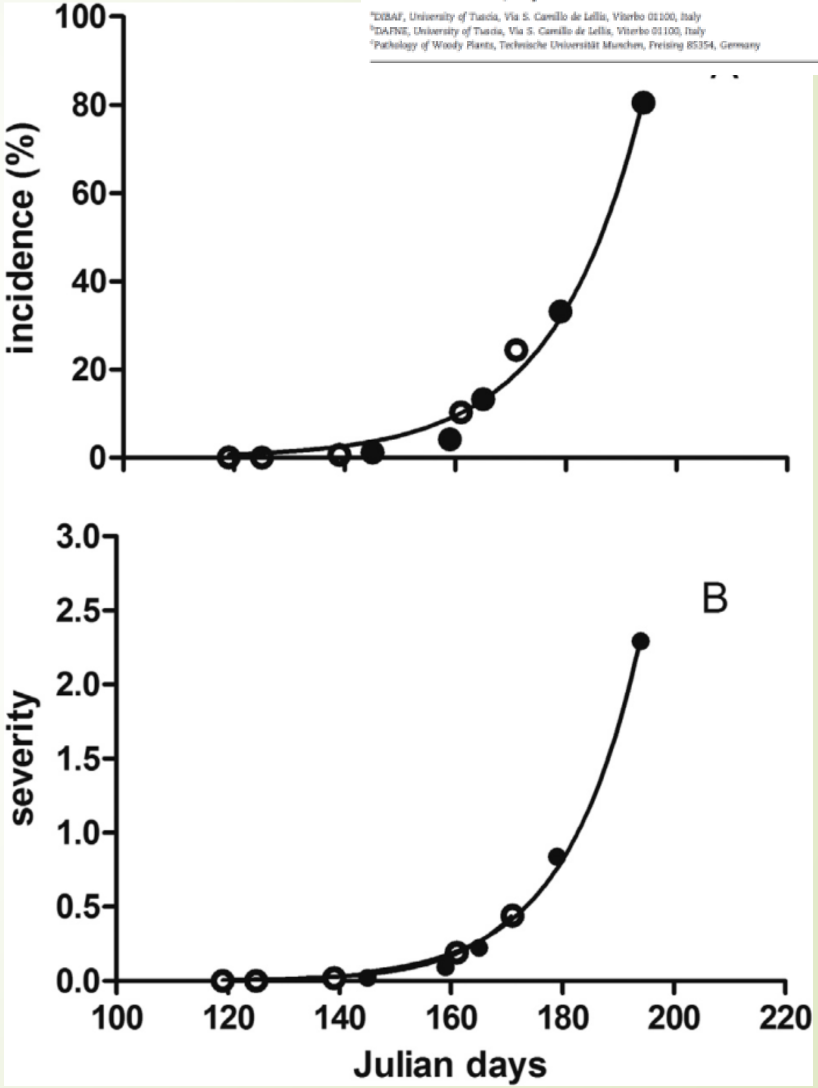
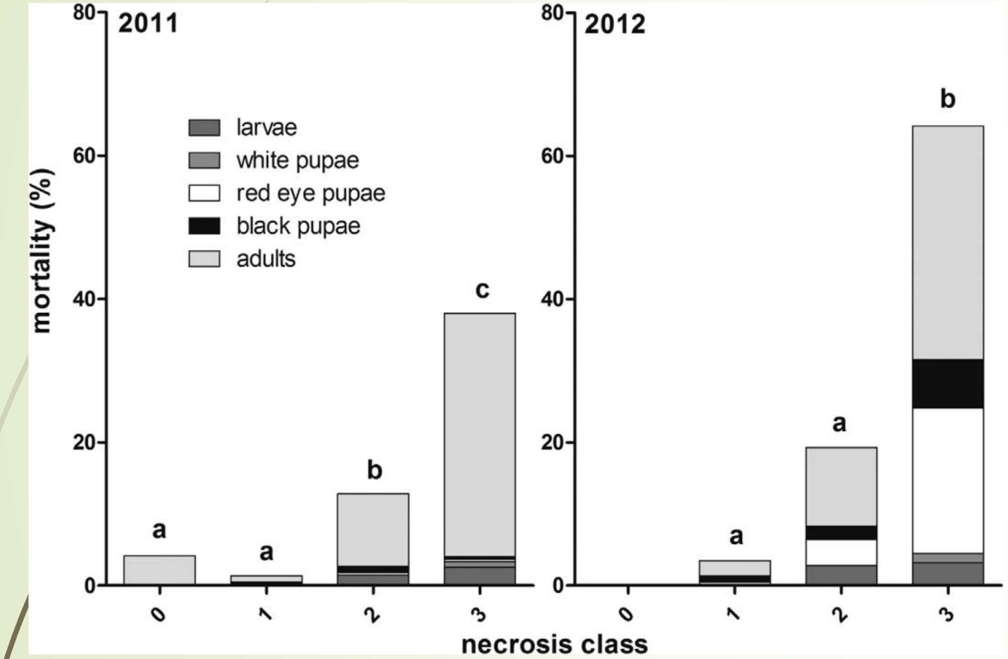


GC and the Chinese Gall Wasp

- GC abundantly sporulates on necrotized galls during early summer(picture from Maresi et al., 2013)

- Such mass of inoculum on galls could be responsible of massive floral infection as evidenced by Shuttleworth & Guest (2017) for inoculum produced by abandoned burrs.

Dryocosmus kuriphilus and *Gnomoniopsis castanea* (GC): direct interaction



- GC act as biocontrol agent of *D. kuriphilus*
- Mortality of the Chinese Gall Wasp development stages is very high in completely necrotized galls even exceeding 60%.
- On the average GC contribute to ca 15-20% of total mortality in natural chestnut areas

Chinese Gall Wasp and chestnut blight: a indirect interaction II

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Emerging new crown symptoms on *Castanea sativa* (Mill.): Attempting to model interactions among pests and fungal pathogens

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- Chestnut blight novel symptom: flagging of terminal shoots
- Dispersed over the crown
- Evident during witer time

Late August 2015

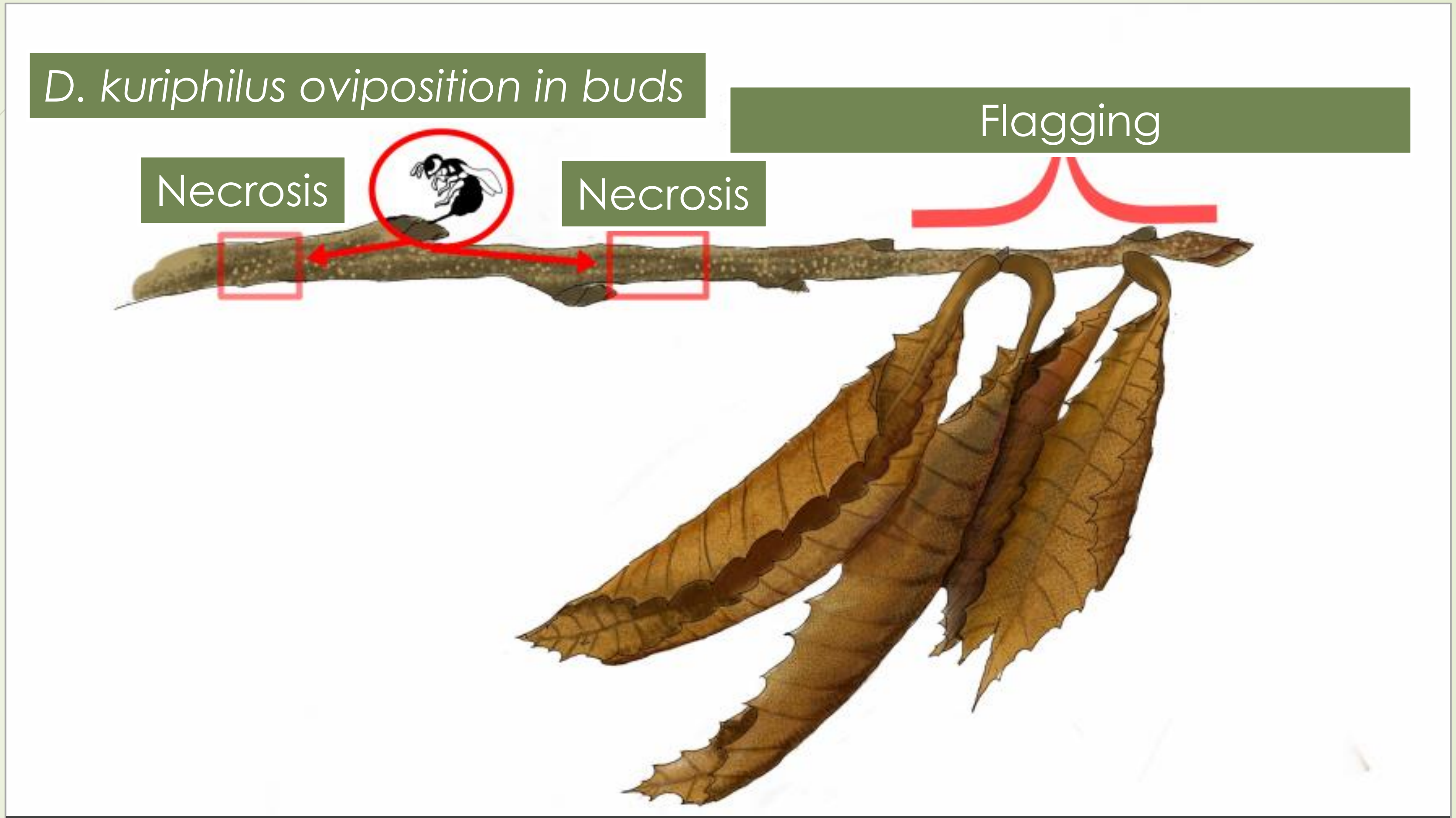
December 2015

Early Spring, 2016

- *Cryphonectria parasitica* resulted highly associated to flagging in absence of cankers (OR 24,41).
- *C. parasitica* was constantly associated with undisclosed buds underneath the flagging symptom.

	Cryphonectria parasitica	
	YES	NO
Flagging	21	19
Control	0	10
Fisher's excact test p = 0.0026 ODDS RATIO = 24.41		

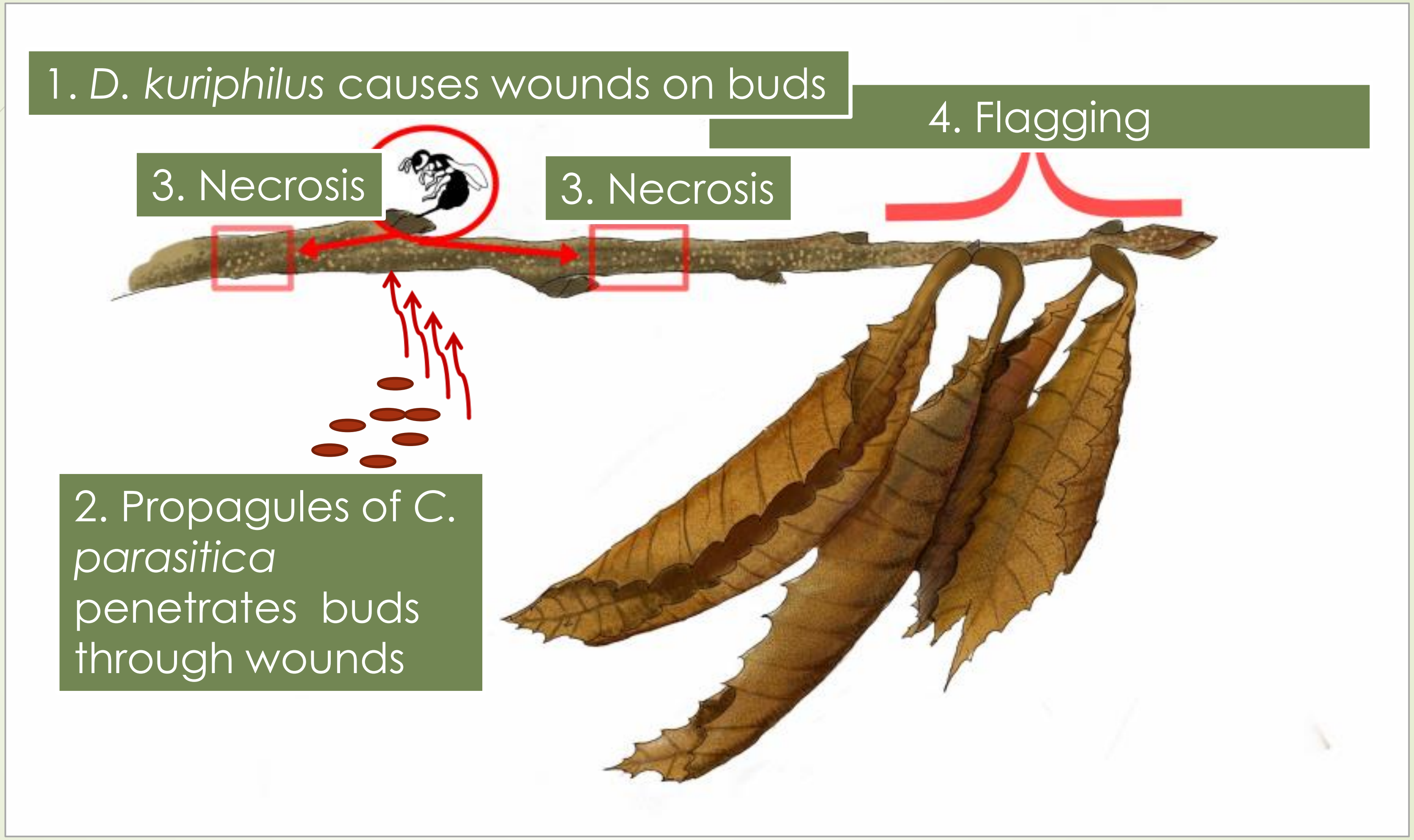
Direct interaction as vector?



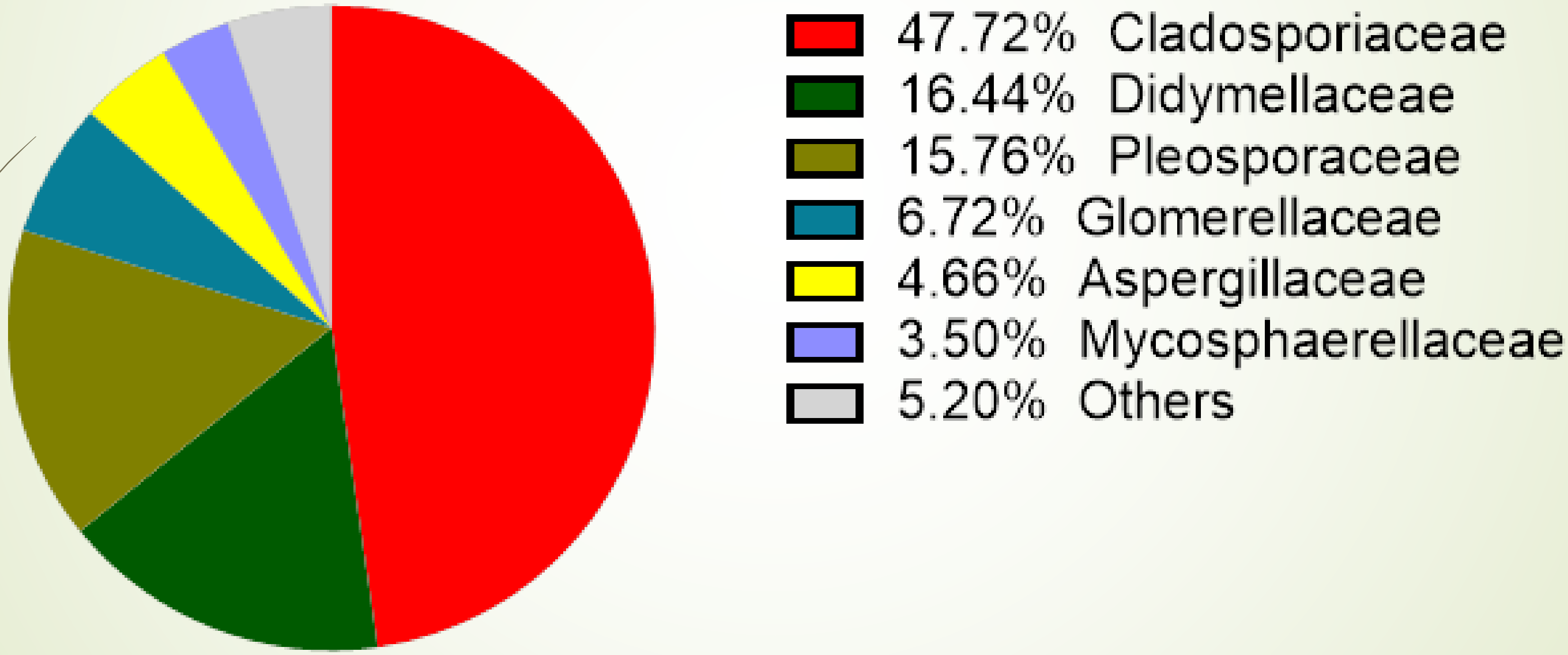
Is C. *parasitica* vectored by Chinese Gall Wasp or the inoculum present in the environment takes advantage of the insect wounding the buds?

Metabarcoding of fungal DNA associated to the body of flying Chinese Gall Wasp adults failed in identifying C. *parasitica*

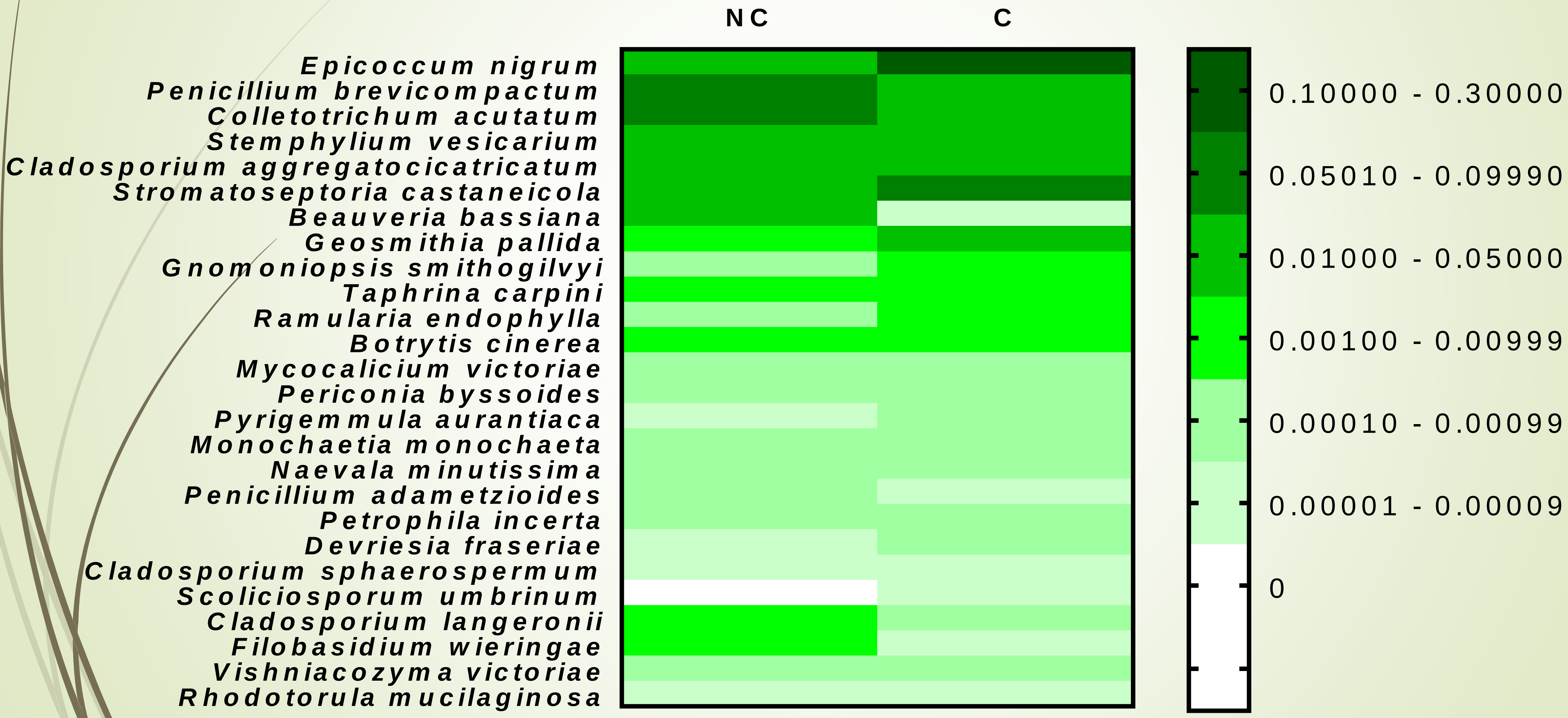
Indirect interaction



90 OTU's formed the core microbiome clustering the 96,72 % (3.463.105) of the reads



Twenty-nine OTU's out of 90 were resolved at species level identifying 26 different fungal species



OTU	Species	Fungus Order	Lifestyle	Occurrence	Reported host range	Reported substrate	Recorded on chestnut
2, 17	*Epicoccum nigrum*	Pleosporales	Endophyte/weak pathogen	cosmopolitan	broad-host range	leaves	no
5	*Penicillium brevicompactum*	Eurotiales	Plant pathogen	cosmopolitan	broad-host range	generic	no
7	*Colletotrichum acutatum*	Glomerellales	Plant pathogen	cosmopolitan	broad-host range	leaves, shoots, buds, fruits	yes
8	*Stemphylium vesicarium*	Pleosporales	Plant pathogen	cosmopolitan	broad-host range	leaves, roots, seeds	no
9	*Cladosporium aggregatocicatricatum*	Capnodiales	Saprotroph	USA, Europe	*Vitis vinifera*, *Asteriscus sericeus*	generic	no
11, 18, 1[illegible]	*Stromatoseptoria castaneicola*	Capnodiales	Plant pathogen	cosmpopolitan	*Castanea* spp., *Aesculus* spp., *Chrysanthemum* sp.	leaves	yes
12	*Beauveria bassiana*	Hypocreales	Entomopathogen	cosmopolitan	broad-host range	insects	not applicable
15	*Geosmithia pallida*	Hypocreales	Plant pathogen	cosmopolitan	*Quercus* spp., *Ulmus* spp.	bark	no
19	*Gnomoniopsis smithogilvyi*	Diaporthales	Plant pathogen	cosmopolitan	*Castanea* spp.	leaves, shoots, fruits, buds	yes
21	*Taphrina carpini*	Taphrinales	Plant pathogen	Europe, Asia	*Carpinus* spp., *Quercus pyrenaica*	leaves	no
25	*Ramularia endophylla*	Capnodiales	Plant pathogen	cosmopolitan	broad-host range	leaves	yes
28	*Botrytis cinerea*	Helotiales	Plant pathogen	cosmopolitan	broad-host range	Stems, twigs, leaves, fruit, pods, stems.	yes

OTU	Species	Fungus Order	Lifestyle	Occurrence	Reported host range	Reported substrate	Recorded on chestnut
38	*Mycocalicium victoriae*	Mycocaliciales	Symbiont/lichen	Europe, USA, Australia			no
59	*Periconia byssoides*	Pleosporales	Endophyte	cosmopolitan	broad-host range	leaves	no
65	*Pyrigemmula aurantiaca*	Chaetosphaeriales	Saprotroph	Europe	woody hosts	bark	yes
74	*Monochaetia monochaeta*	Xylariales	Plant pathogen	cosmopolitan	woody hosts	leaves, stems	yes
121	*Naevala minutissima*	Helotiales	Saprotroph	Europe	*Quercus* spp.	leaves, litter	no
130	*Penicillium adametzioides*	Eurotiales	Saprotroph	Europe, Japan	woody hosts	fruits, various organs	no
152	*Petrophila incerta*	Capnodiales					no
170	*Devriesia fraseriae*	Capnodiales	Saprotroph	Australia	*Maleleuca* sp.	Leaves	no
[illegible]36	*Cladosporium sphaerospermum*	Capnodiales	Saprotroph	cosmopolitan	broad-host range	generic	yes
294	*Scoliciosporum umbrinum*	Lecanorales	Symbiont/lichen			generic	yes
468	*Cladosporium langeronii*	Capnodiales	Saprotroph	cosmopolitan	broad-host range	generic	no
23	*Filobasidium wieringae*	Filobasidiales	Saprotroph/yeast			generic	no
45	*Vishniacozyma victoriae*	Tremellales	Biocontrol agent/yeast			generic	no
238	*Rhodotorula mucilaginosa*	Sporidiobolales	Saprotroph/yeast	cosmopolitan	broad-host range	generic	no

Final remarks

- *Dryocosmus kuriphilus* has indirect interaction with native and alien (old invaders) pathogens of chestnut favouring specific phases of the disease cycle such as inoculum built up (GC) and penetration (CP)
- Apparently It does not act as vector of C. *parsitica* but it is indirectly responsible of the new damages on chestnut caused by the pathogen
- *D. kuriphilus* , as many gall inducing insects, interact with many fungi representing a typical community associated to chestnut, and including pathogens, symbionts and saprotrophs
- A negative direct interaction exist between *Dryocosmus kuriphilus* and the native *Gnomoniopsis castanea,* being the latter an efficient natural bio-control agent of the insect

A new COST action on Insect-fungi interactions?

Thanks for your attention