Foliar, Shoot, Stem and Rust Diseases of Trees - Forest Diseases During Global Crises

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Book of Abstracts

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Genetic diversity of *Cryphonectria parasitica* and *Cryphonectria hypovirus* 1 in the chestnut stands of South Tyrol (Northern Italy)

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Cryphonectria parasitica was responsible for the death of 99% of American chestnut trees, while in Europe, it still represents a threat to the European chestnut, *Castanea sativa*. Natural and human-mediated hypovirulence, governed by the infection of the fungus with *Cryphonectria hypovirus* 1 (CHV-1), has helped to decrease chestnut tree mortality in many European countries. The present study aimed to determine the population structure of *C. parasitica* and its associated mycovirus in South Tyrol (northern Italy), as no such data was available, which was an obstacle to successful biocontrol. We used different molecular markers such as vegetative compatibility (VC) type loci, mating type locus, internal transcribed spacer region (ITS), and microsatellite loci for *C. parasitica* and sequencing of open reading frames (ORFs) of CHV-1 to characterize the local pathogen populations. Twenty-three different VC types were found in the region with a mating type ratio close to 1:1. ITS sequencing and microsatellite analysis also revealed high genetic diversity and indicated at least two independent introduction events to the region. A novel immunoassay-based method helped to detect the presence of the RNA mycovirus and the ORFs analysis allowed identifying a number of hypovirulent strains of potential interest to biocontrol. The results obtained in the study represent an important contribution to the implementation and improvement of biocontrol measures against the pathogen and advance our understanding of the invasive pathogen.
Keywords: Population genetics, chestnut blight, phylogeography

Genetic variability of *Fusarium circinatum* through molecular markers and sequence analysis

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Pine trees are an essential part of European forests, accounting for around 30% of Europe's forest mass. Pine trees provide ecosystem services and wood for construction. However, they are threatened by a fungus, *Fusarium circinatum*, which causes pine pitch canker. This pathogen reduces its growth and sometimes causes its death, especially in nurseries and in the forest on highly susceptible species such as *Pinus radiata*. *Fusarium circinatum* is an invasive alien species in Europe and its exact invasion history is poorly studied. Therefore, this work investigate the possible introduction events of *F. circinatum* in Europe using different molecular markers such as the internal transcribed spacer (ITS) region, transcription elongation factor-1 (TEF1) and calmodulin. Isolates of *F. circinatum* from different Spanish regions were sequenced and compared with sequences from other regions available in the Genbank (NCBI). Preliminary results showed that the Spanish populations of *F. circinatum* are closely related to the Mexican populations. Our study has allowed placing the sequences of *F. circinatum* from Spain in a broader phylogeographic context. This study could modify quarantine measures and help understand the population genetics of the invasive fungus. Understanding the route of entry and invasion of this fungus would help prevent or mitigate future invasions of other forest pathogens.

Keywords: Population genetics, *Fusarium circinatum*, pines, pine pitch blight, phylogeography.
Acknowledgments: RNAi Technologies for the management of Pine Pitch Canker of Pines (RNAi4Fusarium). PID2019-110459RB-I00. Funded by Spanish Ministry of Science.

Assessment of antibiotics towards *Xanthomonas axonopodis* causing bacterial blight of *E*ucalyptus *camaldulensis*

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Eucalyptus camaldulensis is a significant tree species of tropical, temperate, and subtropical regions across the globe. *Eucalyptus* is prone to be attacked by many diseases, but bacterial blight is one of the most devastating one that has a terrible impact at nursery stage. There is a strong association between environmental factors and incidence of bacterial blight. After collecting diseased samples from the field, experiment was conducted in Research Area, Department of Forestry and Range Management, University of Agriculture, Faisalabad (UAF). The major purpose of the current study was to assess the discrete concentrations of antibiotics to cope with bacterial blight. After conducting isolation, identification with pathogenicity tests, antibiotics such as Ceftazidime, Amikacin Sulphate, Ampicillin Sodium, Cefuroxime, Ceftizoxime Sodium, Ampicillin/Cloxacillin, Co-amoxiclav, Ceftriaxone, and Cephradine with one treatment as control were appraised at 100ppm, 200ppm and 300ppm under i*n-vivo,* greenhouse and in-vitro conditions. Ceftriaxone was proved best as it expressed the utmost inhibition zone (12.657mm) at 100ppm, 200ppm, and 300ppm concentrations. While under greenhouse and field conditions, combination of Cephradine and Ceftriaxone exhibited minimum incidence (15.185%) of bacterial blight.

Keywords: Bacterial blight, Eucalyptus, management, antibiotics

Emergence of a *Melampsoridium* rust epidemic on Ironwood and Hophornbeam in Southeastern United States

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In the late fall of 2018, foliar rust (referred to as Ironwood/Hophornbeam Leaf Rust (IHLR)) was discovered in Alachua, Levy, Marion and Gilchrist Counties, Florida on ironwood/eastern hornbeam (*Carpinus caroliniana*) and hophornbeam (*Ostrya virginiana*), both members of the Betulaceae family. Uredinia were observed on leaves and in some cases, samaras, of both species at numerous locations on trees of all age classes. Similar reports across Southeastern United States (Georgia, the Carolinas, Tennessee, and Texas) were communicated to our research group in the following year and European hornbeam (*Carpinus betulus*) has been reported as an additional host, while *Ostrya virginiana* var. *guatemalensis* in El Salvador was also discovered showing signs of uredinial infection. Analyses on morphological data obtained with light and scanning electron microscopy on IHLR and *Melampsoridium* herbarium samples and combined molecular data from the ITS and LSU loci indicate that *i)* the IHLR rust across all samples belongs to the same taxon and is likely overwintering through uredinial stage; *ii)* IHLR is closely affiliated to *M. asiaticum*; *iii)* some taxonomic modifications might be necessary at the genus level.

Interaction between *Melampsora pinitorqua* and *Diplodia sapinea* in Scots pine: do *M. pinitorqua* infections leave the trees more vulnerable to Diplodia tip blight?

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Diplodia sapinea is a conifer pathogen that causes substantial losses for the forest industry. It usually remains latent but causes Diplodia tip blight (DTB) when the host suffers from abiotic stress. Pine twisting rust (*Melampsora pinitorqua*) is a pathogen that infects the pine's annual shoots, causing bending or breaking. Initial surveys suggest that young Scots pines (*Pinus sylvestris* L.) infected by *M. pinitorqua* often have simultaneous infections by *D. sapinea*. Here, we investigate the interactions between these fungi on Scots pine, testing the following hypotheses; DTB is more frequent on individuals severely affected by *M. pinitorqua*; *M. pinitorqua* infections on pine lead to the accumulation of metabolites that *D. sapinea* can utilise for energy; individuals severely affected by *M. pinitorqua*, but showing no DTB, harbour a microbiome that consumes metabolites more efficiently than *D. sapinea*, or produce different metabolites than individuals attacked by both pathogens. Samples for genotyping, metabolite profiling, and analysis of the associated mycobiome were collected and processed. Metabolites facilitating DTB will be identified, and *D. sapinea*'s capacity to metabolise the compounds will be tested *in vitro*. Potential antagonistic members of the mycobiome will be tested regarding their capacity to utilise metabolites favoured by *D. sapinea*.

Conifer needle disease causes dysbiosis of foliar fungal communities and activities

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Understanding complex host tree-microbe interactions in changing environments is important for deciphering mechanisms to improve tree resilience and to develop sustainable strategies for forest disease management. Despite increasing incidence and severity due to climate change, conifer needle pathosystems are understudied which limits our ability to design efficient management strategies and to grasp their potential as threats in forest ecosystems. To understand tree-microbe interactions among emerging needle diseases, we examined (1) the composition of the fungal community within needles and (2) gene expression profiles of the host and the mycobiota within the Lophodermella needle cast pathosystem. Metabarcoding analysis showed that *Lophodermella* pathogens are part of the normal mycobiota in healthy needles, and that fungal diversity significantly decreased with an abundance of *Lophodermella* pathogens in symptomatic needles. Metatranscriptomics analysis revealed marginal mycobiota activity in healthy needles, suggesting a latent lifestyle of *Lophodermella* pathogens. In contrast, the overexpression of pathogenicity-related genes in symptomatic needles suggests a metabolic activity driven by *Lophodermella* pathogens as they dominate the mycobiota and colonize the needle tissue. This study also showed an active plant defense system in healthy and diseased states, although plant defense mechanisms were greatly reduced in symptomatic needles.

Use of natural myco-control products and native fungal species for reduced pest/pathogen presence in forests

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Mediterranean forests are affected by biotic stressors, including a variety of pests and pathogens which weaken and kill forest species. Weakened trees are susceptible to other biotic and abiotic stressors which ultimately places the health and existence of the whole forest ecosystem at risk. Within our work we are interested providing proof of concept on the use of natural myco-control products and native fungal species for reduced pest/pathogen presence in forests, and so the objectives of our work include the identification of possible fungal endophytes that may serve as biocontrol against fungi and or insects, as well as the description of ecosystems diversity in the field. For this purpose, four sites were selected in different areas of the Salamanca region (Western Spain). In each selected site the predominant tree species were different: *Quercus Ilex*, *Quercus pyrenaica*, *Quercus suber* and *Castanea sativa*. At these four different sites, wood, bark, branches and leaves were sampled from 5 healthy trees and 5 sick trees. With these samples a metabarcoding analysis was performed to characterize the fungal populations present in the area, simultaneously, these samples were cultured in petri dishes to isolate as many native fungi as possible. Identification of biocontrol endophytes against fungi and insects will provide us an useful tool for the environmental-friendly management of the Mediterranean forests.

Keywords: myco-control, fungi, metabarcoding, antagonist, mycopesticide.

Acknowledgments: The current work was performed under the umbrela of the MycoRestore LIFE project "Innovative use of mycological resources for resilient and productive mediterranean forests threatened by climate change" financing by EU.

Detection of species of the *Colletotrichum acutatum* complex in *Khaya* spp. through molecular markers in Brazil

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African mahogany is the popular name for trees belonging to the genus *Khaya*. In Brazil, *K. senegalensis*, *K. grandifoliola*, *K. anthoteca* and *K. ivorensis* are the most cultivated species, due to their exceptional wood quality. *Khaya* wood is used mainly in civil construction and furniture industry, being considered an alternative to the South American mahogany (*Sweetenia macrophyla*), an endangered species. The African mahogany planted area has been growing in Brazil in recent years, resulting in an increased demand for seedlings. The occurrence of symptoms like leaf spot and apical necrosis in some plantations in Brazil, prompted isolation and culturing studies in order to identify the fungal pathogens associated with these symptoms in the tree species *K. senegalensis*, *K. grandifoliola* and *K. ivorensis*. Based on micromorphological traits (culture and conidia characteristics), the resulting isolates were ascribed to the *Colletotrichum acutatum* complex. Isolates were tested for their pathogenicity in inoculation assays, by spraying a spore suspension of $2x10^4$ on five replicates of 8 months-old seedlings of *Khaya*. Symptoms were confirmed after 10 days. In order to identify the fungal taxa at a species level, DNA was extracted from pure cultures and the three genic/intergenic regions ITS, b-tubulin and chitin sintetase were sequenced. Comparison of the resulting sequences in public databases revealed high percent similarity (greater than 98.5%) with *C. acutatum*. This is the first report of members of the *Colletotrichum* acutatum complex species related to infection in *Khaya*.

New insights in forest disease control: spray-mediated gene silencing against *Fusarium circinatum* and *Phytophthora cinnamomi*

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Forest diseases significantly affect our life quality by compromising the natural resources obtained from forests. Climate change and globalization are enhancing the threats of pathogens in our forests and their management and control is becoming increasingly complicated. In addition, using chemicals to treat these diseases is ineffective and should be avoided since it damages ecosystems. Spray-mediated gene silencing (SIGS) is a novel approach being developed in plant protection that relies on the natural mechanism known as RNA interference (RNAi). RNAi is a eukaryotic cell system that recognizes RNA sequences and specifically degrades them or prevents their translation. In this way, spraying double-stranded RNA (dsRNA) molecules to target essential genes in pathogens can inhibit virulence, protect plants, and control diseases. The application of this methodology in plant disease control has great potential due to the limitation in the use of harmful fungicides. It is having a great development in the protection of crop plants in agriculture. It is therefore well suited for application also in forestry systems, although it has been little explored so far. In our group we work specifically on controlling/managing pine pitch canker (PPC) diseases that destroy pine forests in Europe and whose causal agent is *Fusarium circinatum*, and also *Phytophthora cinnamomi* which is a pathogenic oomycete that seriously threatens Spanish oaks by drying them out, with severe ecosystemic and economic consequences. So we are developing the SIGS technique with dsRNA of essential genes of these pathogen as a way to reduce their virulence and disease symptoms.

Diversity of *Phytophthora* species involved in aerial infections on mountain vegetation in Europe

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Extensive outbreaks of *Phytophthora*-related diseases on leaves, shoots and fruits of small trees and shrubs have recently been observed in Alpine and mountain regions of Europe. Affected plants showed a complex symptomatology including foliar necrosis, shoot blight and bleeding cankers. Since little information is available about the aetiology of these emerging diseases, between 2019 and 2021, an in-depth study was conducted in 47 sites distributed in the mountainous areas of Italy, Austria and Slovenia, to determine the impact and diversity of *Phytophthora* species on mountain vegetation. A total of 286 *Phytophthora* isolates were obtained from 322 samples collected from 26 plant species. Based on morphology and DNA sequence data, 15 species belonging to 6 clades were identified: *Phytophthora pseudosyringae* (169 isolates), *P. plurivora* (39), *P. ilicis* (20), *P. acerina* (13), *P. alpina* (6), *P. cactorum* (6), *P. gonapodyides* (6), *P. idaei* (4), *P. cambivora* (3), *P. psychrophila* (3), *P. pseudocryptogea* (2), *P. bilorbang* (2), *P. gregata* (2), *P. hedraiandra* (2) and *P. kelmanii* (1). The results highlighted an unexpected biodiversity of *Phytophthora* species, many of which able to survive in cold environments and to cause aerial infections due to the production of caducous sporangia.

Poster

Eutypella parasitica in Europe: review of the situation and recent research

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Eutypella parasitica R.W. Davidson and R.C. Lorenz is the causative agent of Eutypella canker of maple, a destructive disease of maples in North America and Europe. The disease was first reported in Europe in Slovenia (2005), and later from Austria (2007), Croatia (2008), Germany (2016), Hungary (2016), the Czech Republic (2017), Poland (2017) and Italy (2018). *E. parasitica* represents a considerable risk for an extensive area of naturally distributed maples in Europe, where most frequently infects sycamore (*Acer pseudoplatanus*), field (*A. campestre*) and Norway maples (*A. platanoides*). It's findings in Europe agree with the results of a spread risk model, based on the host species index and climate variables. Microscopical observed changes in degraded wood suggest that *E. parasitica* can degrade lignin and could therefore be considered as a white rot fungus. Interestingly, *E. parasitica* was isolated also from visually healthy wood, suggesting it's (partial) endophytic life strategy. *E. parasitica* has a weak impact on the success of tissue colonization with other fungal species and a weak competition with other fungi. From the phylogenetic point of view, preliminary results are showing differences between European and north American populations of *E. parasitica*.

Sooty Bark Disease – An emerging disease of deciduous urban trees in western Washington

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Sooty bark disease (SBD), caused by *Cryptostroma corticale*, has emerged as a forest health concern in western Washington due to the number of new reports, its recovery from novel hosts, and its risk to human health. The fungus is believed to be native to the Great Lakes Region of North America and was first detected on *Acer pseduoplatanus* in Washington State in 1968. It primarily infects maple trees, which are important components of Pacific Northwest (PNW) urban forests. In Tacoma, WA, Seattle, WA, and Portland, OR, Acer spp. consist of 12.4%, 21.6%, and 26.5% of the total mapped street trees, respectively. Bigleaf maple (*A. macrophyllum*) and vine maple (*A. circinatum*) are also important components of natural PNW forests and used extensively in restoration plantings. Since 2020, *C. corticale* has been confirmed on numerous dying deciduous trees in Seattle and neighboring cities including the following 11 potentially novel hosts: *A. campestre*, *A. circinatum*, *A. japonicum*, *A. macrophyllum*, *A. palmatum*, *A. rubrum*, *Aesculus hippocastanum*, *Cornus nuttallii*, *Prunus cerasifera*, *Betula occidentalis*, and *Fagus sylvatica.* More research into the potential impacts and link between the emergence of this disease and the region's longer and hotter summer droughts is urgently needed.

Poster

Coniferous Rust Fungi of Phylogeography and International Quarantine

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EA/Alpine WPBR in the Northern Hemisphere, the primary forest disease is coniferous rust fungi, a genus of heteroecious host plant specific pathogens: *Cronartium* that have coevolved with their hosts. 3 major tree rust species, 12 pine species and 24 Ribes species of white pine blister rust in the in regions of North America and East Asia, and 27 pine species and 28 oak species susceptible to white pine blister rust that was introduced to the US from an external source but, evidence of lineage is lacking and suggests that occurrences of rust floras in virgin forests demonstrate separate changes in evolution based the regional ecosystemtible to pine-oak rust in North America, Europe and East Asia. By studying the phylogeography of tree rust, we can better explain the geographic patterns that may result in specific speciation. After 50 years search at China, Japan, Russia and North America to the international quarantine program of pine rust, I gained the following views: 1, Tree rust with hosts genetic base are Co-evolution; 2, Each pine rust fungus has it specific host; 3, Difficult to determine heteroecious host specific pathogens rust are an object of international quarantine; 4, When the pine export and planted to another rust flora area, if no native alternate host, pine rust does not spread and establish; 5, The archival, epidemic story of WPBR spreading in Western America may be caused by artificial subjects, due to lack of scientific data.

Uncovering viral traffic in forestry ecosystems using machine learning

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Climate crisis and anthropic pressure facilitate the entry of new viruses into forests that don't share co-evolutionary history with them. This situation poses a challenge both to the persistence of natural ecosystems and to the human being itself, due to urban expansion and overpopulation. To better understand this viral traffic, it's mandatory to study how likely viruses are to traverse across life kingdoms. The present work addresses this issue following a Natural Language Processing (NLP) approach to data mining of available literature. First, a semiautomatic selection of relevant publications is performed using a Naïve Bayes classifier trained on a subset of abstracts. Selected abstracts are tokenized into phrases and named entity recognition (NER) is performed using scispaCY models. Afterwards, each sentence is scored according to a sentiment analysis algorithm. Sentences carrying positive and negative connotations are compared by means of co-ocurrence networks. Results so far show that *Rhabdoviruses* are frequently associated with plants and animals in negative sentences. Crop viruses such as TSWV and BYDV also associate preferably with humans in negative sentences, whereas *Arbovirus* and *Bunyaviridae* do so in positive sentences. Thus, the proposed pipeline shows great potential as an early detection tool of candidate virus with cross-kingdom capabilities.

Keywords: artificial intelligence, plant pathogen, globalisation

Distribution and incidence of *Eutypella parasitica* in Switzerland

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Eutypella parasitica was first discovered in Europe in Slovenia in May 2005. In Switzerland, it was first detected in 2014 on a single Sycamore maple (*Acer pseudoplatanus*) in the east of the country. In 2021 five new locations with presence of *E. parasitica* were detected north of the Alps, covering an area from east to north-west Switzerland. Most cankers were found on Sycamore maple with one exception on a Norway maple (*Acer platanoides*). Some cankers appeared to be large (up to 1 m), infections prior to 2014 are thus assumed. Although *E. parasitica* is not a regulated organism in Europe, any new detection is eradicated and surveillance is conducted to detect other infections in the direct vicinity. To clarify the abundance and distribution of this invasive fungal species, a special monitoring has been started 2022. For three sites where the presence of *E. parasitica* was confirmed, 4 to 10 plots (100 m radius) have been surveyed for the presence of *E. parasitica* on different maple species. This study provides first insights into the distribution, abundance and incidence of *E. parasitica* in Swiss forests.

Poster

Monitoring practices and management of fungal diseases occurring in forest and nursery plantations in Zambia: A review

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Teratospheria zuluensis, which causes *Coniothyrium* canker, and *Lasiodiplodia theobromae*, which causes stem canker and die-back on *Eucalyptus* spp, are the most significant and frequent fungal infections in tree plantations in Zambia. *Calonectria pauciramosum* was also detected and shown to induce post-emergence and late damping-off of *Pinus oocarpa* and *Pinus kesiya* in

nursery plant trees in Zambia. Worldwide, diseases caused by fungi pose severe threats to plantation production. Fungicides to control such diseases are expensive and eco-unfriendly. This presentation collates information on fungal infections that affect seedlings in forest nurseries, particularly in Zambia discussing infection patterns, disease symptoms, and control options. We have summarized and reviewed previously proposed biological management approaches, with a particular focus on the use of fungal endophytes as biological control agents (BCAs). This information will facilitate formulating an integrated, continuous monitoring and management approach to reduce the impact of fungal diseases in plantations in Zambia, especially in forest and nursery plantations designated for post-mining landscape restoration.

Keywords: Zambia, fungal diseases, forests, fungal endophytes, biological management approaches

Early *in situ* detection of beech leaf disease using near infrared spectroscopy and machine learning

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We developed an early detection pipeline for beech leaf disease (BLD) using supervised classification models with spectral profiles from infected-presymptomatic and presumably naïve leaves. We also tested if DNA of *Litylenchus crenatae* ssp. *mccannii* (LCM), a nematode associated with BLD infection, was significantly associated with presymptomatic samples. Near-infrared (NIR) spectra were collected from leaves in the field in May, July, and September and analyzed using support vector machine and random forest models. The models accurately predicted presymptomatic leaves (highest testing accuracy = 100%) in the May and July datasets but also accurately discriminated the spectra based on location (highest testing accuracy = 90%). Therefore, we could not conclude that spectral differences were due to pathogen presence alone. However, location was accounted for in the September dataset, and the models accurately predicted presymptomatic samples (highest testing accuracy = 95.9%). Finally, qPCR quantification of LCM DNA showed that there was significantly more LCM DNA in

presymptomatic than naïve samples. Higher numbers of nematodes in presymptomatic samples are presumably responsible for the measured changes in spectral profiles. We propose that this technology has high value for early detection of BLD as well as other forest diseases.

Culture filtrates and/or broth culture extracts of fungal endophytes can decrease spore germination, growth and/or disease incidence of *Phytophthora cinnamomi*

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Holm oak (*Quercus ilex*) is the main component of the tree layer in *Dehesas*, grasslands with scattered trees and well-developed herbaceous understory mainly used for extensive rearing of livestock. This tree species is suffering a severe decline in last decades, which puts the survival of this characteristic Spanish agroforestry system at risk. Among other reasons, root rot by the oomycete *Phytophthora cinnamomi* has been invoked as a possible cause of this decline. The pathogen also affects *Lupinus luteus*, an herbaceous leguminous plant which is also very important in *Dehesas* from an ecological and an agronomic point of view. Fungal endophytes proved effective in reducing the damage caused by the disease and without environmental problems associated to chemical pesticides. Endophytic fungi live in the internal plant tissues, creating a symbiotic or mutualistic association with their host. The direct use of fungal metabolites, usually the main responsible for the protective effect, instead of the living organism might not be so dependent from the environmental conditions and its application might be much easier. In the present study, broth culture extracts or culture filtrates (containing the metabolites) of four fungal endophytes were effective in reducing spore germination, mycelial growth *in vitro* and/or disease incidence of *P.cinnamomi* after their application in *L. luteus* plants inoculated with the pathogen in the greenhouse.

Experimental evidence for bark mediated feedbacks between scale insects and fungi as a driver of beech bark disease development and severity

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Beech bark disease (BBD) is among the most iconic forest diseases of eastern North America. While infested beech trees exhibit elevated mortality rates, beech basal area is maintained via demographic compensation in the understory. BBD arises from the interaction between the tree host (*Fagus grandifolia*), an introduced scale insect (*Cryptococcus fagisuga*), and two species of *Neonectria* fungi (*N. faginata* and *N. ditissima*). While *C. fagisuga* obligately facilitates the establishment of *Neonectria* on naïve hosts in the advancing front, there is accumulating observational evidence that these organisms antagonize one another on long infested trees. We used experimental manipulation of scale insect densities and artificial inoculation of fungal mycelia of six strains of the two *Neonectria* species (and their combination) to elucidate the nature and strength of insect-fungal feedbacks on smooth and rough-barked American beech. We find evidence for bark mediated antagonistic interactions whereby insect establishment is weakened by both fungal scale insect-induced cankering and defect. We also found smaller *Neonectria* lesions on areas of bark with higher densities of scale. These results indicate that local scale negative feedbacks between scale insects and fungi can limit disease progression, and that bark response type is an important mediating factor of BBD development and severity.

Is there a role of terpenoids in systemic induced resistance in Austrian pine?

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Knowledge on tree systemic induced resistance (SIR) (whole plant-level immunity) and its underlying mechanisms remains limited, and is non-existent for tree systemic signaling. Using a spatio-temporal approach to elicit SIR, we induced the lower stems of 4-6-year-old Austrian pine by either wounding or inoculation with *Diplodia sapinea*. After 12 hours, 72 hours, or 10 days, trees were simultaneously challenged with a secondary inoculation of *D. sapinea* 15 cm above the induction. Two weeks post challenge, pathogen-induced trees produced shorter challenge lesions compared to wound-induced and negative controls. Notably, lesions were progressively shorter with increasing time between induction and challenge, demonstrating induction of SIR in a time-dependent manner. We also found that various terpenoids, as well as other volatile compounds, increasingly accumulated with increasing time between induction and challenge. Accumulation of terpenoids and other compounds was characterized by distinct coregulation. Furthermore, α-pinene and a group that includes β-pinene, limonene, benzaldehyde, dodecanol, and n-dodecyl acrylate were negatively correlated with lesion length, suggesting they may be involved in the expression of the SIR phenotype. We will use this system to further dissect the SIR response, including signaling, in ongoing experiments.

Mechanisms of pine disease susceptibility under experimental climate change

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Climate change (CC) makes trees more susceptible to pathogens but our understanding of the physiological and metabolic alterations underlying such phenotype is very limited. We conditioned 2-3-year-old Austrian pine saplings to a simulated CC environment consisting of reduced water availability and elevated temperature. We then challenged the trees with two sister fungal species, *Diplodia sapinea* (aggressive) and *D. scrobiculata* (less aggressive). Longer lesions were observed in CC-treated pines for both sister pathogens three weeks post-inoculation; notably, unlike under control climate, lesions lengths produced by the two pathogens were not significantly different under CC, indicating significantly enhanced susceptibility to the less aggressive pathogen. We conducted a dual transcriptomic analysis and found that, under control conditions, nitrogen, and carbon metabolism, as well as defense-associated pathways, were suppressed by *D. sapinea*, while pathogenic carbon assimilation was enhanced. In contrast, host nitrogen and fatty acid metabolism and defense pathways were enhanced by *D. scrobiculata* infection, consistently with its lower aggressiveness. However, *D. scrobiculata* suppressed carbon and nitrogen metabolism, as well as defense pathways, under CC. We synthesize our results into an integrated model of concurrent tree and pathogen responses that highlights critical host-pathogen metabolic changes under CC.

Butternut Canker Disease – Questions after 50 years of Study

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Butternut canker disease, caused by the presumed introduced fungus *Ophiognomonia clavigignenti-juglandacearum* has induced drastic changes to the distribution and abundance of butternut (*Juglans cinerea*) within its native range. The fungus causes small elliptical cankers and necrosis on all tissues. Cankers coalesce and kill branches and trees of all ages or contribute to eventual mortality caused by other pathogens (e.g. *Armillaria* sp.). The tree species short life-span, failure of forest management to provide adequate disturbance in eastern hardwood forests, and hybridization with the introduced nut tree Japanese walnut (*J. ailantifolia*), have all contributed to its extensive and steep population decline. However, butternut canker disease remains the main obstacle to butternut restoration. In this presentation I will give a brief overview of the butternut

canker pathosystem, discuss what researchers have learned in the 50 years since butternut canker disease was described and present future directions for research in the system and restoration of the species.

Poster

Abiotic and biotic tree stress signatures for urban tree health monitoring and management

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Currently, 50% of the world's population live in urban areas, and this proportion is projected to reach 68% by 2050. In densely populated areas such as the Northeastern United States, even 90% of the population live in urban areas. In these areas the importance of healthy urban canopy cover for public health is highlighted. Urban conditions create a challenging environment for trees while creating beneficial conditions for many pathogens and pests. With the intensifying impacts of climate change, information of the most effective methods to improve tree resilience is needed. Tree resilience is critical for addressing climate change mitigation efforts and tree cover disparity in urban areas. One critical element in tree health management are diagnostic methods that enable more targeted tree care and can help predict tree survival. Using tree stress signatures for timely detection of tree health issues can also help intercept invasive species. This presentation will offer an overview of the factors affecting urban tree health in the Northeastern US, will review the current urban tree health diagnostic methods, and will outline possible directions for further research on urban tree health monitoring and management.

A Reciprocal Sentinel Planting Approach for Assessment of Risk from Invasive Alien Tree Pests

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Invasive alien tree pests (IATP) are among the greatest recognized threats to forest ecosystems worldwide. Prevention and early detection of IATPs are the most effective management strategies in mitigating their impact. It is thus imperative that potential invaders are accurately and rapidly identified. Sentinel plantings can address this issue via the direct establishment and monitoring of target plants in potential source environments with exposure to native insects and pathogens. Through an international collaborative study, we established twin plantations of Asian and European tree species in Columbus, Ohio and Portsmouth, New Hampshire. Equivalent plantations, using identical planting schemes, were established in Alnarp, Sweden, and Florence, Italy with Asian and North American species, and in Nanjing, China with European and North

American species. Sentinel plantations were monitored for symptoms and signs of disease and insect attack for two seasons and fungal and insect species were collected and identified. We observed three potentially novel or otherwise unreported host-insect associations along two potentially novel host-fungal associations at genus level resolution. Species level identifications using multi-gene phylogeny is currently underway to resolve these relationships. We hope the success of this project will help promote further use of sentinel plantings as a tool for risk assessment.

Poster

Developing a rapid-phenotyping solution to fusiform rust disease in loblolly pine

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Fusiform rust is an economically devastating disease of commercial pine stands in the southeastern US. Breeding for resistance has been quite successful in this pathosystem; however, the process of identifying resistant families (i.e. phenotyping) is time-consuming, depends on local disease pressure, and varies by site. In this project, we are presenting a quicker and more versatile phenotyping method for fusiform rust resistance in loblolly pine by using vibrational spectroscopy (VS). These tools allow users to obtain a comprehensive spectral reading from a single scan, with each scan being as unique as a fingerprint. We collected spectra of needles and phloem from 340 loblolly pines at eight progeny test sites across the southeastern US. We used a portable near-infrared (NIR) VS scanner for in-field sampling and collected the same tissues to process them on a more sensitive benchtop Fourier-transformed VS machine for comparison of both tools. We then developed an algorithm that can discriminate between resistant or susceptible families based on their spectrum, allowing us to predict the phenotype of untested trees. Ultimately, our goal is to develop a time-efficient method to directly phenotype loblolly pine recruitment populations for fusiform rust resistance, regardless of the disease incidence in the area.

White Pine Blister Rust 1900-2018: The Impacts of an Exotic Disease in New Hampshire's Changing Climate

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White pine blister rust (WPBR), caused by the exotic fungal pathogen *Cronartium ribicola* J.C. Fisch., infected New Hampshire's (NH) valuable timber species, native white pine (*Pinus strobus* L.), circa 1900. From 1917-1979, NH controlled *Ribes*, the disease's telial host, through quarantines, hand-pulling, and herbicide applications. Disease incidence was reduced and the control program ended in 1980. In 2018, a field study revisited 50 white pine stands with documented infection (1929-1976) and 50 white pine stands mapped as disease-free by the former NH Office of Blister Rust Control. The distribution and incidence of WPBR were compared with historical reports of the disease. Results suggested that: disease incidence in white pine increased since a 1998 statewide study; native *Ribes* populations in several towns were infected with WPBR; the closest *Ribes* to infected trees were less likely to be within the historical 300-yard management zone; historical hazard models were outdated in relation to New Hampshire's climate; and WPBR advanced forest succession. Management challenges include: the ability of *C. ribicola* to adapt to NH's warmer, wetter climate; a longer and earlier infection season; the presence of multiple pathogen strains in NH; and the re-establishment of susceptible native and naturalized *Ribes*.

A next-generation survey of fungi associated with needle cast outbreaks on loblolly pine (*Pinus taeda*)

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Foliar diseases of conifers are caused by fungi and result in the loss or death of needles. Reduced photosynthesis impairs growth and can cause mortality in severe cases. Forest managers throughout the Southeast United States report that needle disease outbreaks on loblolly pine (*Pinus taeda*), the principal timber species in the region, are increasing in frequency and severity. The scale of these outbreaks is concerning to loblolly pine growers, as needle diseases are not traditionally considered a threat to this species. Little is known of the fungi associated with the recent outbreaks. In this work, loblolly pine foliage was obtained from impacted stands throughout the region and examined to identify fungi associated with symptomatic needles using directed molecular diagnostics and DNA metabarcoding. The causal agent of brown-spot needle blight, *Lecanosticta acicola*, was detected among symptomatic needles from Arkansas, Alabama, and Louisiana, but not from states to the east. DNA metabarcoding revealed multiple known needle pathogens among symptomatic needles, which differed by state, and indicated the strong association of opportunistic parasitic fungi with symptomatic needles. These findings provide guidance for future work to isolate and test the pathogenicity of possible causal agents and insight into the complexity of this emerging issue.

The impact of climate change on forest tree diseases: winners and losers in the Mediterranean region

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Environmental changes are occurring on a global scale, but their effects are most pronounced in climate change hotspots. The Mediterranean basin is a climate change hotspot region. Within this area Italy, extending from its southern territories in the centre of the Mediterranean Sea to its northernmost pre-Alpine and Alpine regions, is characterized by a variety of climatic conditions and vegetation types. Surveys conducted in recent years in various forest formations along the Italian peninsula revealed that the enhanced warming trend and irregular distribution of precipitation are strongly impacting forest health, with some fungal pathogens being important contributing factors to forest decline. However, in some stands the incidence and severity of tree diseases was markedly reduced compared to previous years. This positive or negative influence of climate change on some groups of pathogens and the diseases they cause appeared to depend on various factors including microclimate conditions and anthropogenic interference (i.e. silvicultural management and fires). We report here an overview of pathogens that seem favoured or disadvantaged by climate anomalies. Latent pathogens living as opportunistic endophytes seem to thrive under warmer and drier conditions, whereas obligate biotrophic fungal pathogens are impaired and the damage they cause is reduced.

Thousand cankers disease of walnut in Europe: impact, diagnosis and control

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Thousand cankers disease (TCD) causes branch dieback and mortality of walnut, especially black walnut (*Juglans nigra*) in natural forests, plantations and ornamental greenery. The disease is the result of the aggressive feeding by the bark beetle *Pityophthorus juglandis* on walnut trees and the subsequent development of a multitude of coalescing cankers by the ascomycete fungus *Geosmithia morbida* around beetle galleries. First originated in the western US, TCD then spread into the native range of black walnut in eastern US, and more recently arrived in Europe, Italy being currently the only European country where TCD is reported. The impact of this insect-fungus complex has been devastating in some black walnut plantations in the north-central part of the country. Both the symbiont fungus and its beetle vector are regulated as quarantine organisms in Europe (A2, EPPO List), hence the immediate eradication of new TCD outbreaks is a priority. Simple, accurate and repeatable molecular diagnostic protocols were developed for a prompt identification of both TCD complex members from various organic matrices. These assays, based on duplex real-time PCR, SYBR Green qPCR and LAMP, could validly support phytosanitary services in surveillance campaigns. In fact, being both members of the TCD pest complex difficult to identify with traditional methods, the use of molecular tools could assist in the phytosanitary inspection of import/export plant material as well as of plantations, nurseries and urban greenery.

Fungal diversity in American beech bark is associated with beech bark disease severity and climate

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Fungi are hyperdiverse taxonomically, with an estimated 2-5 million species globally. They fill a range of functional roles as pathogens of plants and animals, plant mutualists, and saprotrophs. Decades of research have focused on characterizing diversity of plant-associated fungi — root associates (e.g., mycorrhizal fungi) and leaf associates (e.g., endophytes, pathogens) in particular. However, fungi that live in plant stems have received relatively less attention except in the case of conspicuous pathogens, despite that stem-associated fungi may contribute to tree health and modulate disease severity. Here we characterize fungal diversity in the bark of a dominant North American forest tree (American beech [*Fagus grandifolia*]) afflicted with a widespread stem disease complex (beech bark disease [BBD]) and explore the primary drivers of taxonomic diversity and functional group diversity — asking whether diversity is more strongly associated with tree-level characteristics or climate. Total fungal species richness responded to different pressures than species richness within functional groups (plant pathogens, animal pathogens, saprotrophs). Whereas total species richness in tree bark was most strongly associated with climate, species richness within functional groups responded more to local, tree-level conditions. These patterns suggest that functional capacity tracks changes in the local environment, whereas climate may structure niche breadth overall.

Emergence of *Cryptostroma corticale* in Europe

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The sooty bark disease (SBD), caused by *Cryptostroma corticale*, is an emerging disease affecting maple trees (*Acer pseudoplatanus*) in Europe. The reports have been exponentially increasing since 2000. The distribution of the SBD and conditions of emergences in Europe have not been studied yet. Here, we study the SBD emergence in Europe through pathogen detection in aerobiological samples. We performed a regional study in France comparing two-year aerobiological and epidemiological data; and a continental study including 12 sites from six European countries (Italy, Czech Republic, Switzerland, Sweden, France and Portugal). We also analysed detailed data of the outbreaks in France and Switzerland since 1990 and its link to climatic data. Aerobiological detection of the pathogen was high in countries within the host native area and with longer disease presence, such as France, Switzerland and Czech Republic, and absent in countries where the pathogen has not been reported yet. The disease was frequently reported in France and Switzerland after the severe drought and heat waves of 1990-91, 2003 and especially 2018-20. SBD occurrence was strongly related to the maximal daily temperature of July-August. Aerobiological surveillance can inform the spatial distribution of the SBD, and contribute to early detection in pathogen-free countries.

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Effect of silvicultural treatments on songbird populations and diseases of eastern white pine (*Pinus strobus*) regeneration

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In Eastern USA, forested land mostly privately owned. Obtaining support for silvicultural treatments to improve pest conditions is challenging because private landowners are not solely interested in commercial value of their trees, however, many are interested in wildlife management. The Massabesic Experimental Forest in Maine offers researchers the opportunity to study silvicultural treatments such as patch cuts, group selection, shelterwood, and low-density thinnings established in white pine stands during 2007-2008. Many studies were designed across these vegetative structural conditions including monitoring breeding songbird communities and white pine diseases. Whereas high seedling density is encouraged to reduce damage by white pine blister rust and weevil, dense conditions may increase damage by foliar diseases and Caliciopsis canker. Prior to silvicultural treatments, birds characteristic of early successional habitats were not present whereas birds characterized as forest- and generalist-users were. Following treatment, the proportion of early successional birds increased with the growth of woody regeneration; forest- and generalist-birds were present in regenerating stands and low-density thinnings. The ephemeral nature of early successional habitat fades out around 15 years post-treatment as regeneration grows. Designing silvicultural treatments that increase wildlife diversity is a useful means to encourage landowners to implement treatments that also improve forest health.

Poster

Evaluating heights to blister rust cankers on young western white pine in the Oregon East Cascades with implications for blister rust pruning guidelines

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Along with breeding for genetic resistance, lower branch pruning is a management strategy to reduce adverse effects of *Cronartium ribicola* on young western white pine (*Pinus monticola*; WWP) in portions of the Inland Northwest, USA (NW), including the Oregon East Cascades (OEC). However, information from outside the OEC on heights to blister rust cankers aboveground, and post-treatment effects, have previously informed NW pruning guidelines. In this study, heights to cankers on young WWP were measured in 12 stands (four naturally and eight artificially regenerated) where rust was present during 2015-2021. Incidence of rust on live WWP was 38% (range 10-89%). Differences in rust incidence, number of cankers, and heights to cankers on WWP ≥ 2.54 cm in diameter at 1.37 m were observed among stands ($p < 0.05$). At the stand-level, 90% of mean heights to branch and bole cankers were < 2.31 m. Mean heights to branch and bole cankers in stands were < 1.4 m, but bole cankers were significantly lower than branch cankers ($p = 0.001$). After evaluating the distribution of heights to cankers, pruning via one crown lift to maintain WWP should still be considered a potential management option. Prioritization of stands for treatment was reaffirmed.

First report of *Gnomoniopsis smithogilvyi* causing lesions on sweet chestnut in Ireland

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In mid-autumn 2020 lesions and shoot dieback were detected on Castanea sativa trees during a survey for *Cryphonectria parastica* in Co. Wicklow. The three-year-old trees were part of a genebank scheme over two sites, which had been imported from the United Kingdom. Samples of the affected shoots were examined and isolations were made from the margins of the lesions. DNA was extracted from mycelium and the ITS region was amplified and sequenced with ITS7 primers. Based on morphological features and ITS sequences the identity of the fungus was confirmed as

Gnomoniopsis smithogilvyi. Koch's postulates was confirmed by inoculating 50 three-year-old sweet chestnut saplings, with two different isolates of *G. smithogilvyi* (25 per isolate). After six weeks, necrotic lesions were observed on the inoculated saplings and the fungus was subsequently reisolated from all lesions. No *G. smithogilvyi* was recovered from control saplings. The identity was confirmed by morphology and by sequencing of the ITS region. Furthermore, *G. smithogilvyi* has been found causing cankers on shoots and scions in the UK and India. This is the first report of *G. smithogilvyi* causing cankers on sweet chestnut in the wider environment in Ireland.

A novel threadblight pathogen threatens a wide host range of hardwood and conifers in the southeastern United States

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Since the fall of 2019, mortality & blight symptoms and white mycelium were identified on *Afrocarpus falcatus* and *Ligustrum japonicum* in Gainesville, Florida and subsequently on numerous hardwood and conifer near Tallahassee and Torreya State Park. To determinate the causal agent, isolations, *in-vitro* and greenhouse experiments were performed. We identified an unknown basidiomycete based on morphological characteristics and sequences of the ITS and LSU-rDNA. A nucleotide alignment of diverse Agaricales and phylogenetic analyses were performed. Based on our analysis we determined that the unknown basidiomycete is related to resupinate and cyphelloid fungi in the Cystostereaceae family. To determinate the pathogenicity of the unknown fungus, *in-vitro* (*Ligustrum* leaves on water agar medium) and greenhouse inoculations (*Ligustrum* sp., *Afrocarpus falcatus* and *Quercus nigra)* were carried out. In both experiments, leaves were inoculated with mycelium plugs of the new basidiomycete, and water agar as a negative control. Blight and white mycelium were present, and symptoms and signs were consistent among replicates. This study highlights a significant broad host range for a newly

discovered fungal taxon which may represent a new introduction into the region. Implications for management and future research needs will be discussed.

Incorporating host-pathogen interactions into predictions of qualitative and quantitative resistance to fusiform rust

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Fusiform rust, the destructive disease that is caused by *Cronartium quercuum* f.sp. *fusiforme* (Cqf), affects pine species across the southeastern United States. Breeding for resistance to Cqf has effectively led to improved pine selections by focusing on the qualitative or gene-for-gene resistance to the pathogen. We are developing an integrative approach that includes both pathogen and host genomics to improve both qualitative resistance and quantitative resistance. Screening elite loblolly pine families with Cqf isolates from ten geographically dispersed populations provided data on gall presence and absence (qualitative) as well as gall length (quantitative) demonstrated significant host by pathogen interactions. Integrating this phenotype data with genotypic data from resistance genes in the host and whole-genome sequencing from basidiospores used in the inoculations as well as pycnia obtained from resulting galls will be used to dissect rust resistance mechanisms. Combining this data with existing marker and phenotyping information from previous studies will be used to evaluate changes in genomic prediction model accuracy and marker trait associations.

First report of *Melampsora epitea* causing stem cankers on willow in Alberta, Canada

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In June, 2021, an unknown rust disease with atypical canker symptoms was discovered on laurel leaf willow (*Salix pentandra*) near Slave Lake, Alberta. Bright yellow urediniospores were observed on uredia that were present on catkins, leaves and stems. All *Melampsora spp.* previously reported in Canada are recorded as infecting leaves; therefore, further investigation using DNA sequence data from the internal transcribed spacer ribosomal RNA region was conducted. Sequencing and BLAST analysis of the material from the sample resulted in a 99.3% sequence identity match to *Melampsora epitea* "Mel J" collected from *Larix larcina* in New York State. To further assess the relationship between this specimen and other *Melampsora* spp. collected from Canada, herbarium samples of willow leaves infected by *Melampsora* spp. were retrieved from the mycological herbariums located at the Laurentian Forestry Centre (QFB) and the Northern Forestry Centre (CFB, WINF). DNA extracted from these herbarium specimens was used to conduct a phylogenetic analysis and it was found that herbarium sample WINF7356 (described as *M. abieti-capraerum* from Manitoba) had 100.0% match with the Alberta sample. Additionally, specimens WINF11892 (*Melampsora* sp. from Manitoba) and CFB8931 (*Melampsora* sp. from the Yukon) had 99.0% sequence identity with the Alberta sample. From these results we conclude that the rust discovered in Slave Lake, AB, is *M. epitea*, and that this is the first report of a stem infecting form of the pathogen that has been collected from Canada. With the current emphasis on willows for bioenergy production in Canada, growers must remain vigilant for this pathogen and the damages it could cause to willow plantations.

Evaluation of the expression of plant defense response genes in *Eucalyptus*

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The genetic investigation of plant's defense mechanisms against stress is an important factor for the discovery of response pathways. A *Eucalyptus* controlled cross produced a progeny with a malformation phenotype exhibiting low growth, tissue malformation and mortality in the first months of development. Leaf, root and stem tissues of anomalous and healthy plants were analyzed based on qPCR for five genes associated with pathogenesis-related (PR) proteins. All selected genes are related to plant defense pathways, such as thaumatin-like, pathogen related proteins, chitinases and cupin genes. The results point to high expression rates of all genes in the anomalous phenotype suggesting, as indicated by the Gene Ontology analysis, responses to plant defense mechanisms, including against pathogens. Complete parental sequencing, as well as offspring, may indicate the metabolic pathways or other genes associated with the defense mechanisms in the anomalous phenotype. These could serve as the basis for future pathogen inoculation tests and infection response studies, with possible indication of targets for plant disease control.

Spray-induced gene silencing (SIGS) using organic and inorganic nanoparticles provides a steady RNAi effect against fungal plant diseases

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A broad range of fungal plant diseases are responsible for severe crop and forestry losses around the world. Fungicides are routinely used to control these diseases in crops but they are banned for use in forestry. Spray-induced gene silencing (SIGS) is an alternative eco-friendly fungal disease management strategy that has emerged in recent years based on the natural phenomenon of cross-kingdom RNA interference (RNAi). It involves the capacity for fungal pathogens to deliver small RNAs (sRNAs) to host plants during the infection process to silence host defense genes, and the capacity for host plants to send sRNAs to the fungal pathogens to inhibit their virulence genes. In light of this discovery and the subsequent discovery that several fungal pathogens can efficiently uptake environmental sRNAs to silence fungal genes with complementary sequences, SIGS has proven to be effective in controlling several fungal diseases on pre- and post-harvest plant material. In spite of this, the durability of the RNAi effect is limited by the stability of the double-stranded RNA (dsRNA) after spraying as well as its uptake efficiency by fungi. In the present study, we used organic (lipid-based) and inorganic (nanoclay-based) nanoparticles to create coated-dsRNA sprays against *Botrytis cinerea*, targeting key virulence-related genes (Dicer-like, *DCL1* and *DCL2*) and essential fungal growth genes (*VPS51*, *SAC1*, *DCTN1*), to control gray mold disease on a range of plant materials, including leaves, flowers and fruits. As a result, nanoparticles ensured a steady RNAi effect and higher silencing activity as compared to naked dsRNA applications over time, providing more opportunities and broader applications for sequence-specific SIGS technologies to manage fungal plant diseases. SIGS is booming and in continuous development in the agricultural sector but requires a great research effort in forestry, where despite being a promising technology, it is still unexplored.

Antagonist effect of nematophagous fungi against pine wood nematode (*Bursaphelenchus xylophilus*)

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Bursaphelenchus xylophilus poses a serious threat that endangers conifers forests worldwide. This pathogen, commonly known as Pine Wood Nematode (PWN), causes sudden decay of affected trees, leading to the so-called Pine Wilt Disease (PWD) and finally to the death of the individuals. Thus far, disease control has been focused solely on insect vectors (*Monochamus spp.).* Specifically, the Forest Entomology Team of the UVa patented the insect attractant (Galloprotect 2D) and developed an attract&infect method based on the use of *Beauveria spp.* (entomopathogenic fungi) to reduce vector populations. However, the control of the pathogen itself is required for effective integrated disease management. For this purpose, the potential nematophagous effect of 8 fungal species was tested, among them two *Beauveria* species, seeking to test whether the reproduction rate of the nematode was reduced in their presence. This *in vitro* experiment has achieved encouraging results, since several species showed a strong antagonist effect on the nematode reproduction. In fact, *Beauveria* species and *Trichoderma citrinoviridae* not only resulted in a null reproduction rate, but also a nematicidal effect. Therefore, a truly effective method of biological control based on *Beauveria* species might become applied for a simultaneous management of the nematode and its vector.

Persistence of the Swiss Needle Cast (*Nothophaeocryptopus gaeumannii*) outbreak in Oregon: 1990s to the present

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An outbreak of the Douglas-fir foliage disease, Swiss needle cast (caused by *Nothophaeocryptopus gaeumannii*), began in coastal Oregon plantations in the mid-1990s and has persisted since that time. Long-term monitoring using aerial detection surveys and ground based disease severity and growth-and-yield plots have shown that the disease is most significant within 30 km of the coast. Although hectares observed with symptoms from aerial survey has varied from 53,048 ha in 1996 to a peak of 238,700 ha in 2015, the geographic area of the epidemic near the coast has remained remarkably stable. Factors which influence disease expression include winter or non-growing season temperatures (warmer is better for disease), leaf wetness during spore dispersal in late spring to mid-summer, abundance of young plantation Douglas-fir trees within 30 km of the coast, and seed source genetics (local is better). Disease severity decreases with increasing elevation and distance from the coast, resulting in a gradient of disease impacts. Young forest plantations (<30 yrs old) appear to be much more susceptible to disease than mature and old-growth Douglas-fir, implying forest structure, tree age, and microenvironment of plantation trees is important in disease epidemiology. Disease may increase at higher elevations with global warming.

Visible scorch symptoms in conifers following the June 2021 heat dome event in Oregon.

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During late June and early July 2021, the Pacific Northwest of North America experienced a heat dome event. Although Oregon and Washington States, USA, were at the southern edge of the heat dome, we experienced all-time record-breaking temperatures for 3 – 5 days in excess of 45° C. Visible symptoms of scorch began to develop shortly after the event, especially in coastal Oregon. The US Forest Service Cooperative Aerial Detection Survey flew the region in July and August and mapped ~93,000 ha (229,000 acres) of visible scorch in western Oregon and Washington. This is perhaps the largest documented scorch event in history. We went to the field in coastal Oregon and some inland locations to determine the impacts on conifer trees. Scorch was consistently associated with south and west facing aspects and trees along edges and on steep south and west slopes. We found scorch on *Tsuga heterophylla*, *Pseudotsuga menziesii*, *Picea sitchensis*, and *Thuja plicata*. Scorch varied from dead needles along the exposed portion of the crown and upper sides of branches, to tip death and fine branch dieback. Although scorch was common in more mesic areas of coastal forests, inland forests which experienced higher temperatures did not show visible scorch.

Wildfire smoke as a potential transport mechanism for forest pathogens

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The term pyroaerobiology has been recently used to describe the study of living microbes that are found in smoke produced from wildland fires. The diversity, origin, function and potential impacts of these microbes is currently the focus of several ongoing studies. Previous studies have demonstrated high viability of microbes in smoke and fuels consumed directly impact the patterns of microbial diversity detected in smoke plumes. Using Illumina sequencing of the ITS2 region from smoke samples obtained from drone-based filters in 2021, we observed a wide diversity of forest pathogens. These include multiple important taxa including *Heterobasidion*, other numerous Basidiomycota that cause decay and rust fungi. Although viability has not yet been determined, these results suggest wildfire smoke may play an important, unexplored role in the long-distance dispersal of forest pathogens. If so, future efforts to understand tree disease epidemics in the landscape should include wildfire as a component in pathogen dispersal.

Eutypella Canker of Maples: A North American research retrospective

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Perennial cankers caused by *Eutypella parasitica* are a characteristic feature of maples in northern hardwood forests of the eastern United States and eastern Canada. Decades-old field surveys and experimental studies in this region revealed fundamental information regarding disease development and impact, especially on sugar maple (*Acer saccharum*). Incidence of Eutypella canker varies widely, both within stands and across the region. Ascospore discharge is initiated following wetting of canker surfaces bearing perithecia. But because windborne ascospores are ejected in octads, the distance of their dissemination is limited and spatial distribution of diseased trees may be clustered. Infection frequently is attributed to fresh trunk wounds exposing sapwood or to branch stubs. Colonized tissues include xylem, vascular cambium, and phloem. Unless

secondarily colonized by more capable decay fungi, defective wood typically does not extend far beyond the margins of cankers. Cankers on lower portions of trunks and these cankers often expand for decades on large trees, causing significant impact to the most commercially valuable portion of trees. Removal of the inoculum source by felling the cankered trees (living or dead) and placement of the canker surface bearing perithecia toward the soil effectively minimizes occurrence of Eutypella canker and losses from this disease.

Cronartium pini epidemic in Northern Scandinavia. Potential for utilizing resistance in Scots pine

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An epidemic of Scots pine blister rust caused by *Cronartium pini* has recently tormented Scots pine forests in northern Scandinavia. There are two life cycle variants of the fungus one that is autoecious on pine and one that alternates with a wide range of herbs, in Northern Scandinavia most prominently with *Melampyrum sylvaticum*. Resistance against the rust is present in the host population but field trials with breeding material has not been able to cover the full range of host genotypes used in forestry. Therefore, work has been initiated to do parental tracking in affected stands to trace resistance and susceptibility in parental trees. In this way host genotypes that are not present in earlier offspring trials are reached and phenotyped under relevant disease pressure. Furthermore, screening of breeding material using controlled infections with basidiospores under laboratory conditions has been initiated. Conditions for cultivating the rust on alternating host material, and infection procedures on pine host seedlings has been established in a pilot scale.

The Role of Host Drought Response in White Pine Blister Rust

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Invasive fungal pathogens have the potential to thrive in ecosystems where the hosts have not coevolved resistance. We have yet to understand how climate change and associated drought in western forests will change interactions between invasive species and their native hosts. *Cronartium ribicola*, the non-native rust fungus that causes the lethal disease white pine blister rust (WPBR) in five-needle pines, has spread through many ecosystems in North America, aided by high fecundity and wind dispersed spores. Though many tree species become more vulnerable to native pathogens during water stress, and five-needle pines have mechanisms to mitigate drought effects, little is known about the interactive effects of drought on native five-needle pines and the invasive *C. ribicola*. As water availability changes, carbon storage will likely also change affecting traits including growth, and phytochemical production. As a biotroph, *C. ribicola* depends entirely on a living host to grow, raising questions about the role of host health in infection and disease progression. Our hypothesis is that drought stressed trees will be a reduced food source for the pathogen and will outweigh any advantages associated with reduced host defenses, leading to lower infection rates and slower overall growth of *C. ribicola* within the drought stressed hosts. We examined drought response and disease progression in the severely droughted, moderately droughted and well-watered *Pinus flexilis* challenged with *C. ribicola*. These results provide an understanding of how host-pathogen interactions within the WPBR pathosystem may change in western forests as drought induced by climate change continue to occur.

Charcoal canker disease on *Eucalyptus gomphocephala* in North Africa

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Tunisia is located in North Africa and is known for its natural and planted forests (coniferous, deciduous wood, and maquis). *Eucalyptus* species have been introduced in Tunisia since 1957. Nevertheless, this non-native genus is threatened by several pest species (pathogens and insects). Among diseases, charcoal canker caused by *Biscogniauxia* spp is a worldwide distributed and well known as a significant fungal disease of oak species and other hardwood hosts. In Tunisia, this fungus was described on *Quercus suber* and shrub Mediterranean species. Since June 2021, serious dieback was noticed on *Eucalyptus gomphocephala* in the forest of North-western Tunisia. A number of 1400 *E. gomphocephala* trees showed charcoal canker symptoms in a surface area of 15 ha representing a disease incidence of about 46%. Stroma samples were collected and perithecia containing ascospores were observed confirming the occurrence of *Biscogniauxia* spp. These symptoms were not observed on *E. camaldulensis* despite its presence in the same area. For better management of the forest, dead trees were cut and incinerated.

Keywords: *Eucalyptus,* pathogen, Tunisia, Forest

Dual RNA-Seq analysis of pine-*Fusarium circinatum* interactions reveals molecular mechanisms underlying host resistance

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Fusarium circinatum causes one of the most devastating diseases of conifers worldwide, the pine pitch canker (PPC). The development of pine resistant varieties offers an excellent opportunity for disease management. This study aimed to identify differences in the molecular responses of the highly susceptible *Pinus radiata* and the relatively resistant *Pinus pinea* to *F. circinatum* at an early stage of infection. The use of a dual RNA-Seq approach also allowed to evaluate pathogen behavior when infecting each pine species. The transcriptome profiling of *P. pinea* revealed an early perception of the pathogen infection together with a strong and coordinated defense activation through the reinforcement and lignification of the cell wall, the antioxidant activity, the induction of PR genes, and the biosynthesis of defense hormones. The susceptible species, in contrast, showed a weaker response, possibly explained by the impaired perception of the fungal infection that could have led to a reduced downstream defense signaling. While in *P. pinea F. circinatum* focused on the degradation of plant cell walls, active uptake of the plant nutrients was showed in *P. radiata*. These insights improve our understanding of the pine-*F. circinatum* interaction and can promote the development of better approaches for breeding disease-resistant programs.

Poster

Isolation and identification of fungi from loblolly pine needles affected with needle cast

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Large areas of southeastern loblolly pine (*Pinus taeda*) forests have shown symptoms of needlecast disease: yellow/brownish spots in the needles, scorching of the distal part of the needles, which are later cast, and reinfection of newly emerged needles. In loblolly pine, needlecast is considered a secondary disease attributed to different fungal species, such *as Lecanosticta acicola, Dothistroma* sp. *Lophodermium australe* and *Rhizosphaera pinicola*. In this study, we obtained fungal isolates from needles collected from Arkansas, Mississippi, Louisiana, Georgia, and Florida, and identified them by Sanger sequencing of PCR products, followed by BLASTn analysis against the NCBI fungal database. Ten isolates were recurrent in two or more sites, of which three (*L. australe*, *Australosphaerella sp*. and *R. pinicola*) were the most common across sites. Testing for Koch's postulates on loblolly pine seedlings using these isolates along with *L. acicola* will help better understand potential causes of needlecast in southeastern United States and help guide future detection and mitigation of this disease.

Benchmarking a fast and simple on-site detection assay for the oak wilt pathogen *Bretziella fagacearum*

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Oak wilt is a vascular disease of oak trees caused by the fungus *Bretziella fagacearum* that can lead to the death of healthy trees in a matter of months. While the disease is currently only found

in the United States, it has been reported within just a few hundred meters of the Canada-USA border. The necessity to develop an on-site, quick and reliable method to detect *B. fagacearum* is now becoming more and more pressing in order to limit the establishment and spread of oak wilt disease in Canada. In this study, we developed and validated a new qPCR TaqMan assay that can, with great specificity and sensitivity, detect the pathogen in a laboratory setting. Using this test as a reference, we also developed and validated a new RPA-DETECTR assay that can be easily deployed on-site to detect, within 1 hour and using minimal laboratory equipment, the presence of *B. fagacearum* in a variety of environmental samples. We also optimised this new RPA-DETECTR protocol for end users with or without previous laboratory experience, making this assay a potential tool for everyone to mitigate the spread of oak wilt.

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