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Plenary session 1

On the long-distance train to Green Deal - next stop: Insect Semiochemicals

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Abstract

Our time-travel starts in the 50ies of the XX century, when the field of chemical ecology was gaining recognition. We alight briefly to look at the methodologies that render possible the detection, capture, separation and identification of compounds acting as insect semiochemicals. Yet, our train-timetable allows for a couple of landmarks only to be sighted on the way. Green Deal being our chimera destination, we focus on the ambitious vision “Working with nature to protect our planet and health”. Regarding forestry, the success of close to nature friendly practices, aimed at raising forest resilience and adaptation, depend upon biodiversity restoring. Thus, we next discuss a few pest management strategies comprising semiochemicals, applicable to insects causing economic damage to forest crops. Among the innovative tools under development, the conjugated use of semiochemicals and semiophysicals, as well as a deep insight into the compounds metabolomics, appear promising. Multi-purpose management strategies can enable *e.g.* population monitoring and early detection of invasive species, further to reduction of the species effectives. Still, securing compatibility between new and classical techniques, such as biological control, should not be disregarded. According to the World Economic Forum, by 2030, business opportunities arising from conservation, restoration and sustainable management of the world´s forests, will create 16 million jobs and generate €190 billion. No doubt, the transition from Integrated Pest Management (IPM) to Integrated Ecosystem Management (IEM), is now the challenge to be pursuit.

Keywords: Insect chemical ecology, pest control strategies, Integrated Ecosystem Management

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Plenary session 2

The role of fungi in shaping the ecology and behavior of bark and ambrosia beetles

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Abstract

Symbioses between fungal ectosymbionts and wood-boring weevils have emerged repeatedly in the last 100 million years. In the most famous bark and ambrosia beetles (Scolytinae and Platypodinae), fungal symbioses range from facultative to obligate mutualisms and involve fungal services such as direct nutrition, decomposition, the detoxification of host plant tissues and virulence to the tree-hosts. Mutualistic fungi, on the other hand, profit from their host's dispersal and protection and may include the maintenance of beneficial fungal communities through active partner choice and promotion as well as the inhibition of fungal competitors and pathogens.

In this talk I present data on (i) the ecological factors leading to the repeated emergence of fungal symbioses in wood-boring weevils and (ii) the consequences farming has had on the morphology and social systems of the beetles. Furthermore, by presenting exemplary cases from our research, (iii) I highlight the diversity of fungi in bark and ambrosia beetles, what crucial and diverse roles these symbionts play in the majority of species and the techniques these beetles employ to maintain beneficial fungal symbionts. Finally, (iv) I present some major open questions concerning the biology and applied management of bark and ambrosia beetles.

Plenary session 3

Preparedness and response to introduced tree pests and pathogens in Britain

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Abstract

The introduction of exotic pests and pathogens which can be a threat to trees and to forest ecosystems in new environments, has grown exponentially in recent years. Most of these introductions are linked to the plant trade and the movement of people, they are unintended, and their establishment can be favoured by climate change. Plant biosecurity is now more important than ever and the National Plant Health Services work to minimise the risk of these introductions in each country. These interventions are risk-based to ensure that the effort is commensurate with the overall costs and benefits to society. To maximise the chances of early detection of plant pest and pathogens in the UK, the Plant Health Risk Register was developed with the aim of proactively assessing and prioritising the risk and preparing appropriate contingency plans. The government's approach to respond to pest and disease threats focuses on a biosecurity continuum, with pre-border, border and inland activities. This means we need to work internationally to reduce the likelihood of pests and pathogens arriving, checking at borders to reduce the opportunities of their introduction and having early warning systems post-border to detect possible introductions early and reduce the chances of establishment. Inland activities include active surveillance undertaken by official bodies who lead targeted surveys and are trained and equipped to detect regulated pests and pathogens. As an early warning system and to build upon official surveys, general surveillance and citizen science projects are also used. Tools have been developed to aid the reporting of pest and pathogens by land-owners and citizens in Britain. Teams of pathologists and entomologists work verifying the reports submitted. Working together with a common approach to plant health from government and citizens can be an effective approach, and provides an early warning system for the detection of pests and pathogens.

Keywords: biosecurity, diseases, surveillance, citizens

Plenary session 4

Global tree health in the future: A world of challenges and opportunities

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Abstract

From a scientific perspective, the study of tree disease is relatively young. This is specifically from a perspective relating to damage due to pests (including microbial pathogens and insects), for which the first experimental studies date back to the latter part of the 19th Century. Interest in and concern regarding this subject was vigorously stimulated as biological invasions due to accidentally introduced pests emerged in natural forests across the boreal region. Classic early examples were invasions by the chestnut blight fungus and the spongy moth, interestingly both of which were included in Elton's landmark 1958 book, which marked the emergence of the field of invasion biology. A substantial body of research relating to the identification, biology, ecology and management of tree pests has emerged since the early 1900's; this in itself a tribute to many gifted and passionate entomologists and pathologists. Yet from a broad perspective, there is much to be learned regarding the future health of the world's forests. New pest invasions have continued to occur at an alarming rate and there is little evidence that we have been particularly successful at halting this process. Most research on tree health issues has focussed on problems in the so-called developed world. Unfortunately, comparatively little attention has been paid to those in resource-poor countries. Yet these problems, as has been vividly learned from the SARS CoViD-2 pandemic, are globally interconnected and will be effectively managed only from that perspective. Likewise, future solutions to tree health problems, as is true for animal health, will lie in an aggressive application of emerging technologies, including those both in the biophysical and social sciences. The world's forests face substantial and growing health challenges. Yet the opportunities to deal with them are both formidable and exciting. As tree health specialists, the future of the world's forests must be our responsibility.

Keywords: entomology, plant pathology, tree disease, invasion biology

Session 1 General session on forest pathology and entomology

Oral communications

Digital Forest Fungarium: A data mining model of knowledge discovery for forest disease and prediction

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Abstract

The current era has seen an exponential surge in the volume and diversification of all types of information, dubbed "information explosion" by some. A number of studies and studies in the scientific literature have discussed the value of biological specimen collections, such as plant disease herbaria and plant pathogen live cultures. Herbaria are collections of biological specimens in which dead, dried, pressed, or preserved plants and fungi, as well as information on the specimens, are permanently archived. Forest Research Institute Dehradun housed nearly 12000 specimens of forest disease and fungi which are preserved. Plant disease herbaria are uncommon in the country due to the nature of specimens and the lack of knowledge about plant disease taxonomy. Collections of forest diseases and fungus, as well as specimens in the form of herbaria, are extremely helpful in identifying various specimens and plant diseases. Baseline information on forest tree disease, pathogens, host plants, location, and disease or pathogen collection period can be found in the forest disease herbarium or Fungarium. Using data of fungarium, we created a data mining or knowledge discovery tool that allows us to forecast the model of forest disease forecasting in a specific forest region. The disease prediction model for plants was created using association rule mining, classification, and online analytical processing (OLAP). This work discusses the concept, design, and implementation of a relational database software database of plant disease specimens or a forest pathology herbarium, as well as a data mining model for plant disease forecasting using data mining methods.

Keywords: Forest Fungarium, plant disease, data mining

Botryosphaeriaceae associated with three native tree species in a subtropical steppe region of South Africa

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Abstract

The Botryosphaeriaceae include various endophytic species, some of which are important latent pathogens that can cause disease on various indigenous and exotic trees. Little is known regarding the diversity and occurrence of these fungi on indigenous *Berchemia discolor* (brown ivory) (Rhamnaceae), *Lannea schweinfurthii* and *Sclerocarya birrea* (marula) (both Anacardiaceae) in South Africa. These trees play a role in the socio-economic well-being of small-scale farmers and people living in rural communities. They are valued for their medicinal properties and nutritional values, and B. discolor and *S. birrea* are used in the production of liquor. Our study aimed to explore the diversity of Botryosphaeriaceae on symptomatic and asymptomatic branches of these trees at sampling sites in agricultural and natural ecosystems. Thirteen species in the Botryosphaeriaceae were identified based on analyses of DNA sequence data of the ITS rDNA region and portions of the β-tubulin, TEF-1α and Rpb2 genes. The fungi identified included four potentially new species, designated as *Alanphillipsia* sp. 1, *Dothiorella* sp. 1, *Oblongocollomyces* sp. 1 and *Oblongocollomyces* sp. 2, which await morphological descriptions and formal naming. In addition, isolates of *Diplodia allocellula*, *Dothiorella brevicollis*, *Do. diospyricola*, *Do. dulcispinae*, *Do. viticola*, *Lasiodiplodia crassispora*, *L. exigua*, *L. gonubiensis*, *L. mahajangana*, *L.. margaritacea*, *L. pseudotheobromae* and *Neofusicoccum parvum* and various isolates with uncertain identity, belonging to Botryosphaeria and Dothiorella were identified. Some species occurred on both symptomatic and asymptomatic branches of the three trees in natural and agricultural ecosystems, and thus providing support for them being able to survive as both endophytes and pathogens. Overall our results showed that native tree species are rich reservoirs for the Botryosphaeriaceae in the sampling sites.

Keywords: native tree health; tree pathogens; tree pathogen systematics; tree endophyte systematics; tree endophytes

Pathological impacts on tree health due to effects based on climate change in Southwest Germany

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Abstract

Recent Surveys and reports on forest diseases show that numerous rare leaf, shoot and bark diseases cause damage for the most important forest tree species after years characterized by the consequences of drought and heat.

Since the drought events and hot summer temperatures between 2018-2020, the most attention has been drawn to the presence of *Biscogniauxia nummularia* on beech trees, which has led to a rapid increase in the amount of damaged wood in beech forests, the consequences of the drought and heat of previous years were still very pronounced in 2021. For this reason, many beech trees continued to be felled last year due to infestation by pests and the dangers posed by dying tree components at around 11,000 ha in 2021. With a focus on the northern Upper Rhine Plain, pines have been affected by a complex disease pattern for years. The reported damaged area was 6,200 ha for Southwest Germany. There, drought stress is often decisively exacerbated by chronic mistletoe infestations. In addition, Diplodia shoot dieback played a significant role in the damage incidence. Douglas-fir shows regionally striking vitality losses and mortality on the basis of conspicuously sparse crowns, The invasive Douglas-fir gall midge, which originates from North America, has spread noticeably. In April 2022, plantations show widespread damage due to frost-dryness and physiological reddening of the needles after unexpected intense solar radiation and daily temperature fluctuations nearly up to 20°C on frozen or dry soils. The damp and cool early season of 2021 caused many rare leaf and shoot diseases to become effective. Damage by *Cristulariella depreadans* on maple, *Stromastoseptoria castaneicola* on sweet chestnut and *Mycoshaerella laricina* on larch attracted attention to the surveys. These leaf pathogens have not led to any significant damage reports in the last 10 years. The hornbeam showed damage by bark pathogens such as *Anthostoma decipiens* and *Cryphonectria radicalis* on predisposed sites. The losses for maple caused by infestation of *Cryptostoma corticale* continues to increase. Damages after summer floods in July 2021 occurred mainly due to previous infestations by Armillaria gallica on tested sites. Additionaly slime flux caused by *Phytophthora plurivora* on maple and Phytophthora cambivora beech increase loss of timber on affected sites.

Overall, the vulnerability for many major tree species in the forest areas of Southwest Germany has increased due to the presence of a wide variety of fungal species together with the extreme stresses caused by heat and drought events and must be considered for future silvicultural planning and tree species selections.

Keywords: Drought, Heat, Fungi, beech, maple

REINFFORCE common gardens : crown discoloration, defoliation and mortality response under Atlantic climate gradient from UK to Portugal for 35 forest tree species

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Abstract

With the ongoing climate change, all ecosystems and specially forest will undergo rough conditions. The temperature will globally rise, but the most impactful rise for forest will be the occurrence and intensity of critical climate conditions. We know that trees can endure rough conditions by stopping their metabolism and therefore avoid taking too much damage, but we also know that there is a threshold beyond their resistance. This threshold will be more and more reached in the future and will cause tree damage and lower the resistance to pathogens.

With the present study we aimed at evaluating the sanitary status along a climatic gradient thanks to a large network of arboretum distributed on the European Atlantic coast (from 56.53 to 37.76 latitude). Tested hypotheses are: (i) Is there a link between tree crown damage and climate conditions? (ii) Does every species have the same phenological /sanitary response regarding climate? And (iii) is there one species who does not suffer crown damage whatever climate is?

In order to answer to these questions, we used the REINFFORCE network (with 38 arboretum and 35 species in each site, see https://reinfforce.iefc.net) to perform a field data collection during summers 2019 and 2021. The sanitary campaign focused on 3 main crown parameters: Crown defoliation (%) Crown discoloration (%) Crown mortality (%). We gave a score form 0,5 (low damage – 0-10%) to 4 (high damage – 90-100%) for at least 6*3 trees of each species in each arboretum. Using mixed models with these scores and climatic variable from B4EST downscaling tool, we could evaluate sanitary responses of the 35 REINFFORCE species along the climatic gradient.

The preliminary result seems to highlight that some climatic variables have a strong negative effect for several species (degree days above 5°C) on tree crown damage. Moreover the interaction with tree damage and climate conditions change regarding different species, for example *Quercus suber* crown mortality is higher with high precipitation values and on the opposite Sequoia sempervirens crown mortality is higher with low precipitation values. One more striking result is that the *Cedrus atlantica* seems to have less crown damage in dry condition, putting him on top of the list for climate change adapted species.

Finally this study supported by B4ESt end HOMED EU projects show that good results can be highlighted regarding tree damage influenced by climate variables and open the road to promising studies with more data and more focused on specific agents.

Keywords: Climate change, Sanitary status, Gradient, Tree Crown, REINFFORCE network, pine eucalyptus, oaks, adaptation, tree health

Collateral damages: Military invasions beget biological invasions

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Abstract

War is a key elements of world history and it often involves invasion of enemy territories. Biological invasions are frequently associated with human invasions; there are many examples of species that were either intentionally or accidentally translocated during European colonization of distant lands. Much like colonialism, military activity is intimately associated with the occurrence of biological invasions. In particular, world wars that moved thousands of people, supplies, and foodstuff among continents, have resulted in the global spread of several plant, insect and pathogen species and even vertebrate animals. However, lesser military actions often require huge movements of troops and related material over a short time between distant continents or geographical areas, creating ideal conditions for the accidental transport of species. Here we report several examples of intentional and unintentional introductions of non-native species associated with military operations, including those in which invasive pests were used as weapons. Considering the devastating effect of wars, the introduction of invasive species could be considered minor collateral damage, but many invasions have profound and lasting effects on ecosystems and economies of invaded regions. Regulation of military practices that promote invasions through existing international conventions may not be possible.

Keywords: hitchhiking organisms; invasibility; military invasion; military supply; unregulated pathway of introduction

Biogeographic and taxonomic patterns of historical insect invasions worldwide

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Abstract

Thousands of insect species have invaded regions beyond their native ranges and many of these have had substantial impacts. Here I provide an overview of both taxonomic and biogeographic patterns of insect invasions based on an analysis of a comprehensive database of insect invasions in 11 different world regions. Results indicate that certain insect groups (e.g., certain families such as the Aphididae and certain orders such as the Hemiptera) tend to be over-represented in non-native insect assemblages. Those patterns appear to related to life history traits that either cause those species to become associated with invasion pathways or traits that facilitate the establishment of arriving populations. Furthermore there distinct biogeographic patterns of certain geographic regions tending to host more non-native species than others and this may have to do with features of the habitat that create more ecological niches or socioeconomic patterns that facilitate propagule pressure.

Keywords: biogeography, socioeconomic, pathway, invasibility, propagule pressure

Mechanical damage reduces Dutch elm disease symptomatology in *Ulmus minor* (Mill.) trees

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Abstract

The activation of the defence system caused by insect or herbivore wounds on trees generates a local response that prevents the entry of microbes, but also a systemic response mediated by hormones such as jasmonic and salicylic acids. In the mid-term, anatomical responses, such as the production of protection wood or parenchyma proliferation, block or slow down the spread of microbes. In the present work, we test the hypothesis that mechanical wounds made in adult elms stimulate the defence against the vascular fungus *Ophiostoma novo-ulmi*, the causal agent of Dutch elm disease (DED), through activation of hormonal pathways and anatomical changes. Trees from three *Ulmus minor* (field elm) clones with high (VAD2), moderate (TOTO1.10), and low (MDV1) resistance to DED were slightly wounded in the trunk, and two weeks later half of them were inoculated with *O. novo-ulmi*. These trees were compared with non-wounded trees inoculated with sterile water or *O. novo-ulmi*. A significant reduction of foliage wilting produced by *O. novo-ulmi* was observed in TOTO1.10 and MDV1 clones when these trees were previously wounded. VAD2 trees showed very few DED symptoms as expected. Different hormones were analysed by HPLC-ESI-MS/MS in the three clones 21 days after inoculation. An increase in salicylic acid concentration in response to *O. novo-ulmi* inoculation was seen in TOTO1.10 and MDV1. In addition, in MDV1, a reduction in abscisic acid concentration was observed in response to wounding that was more pronounced in those wounded trees also inoculated with the pathogen. Moreover, anatomical measurements made in these trees one year after the inoculation showed a reduction of mean xylem vessel area in wounded MDV1 trees inoculated with *O. novo-ulmi* compared to unwounded trees, which could hinder pathogen spread. Wounding seemed to promote a priming effect that helped DED-susceptible elms to deal better with DED.

Keywords: Wounding; Priming; Dutch elm disease; Hormones

The Right Chemistry – can host chemicals regulate the capacity of endophytes to antagonize Dutch elm disease pathogens?

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Abstract

Dutch elm disease (DED) is a vascular wilt disease that has killed millions of elm trees (*Ulmus* spp.). The causal agents of DED are fungi in the genus *Ophiostoma*. Measures to control the disease are limited and new, nature-based solutions to protect elms against DED would thus be needed. In earlier in vitro studies, some endophytic fungi isolated from elms were found to antagonize DED pathogens through chemical antagonism or nutritional niche competition, suggesting that these endophytes could have potential as biocontrol agents. We hypothesized that chemical signals from the host influence the pathogens and endophytes differently, potentially changing the outcome of their interaction. To test this, we cultured three *O. novo-ulmi* strains and an antagonistic endophyte (Fusarium sp.) on agar-media amended with water extracts of bark and sapwood of elm or three non-host tree species (oak, ash, or beech). The absorbance of the extracts was scanned (200-400 nm) to study the differences in the chemical profiles. The mycelial cells from different growth media were collected and their capacity to utilize nitrogen-based compounds was studied using phenotype microarrays. The results showed that all three pathogen strains grew fastest on the elm-extract medium whereas their growth on the non-host media was suppressed. In contrast, the growth of the endophyte was less affected by the wood and bark extracts that were rich in phenolic substances. Results from the phenotype microarrays indicate that the nutrient-utilization profiles of the DED pathogens and endophytes overlapped, and the endophyte could utilize a broader range of chemicals than the pathogen. The results suggest that certain chemical traits of the host trees could promote the growth of the antagonistic endophyte species. Better understanding of the importance of host chemical signals for fungal growth can thus open new avenues for promoting tree health using nature-based solutions.

Keywords: Biocontrol, Dutch elm disease, endophytic fungi, antagonism, phenolics

Species diversity and distribution of the genus *Cryphonectria*: hidden diversity and wide distribution in Europe

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Abstract

Members of Cryphonectria (Diaporthales) are known to infest important broadleaf tree genera of Fagaceae and Betulaceae in forests, orchards, and urban areas. In this presentation, we want to report on a knowledge gap in the distribution of neglected Cryphonectria species in Europe. In the shadow of the invasive and highly aggressive *Cryphonectria parasitica* originally from East Asia, *Cryphonectria carpinicola*, *Cryphonectria naterciae*, and *Cryphonectria radicalis* have long been overlooked and little attention has been paid to their study. To create the current European map of these three species, we reviewed the literature and searched in published lists of herbaria and living collections. We also sequenced the ITS barcode of specimens from our living collection from Europe, the Pontic Mountains (Turkey), and the Caucasus (Georgia and Azerbaijan), which contains mainly 10–30-year-old isolates, but some dating back to the early 1950s. Dry specimens from herbaria dating back to the 1980s were generously provided by curators and also barcoded to link both living cultures and dry specimens from herbaria to DNA data. Where necessary, we re-identified historical and geographic rarities and integrated the results into a European distribution map of *Cryphonectria* fungi. This study reveals a previously unappreciated diversity and wide distribution of *Cryphonectria* species in Europe.

Keywords: biodiversity, species distribution, culture collection, pathogen

Genetic Characterization of *Cryphonectria parasitica* and its associated mycoviruses in Cantabria (Northern Spain)

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Abstract

European chestnut (*Castanea sativa*) is an economically significant forest tree grown mainly in Mediterranean forests, including Spain. The tree is endangered by a fungal pathogen, *Cryphonectria parasitica*, that causes chestnut blight. The most efficient way to control *C. parasitica* is by applying biocontrol through a hyperparasite, *Cryphonectria hypovirus* 1 (CHV-1). The virus exploits the hyphal anastomosis of the fungus to spread itself in the host populations. However, the fungal non-self-recognition system restricts the fungi from fusing with other strains belonging to different vegetative compatibility (VC) types, leading to low or no virus spread. Therefore, it is essential to know the VC types in an area to apply biocontrol measures in a region successfully. Thus, the current study aimed to survey previously unexplored populations of *C. parasitica* in Cantabria (Northern Spain) and study the different VC types present in the region. The other aim of the study was to investigate the occurrence of CHV-1 in the region and find additional mycoviruses that might be present in the region. All chestnut growing areas are being sampled to isolate the pathogen and perform the characterization. Preliminary results show a high degree of variability of the fungal isolates. This study would lead to the potential biocontrol of a devastating disease and avoid economic and ecological losses.

Keywords: Mycoviruses, biological Control, chestnut blight, *Castanea sativa*, fungal-viral interactions

Molecular research in Holm oak (*Quercus ilex* L.): why, what for, and how

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Abstract

The research group "" Agroforestry and Plant Biochemistry, Proteomics and Systems Biology"" at the University of Cordoba started, in 2004, a research line focused on the study of the molecular biology of Holm oak (*Quercus ilex* L.). The main objectives have been optimize molecular techniques, mainly omics, as well as generate knowledge about key aspects of its biology focused on the variability and, genetic structure of individuals and populations. Omic techniques such as the transcriptomics, proteomics and metabolomics have been developed in different organs (leaves, embryos, roots and acorns), and in response to biotic (presence of Phytophthora cinnamomi) and abiotic (drought) stresses. Currently, we are advancing in the methodology involved in the development of a first draft of the both genome and methylome of the species. The aforementioned techniques are combined with other morphological, physiological and biochemical ones in the direction of Systems Biology to deepen in the study of variability, biodiversity, seed recalcitrance, traceability, responses to biotic and abiotic stresses associated with the decline syndrome and climate change conditions.

To date, we have a list of putative genetic, epigenetic, transcript, protein and metabolite markers related to inter- and intra-population variability, stress response and phytochemical characterization of acorns. We have identified genes and gene products that are indicators of variability and involved in the response to stresses.

Keywords: Holm oak, *Quercus ilex*, variability, omics approaches, molecular markers, biotic and abiotic stresses

Does perennial decrement of stem diameter occur in live trees? Preliminary results in mixed cork and holm oak woodlands

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Abstract

Stem diameter growth in the living trees refers to the invariably increase in dimension over a given period. However, reversible stem diameter decrease could occur, related to water movement in the vessels within the wood (in a daily basis) and to tree water deficit and depletion of stored water (in a seasonal basis). Recently, perennial decrement of stem diameter size has been related to a tree declining pathway, eventually, resulting in tree death. In this study, in southern Portugal (Ourique), in endangered mixed evergreen cork oak (*Quercus suber* L.) and holm oak (*Quercus rotundifolia* Lam.) woodlands, infested with *Phytophthora cinnamomi* Rand, we compared stem diameter intra-annual growth dynamics of 15-paired holm and cork oak trees for one full growing season, based on band dendrometers recordings. Our focus was to find species-specific stem diameter growth patterns and trends in possible declining pathways. Our results showed that perennial decrement occurs, and it is species-specific. Holm oaks were much more sensitive to water deficit and, on average, decreased continuously their stem diameter by -1 mm.yr-1, with highest decrease rate between December, in the winter previous to the growth year, and March, in the onset of the growth season (-0.5 mm.yr-1), and with a decrease rate of -0.3 mm.yr-1 in the summer (drought) season. In contrast, cork oaks showed a negligible stem diameter decrease rate of -0.1 mm. yr-1, only in winter previous to the growth year. The highest increase of stem diameter, occurred in late spring-early summer season, between May and July (1.4 mm.yr-1), for an annual net stem diameter increase of 2.6 mm.yr-1. These preliminary results revealed that holm oaks can survive during a growing season with a continuous decrease of their stem diameters. In addition, species-specific adaptations to limited soil water availability, possibly interrelated to *P. cinnamomi* infection, would explain the asynchrony in intra-annual growth dynamics.

Keywords: Band dendrometers; Mediterranean oak forests; *Phytophthora cinnamomi* Rand; Tree mortality; Climate change

Phosphite applications to control *Phytophthora* infections of *Quercus ilex* and *Q. suber* seedlings under different climate scenarios and watering regimes

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Abstract

Phosphite has been extensively used for reducing the impact of *Phytophthora* infections on forests and crops. Future climate scenarios might not only affect host-*Phytophthora* interactions but also might influence the efficacy of phosphite for controlling *Phytophthora* attacks. The present study aims to assess the effects of preventive applications of phosphite on the Mediterranean tree species *Quercus ilex* and *Q. suber* infested with *P. cinnamomi* under two climate scenarios: current climate conditions and future climate predictions for 2100. Plants were subjected to either normal irrigation or drought conditions and a heat wave of 15 days was also applied. During the experiment, regular assessments of mortality rates and measurements of gas exchange, chlorophyll fluorescence and content of pigments were performed. At the end of the trial, the root systems of all plants were examined. Mortality of non-infested phosphite-treated plants occurred only under drought conditions in both present and future climate scenarios. Death of phosphite-treated *Q. ilex*-infested plants appeared under both water regimes in the present climate scenario and only under drought conditions in the future climate scenario. Mortality of treated *Q. suber*-infested plants was observed only under drought conditions and the current climate. Under current climate scenarios, both phosphite-treated infested tree species showed a higher content of chlorophyll and higher values of maximum quantum yield of PSII for drought regime, while under future climate scenarios those trends were less prominent. Under both climate scenarios, phosphite-treated infected plants showed significantly increased values of net CO_2 assimilation rate for the drought regime immediately after the heat wave started, although this increase was small. Phosphite treatment was proved to be effective to control *P. cinnamomi* infections of *Q. ilex* and *Q. suber* under future climate scenarios.

Keywords: root pathogen, climate change, disease management, ecophysiology, drough

Phytophthora spp. vs *Fagus sylvatica* in Europe

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Abstract

During the last few decades, we observe decline of a common broadleaved tree species, Fagus sylvatica, in European temperate forests. Initially, this wide spread in Europe tree was considered as being resistant to pathogens and pests, but a recent widespread decline and death of not only juvenile but also mature individuals across Europe and North America caused a great concern. Surveys in natural beech stands and nurseries indicated that the soilborne species *Phytophthora plurivora* and *P. cambivora* are likely driving European beech decline. Recently, it was shown that the genotypes of *P. plurivora* recovered from the environment (soil and water) are more diverse than those found in lesions on trees, suggesting a recruitment from standing variations, and supporting the notion that the aggressive strains may newly evolve. Little is known about *P. cambivora* contribution into the epidemiology. Disease severity likely results from a combination of factors, with the genetic makeup of the host and the pathogen being key determinants for the infections under favorable climatic conditions. In this project, I investigate the molecular epidemiology of *Phytophthora plurivora* and *P. cambivora* by performing whole-genome sequencing of isolates recovered from the soil and from tree lesions. Here I tell about the project and will show preliminary results. Draft genome of both pathogens were published previously (Vetukuri et al. 2018, Feau et al. 2016), here I will present preliminary de novo assembly results of the *P. plurivora* and *P. cambivora* genomes using long-read DNA sequencing technologies (PacBio and Nanopore), Illumina short reads and Hi-C sequencing.

Keywords: Molecular epidemiology, forest pathogen, population genomics

Exploring the infection biology and ecology of *Gymnopus fusipes*, a fungal root rot pathogen of woodland trees

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Abstract

Gymnopus fusipes (syn. *Collybia fusipes*), a basidiomycete fungus commonly found in woodlands across Europe, is a slow growing primary pathogen, with the ability to destroy whole root systems, which has been linked to episodes of oak decline in Europe and the UK. A systematic review of 96 publications revealed that *G. fusipes* is associated with numerous species of tree host, mainly Quercus spp. and is suggested to have a presence across the northern hemisphere, although a lack of molecular work and occasional spurious citation in the small amounts of focused literature makes this unclear. Orange lesions on large central roots are characteristic of *G. fusipes* infection and are often accompanied by white mycelial fans. Above the ground level, typical decline symptoms (such as poor crown condition and presence of fruiting bodies) are not always correlated with infection status, leading to ineffective detection and diagnosis, leaving young and mature trees at higher risk of being wind thrown due to a lack of anchoring roots. To address the paucity of information on the infection biology and ecology of *G. fusipes*, the current study focused on optimisation of culture dependant methods and molecular analyses, with the main aim being to develop a molecular diagnostic to aid in accurate detection and diagnosis of this species in environmental samples. Data revealed that optimal growth of *G. fusipes* occurred after 28 days on half-strength malt extract agar, when incubated at 25 ˚C. DNA extraction methods were optimised, and 5 housekeeping genes were used in an MLSA to create a phylogeny of 20 *G. fusipes* isolates. A qPCR-based diagnostic, targeting the 18SrRNA gene was developed and demonstrated high specificity for *G. fusipes* in infected woody tissue and fruiting bodies. Future work aims to investigate infection at a molecular level by comparing transcriptomes of *G. fusipes* at different stages of infection. The data presented in this study are crucial to understanding the spread, ecology, and infection biology of this important root rot pathogen.

Keywords: *Collybia fusipes*; *Gymnopus fusipes*; Molecular diagnostic; Oak decline; Root rot

Two distinct NRPS-independent siderophore synthetase gene clusters identified in *Armillaria* species

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Abstract

Siderophores are important for ferric iron solubilization, sequestration, transportation, and storage, especially under iron-limiting conditions such as aerobic conditions and in environments with high pH. These low molecular weight protein molecules are produced by either the non-ribosomal peptide synthetases (NRPS)-dependent siderophore pathway or NRPS-independent siderophore (NIS) synthetase pathway. Outcompeting or inhibition of plant pathogens, alteration of host defence mechanisms such as induced systemic resistance, and alteration of plant-fungal interactions have been associated with fungal siderophores. To understand these mechanisms in fungi, studies have been conducted on siderophores synthesised by ascomycetes with limited focus on the basidiomycetes. *Armillaria* (Basidiomycota) includes several species that are pathogens of woody plants and trees important to agriculture, horticulture and forestry. The aim of this study was to investigate the presence of NIS gene cluster(s) in genomes of *Armillaria* species using a comparative genomics approach. Iron-dependent growth and siderophore biosynthesis in strains of selected *Armillaria* spp. in vitro was also evaluated. Two distinct NIS gene clusters were identified in all the genomes. All NIS genes identified putatively encode Type A' NIS synthetases with IucA/IucC and Fhuf-like transporter domains at their N- and C-terminals respectively. The effect of iron on culture growth rate varied among the strains studied. Bioassays using the CAS assay on selected *Armillaria* spp. revealed in vitro siderophore biosynthesis by all strains. The results of this study sheds light on the arsenal of tools that Armillaria species allocate to iron acquisition and/or storage. The information generated from this study may in future aid in developing molecular based methods to control these pathogens.

Keywords: tree pathogens; gene clusters; NIS synthetase; secondary metabolites

Bac-Stop – A multidisciplinary research project to address a complex, emerging decline disease called Acute oak decline, in the UK

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Abstract

Acute oak decline (AOD) is an emerging disease of native oak trees (*Quercus robur* and *Q, petraea*) in the UK. It is characterised by weeping patches on stems of trees usually older than 50 years. In internal live bark tissues, the phloem and sapwood tissues underlying the weeping patches are macerated by a pathobiome of bacteria, in which *Brenneria goodwinii* appears to have a key role. The larval galleries of the native beetle *Agrilus biguttatus* are always associated with the necrotic patches. Many trees die once affected, but some are able to callus over the lesions. It is known that *A. biguttatus* can only attack weakened trees and predisposition is considered an integral part of Decline diseases, especially AOD. As this is a serious disease in the UK that causes death of an iconic, keystone trees species, preventative or remediative management is sought. Since predisposition drivers and a native beetle are involved in the disease, and oak is keystone and iconic, social and ecological considerations are vital to management planning. In this paper we discuss the key research questions we address using multidisciplinary, integrated approaches and share preliminary results of this project.

Keywords: Oak, Decline diseases, AOD, multidisciplinary, integrated research approaches

Exploring values for oak and acceptability of different management options in the face of Acute Oak Decline

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Abstract

In Britain oak is a valued species because of the ecosystem services it provides and its importance in the fabric of British culture. The Bac-Stop (Bacteria: Advancement of Control and Knowledge to Save Threatened Oak and Protect them for Future Generations) project is interdisciplinary and focuses on Acute Oak Decline (AOD) and stopping the spread of pathogenic bacteria that is causing damage and death to native British oak trees. One aspect of the project is exploring the values, knowledge, attitudes and support for various management and surveillance options of AOD by land managers, publics and scientists. A mixed methods approach was undertaken, this included a representative survey of the United Kingdom public (n=6,000), interviews with managers of oak (n=21), and interviews with a sample of scientists participating in three oak related research projects, including Bac-Stop, (n=10).

We found that native oaks were highly valued particularly by land managers involved in managing oak in woodlands and parklands. A distinction could be made between their professional values for oak such as biodiversity, timber, historical importance, and their personal values which highlighted more emotional connections and a love of oaks. However, in the public survey only 6% stated oaks were their favourite tree and only a further 16% agreed that they value oaks more than other trees, whereas 66% valued oaks the same as other trees. The public survey found that the most acceptable methods for managing AOD are improving soils, increasing tree watering, and using native species to feed on or parasitize beetles on at-risk trees.

Management responses to AOD need to be cognisant of different value systems held by a variety of stakeholders. Therefore, understanding the values of a variety of stakeholders and their acceptance of different management options to deal with AOD is important as difficult choices will need to be made concerning the deployment of resources to increase oak resilience.

Keywords: Acute Oak Decline; Values; Acceptability; Stakeholders; Management options

Lessons learned from an international reciprocal sentinel planting project

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Abstract

Prevention and early detection of invasive alien tree pests are the most effective management strategies in mitigating their impact. 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 achieved some level of success by recording three potentially novel or otherwise unreported host-insect associations along with two potentially novel host-fungal associations at the genus level. Fungal species-level identification using multi-gene phylogenies is currently underway to resolve these relationships. However, international collaborations can be quite difficult to implement and manage. We will present and discuss some of the constraints that are likely to confront any group attempting similar projects, ranging from logistical challenges to basic bureaucratic obstacles related to international, inter-institutional communication and fund management.

Keywords: Invasive, alien pests, sentinel planting, risk assessment

First report of Elsinoë scab and shoot malformation disease on *Eucalyptus* in South Africa

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Abstract

A serious new disease on *Eucalyptus* was detected in plantations in the KwaZulu-Natal Province of South Africa during the summer of 2021/2022. Initial symptoms of the disease are scab-like spots on leaves, petioles and young branches. On highly susceptible *Eucalyptus* genotypes, leaves and shoots may die. Shoot and leaf malformation leads to a “feathering” appearance in the canopies of heavily affected trees. Samples were collected from symptomatic trees and isolations made directly from developing spots and scabs onto malt-extract agar containing Streptomycin. Resulting pure cultures were identified based on phylogenetic analysis of DNA sequence data for the ITS region. Based on these results, the fungus was identified as a species of *Elsinoë* (Elsinoaceae, Myriangiales), closely related to but distinct from the recently described *Elsinoë necatrix* that causes a serious scab and shoot malformation disease on *Eucalyptus* in Indonesia. This is the first report of a scab and shoot malformation disease on *Eucalyptus* outside of Indonesia. Field surveys of diseased trees have shown significant variation in the susceptibility of different Eucalyptus genotypes. Currently, the most severely affected genotypes include a *Eucalyptus grandis* x *nitens* hybrid variety and *E. grandis*. The disease has also been observed on *Eucalyptus grandis* x *urophylla* varieties and very mild infections on *E. dunnii*. The most severely affected variety has displayed stunted growth and tree mortality could follow if multiple seasons of infection occur. Studies are underway to develop management strategies for this new disease including selection of tolerant Eucalyptus genotypes as well as identification of climatic zones where the disease might be avoided.

Keywords: Eucalyptus scab disease, shoot malformation

Saproxylic beetle diversity in dead wood is lower in non-native than native tree species

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Abstract

Non-native tree species are widely used in forest plantations. This may have negative consequences for biodiversity. Hitherto, most studies have compared species diversity between native and non-native forest stands, which makes it difficult to separate the impact of tree species per se from stand characteristics. Our study, conducted in the south of Sweden, compares saproxylic beetle diversity across different nutritional groups, in dead wood of two native and four non-native tree species after one and three seasons. Mean species richness per log was lower in non-native (lodgepole pine: 10.7, Sitka spruce: 8.5, Douglas fir: 7.1, Japanese larch: 9.4) than in native tree species (Norway spruce: 12.0; Scots pine: 12.3). The difference in species composition among tree species was strongly driven by bark and wood consumers in the first season, while for predators and fungivores, the differences were smaller. Species composition differed more after one season. Dissimilarity in beetle species composition was positively correlated with phylogenetic distances between the tree species. Species richness was lower in non-native tree species that are only remotely related to native trees species. Of the studied non-native tree species, lodgepole pine was more closely related to native tree species and consistently harboured higher species richness. Although non-native tree species also harbour saproxylic beetle communities, the use of non-native tree species, especially those only remotely related to native tree species, reduces local diversity of saproxylic beetles. Thus, for biodiversity conservation, an extensive use of non-native tree species is not recommended as this increases the risk of losing forest biodiversity, especially when they are only distantly related to native tree species.

Keywords: Exotic tree species, *Larix*, Phylogeny, *Pinus*, *Picea*, *Pseudotsuga*, Saproxylic beetles, Species richness

Annual flight activity of *Dioryctria mendacella* (Lepidoptera; Pyralidae) in southern Portugal

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Abstract

The pine cone moth, *Dioryctria mendacella*, is one of the most important pests affecting cones of Mediterranean pines in southern Europe. The larvae cause damages to cone tissues and seeds resulting in deformations, abnormal growth, and abortion. Despite its pest status, the biology of *D. mendacella* is largely unknown, namely the annual flight activity of the adults.

We studied the seasonal flight activity of *D. mendacella* in Canha (southern Portugal) using traps baited with the sexual pheromone. Fifteen white plastic Delta traps were placed on the lower branches (3-4 m above ground) of grafted *P. pinea* trees distant ≈20 meters apart. Pheromones were changed every four weeks during one year, from 18 March 2021 to 24 March 2022, and insects were collected weekly.

We captured a total of 3.555 individuals during a prolonged flight period from February to early December. Initial captures were relatively low but subsequently increased during the summer months, peaking in August and September. The highest catches occurred on late September when 332 males were caught during a single week. In the autumn, captures decreased sharply until ceasing in early December. Absence of captures occurred during 11 weeks of the year, from early December to late February. All specimens were identified as *D. mendaccella*, and by-catch captures consisted mainly of flies (Diptera).

Overall, we found that the Delta traps baited with the pheromone were effective and specific in capturing adult moths of *D. mendacella* throughout the year, and our results support the occurrence of two (or more) annual generations for this pest, with overlapping/sister generations possible. The use of traps baited with sex-pheromones offers the possibility of studying in detail the biology and population dynamics of this pest, and monitor its population levels between years and locations, required for integrated pest management strategies that should include silvicultural, mechanical and biological control methods.

Keywords: Flight phenology; Pheromone-baited traps; Pine cone moth; *Pinus pinea*; Portugal

Capture efficiency of pitfall traps based on sampling strategy and the movement of arthropods

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Abstract

A major challenge in ecological field studies is to reliably estimate the population abundance of a species. In the case of insect monitoring, pitfall traps are usually installed to obtain trap counts. Translating these counts accurately to obtain population estimates and compare across field studies is a difficult task, since trap catch patterns depend heavily on many factors, including experimental design and sampling strategy. The issue becomes more complicated if experiments are to be replicated under supposedly controlled conditions. In this talk, our focus is on the spatial arrangement of multiple pitfall traps, the movement behaviour of insects and the subsequent effect on the trap count dynamics. We consider four different types of trap placements that are commonly used, such as: rectangular (grid), transect or nested cross arrangements and traps randomly placed. We model the movement of an insect population over space by simulating the movement path of every individual independently using the Correlated Random Walk (CRW). By investigating trap count patterns for these distinct movement types alongside different trap placements, we present a novel study with a theoretical analysis on the impact of sampling strategy on capture efficiency.

Keywords: Insect monitoring, Pitfall trapping, Random Walk, Sampling strategy, Spatial arrangement

The Fan-trap, an inexpensive, light and scalable insect trap for monitoring surveys and experimental use

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Abstract

Monitoring is an important component of pest management, both to prevent or mitigate outbreaks of native pests, and to check for newly introduced organisms. Monitoring surveys often rely on trap networks, especially when the target species respond to semiochemicals. Many trap models are available for this purpose, but they are bulky in most cases, which raises transportation and deployment issues, and they are relatively expensive, which limits the size and accuracy of any network. To overpass these difficulties, entomologists have often used recycled material, such as modified plastic bottles, producing cheap and reliable traps but at the cost of recurrent handywork, not necessarily possible for all end-users (e.g., for national plant protection organizations). These bottle-traps have allowed very large surveys which would have been impossible with standard commercial traps, and we illustrate this approach with a few examples. Even though individual traps can be easily transported in the field (thirty of them, stacked, fit easily in a backpack), they ship less easily in large numbers, because they are three-dimensional. Here we present, under a Creative Commons license, the blueprint of a foldable model, laser-cut from a sheet of polypropylene, that can rapidly be produced in large numbers, ships easily because it is flat when unfolded, and could be transported and deployed in the field with very little efforts. Our first comparisons show that fan-traps are as efficient as bottle-traps, and we describe two cases where they are being used for monitoring and the assessment of population expansion.

Keywords: Monitoring; Surveys; Traps; Semiochemicals

Will the trees get bored? The social dimensions of managing multiple threats to ash in the British landscape

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Abstract

Ash trees in many parts of Europe are already under threat from ash dieback (ADB) and the additional introduction of Emerald Ash Borer (*Agrilus planipennis* EAB) may signal the end for European ash. Contingency plans across Europe recognise the need to prepare for the arrival of EAB but surveillance for EAB and management of ash by land managers in the context of the already well established ADB is likely to be much more challenging because of the uncertainties surrounding ADB and EAB interactions. In Britain, ADB has had devastating effects on ash trees resulting in significant social, economic and ecological impacts. Policy concerns about how land managers may react and whether wider society is prepared for the drastic action that will be required should EAB reach our shores including, potentially, the complete loss of ash requires urgent research. We present findings from an interdisciplinary project focussing on the uptake of surveillance and management options for EAB. Experience of ADB is likely to affect land managers perceptions of risk and their decision-making regarding management options for EAB but the values that people hold for ash will also influence whether, how and why they will act. Using data from a survey (n=368) and semi-structured interviews (n=43) with managers of ash trees, we explore whether current experiences with ADB will inform future behaviours. For example, survey results reveal that land managers who stated ash trees were personally important to them and expressed concerned that EAB posed a risk to ash across Britain were more likely to participate in surveillance activities. The purpose and location of ash matters and participants who stated that ash trees were important for a 'connection to nature' were less likely to carry out all possible EAB management options. Interviews revealed strong connections to the remaining ash but there is scepticism around the effectiveness of current measures to manage the spread of EAB and whether it is indeed possible to save the remaining European ash.

Keywords: Emerald Ash Borer, Ash Dieback, land managers, values, decision-making

Diversity in *Thekopsora areolata* the causal agent of cherry spruce rust

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Abstract

Cherry spruce rust causes huge yield losses in Norway spruce seed production in Fennoscandia. The causal agent, *Thekopsora areolata*, is a macrocyclic heteroecious fungus with all five spore stages which uses two host plants *Prunus padus* and *Picea abies* to complete its life cycle. In this study, we analyzed the spore dispersal, genotypic diversity and population structure to understand infection and epidemiological properties.

We show that basidiospores, which infect pistillate cones of *Picea* spp., dispersal coincided with multiple rain events during spring. A high genotypic diversity without population structure was found in *T. areolata*, which suggests predominantly sexual reproduction, random mating and high gene flow within and between populations in Fennoscandia. Furthermore, analyses of genotypic diversity within cones and haplotype inference suggest that each pistillate cone is infected by several basidiospores during high disease pressure. In addition, it was shown that genotypic diversity in *T. areolata* varies between years and increase with disease occurrence. The results contribute with new knowledge to the understanding of the infection, colonization, sexual reproduction process and disease epidemiology of *T. areolata* in Norway spruce.

Keywords: Fungal pathogen, Rust fungi, Norway spruce, Population genetics, Seed orchard

Transcriptional responses to different host diets in six-toothed bark beetle, *Ips sexdentatus* (Curculionidae; Scolytinae)

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Abstract

Phytophagous *Ips* spp. can cause extensive damage to their host and exhibit diverse mechanisms to overcome novel host adaptation and specialization. A diet switches are often important for beetles when no or limited preferred host is available. A novel host also provides enemy-free space, which is ecologically advantageous. It is interesting to evaluate the impact of diet switches on the gut gene expression in bark beetles. We investigated the effects of host switch (Pine to Norway spruce) responses to *Ips sexdentatus*. Growth rate and survival were increased when beetles were reared exclusively on spruce compared to pine. However, based on comparative gene expression analysis, >1000 differentially expressed genes (DEGs) were identified in spruce-feeding beetle gut, including genes related to digestion, detoxification, transporter activity, growth, signaling, and stress response. However, only 299 DEGs were identified in F0 Pine vs F1 Pine fed beetles comparison, signifying expression differences between generations. The genes encoding transporters, Multidrug resistance protein 4 (MRP) and trehalose transporter (TRET), were induced by spruce feeding, suggesting that this could play a role in pumping a wide variety of endogenous and xenobiotic compounds (host chemicals) and antioxidants against stresses. In other cases, UDP-glucuronosyltransferases (UGTs) gene over-expression was maintained in spruce-fed beetles. RT-qPCR and enzymatic assays further validated our findings. In conclusion, transcriptional plasticity in genes related to digestion, detoxification, transporter activity, and stress response plays a crucial role in the diet switch responses of the *I. sexdentatus* and provides vital clues for beetle adaptive potential on a different host.

Keywords: *Ips*, diet switch, differential gene expression (DGE), Detoxification, bark beetles, host adaptation

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

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Abstract

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.

Keywords: Fungal epidemic, Pine resin top disease, *Pinus sylvestris*

Hormonal profiling reveals novel insight into pine defence against the biotrophic rust fungus, *Cronartium*

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Abstract

Plant immune responses to pathogens are regulated by key defense hormones that include salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). The activation of defense signalling pathways mediated by hormones in the host depends on the lifestyle of the pathogen. Generally, an SA-dependent signalling network is activated in response to biotrophs while the network regulated by JA and ET is activated following invasion by necrotrophs.

Cronartium harknessii (J. P. Moore) E. Meinecke, is a rust pathogen found across much of North America that causes western gall rust. Attack by *C. harknessii* in pines leads to development of galls on stems and branches, and subsequent distortion in growth. Jack pine (*Pinus banksiana* A. B. Lambert) and lodgepole pine (*Pinus contorta* Douglas ex Loudon var. *latifolia* Engelm.), exhibit quantitative resistance to *C. harknessii*, with jack pine exhibiting relatively higher resistance than lodgepole pine. Understanding intra- and interspecies molecular mechanisms for resistance against *C. harknessii* in lodgepole and jack pines is relevant for effective resistance breeding. In this study, we examine hormone responses in quantitively resistant (QR) and quantitatively susceptible (QS) families of lodgepole and jack pine following inoculation with *C. harknessii*. Given that rust fungi are generally considered to be obligate biotrophs, we hypothesized that hosts would exhibit increased SA upon *C. harknessii* challenge. SA and JA as well as their derivatives were quantified using high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS). Contrary to our hypothesis, the biotrophic-associated SA-mediated signalling network was not invoked in response to the fungus. However, JA was significantly induced following inoculation with *C. harknessii*. Furthermore, we observed a significant increase in JA in QS lodgepole pine compared to QR jack pine. Considering the marked difference in JA between QS lodgepole and QR jack pine, and that jack pine is relatively more resistant than lodgepole, our findings raise the possibility that JA is been induced by *C. harknessii* to suppress the host defense.

Our result provides new insight for genomic studies in identifying the genetic mechanism for resistance against *C. harknessii* in pines, and also sheds more light on the role of JA in conifer defense against biotrophs.

Keywords: Jack pine, lodgepole pine, *Cronartium harknesii*, salicylic acid, jasmonic aci

Transitions of foliar mycobiota community and transcriptome in response to pathogenic conifer needle interactions

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Abstract

Profiling the host-mycobiota interactions in healthy vs. diseased forest ecosystems helps understand the dynamics of understudied yet increasingly important threats to forest health that are emerging due to climate change. We analyzed the structural and functional changes of the mycobiota and the responses of *Pinus contorta* in the *Lophodermella* needle cast pathosystem through metabarcoding and metatranscriptomics. When needles transitioned from asymptomatic to symptomatic, dysbiosis of the mycobiota occurred, but with an enrichment of *Lophodermella* pathogens. Many pathogenicity-related genes were highly expressed by the mycobiota at the necrotrophic phase, showing an active pathogen response that are absent in asymptomatic needles. This study also revealed that *Lophodermella* spp. are members of a healthy needle mycobiota that have a latent lifestyles suggesting that other pine needle pathogens may have similar biology. Interestingly, *Pinus contorta* upregulated defense genes in healthy needles, indicating response to fungal recognition, while a variety of biotic and abiotic stresses genes were activated in diseased needles. Further investigation to elucidate the possible antagonistic interplay of other biotic members leading to disease progression and/or suppression is warranted. This study provides insights into microbial interactions in non-model pathosystems and contributes to the development of new forest management strategies against emerging latent pathogens.

Keywords: biotrophic, dysbiosis, foliar pathogen, *Pinus contorta*, plant defense

Metabarcoding to inoculation: diagnosing emerging needle diseases of loblolly pine (*Pinus taeda*)

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Abstract

Needle pathogens of conifers cause disease by infecting foliage, resulting in the death or premature abscission of needles. The loss of foliage reduces growth and vigor, and repeated defoliation may result in mortality. Since 2016, public and private forest managers in the southeast United States have reported increasing frequency and severity of needle disease outbreaks on the region's principal timber species, loblolly pine (*Pinus taeda*). Pine forest managers have voiced concern over the scale of recent outbreaks, as needle diseases are not traditionally considered a threat to loblolly pine. The causal agent of these outbreaks, however, is not yet known. In this research, loblolly pine foliage was collected from affected stands and analyzed to identify fungi associated with symptoms of needle disease. We employed targeted molecular diagnostics and DNA metabarcoding in parallel to isolating possible pathogens in culture. *Lecanosticta acicola*, the native causal agent of brown-spot needle blight, was detected among symptomatic needles from western portions of the loblolly pine range, including Louisiana, Mississippi, Alabama, and Arkansas, but not from stands in eastern states. Fungal ITS1 metabarcoding revealed multiple needle pathogens in symptomatic needles, which differed by state, and identified associations between opportunistic parasitic fungi and symptom presentation. Isolates of multiple possible pathogens, including *L. acicola*, *Lophodermium* spp., and *Rhizosphaera* spp. were retained for future pathogenicity trials using multiple families of loblolly pine and slash pine (*P. elliottii*). The results of this work will provide insight into which fungal pathogens may be driving the recent outbreaks of needle disease on loblolly pine and guide future work to develop management strategies.

Keywords: needle blight; needlecast; planted forest; emerging disease

Genotype impact on the response of *Picea abies* to the artificial inoculation of H*eterobasidion parviporum*

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Abstract

Norway spruce (*Picea abies*) is one of the most economically important tree species in Europe. However, it is highly susceptible to root rot fungi *Heterobasidion parviporum*. The projected climate change will benefit the pathogen as the tree host is weakened, e.g., by extended drought. Resistance breeding has been established to improve forest health, and there are several root rot genetic markers suggested for the resistance of Norway spruce. This study aimed at comparing the resistance (here defined as necrosis length) of Norway spruce families and genotypes against two different strains of *H. parviporum* under drought conditions. We inoculated 444 trees with two different strains of *H. parviporum*; 222 plants were inoculated as mock control, while 134 plants remained non-treated. The experiment lasted for twenty-eight weeks, and during this period, half of the trees were exposed to sixteen-weeks drought conditions, after which they were harvested. The visible necrotic lesion length and width in phloem and sapwood were measured. Our results show that the family (and genotype within) has an impact on the necrosis length, which is related to the aggressiveness of the fungal strains. Under drought conditions, the necrosis increased only in horizontal directions in phloem and sapwood. Similarly, the growth was not disturbed by abiotic stress, indicating that the stress level (drought) was too low in this setting. The knowledge gained in this study could improve forest health in the changing world by understanding the response of Norway spruce to pathogenic attacks under additional stress at the family level. This understanding could be strategically used in forest breeding to improve the resistance of Norway spruce trees against root rot.

Keywords: drought stress; fungus-host relationship; genotypic variation; necrosis

A field-ready LAMP assay to detect *Fusarium circinatum* from host tissue

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Abstract

The pine pitch canker pathogen *Fusarium circinatum* is endemic to the Southeast United States and Mexico. The fungus has been inadvertently introduced into various countries of the world and represents a growing threat to Pinus spp. in natural and planted forests globally. Pine pitch canker causes mortality of seedlings in nursery beds and results in canker on established trees resulting in a decline in forest health and productivity. While the disease causes characteristic canker symptoms with resin bleeding and flagging on mature trees, infected planting stock can remain asymptomatic until planted in the field. For this reason, rapid and accurate molecular diagnostic tools are needed to screen for the presence of the pathogen on-site, at ports of entry, in nurseries, and in planted forests. In this study, a field-ready molecular test was designed to detect *F. circinatum* DNA using Loop-mediated isothermal AMPlification (LAMP). LAMP primers and a fluorescent assimilating probe were designed to amplify a target region that was identified in *F. circinatum* whole genome sequences as unique to the pathogen. The assay was demonstrated to detect *F. circinatum* DNA from an extensive culture collection, which represents the genetic diversity present throughout its expanding range. The test is rapid and sensitive, amplifying target DNA in as little as 12 minutes and from as few as ten cells. The assay can be readily deployed in the field, as it may be coupled with a simple, pipette-free DNA extraction technique and a portable, battery-powered instrument. This crude DNA extraction method was shown to effectively process infected host tissue samples for amplification by LAMP. The tools developed in this research can now be integrated into diagnostic and surveillance programs and they represent an opportunity to reduce the global spread and impact of pitch canker.

Keywords: Point-of-care diagnostics; crude extraction

RNAi: An emerging approach to control pine pitch canker caused by *Fusarium circinatum*

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Abstract

Fusarium circinatum is an invasive ascomycete fungus that causes pine pitch canker (PPC) disease in Pinus and other coniferous tree species. The PPC pathogen is a ubiquitous forest pathogen of global significance due to high socio-economic impacts. Injudicious use of chemicals to combat PPC does not only leave the environmental residual effects, but it also induces fungicide-resistant pathogenic strains. In our present studies, we demonstrate the use of RNAi (RNA interference) to control the pathogen. RNAi is a mechanism that is induced by double-stranded RNA (dsRNA) in which expression of gene is inhibited or silenced through the degradation of mRNA. Due to the sequence - specific knockdown of targeted genes followed by delivery of dsRNA, RNAi offers an alternative biological control method to chemical pesticides without affecting non-targets. The uptake of exogenous dsRNAs targeting essential genes by fungi can lead to reduction in pathogenicity of fungus through foliar application of dsRNA to plant surface, thus focusing on the use the Spray induced gene silencing (SIGS) in our present studies. SIGS is accomplished by the production of microbial-based double-stranded RNA (dsRNA) of genes of interest using an inducible plasmid for the dsRNA production in the HT115 *Escherichia coli* strain which is deficient in RNaseIII that allows dsRNA molecule preservation. For a quick effectivity test of our pool of dsRNA molecules that target several fungal essential genes, dsRNA was applied to Spinach and *Arabidopsis* detached leaves. Then, we selected the dsRNA molecules with the best inhibition of fungal growth that causes weaker infection symptoms on the leaves to be use in SIGS on *Pinus radiata* seedlings. The application of the selected dsRNA molecules on the pine seedlings resulted in the inhibition of fungal growth. Hence, the studies illustrated that the use of target-specific dsRNA through SIGS is an eco-friendly and sustainable method for management of PPC pathogen.

Keywords: Microbial-based dsRNAs (double-stranded RNAs) production; PPC (Pine Pitch Canker); RNAi (RNA interference); SIGS (Spray-induced gene silencing).

Laminated root rot alters community composition but not forest productivity in the Pacific Northwest, USA

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Abstract

Laminated root rot (LRR; caused by *Coniferiporia sulphurascens*) is the most damaging root disease of Douglas-fir (PSME, *Pseudotsuga menziesii*) in the Pacific Northwest USA and southwestern Canada. LRR influences on tree mortality and forest productivity have been well-documented in young commercial Douglas-fir forests; however, much less is known about the role of LRR in forest dynamics of older stands (>50 years). The objective of this study was to improve fundamental understanding of the role that LRR plays on forest community composition and forest productivity in Douglas-fir forests of the Pacific Northwest using long-term permanent forest demography plots that are part of a research plot network in NW Oregon and SW Washington State, USA (https://pnwpsp.forestry.oregonstate.edu/). Tree diameter and mortality measurements were made (5-10 year intervals) at 16 plots among five sites established between 1910-1930 in western Oregon and Washington, USA. Each plot was surveyed to assess dead trees for evidence of LRR, LRR-suspected dead trees were cultured, and fungal isolates were identified using DNA sequencing method in 2019-2021. The LRR pathogen was confirmed in 13 of 16 plots. The preliminary analyses showed that elevated LRR incidence resulted in 1) greater PSME tree density decreases; 2) reduced aboveground live biomass (AGB) accumulation in PSME and increased AGB accumulation in other species, with a 20% reduction in PSME AGB-weighted dominance; and 3) no changes in ecosystem AGB accumulation rates. These results imply that LRR may accelerate late-seral species establishment, growth, and thus replacement of PSME through forest succession, but may not alter overall AGB dynamics from 50 to 150 years in stand age. This research will provide information to natural resource managers about LRR's influence in second-growth, mature, and old-growth forests of Oregon and Washington, USA and provides further testament to the need for long-term forest demography plots.

Keywords: *Coniferiporia sulphurascens*, *Pseudotuga menziesii*, root rot pathogen, forest composition, forest productivity

Enhanced virulence of Diplodia tip blight pathogen effected by host switch poses threat to conifer

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Abstract

While climate change is causing stress to most forests in Europe through increased temperatures, reduced precipitation and adverse weather conditions, conifer trees' defense systems are weakened and thus more susceptible to pests and pathogens. One of the major causal agents of shoot blight in conifers, and especially Scots pine (*Pinus sylvestris*), is the *Diplodia* tip blight fungus *Diplodia sapinea* (≡ *Sphaeropsis sapinea*). Instead of remaining in its endophytic life stage, the warm-loving fungus emerges through Europe further north, switches to its pathogenic stage and thus causes disease outbreaks in new areas. Additionally, the host spectrum is expanding: more often symptomatic broad-leaved tree species are also confirmed to be the hosts of *D. sapinea*. The virulence of *D. sapinea* isolates from symptomatic, non-symptomatic and non-pine hosts was tested in a greenhouse study on 3-year-old Scots pines under normal and drought conditions. Isolates that had undergone a host switch caused the highest necrosis, indicating that this resulted in the increased virulence of these strains – a scenario that can increase in the near future and lead to more damages in conifer-dominated forests. In order to track the movement of the fungus and the population genetic structure, newly developed microsatellite (simple sequence repeat)-markers were amplified for *D. sapinea* populations collected from Germany and Finland. The genetic diversity of *D. sapinea* in these populations was characterized by the number of alleles and haplotypes as well as the clonal fraction and genetic structure. The study revealed 13 haplotypes from 28 samples from three German populations and eight different haplotypes from 23 samples from southern Finland. Furthermore, three haplotypes were shared between the German and the Finnish populations. The high levels of genetic diversity and especially the high number of private haplotypes most likely indicate both: that *D. sapinea* has lived endophytically in Finland already for a long time and that it might have been introduced multiple times. Additional sampling from neighbouring countries is needed to validate these hypotheses. The results of our studies highlight the importance of new research on this pathosystem at the continental level to develop new measures to hinder further outbreaks of Diplodia tip blight.

Keywords: *Diplodia sapinea*; emerging pathogens; host stress by drought; microsatellite (SSR) markers; *Sphaeropsis sapinea*

Posters

Effects of an *Eucalyptus*-based herbicide on symbiotic microbial communities of agricultural soils

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Abstract

Eucalyptus globulus is a highly inflammable invasive species, widely spread in Portuguese forests. Although the replacement of eucalyptus with native plant species is of great importance, several economic, social, and political factors cause difficulties in its implementation. For this reason, alternative solutions to reduce the spontaneous expansion of eucalyptus area in Portugal are needed. Eucalyptus leaves have phytotoxic potential and their use as an environmentally friendly herbicide could help to reduce the eucalypt biomass in forests. For this reason, an eco-friendly herbicide, based on eucalyptus sprouts (EucaBio_solid), was created and its impact on microbial communities of agricultural soils was evaluated. For that, different concentrations of EucaBio_solid (0%, 2.5%, 5%, and 10%; w/v) were used in pot assays filled with agricultural soils. Soil samples were collected before oat seed sowing and during plant development. DNA extraction was performed for these soil samples and microbial communities (fungi and bacteria) were analyzed by high-throughput sequencing using Illumina platform. The effect of EucaBio_solid on the microbial community was evaluated considering the different concentrations applied to the soil. Also, different concentrations of the same eco-friendly herbicide in the liquid format (EucaBio_liquid) were used to evaluate antimicrobial activity. A zone inhibition test was performed, in vitro, using different species of bacteria and fungi. At the end, this work helps to validate EucaBio_solid as a sustainable alternative to synthetic herbicides.

Keywords: Eco-friendly; *Eucalyptus globulus*; herbicide; microorganisms

Characterisation of Hornbeam Decline in England (UK)

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Abstract

In recent years there have been several reports of declining common hornbeam trees (Carpinus betulus) in Europe (Italy, Austria, Switzerland, Germany and Georgia) as well as Iran. Decline is characterised by cankers on the trunk and branches of hornbeam trees, which have been attributed to two causal fungi, *Anthostoma decipiens* and *Cryphonectria carpinicola*. The latter was described as a new species in 2021 and pathogenicity tests confirmed that C. betulus is a major host species for *C. carpinicola*. In 2022, Forest Research pathology teams were alerted to possible cases of hornbeam decline in the England (UK). Isolation of fungi from diseased branches, sequencing of barcoding loci (ITS, TUB, LSU, ACT, EF) and multigene phylogenies confirmed the presence of different fungal species: *C. carpinicola*, *Melanconiella spodiaea* and novel species assigned to the genus *Cytospora*. This is the first report of *C. carpinicola* in Britain, *M. spodiaeais* known in the UK and is the causal agent of leaf spot in hornbeam, and the novel *Cytospora* sp. needs further work to be described. Ongoing pathogenicity trials on hornbeam saplings with individual and combinations of these fungi will reveal insights into their role in the decline of hornbeam trees in England.

Keywords: *Cryphonectria carpinicola*, *Anthostoma decipiens*, *Cytospora*

Evaluation of the damping effect of trees on the thermal sensation according to their distribution and placement in an urban area

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Abstract

Over-urbanisation and continued population growth reduce the capacity of trees to provide ecosystem services, including climate regulation. As a consequence, many of the surrounding areas suffer from high levels of wind chill. This research work focuses on the evaluation and analysis of the distribution and placement of trees to reduce the thermal sensation in the residential area "Unidad Vecinal 3" in the district of Cercado de Lima, Peru. To carry out the study, changes in thermal sensation were modeled using ENVI-met software, determining the distribution of this variable in areas with different tree distributions. Then, a field evaluation was carried out to contrast the data. Thermocouples were installed in different buildings located in the study area. The results are related to the link that exists between the damping effect of trees concerning variations in the ambient temperature of Lima, Peru.

Keywords: ENVI-met, thermal sensation, positioning, cushioning effect, distribution

Screening of eucalyptus clones for resistance to gall wasp (*Leptocybe invasa*)

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Abstract

Eucalyptus is the most widely planted exotic species in the tropics, and play important roles in reforestation and production of timber, pulp, potential bioenergy feedstock, and other forest products. The gall wasp, *Leptocybe invasa* (Hymenoptera: Eulophidae), native from Australia, is a worldwide pest in *Eucalyptus* plantations. *L. invasa* was introduced in Brazil in 2008, where it exhibits the behavior of endophytic oviposition, inducing the formation of galls on leaves and petioles of eucalyptus trees, with increased frequency of leaf distortion, drying, and defoliation of new branches. The existence of native and exotic parasitoids in planted *Eucalyptus* forests, as well as the introduction of exotic parasitoids, have shown promise for the biological control of *L. invasa*. However, the most effective control method widely used in commercial *Eucalyptus* planting areas is the use of resistant genotypes. Therefore, the objective of the present study was to evaluate the resistance of *Eucalyptus* spp. clones to *L. invasa*. The selection was carried out in field, in Açailândia (Maranhão State), Brazil. Resistance to *L. invasa* was evaluated in 617 clones of *Eucalyptus* spp. The the experimental network was in a randomized block design (DBC) with 6 plants per plot and four replications (blocks), spaced 3.0 × 3.0 m. The evaluations took place monthly until the planting reached 1 year of age, evaluating the four central plants of each treatment. For the border, two clones susceptible to the gall wasp were used planting in double lines between the blocks and in a quadruple line around the contour of the experimental area. As controls, the same four wasp-resistant and four wasp-susceptible clones were used in each test. The following parameters were evaluated: number of galls per plant; size of galls; predominant position of galls; loss of apical dominance; and emergency hole. Based on these parameters, the clones were classified as resistant or susceptible to gall wasp. 78% of the clones evaluated were classified as resistant (R), 14% were susceptible (S), and 8% were moderately R/S. The high number of resistant clones found in this work suggest that the genetic basis is formed by key eucalyptus species in relation to gall wasp resistance, and therefore, there is potential for selection and planting of resistant materials in the short term and to increase resistance through breeding to reduce the impact of this insect over the long-term.

Keywords: Phenotyping, field test, eucalyptus, pest, integrated pest management

Fungal communities associated with nursery-grown *Pinus sylvestris*: implications for disease management

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Abstract

In forest nurseries, intensive management practices (e.g., extensive monocultures, dense cultivation, shortage of beneficial organisms) may often stress tree seedlings into a predisposition to fungal diseases. Fungal pathogens are often controlled using various management methods, including cultural practice and fungicidal applications. Knowledge of fungal communities would improve preventative and directed control methods, and ultimately reduce the use of chemical treatments. The aim of this study was to establish occurrences and shifts within local fungal communities, and to identify common pathogens associated with *Pinus sylvestris* seedlings in Swedish forest nurseries. Four microbial treatments were used to investigate their effects on seedling growth and disease control. Treatment and sampling of symptomless needles was conducted at four forest nurseries every third week during the growing season. Diseased seedlings were also collected, and the pathogen was then morphologically identified. DNA was extracted from collected needles, followed by amplification and high-throughput sequencing of the ITS2 region. Results showed distinct differences in fungal communities between northern and southern nurseries as well as significant changes in fungal community composition over time. The fungal pathogens *Cladosporium* sp. (15.1%), *Phoma herbarum* (14.5%), *Alternaria alternata* (5,5%), and *Botrytis cinerea* (5.4%) were among the most abundant fungal species. A low number of disease incidents were reported (infection rate < 0.6%). However, a few seedlings infected with *Diplodia sapinea* were reported during the study period. Microbial treatments were found to have neither a positive nor a negative impact on seedling growth or survival. Ultimately, several distinct fungal pathogens were identified, and fungal communities were shown to become more homogeneous throughout time. This information will be useful to improve disease control strategies in forest nurseries.

Keywords: Community composition, Fungal pathogens, Metabarcoding, *Pinus sylvestris*, Tree seedlings

Diversity assessment of Aquatic Beetles (Dytiscidae) along the Altitudinal Gradient and its relationship with the water physiochemical parameters at Tashiding, Tshendagang and Gozhi Geogs, Dagana

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Abstract

As all other aquatic beetles Dytiscidae play a vital role in the ecosystem as they purify water and serve as the prey for many other organisms. This family of aquatic beetle is a predator, which feeds on small organisms such as tadpoles, worms or fingerlings. They themselves are also the food to the fishes. They are sensitive to the environmental change. Therefore, they are considered as the indicator of biodiversity and conservation assessment. The study on diversity of Dytiscidae and its relationship with physiochemical parameters of water applied the stratified random sampling method. This study assessed the diversity of Dytiscidae in three Geogs in Dagana along the altitudinal gradient. The specific objective of the study was to determine the habitats used by different genera recorded. Furthermore, the relationship of Dytiscid diversity and taxon richness with the physiochemical parameters of the lentic and the lotic water bodies along the altitudinal gradient were determined. Specimens were collected in lotic (stream) and lentic (marshy area and pond) systems in the month of February 2021. In total 664 individuals were collected and five subfamilies of Dytiscidae, 12 genera and 17 species were identified. Pearson's correlation showed moderately negative correlation of diversity and taxon richness with altitude, temperature, resistivity and pH ($p < 0.05$). Canonical correspondence analysis (CCA) found temperature as an effective determining factor in distribution of Dytiscidae. Kruskal Walllis test among different habitats and altitudinal strata showed significant difference in Dytiscids diversity and taxon richness ($p < 0.05$). The Diversity and richness showed a decreasing trend as the altitude increases. The highest diversity was found in marshy area ($H = 2.36$) and second altitude stratum [800 – 1200 masl] ($H = 2.07$) in the current study area. Therefore, the best conservation strategy of Dytiscidae could be to protect these areas before they are degraded.

Keywords: Coleoptera, diversity, Dytiscidae, elevation, physiochemical parameter.

Rapid and reliable molecular detection of *Diplodia sapinea* on pine trees

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Abstract

The Ascomycete *Diplodia sapinea*, the causal agent of shoot tip dieback of pine trees, is one of the major wordwide spread plant pathogen. In the last years, as a consequence of global warming, this pathogen is widely spreading in northern Europe. The fungus can persist asymptomatically for long periods of time in the host plants, becoming pathogen as environmental condition turn more favorable. In this study we are presenting two sensitive molecular methods (qLAMP and qPCR) to detect fungal pathogens in DNA from pure mycelium and from naturally infected woody plant tissues. Methods were designed on the mating type genes using specific probes and primers with high specificity and sensitivity, no cross-reactivity with other species of Botryosphaeriaceae family was ever observed. qLAMP and qPCR assays are useful tools for a rapid and sensitive detection of D. sapinea in plant material and for routine phytosanitary inspections.

Keywords: qLAMP; qPCR; fungal pathogen; *Pinus*

Effects of wind disturbance management practices on lower stem and root feeding beetles of longleaf pine ecosystems

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Abstract

Hurricane Michael made landfall in Northwest Florida in October 2018, devastating forests, timberland, agricultural land, and infrastructures as it moved northeastward across the southeastern USA. Adding to the massive initial damage, another long-term effect that concerned forest landowners was the large number of weakened plants and amount of coarse woody debris left behind, which created an optimal environment for secondary forest pests, such as lower stem and root feeding beetles. This project aimed to better understand the impacts of different management strategies implemented after hurricane damage on lower stem and root feeding beetles of longleaf pine ecosystems. In 2019, we established a research project at the Jones Center at Ichauway, a restored longleaf pine ecosystem in southwest Georgia that was directly impacted by Hurricane Michael. We sampled 15 plots within sites managed using three silvicultural approaches in response to the hurricane: (i) no salvage logging and delayed prescribed burning in Spring of 2020, 17 to 19 months after the hurricane; (ii) no salvage logging but prescribed burning in Spring of 2019; and (iii) salvage logging immediately following the hurricane and prescribed burning in Spring of 2019. For the two years following Hurricane Michael, we characterized the seasonal patterns of flight activity of beetles within the genera *Dendroctonus*, *Hylastes*, *Hylobius*, and *Pachylobius* within each plot and found that while beetle abundance varied across seasons in both years, silvicultural treatment affected abundance only in the second year, and only for some of the species. We also quantified the number of propagules of ophiostomatoid fungi vectored by *H. pales* and *P. picivorus* during their flight peak in Fall 2020 and found that treatment did not affect their numbers. Our results suggest that lower stem and root feeding beetles or their symbiotic fungi are not overall affected by management strategies to reduce coarse woody debris.

Keywords: Blue-stain fungi; Flight activity; Hurricane Michael; *Pinus palustris*; Root-feeding beetles

Leaf spot and anthracnose diseases of pedunculate oak (*Quercus robur* L.) in the Mura-Drava-Danube Biosphere Reserve

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Abstract

The Mura-Drava-Danube UNESCO Biosphere Reserve represents one of the best-preserved wetlands in the Danube River basin area. It is among the largest riverine protected areas in Europe; hence it was named the "Amazon of Europe". Recently, in the Interreg Europe project “Resilient riparian forests as ecological corridors in the Mura-Drava-Danube Biosphere Reserve” (REFOCuS) the spread of emerging pests and diseases was assessed among other activities in the Biosphere Reserve. Hence, in summer of 2019 leaves of *Quercus robur* with symptoms such as leaf spots and anthracnose were collected in three countries, i.e., Serbia, Slovenia, and Austria. The aim of this study was to identify the causal agents of the observed leaf diseases. Fungal cultures were obtained from symptomatic leaves and identified using morphological characteristics and phylogeny of the ITS rDNA, TEF 1-alpha, β-tub2, LSU, ACT, RPB2, and CAL genes. The fungi were identified as *Tubakia dryina*, *Tubakia dryinoides*, *Didymella macrostoma*, and *Apiognomonia errabunda*. Pathogenicity tests were conducted using 1-year old *Q. robur* plants in the greenhouse and results revealed that the isolated fungi caused symptoms of leaf spots and anthracnose on inoculated leaves, like those seen in the field. To our knowledge, this study represents the first report of *Tubakia* leaf spot disease caused by *T. dryina* in Serbia and the first report of *T. dryinoides* as pathogen of *Q. robur* in Slovenia, Austria, and the world. Moreover, it is the first finding of *D. macrostoma* causing leaf spot disease on *Q. robur* anywhere in the world. The study also confirmed that leaf anthracnose of *Q. robur* in Slovenia and Austria is caused by *A. errabunda*. Leaf diseases can reduce the vigor of trees and make them susceptible to other pathogens and insect pests. Therefore, the results of this study represent valuable information to provide management guidelines to boost riparian forest resilience and counteract forest decline.

Keywords: Riparian forests, leaf spot diseases, Tubakia leaf spot, oak anthracnose

Pesticide application to control *Gonipterus platensis* in *Eucalyptus* plantations: monitoring the effects on honey bees

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Abstract

Honey bees (*Apis mellifera iberiensis*) are well known pollinators, commonly reared in agroforestry ecosystems and whose colony development can be frequently monitored, making them good model species for several studies. In Portugal, many beekeepers place their hives in forest areas, mainly due to the amount of resources provided by floral species. Due to its economic value especially for pulp mills, *Eucalyptus* plantations occupy a significant part of Portuguese forest areas, and its flowers comprise relevant sources of pollen/nectar for honey bee colonies. Eucalyptus are susceptible to pests, such as the eucalyptus weevil *Gonipterus platensis*, which can lead to high productivity losses. Many strategies have been developed to control this pest, including insecticides – by applying a neonicotinoid: acetamiprid –, and the assessment of potential effects on non-target organisms such as pollinator insects is mandatory. Here, we intend to perform a multifactorial assessment of the health status and development of honey bee colonies in 2 *E. globulus* plantations: 1 control (no pesticide application) and other with pesticide application. An apiary with 5 hives was installed in each of the selected areas, that were monitored before and after a single application of acetamiprid (40g a.i./ha). Colony health and development, use of resources and pesticide residues accumulation were assessed. Results showed that acetamiprid application didn't change the health status/development of the colonies, which could be explained by the low levels of the residues of active substance detected only in pollen. The landscape also offered a low amount of resources, leading the honey bees to forage outside the spraying area at the time of the pesticide application, which can explain the low levels of a.s. residues found. Here, we provide a contribution for improved pesticide application strategies in intensive production systems, in order to minimize the risk of pesticide exposure for honey bees.

Keywords: *Apis mellifera iberiensis*; *Eucalyptus globulus*; *Gonipterus platensis*; neonicotinoids; risk assessment

Best practices for management *Eucalyptus* Longhorned Borers, in Portugal

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Abstract

Eucalyptus Longhorned Borers (ELB) are among the most important pests of eucalypt trees. In Portugal, two species can be found, *Phoracantha semipunctata* and *P. recurva*. These are responsible for severe damage to eucalypt stands located in the southern and inland regions, especially after hot and dry years. The current strategy to mitigate ELB is focused on monitoring, placing log traps, and performing sanitary fellings. Traditional monitoring methods are costly and time-consuming. Multispectral imagery captured using Unmanned Aerial Vehicles (UAV) has been tested in recent years with satisfactory results to detect and pinpoint dead trees that require felling and early detection of infested trees. Log traps are used for both monitoring ELB and improving local parasitism by the egg parasitoid Avetianella longoi, whereas sanitary fellings directly reduce pest populations. However, adequate cost/ benefit assessments are lacking. To address this, since 2021, several pairs of plots have been installed in central and southern regions of Portugal. Each pair of experimental plots is installed in places with the same genetic material, exposure, and slope, aged between two and four years, and in the first rotation, with at least 500 trees and separated by at least 500 meters. In the treatment plots, control methods are being applied (log traps and sanitary fellings) within the plot and in a buffer area of 250 meters surrounding it, while there is no intervention in the control plot. Long term phytosanitary assessments will be carried out, and all the silvicultural operations will be monitored. In addition, to support this study, multispectral imagery will be used to validate the use of this remote detection tool to detect and quantify areas affected by ELB. This work will allow us to develop a set of robust tools capable to mitigate the damage caused by ELB. Moreover, it will allow us to quantify and validate the benefits associated with the currently available control methods for ELB on a long-term basis.

Keywords: *Eucalyptus globulus*, *Phoracantha semipunctata*, *P. recurva*, unmanned aerial vehicles, cost / benefit analysis

Impact of *Heterobasidion* spp. on Norway Spruce radial increment on drained peat soils

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Abstract

Forest ecosystem productivity and accordingly economic value is highly affected by pests and pathogens. Saprotrophic fungus *Heterobasidion* spp. is known to cause root and butt rot, resulting in possible growth decrease, timber quality reduction and even tree die-off, therefore the aim of the study was to evaluate the impact of *Heterobasidion* spp. on Norway Spruce radial increment on drained peat soils. Data was obtained from five Norway spruce stands in Latvia, with total of 226 increment cores collected, 116 from healthy trees and 110 from Heterobasidion spp. infected trees. The cores were sanded and measured using LINTAB6 with accuracy 0.01mm. The cumulative radial increment was assessed using Gompertz growth model. The results indicated that four from five analysed stands had statistically significant differences in tree radial increment between infected and not infected trees for the analysed 85-year period. Analysis of annual rings highlighted the interannual variation, which is affected by various factors, including meteorological. Higher radial growth at early age for infected trees indicated that trees with higher growth rates possibly are more susceptible to *Heterobasidion* spp. Yet the radial growth reduction later occurs likely due to pathogen development. When radial increment was analysed cumulatively, four out of five analysed sites showed higher values of diameter for infected stands. In conclusion, faster growing Norway spruce trees on drained peat soils might have higher potential of being infected by *Heterobasidion* spp. and therefore subjected to possible radial growth reduction.

Keywords: Drained peat soil; *Heterobasidion* spp; Norway Spruce; Radial growth.

Are bacteria isolated from tree bleeds the causative agents in lesion formation?

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Abstract

Acute Oak Decline (AOD) is an emerging disease affecting many oak trees across the UK, with a number of causal factors implicated including the native bark-boring beetle Agrilus biguttatus and AOD-associated bacteria *Brenneria goodwinii*, *Gibbsiella quercinecans*, and *Rahnella victoriana*. The vertical arrangement of the bleeds on the tree trunks is an important characteristic symptom of AOD, and the fluid can be assessed for AOD-associated bacteria using a multiplex qPCR. However, stem bleeding is a commonly associated symptom of pathogen infection of trees, and in the UK, the underlying cause of a number of stem bleed symptoms on non-oak species, are yet to be determined. The aim of the work presented here was to find out if the AOD-associated bacteria are present in the stem bleeds of other broadleaved forest species and if they are pathogenic to non-oak tree species. To do this, symptomatic tree bleeds were swabbed and tested for the presence of the AOD associated bacteria using a multiplex qPCR and traditional culturing methods. Lime trees, at a number of sites yielded *B. goodwinii* and *Phytophthora plurivora* as well as a number of other bacterial species. In this trial these bacteria alongside *B. goodwinii* were inoculated into billets of Lime (Tilia x moltkei) to assess lesion formation and lesion area, and were re-isolated at the end of the trial to fulfil Koch's postulates. Lesion area did not differ significantly from controls when only single bacterial strains were used, but an increase in lesion size was seen when two bacterial strains of the same species were co-inoculated. In conclusion, we found *B. goodwinii*, a bacteria associated with bleeds and lesion formation on oak can cause lesion formation on lime.

Keywords: Bacteria, bleed, *Brenneria goodwinii*, Lime, Tilia

Impact of bark stripping on stem volume growth of Norway Spruce

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Abstract

Norway spruce is one of the species affected by bark stripping (browsing) by cervids. Due to growing population density of most cervid species, the probability and intensity of such damages are increasing. Scars in tree bark are known to get infected by fungi, causing stem rot and consequently reducing of timber quality. Several studies have been conducted to evaluate effects of bark stripping on tree radial growth. Aim of the study was to characterize the dynamics of Norway spruce trunk volume growth post bark stripping damages, thus gaining more complete understanding on the impact of this type of damages. The data were obtained from two Norway spruce stands, where bark stripping had occurred 6 – 12 years ago. The samples (cross-sections) from 40 trees were collected, 20 damaged trees and 20 control trees with the distance between cross-sections 1m, starting from the root collar and including also 1.3m height. The disks were sanded and scanned, tree ring widths for each stem disk were measured in 16 different directions, using WinDendro. High variation in annual growth reaction, characterized both by the calculated area of annual rings close to the damage itself, as well as stem volume increment, was observed. Majority of trees displayed significant growth reduction. Implications of these results will be discussed in context of the findings of the country-scale browsing damage monitoring.

Keywords: Norway spruce, bark stripping, stem volume, radial growth

New species of *Neopestalotiopsis* genus associated with diseased *Eucalyptus globulus* and *E. nitens*

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Abstract

Eucalypts are among the most widely planted hardwood timber trees in the world. In Portugal, *Eucalyptus* are exotic species, being *Eucalyptus globulus* the most important, regarding land usage and commercial use for papermaking industry. This species was introduced into Portugal in the nineteenth century, but only in the middle of the twentieth century did the great increase of plantations occur. In the following decades, eucalypt plantations were relatively free of pests and diseases. However, since the 1970s, several pests appeared, firstly insects and then fungi. Leaf diseases caused by *Mycosphaerella*, *Teratosphaeria* and *Quambalaria* species, and canker diseases caused by *Teratosphaeria gauchensis*, *Quambalaria eucalypti* and various species in *Botryosphaeriaceae* have been reported to cause important losses in eucalypt plantations in Portugal. In the last years, a disease syndrome associated with pestalotioid fungi has been detected in nurseries and young eucalypt plantations. From diseased plants (leaf necrosis, stem girdling, cutting dieback), twenty-seven isolates of pestalotioid fungi were recovered. Phylogenetic analysis based on internal transcribed spacers, partial translation elongation factor 1-α gene and partial β-tubulin gene sequence data grouped the isolates in five separate clades. Combining morphological, cultural and molecular data, five new species of *Neopestalotiopsis* are described, namely, *Neopestalotiopsis eucalyptorum*, *Neopestalotiopsis hispanica*, *Neopestalotiopsis iberica*, *Neopestalotiopsis longiappendiculata*, and *Neopestalotiopsis lusitanica*. To our knowledge, this is the first report of *Neopestalotiopsis* species causing leaf and stem necrosis on *Eucalyptus* species in Portugal.

Keywords: Coelomycetes, Taxonomy, Plant pathogens, Phylogeny, Sporocadaceae

Novel *Calonectria* species associated with a serious leaf disease on native *Ilex paraguariensis* in Uruguay

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Abstract

Ilex paraguariensis (locally known as yerba mate) is a native Latin American tree, which is culturally important and the source of the most popular tea-like infusion, primarily in Argentina, Brazil and Uruguay. A severe leaf spot disease appeared on *I. paraguariensis* trees in April 2018 in Uruguay. The aim of this study was to determine the cause of this disease and to consider whether this might be related to similar symptoms on non-native *Eucalyptus* that have been extensively planted in the area. Five native populations of *I. paraguariensis* were sampled and symptomatic leaves were collected for laboratory study and to make isolations. Profuse *Calonectria* sporulation was observed on the diseased leaves and 141 isolates were obtained and identified based on morphology and analysis of sequence data for the TEF1-α, β-tubulin, Histone, Calmodulin, Actin and RPB2 gene regions. Preliminary results showed that at least two novel *Calonectria* species are associated with this disease, and these are not amongst those species of *Calonectria* known from *Eucalyptus*. Studies are currently underway to describe these new *Calonectria* spp. Furthermore, to determine whether they are likely native fungi that have evolved together with *I. paraguariensis*, or whether the disease could be caused by pathogens introduced from a currently unknown source.

Keywords: Leaf diseases, native forest surveillance, *Cyllindrocladium*

BAC-STOP Work Package 4: Identification of bacteria associated with non-oak stem bleeds

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Abstract

BAC-STOP is an interdisciplinary project that aims to advance knowledge to achieve practical management of Acute Oak Decline (AOD). AOD is characterised by dark vertical stem bleeds which are caused by a polybacterial pathobiome dominated by *Brenneria goodwinii*, *Gibbsiella quercinecans* and *Rahnella victoriana*. Closely associated with the stem bleeds are larval galleries of the native bark boring buprestid *Agrilus biguttatus*. BAC-STOP Work Package 4 specifically addresses whether bacteria associated with canker formation in AOD are also present in non-oak species in the UK. To this end, Tree Health Officers, researchers, and the public took swab samples of non-oak stem bleeds, from which AOD associated bacteria were detected via a multiplex qPCR assay. Swabs were also streaked onto Eosin Methylene Blue and nutrient agar plates, and bacteria were identified by sequencing barcoding genes and subsequent BlastN analysis against Genbank sequences. In total, we received 80 swab samples were received from 52 trees representing 12 non-oak tree species. AOD associated bacteria were detected from stem bleeds of non-oaks with both the multiplex qPCR assay and bacterial isolations. In addition, a diverse range of species from Gram positive and negative families were isolated with most isolates belonging to Enterobacteriace and Pseudomonadaceae. Two new *Raoultella* spp. were detected from stem bleeds from lime (Tilia X europaea) and a tulip tree (*Liriodendron tulipifera*), and a novel *Erwinia* sp. was characterised from stem bleeds from lime and London plane (*Platanus* X acerifolia) trees. This research addresses a key knowledge gap on the host range of AOD lesion-forming bacteria, and their potential to cause necrosis of other tree species.

Keywords: BAC-STOP, Acute Oak Decline, bacteria, swab

Management strategies to control pine processionary moth outbreaks in North-Western Italian Alps

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Abstract

In recent years, forests have been endangered by several phytosanitary threats, including outbreaks caused by insect pests such as the pine processionary moth (PPM). *Thaumetopoea pityocampa* (Denis and Schiffermüller, 1775) (Lepidoptera, Notodontidae) is considered one of the main insect defoliators of conifers in Southern Europe and North Africa. The PPM caterpillars are severe defoliators, causing significant pine tree health decline. Larvae feed on a wide range of *Pinus* species, especially *Pinus nigra* Arnold and *P. sylvestris* L. From the 3rd instar onwards, they possess urticating hairs having serious social impacts, and associated health problems in humans and domestic and farm animals in urban and suburban areas have been reported. The Interreg V-A Italy-Switzerland 2014/2020 cooperation program "Linee Guida per il monitoraggio e la gestione delle emergenze fitosanitarie nelle foreste delle Alpi centro-occidentali (MONGEFITOFOR)" is a project with the aim to provide management guidelines to contain PPM outbreaks. Surveys were carried out in the 3-year period 2020-2022 in Aosta Valley (NW Italy) to assess PPM population by using pheromone-baited funnel traps and to compare the effectiveness of different control strategies. *Bacillus thuringiensis* var. kurstaki (Btk), mating disruption, and trunk injections were performed. Their effectiveness was evaluated by assessing the number of nests and larval mortality versus control plots. Trunk trap devices were investigated as well, using adhesive barriers and collars. For most of the control strategies, results have shown significant reductions of nests and increased larval mortality in treated sites. Regarding trunk trap devices, whereas adhesive barriers were easily overtaken by larvae, only collars proved to be effective in trapping a high number of caterpillars during the descent along the trunks. These results provide useful data for the management of PPM in forestry and urban environments.

Keywords: *Thaumetopoea pityocampa*; seasonal flight activity; control strategies; forest insect pest

Comparison of snow molds between Japan and Europe and North America: Similar but different

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Abstract

Snow molds are fungi that cause shoot blight or seedling death in various conifer species under snow. In Japan, *Racodium therryanum* and *Gremmenia* (*Phacidium*) *abietis* are two major snow molds that have been vigorously studied until 1990. However, due to a lack of molecular biology techniques at that time, the initial identification of these fungi was based only on symptomatology and morphological observation. In this study, we sought to clarify the taxonomic placement of *R. therryanum* and *G. abietis*, as well as their homology with snow molds in Europe and North America, combining morphological observation and phylogenetic and population analyses.

Previous studies have pointed out the similarities between *R. therryanum* and *Herpotrichia juniperi*, the causal agent of brown felt blight in Europe and North America, although those studies could not identify the species due to a lack of sporulation. We conducted a phylogenetic analysis using ITS, LSU, rpb, and tef. In the concatenated phylogenetic tree, *R. therryanum* and *H. juniperi* composed a clade with a high bootstrap value, indicating that *R. therryanum* is phylogenetically *H. juniperi*. Moreover, population analysis based on 7 microsatellite markers and another phylogenetic analysis using ITS, tef, and tub showed that *H. juniperi* in Japan includes three genetically differentiated intraspecific groups, two of which were allied with cryptic species reported previously in Europe. *Gremmenia abietis* is a causal agent of snow blight in Japan and North America (cf. related species *Gremmenia infestans* is observed in Europe). We collected two fresh G. abietis samples in 2021 in Japan and carried out morphological and phylogenetic analyses. The sizes of ascus (88.0–133.4 × 12.4–18.3 µm) and ascospore (16.1–22.5 × 6.3–8.7 µm) corresponded with those of *G. abietis* in previous studies. However, in a phylogenetic tree based on ITS and rpb, our isolates were included in neither genus *Gremmenia* nor *Phacidium* but located in *Xenosphaeropsis* (Phacidiaceae). Our results indicate similarities as well as differences in snow molds between Japan and Europe and North America. This fundamental knowledge will contribute to a comprehensive understanding of snow molds and related diseases.

Keywords: Phylogenetic analysis; Snow blight

Stakeholder awareness, detection and management of new and emerging tree pest and diseases in Europe – a survey

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Abstract

New tree pests and diseases in Europe are increasing in number and range, with impacts on biodiversity and forest services. Stakeholders are well placed to detect and manage new and emerging tree pests and pathogens (PnPs), so their collaboration is valuable in reducing these emerging threats. We conducted an online survey among different groups of stakeholders in 15 European countries (researchers, managers, owners, advisors, forestry authorities, forest health surveyors and civil society) to find out about their knowledge and concerns regarding new and emerging PnPs. We also asked about particular tools and methods they currently use for detection, identification and management of PnPs, and what additional tools they would wish to have available. We found that pests on the European list of 'priority pests' were better known than those not listed. Stakeholders working in urban environments were more aware of PnPs compared to those working in rural areas. The number of PnPs present in a country did not have an impact on the probability of knowing them. Respondents were well aware of the 18 most frequent PnPs in Europe and listed a further 37 pest species and 21 pathogen species. Use of tools differs significantly between stakeholder groups with regard to detection and identification of PnPs but not for management. Most stakeholders would like to have access to more tools and methods for detection identification, and management of PnPs. They called for increased long-term citizen science monitoring, access to maps showing spread and range of new PnPs, smartphone apps for new pest identification, handheld detection devices, eDNA applications, drone monitoring, non-destructive metabarcoding, development of mixed forests, less movement of nursery stocks, and information and data sharing between organisations, to help facilitate better forest health across Europe.

Keywords: Detection, invasion, management, stakeholders, survey

Developing a monitoring capacity for native and exotic *Agrilus* spp. in Belgium

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Abstracts

The aim of this work is to develop monitoring tools for native *Agrilus* species (e.g. *A. biguttatus*, *A. viridis*, *A. sulcicollis*, *A. sinuatus*) and quarantine species (*A. anxius*, *A. auraguttatus*, *A. bilineatus*, *A. planipennis*) in Belgium. This project is linked to Euphresco's project 2020-A-337 "Developing and assessing surveillance methodologies for *Agrilus* beetles" In a first step, a field study in Belgium aimed at measuring the visual attraction of Belgian native *Agrilus* species to different types of traps. Commercial traps (green Lindgren funnel-traps from Chemtica, in Oklahoma and a Hungarian green funnel-trap model from Csalomon), and green, locally-produced bottle-traps (with or without a decoy consisting in a dead *Agrilus planipennis* adult). The traps were deployed over two seasons in beech and oak stands, and in pear orchards (for *A. sinuatus*). During the first season (2021), the Hungarian traps caught more beetles than the other traps. Catches with bottle-traps were too low to determine the impact of the decoys. During the second season (2022), the bottle-traps were replaced by fluorescent yellow, and by green Fan-traps locally-produced under a Creative Commons license (with or without a decoy). The experiments of the second season are still in progress. Fan-traps, with or without decoys were also deployed in France in 2022, to replicate our tests in Belgium. And, finally, to test them as a tool for monitoring quarantine species, and in particular *A. planipennis*, Fan-traps were also sent in 2022 to Canada and the USA, so that they could be compared with the multi-funnel traps and sticky prism traps used locally. These experiments, too, are in progress.

Keywords: *Agrilus*, monitoring, surveys, traps

Pre-emergence matings in twelve species of Scolytinae

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Abstract

Most Scolytinae are exogamous, and mating with a non-sibling usually takes place after emergence, in a new host tree. However, ca 1600 species are endogamous. The females, fertilized by a sibling in the natal gallery, start a new colony on their own. Inbreeding associated with solitary foundation is a major asset for invasive species. However, these categories are not always clear-cut. The literature reports pre-emergence mating, with a sibling or with a neighbor from another colony on the same host tree, among the females of six species of Scolytinae known to be exogamous (*Dendroctonus ponderosae*; *Dendroctonus valens*; *Ips grandicollis*; *Ips pini*; *Orthotomicus erosus*; *Tomicus piniperda*). In the study presented here, we analyzed and compared the rate of pre-emergence mating of a further twelve species of exogamous Scolytinae trapped or collected after emergence from infested logs, in spring after overwintering, in several European countries: *Gnathotricus materiarius*; *Hylastes ater*; *Hylastes attenuatus*; *Hylastes cunicularius*; *Hylurgops palliatus*; *Hylurgus ligniperda*; *Ips sexdentatus*; *Ips typographus*; *Polygraphus poligraphus*; *Trypodendron domesticum*; *Trypodendron lineatum*; *Trypodendron signatum*. We dissected the females' spermathecae and found pre-emergence mating in eleven out of the twelve species, with a rate of females fertilized before emergence ranging from 4% to 100%. This ongoing study has allowed us to document an unexpected aspect of the reproductive behavior of many Scolytinae which might modify our views of their ecology and invasive capacities.

Keywords: Scolytinae, Pre-emergence mating, Invasive capacities

Detection of *Phytophthora* pathogens in forest nurseries using different methods

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Abstract

Most *Phytophthora* species are known global plant and forest pathogens that cause significant economic losses in agriculture, horticulture and forestry and are major threats to natural ecosystems and biodiversity. A key factor in aggressive behavior and the onset of epidemics is the increased incidence of temperature extremes supporting asexual sporulation and zoospore production. Forecasts of global warming could lead to increased susceptibility of oaks and beeches in Europe, where the pathogen could spread north and east from areas where there are already species that cause whole plant death. However, *Phytophthora* is often not detected which leads to misdiagnosis.

Traditional method of isolation and detection of these pathogens is so-called baiting, where the soil is taken from the research area; the soil is subsequently flooded with water in laboratory and the water surface is covered with beech or oak leaves. The pure cultures are isolated from leaves after a few days. These cultures are then subjected to DNA extraction and sequencing to determine the species present in the soil. This method is quite time consuming and can take several weeks. We took this traditional baiting method and we opposed it to the NGS method to find the differences between the two methods. We focused mainly on the differences in time needed for the detection and the difference in the number of detected species.

Thanks to this research, we have obtained a new and modern methodology for the detection of pathogens of the genus *Phytophthora* using the Next generation sequencing, which is faster and more accurate and will help us detect a larger number of species. We have also mapped the occurrence of these pathogens in forest nurseries in the Czech Republic and thus have a better overview of the risks that the occurrence of these pathogens may bring for forestry in the Czech Republic.

Keywords: *Phytophthora,* Next generation sequencing Detection, Baiting, Forest nursery

Integrating population density with seed damage by *Leptoglossus occidentalis* gives new insights into the impact of the seed bug

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Abstract

The production of pine nuts is one of the most important non-wood forest products from stone pine (*Pinus pinea*) plantations in Portugal. In the last decade, a decrease in seed yield has been observed. The invasive bug *Leptoglossus occidentalis* (Hemiptera: Coreidae), which originated in North America and was first detected in Portugal in 2010, has been causing major concern among pine nut producers. Both nymphs and adults of this species feed on the pine seeds of different conifers and have been considered responsible for the pine seed yield decrease in several Mediterranean countries. Still, other possible causes, namely native emergent pests and climate (extremely high temperatures and drought) have been pointed out as possible causes. The +PINHÃO operational group, which brings together researchers, producers, and forestry associations, aimed to study the impact and possible control methods of the main biotic agents of the pinecone and pinyon pine, namely the seed-feed bug. For 5 years (2015-2019) we monitored the seed bug populations and the damage in pine seeds, after pine cone harvest, in two umbrella pine stands in central Portugal. In addition, we compared pinecones from fertilized and irrigated trees. The damage assigned to *L. occidentalis* varied over the years ranging from < 2% to more than 20%, which were related to fluctuations in the bug population in the same period. A positive correlation was recorded between pine seed loss and average insect density in the field. Irrigated trees also showed a higher pine seed loss assigned to the seed bug. Other causes of see loss, which could be due to climate or non-fertilization, varied between 5 and 10%. This approach allowed quantifying the impact of the insect and confirmed its importance in the decrease in pine seed yield reported in the last decade.

Keywords: *Leptoglossus occidentalis*, Portugal, pine nut production, *Pinus pinea*

Session 2 Tree health in urban forests

Oral communications

Effects of urban heat islands on forest trees and arthropods

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Abstract

Urban heat islands can increase forest air and soil temperature and reduce soil moisture especially when combined with greater climate change. Land managers should consider the surrounding urban density when trying to determine which plants and animals can persist in urban forests. As forests are fragmented or encroached upon by urbanization the forest environment will change and become more hospitable for some species and less hospitable for others. Overall, there is insufficient research focused on urban-forest interfaces and the consequences of urbanization for plants and animals within forests.

Keywords: urban forest, climate change, urban heat island, conservation, tree pests

Modelling the spread of *Trioza erytreae* according to the landscape in both urban and peri-urban environments using trees as agents for an Agent-based model

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Abstract

Urban trees are an important part of urban landscapes worldwide. These offer a variety of important ecosystem services beneficial for human society. Yet, they are also linked with the introduction and establishment of invasive pest species, enabling their naturalization and later widespread.

The African citrus psyllid, *Trioza erytreae* (Del Guercio), is a small sap-sucking hemipteran insect, native to tropical Africa whose host species are mostly rutaceous trees, including citrus trees. It is one of the two known vectors of the citrus greening disease and was recently found in Europe in 2014, both in Portugal and Spain.

Urban trees are even more relevant in the context of this pest species in the Iberia Peninsula, as both lemon and orange trees are commonly present in urban and peri-urban environments in courtyards and as street trees. Consequently, the citrus trees of residential areas are an important reservoir and pathway for the spread of the psyllid, including the major citrus production areas, as well as a shelter from pest management actions (e.g., insecticide spraying).

In this work we have developed an agent-based model to simulate the spread and infestation of the African citrus psyllid in both urban and rural landscapes. The density of citrus trees in the urban residential areas were gauged using observational sampling of Google Street View imagery. The model's agents are the citrus trees, which along with the use of key biological and ecological traits, are integrated within an epidemiological approach, for understanding the effect of the landscape's heterogeneity towards the species' dispersal, namely the dispersal speed. Additionally, we aimed at evaluating the effectiveness of some management actions against the psyllid, providing a realistic and useful model that might be easily parametrized to other species and conditions, such as the citrus greening disease itself.

Our results confirm the importance that urban trees can have in the invasive process of pest species, such as *T. erytreae*. Additionally, it provides additional information regarding the pest species population dynamics, vital to its management. Our work also provides a different approach for agent-based modelling of tree invasive pest species, serving as a relevant basis for future research work related with this and other species.

Keywords: Agent-based modelling, Insect Invasions, Human-mediated dispersal, *Trioza erytreae*, Urban trees.

The role of *Verticillium dahliae* in the biocontrol of the invasive *Ailanthus altissima*: work progress in Central Italy

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Abstract

Natural infections of *Verticillium* Nees spp. on *Ailanthus altissima* Mill. (Swingle) have suggested to consider the biological control as a promising strategy to counteract this invasive plant, which is otherwise difficult to control by traditional mechanical and chemical treatments. In Italy, *Ailanthus* decays caused by *V. dahliae* Kleb. have been reported in urban and peri-urban areas (Tuscany) as well as in forest environments (Trentino Alto Adige). After reporting a first Verticillium wilt disease in Tuscany (registered as strain VDGL16), a pilot study was performed in controlled conditions to firstly elucidate the plant-pathogen interactions at the physiochemical level. A second experiment set in Pisa city center (Tuscany, Italy) showed that artificially inoculated adult trees were severely compromised by disease and were killed after three years from inoculum. By using FISH techniques, *V. dahliae* was detected in residuals of infected *Ailanthus*, posing attention on the potential risk to diffuse *Verticillium* propagules and contemporarily guaranteeing a long-term biocontrol. In 2021, according to the plan of management of fluvial systems in Tuscany, *A. altissima* inoculations using strain VDGL16 were also performed in three sites in the Florence countryside. Also here, disease progress was rapid and effective, with the absence of non-target effects, suggesting a host-specialization of the strain VDGL16.

Keywords: bioherbicide, wilt disease, eco-friendly, management, specialization

Invasion of *Popillia japonica* in urban forest habitats of Northern Italy

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Abstract

The Japanese beetle *Popillia japonica* Newman (Coleoptera: Scarabaeidae) is a highly polyphagous pest originated from north-eastern Asia and introduced and established in Northern Italy, between Lombardy and Piedmont regions, since 2014. The infested area increased over time at a constant rate, and it covers now more than 15,000 km^2. The reason for this rapid expansion is linked to various drivers, among which the high suitability of the region. The beetle requires broadleaved tree species for maturation feeding and roots of various plants for larval development. The proximity of those resources does maximise the performance and facilitate the spread. Indeed, urban forested areas and irrigated hay meadows are widely represented in the infested area. They represent key habitats for the development and spread of the species, providing high-quality nutritional resources for both larvae and adults. The high suitability of the territory for the beetle has hindered the success of all control actions officially implemented by the plant health authorities, and *P. japonica* in Italy is now considered not eradicable. Therefore, a systems approach strategy aimed at controlling the pest is required for preventing the establishment of the species in new areas and for reducing pest population pressure. An outline of the systems approach developed for *P. japonica* is presented.

Keywords: Coleoptera; Invasive alien species; Pest management; System approach

Tree density and diversity control the risk of exotic pest damage in urban forests

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Abstract

Alien invasive pests are a growing threat to urban trees and the services they provide to city residents. Knowing that chemical insecticides are best avoided in cities, environmentally friendly and effective pest control methods are increasingly needed. Biological invasion theory predicts that the risk of establishment and damage of non-native species depends on the amount and diversity of resources available in the new habitat, with these drivers operating with different intensity and direction at different spatial scales.

We tested these assumptions by focusing on two species of invasive alien insects, the horse chestnut leafminer and the oak lace bug in the city of Bordeaux, France. We sampled target trees along two independent gradients of host tree density within a buffer of few hundred meters and the species diversity of nearby trees. We measured levels of herbivory caused by exotic pests and estimated the abundance or activity of their natural enemies.

We showed that the rate of damage tended to increase with local canopy cover while predators or parasitoids were more influenced by the species diversity in the close vicinity of the sampled trees. Our results pave the way for the management of exotic insect pests in cities based on the manipulation of the spatial distribution and diversity of urban tree species.

Keywords: control; invasion; pests; spatial; urban

Saving the urban elms through vaccination treatment – is associated biodiversity affected?

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Abstract

Elms (*Ulmus* spp.) are appreciated urban trees and provide habitat and nutrition to many organisms, some of which are dependent on elms. Unfortunately, Dutch elm disease (DED), caused by fungi in the genus *Ophiostoma* has become a major hindrance to the use of elms in urban environments. To protect elms, a biological control product based on a weak pathogen has been developed. It is administered as annual, preventive injections and in several cases, good results in disease protection have been obtained. The effect of the vaccination treatment is likely to stimulate the defensive responses in elms. In earlier studies, higher resistance to DED has been accompanied by reduced diversity of endophytic fungi in elm bark, possibly because the same resistance mechanisms that suppress the pathogenic fungus may also affect other fungi. In this pilot study, we investigated the possible effects of vaccination treatment on the epiphytes (lichens, mosses) and endophytic fungi in elm bark. The epiphyte coverage was mapped on bark sections (400 cm2) of 17 U. glabra 'Camperdownii' trees, vaccinated annually between 2018-2021 and on as many conspecific, unvaccinated trees. For isolation of endophytes, bark samples were collected at 130 cm height from unvaccinated (n=15), continuously vaccinated (n=15) and previously vaccinated (n=15) elm (U. glabra and U. minor) trees. Epiphyte coverage tended to be lower in vaccinated trees as compared to unvaccinated trees. All samples yielded mycelial growth, suggesting that vaccination did not affect the frequency of fungal infections in the elm bark. The isolated fungi were classified into over 100 morphological groups, with a large proportion of singletons. The highest number of morphological groups was found in elms that were previously vaccinated. The number of singletons was highest in the continuously vaccinated elms. The results indicate that vaccination treatment can affect the dynamics of epiphyte and endophyte communities in elms.

Keywords: Biological control, biodiversity, *Ulmus* spp., endophytic fungi, epiphytes

The astonishing species richness of urban trees in Switzerland – implications for monitoring invasive alien species

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Abstract

Forest tree species composition in Central Europe and North America is often described well by forest inventories on different scales, but in comparison, few is known about urban tree species composition. However, urban tree composition strongly influences urban climate regulation, human health, urban biodiversity and biosecurity.

In Switzerland, urban tree inventories are mainly organized and maintained by individual municipalities, but not shared on a national level. As part of a project where we estimate the probability of establishment of priority quarantine forest pests, we collected and combined urban tree inventories from 30 municipalities, with data from around 450’000 trees recorded by municipalities. Urban tree inventories contain a very high species diversity with representatives of more than 130 genera and 1500 species, while the Swiss National Forest Inventory (NFI) so far has observed around 76.000 trees with a DBH of more than 4 cm, consisting of 146 woody species on all sample plots, of which 72 tree species. However, the 10 most common tree species in the urban dataset were native species.

We used the combined dataset of the municipalities and the NFI to explore the suitability of urban trees and forest trees surrounding urban areas for the establishment of priority quarantine forest pests in Switzerland, since most first observations of invasive alien forest are generally made in urban areas. We found that all tree genera of forest trees in a circle of 10km around cities were well represented within the corresponding urban tree inventories. Furthermore, we found that urban tree inventories also included native genera, which were not present in the surrounding forests. This means that monitoring arrival of invasive forest pests should be mainly done in urban areas, and tree species composition in cities should be a more important criterion for host availability than tree species composition of surrounding forests.

Keywords: Biodiversity; Biological invasions; Tree inventories; Urban ecology

Posters

Horse Chestnut (*Aesculus* spp.) disease symptoms are increasing due to several opportunistic pathogens in addition to the Leaf Blotch pathogen *Guignardia aesculi*

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Abstract

Horse Chestnut trees (*Aesculus hippocastanum*) represent one of the most familiar ornamental trees in urban areas in Germany. Trees are aesthetically valuable and essential with their vast canopies and ecological values. Rising temperatures due to climate change can benefit several fungi over their host tree. The invasion of non-native pathogens leaf dieback, e.g., tree mortality, can increase due to the leaf miner (*Cameraria ohridella*) and leaf blotch (*Guignardia aesculi*). Though, we lack knowledge of the role of opportunistic species in the lately worsening health conditions of Horse Chestnut trees. Investigating all fungal species that may be responsible for causing symptoms additionally to the leaf blotch pathogen, symptomatic (e.g., necrotic) Horse Chestnut leaves from several locations in Göttingen, Germany, were collected. A high fungal diversity of Ascomycetes could be isolated and identified with several molecular markers (i.e., ITS4, LR6), such as the potential pathogens *Biscogniauxia nummularia*, *Colletotrichum* spp., *Alternaria* spp., and *Fusarium* spp. A reputed interaction between 14 isolated fungi and *G. aesculi* was investigated by antagonism assays with 27 combinations. The results suggest that most fungi do not antagonize each other, while some do; thus, a co-existence on the leaves is possible. Further, these fungi' role in causing the symptoms on the leaves was tested on 250 3-year-old Horse Chestnut trees by infecting them with the spore-phytogel-layer method, followed by fungal re-isolations and necroses measurements. The results confirmed that several fungi are responsible for the described symptoms. Horse Chestnut trees may suffer from various opportunistic fungal pathogens that can decrease their hosts' health. The situation may worsen with climate change and a rising number of alien species. The study was conducted in central Europe; the preliminary outcomes suggest that habitually some warm-loving fungi such as *Colletotrichum* spp. can spread to temperate climate zones. However, more tests and observations are required to investigate pathogens originating from tropical and sub-tropical areas and their possible connection to increasing tree disease outbreaks within Europe.

Keywords: Horse Chestnut, Opportunistic fungal pathogens

Session 3 Parasitic flowering plants in forests

Oral communications

Leveraging parasitic flowering plant collections to understand and monitor impacts of global change

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Abstract

Leveraging parasitic flowering plant collections to understand and monitor impacts of global changeThe widespread digitization of natural history collections, combined with novel tools and approaches is revolutionizing biodiversity science. Herbarium specimens that by their very nature capture multispecies relationships, such as certain parasites, fungi and lichens, hold great potential to provide a broader and more integrative view of the ecology and evolution of symbiotic interactions. The potential is even greater in the case of species that act as keystone players in natural and disturbed ecosystems, such as most parasitic flowering plants. In this context, to achieve the full potential of parasitic plant collections and help motivate the next generation of global change research, we assessed the representation of these peculiar plants in herbarium collections worldwide. Nearly 1.02 million records housed in 950 institutions were gathered from the Global Biodiversity Facility. Our analysis shows that over 40% of the freely available records include images and geographical coordinates. Although apparently low, this percentage is well above the general proportion reported for animal parasite collections, highlighting that parasitic plant specimens are primed for further exploration. This presentation will focus on how data and metadata obtained from parasitic plant specimens can inform analyses of co-distribution patterns, functional traits, changes in species plasticity, chemical ecology of tripartite interactions (e.g., host–parasite–herbivore), and molecular data critical for species conservation. I will also explore how the historic nature and sheer size of global herbarium collections can provide the spatiotemporal breadth essential for investigating organismal response to global change using parasitic plant specimens.

Keywords: global change biology, herbarium, natural history collections, parasitic plants, species interaction

Geographic context outweighs habitat disturbance effects in explaining mistletoe population genetic differentiation

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Abstract

Genetic differentiation depends on ecological and evolutionary processes that operate at different spatial and temporal scales. While the geographical context is likely to determine large-scale genetic variation patterns, habitat disturbance events will likely influence small-scale genetic diversity and gene flow patterns. Therefore, the genetic diversity patterns that we observe today result from the combination of both processes, but they are rarely assessed simultaneously. We determined the population structure and genetic diversity of a hemiparasitic mistletoe (*Tristerix corymbosus*) from the temperate rainforests of southern Chile to determine the effects of the geographic context and habitat disturbance at a regional scale and if it is affected by the abundance and occurrence of its seed disperser mutualist (the arboreal marsupial *Dromiciops gliroides*). We genotyped 359 mistletoe individuals from 12 populations using SNPs, across three different geographic contexts and four disturbance conditions. We also used camera traps to estimate the abundance and occurrence of the seed disperser. Our results suggest that genetic differences among populations are more related to the geographical context than to habitat disturbance. However, as disturbance increased, D. gliroides abundance and occurrence decreased, and mistletoe inbreeding index (FIS) increased. We also found highly uneven gene flow among study sites. Despite the high levels of disturbance that these temperate rainforests are facing, our results suggest that mistletoe genetic differentiation at a regional scale was more influenced by historical events. However, habitat disturbance can indirectly affect mistletoe population genetic differentiation via the seed dispersal process, which may increase inbreeding levels in the long term.

Keywords: continental island; habitat disturbance; gene flow; glacial refugia; temperate rainforest

Functional anatomy of parasitic woods: A hydraulic perspective on 13 genera of neotropical Loranthaceae

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Abstract

Loranthaceae is one of the largest parasitic plant lineages, having both root and stem parasites. Although both obtain nutrients directly from their host's vascular system, there are differences in conditions in which the nutrients are obtained depending on the parasitized organ. In order to the flux of water to happen, plants establish a negative water potential gradient in their xylem from roots to leaves This means stem parasites have more difficulty to obtain water and nutrients from their hosts, operating under more negative water potentials to do so, having greater risk of developing hydraulic failures. This work aimed to investigate the differences between root and stem hemiparasites concerning wood anatomy and plant hydraulics. We selected 13 American genera of South Loranthaceae for wood anatomy and hydraulic comparison, including the early divergent Gaiadendron (root parasite) and many different genera of mistletoes with varying haustoria and plant morphotypes. All samples were sectioned using classical anatomy techniques and analysed with light microscopy. Quantitative anatomy measurements were taken from the sections and used as basis for the calculations of the hydraulic properties. Mistletoes showed some classically xeromorphic-associated features such as thicker vessel and fibre wall. We also found that Gaiandendron had greater average vessel diameter and hydraulic weighted vessel diameter (HWD) than most mistletoe species, but lower vessel density. According to Hagen- Poiseuille's law, the water transport efficiency scales to the fourth power of the conduit's width, thus we expected to find the specific conductivity of Gaiadendron to be much higher than the conductivity found for mistletoes. As expected, Gaiadendron had in general higher conductivity (Kps) than most mistletoes, although there was considerable variance between species. Surprisingly, Tripodanthus the greatest values of conductivity al all species, surpassing by far all other mistletoe species and contradicting the original prediction. Furthermore, mistletoes generally had larger pit chambers and pit fractions, what could indicate more permeable and efficient intervascular water transport. This, at least in theory, should lower the overall resistivity of the main bottleneck in the system.

Keywords: mistletoe hydraulic architecture, xylem hydraulics, functional wood anatomy, Santalales

Transformation of western hemlock (*Tsuga heterophylla*) tree crowns by dwarf mistletoe (*Arceuthobium tsugense*, Viscaceae)

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Abstract

Dwarf mistletoes (*Arceuthobium* species) are arboreal, hemiparasitic plants of conifers that can change the structure and function of the tree crown. Hemlock dwarf mistletoe (*Arceuthobium tsugense* subsp. *tsugense*) principally parasitizes western hemlock (*Tsuga heterophylla*) and effects 10.8% of all western hemlock trees in Oregon, USA. In this study, we climbed 16 western hemlock trees (age 97–321 years, height 33–54.7 m) across a gradient of infection (0%–100% of branches infected) and measured occurrence of all dwarf mistletoe infections, dwarf mistletoe caused deformities, foliage, branch and crown metrics, and sapwood area. We then modelled over 25 different response variables using linear and generalized linear models with three metrics of severity as explanatory variables: total infection incidence, proportion of all live branches infected, and proportion of all live, infected branches with 33 per cent or more foliage distal to infection. A strong effect of dwarf mistletoe intensification was the reduction of branch foliage and an increase in the proportional amount of foliage distal to infections, with severely infected trees having the majority of foliage distal to infections. Increasing severity led to an apparent crown compaction as crown volumes decreased and became increasingly comprised of deformities. Sapwood area was unrelated to infection severity. Branch length and diameters were unrelated to increasing infection severity despite severely infected branches supporting 1–70 infections. The most severely infected tree had 3,615 individual plants in the crown. Our results suggested that shifts in crown structure and branch deformation, foliage amount, and foliage distal to infection, reflected a likely reduction of capacity for tree growth that coincided with a hypothesized increase in resource demand by dwarf mistletoe plants as infection severity intensified. (published in Forest Pathology 2020; https://doi.org/10.1111/efp.12664)

Keywords: *Arceuthobium tsugense*, dwarf mistletoe, tree crown deformation, *Tsuga heterophylla*

How the European mistletoe attaches to its host—Biomechanics and functional morphology of their persistent connection

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Abstract

The hemiparasitic European mistletoe (*Viscum album*) is one of the most common and visually prominent parasites in Europe. It can reach an age of over 20 years, with its evergreen plants growing up to two meters in diameter. Despite the resulting additional weight and wind loads, mechanical failure of the mistletoe-host connection has never been described in nature. While biological research on mistletoes to date has focused mainly on their morphology, anatomy, ecology, and interaction with the host, the goal of our work was to biomechanically characterize this persistent interface and analyze its underlying functional morphology. For this purpose, we mechanically tested intact mistletoe-host samples and sliced samples through the attachment site under tensile loading until failure, determined local strains using digital image correlation, and quantified the area and roughness of the resulting fracture surface by digital microscopy. We complemented these analyses with morphometric measurements, light microscopic imaging, and X-ray microtomographic scans of mistletoe-host samples of various ages. Our results showed that the functional and structural integrity of the mistletoe-host connection is independent of age and can thus be maintained throughout its entire lifetime. This is facilitated by an increase of the fracture surface, evident by a larger roughness compared to pure host wood samples, and a redundancy of the sinker anchorage, apparent by pre- and post-failure events in the resulting force-displacement curves. 3D imaging revealed that the mistletoe grows several centimeters acropetally and basipetally along the host branch already at a young age, and that several small sinkers of the young mistletoe merge into a main sinker during synchronous growth with the host. Cellular and biomechanical gradients along the interface smooth the transition of mechanical properties between the two species. Further studies will abstract the biological functional principles by using simulation approaches in order to translate them into an engineering-compatible language for a transfer to technical joining of materials systems.

Keywords: Biomechanics; Mistletoe-host interface; Segmented microCT analysis; Tensile testing; *Viscum album*

Fruit and seed dispersal of *Phoradendron quadrangulare* (Santalaceae) by birds in urban areas of the city of São Paulo: a parasite may enhance the conservation of a threatened bird

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Abstract

This project aimed to investigate the dispersal of the mistletoe *Phoradendron quadrangulare* (Santalaceae) parasitizing the exotic tree Melia azedarach (Meliaceae), as well as the diversity visiting of birds. We compared two areas in Sao Paulo: i) city streets that presented almost no trees; ii) areas presenting a greater number of trees and shrubs and that could be considered an "urban park"— the "Cidade Universitária Armando de Salles Oliveira (CUASO)". To perform this comparison, focal observations were conducted, a method which consists in directly observing a plant or a set of plants and recording any events of interest. Around 80 hours of observation were carried out during 2019 and 2020, during the months of fructification (from May to September), recording the birds that either visited or consumed fruits of the host and/or parasite pair. A total of 21 species of birds were identified. The most observed visiting birds of the host tree were: Sayaca Tanager (*Tangara sayaca*), Rufous-bellied Thrush (*Turdus rufiventris*), Great Kiskadee (*Pitangus sulphuratus*), and the Picazuro Pigeon (*Patagioenas picazuro*). From the abovementioned species, the main consumers of host fruits were the Rufous-bellied Thrush (*T. rufiventris*), Sayaca Tanager (*T. sayaca*), and the Great Kiskadee (*P. sulphuratus*). As for *P. quadrangulare*, although the Sayaca Tanager (*T. sayaca*) was the second most frequent visitor, only one species stood out as consumer of fruits: the Golden-Rumped Euphonia (*Euphonia cyanocephala*), a threatened bird, which was observed during the entire period, except for the month of May. Our observations make *E. cyanocephala* a strong candidate for dispersing the parasite and highlights the fact that the mistletoes may be key resources for some species. Especially in megacities like São Paulo, which lack high densely forested areas, the presence of *P. quadrangulare* might assist Golden-Rumped Euphonia conservation efforts, making it a crucial species to protect.

Keywords: ornithochory; parasitic plants; Santalales; mistletoe

Functional roles of mistletoe in a warming world

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Abstract

I summarize the mechanistic basis and functional significance of mistletoe's pervasive influence on productivity and diversity, synthesising recent findings on the responses of these parasitic plants to drought, heat waves and fire. Although representing just one per cent of all angiosperms, the ecophysiological traits associated with parasitism confer pronounced impacts on their hosts and disproportionate influence upon community structure, composition and broader ecosystem function. Recent work on pollinators, seed dispersers and litter-dependent detritovores has advanced our understanding of how mistletoes modulate animal communities via their extended and complementary phenology and the additive role of generalist interactors (pollinators and seed dispersers). Trade-offs between maximising efficiency in obtaining water from hosts and sensitivity to water stress underlie range shifts, host switching and increased reliance upon mistletoes by animal communities for nutritional and microclimatic resources, with direct and indirect impacts from anthropogenic climate change already apparent.

Keywords: Climate change, phenology, drought, fire

Posters

Germination of the mistletoe *Phoradendron quadrangulare* (Santalaceae): effects of substrate, fruit coat and hosts

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Abstract

Regardless of mistletoe seeds typically showing high germination rates and germinating on diverse substrate, it is not clear why some seedlings grow haustoria in some tree species and not in others. We performed two experiments to analyze the germination, survival, and attachment rate of *Phoradendron quadrangulare*: one experiment testing the presence/absence of fruit parts and other comparing the germination success among substrates and tree species. 700 seeds were set to germinate in two different substrates: i) trays with filter paper, where 200 seeds were still attached to the squeezed fruit coat and 200 seeds that had the fruit coat removed. ii) 300 seeds in three potential host species, *Handroanthus chrysotrichus*, *Melia azedarach*, and *Schinus molle*. Our hypotheses were that pericarp removal positively affects germination rate and that host species affects seed/seedling survival. Germination and mortality rates were compared by chi-square tests. Our results showed that the germination rate after 50 days is 37-45% in trays and 19-32% on host trees. The removal of the fruit coat resulted in twice the mortality (12%), rejecting the 1st hypothesis. The germination rate on *M. azedarach* was the greatest of all hosts (86% after 50 days), while the mortality was the lowest (4% after 50 days). Germination rate on *H. chrysotrichus* was 67% and mortality rate were 33% after 50 days. In *S. molle*, the germination rate was 20% and the mortality rate was 41%. The development of adhesive disc occurred first in seedlings on *M. azedarach*, in 41% of the seedlings after 100 days, in *H. chrysotrichus*, in 32% and in 14% in S. molle. Our results support what has been observed in nature: *M. azedarach* gets massively parasitized, while *H. chrysotrichus* is rarely parasitized and *S. molle* is never parasitized, suggesting that the bark physical/chemical properties might affect seed development.

Keywords: Embryo, mistletoe, pericarp, seedling, Santalales

First report of the *Cuscuta* sp. in *Eucalyptu*s clonal propagation.

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Abstract

Cuscuta sp. is a parasitic plant of the Convolvulaceae family and widely distributed around the world. In Brazil, it has found to be problematic particularly for ornamental and horticulture plants. This work reports the first recorded occurrence of this holoparasite plant in *Eucalyptus* hedge gardens during the clonal propagation process. The first occurrence was found in mini-hedges of a *Eucalyptus* clone (*Eucalyptus urophylla* var. *platyphylla*) at a nursery located in Monsenhor Gil, Piaui state. Following the first occurrence, the parasitic plant was found in another regional nursey in Dom Eliseu, Pará state, where eucalypt plants from the previously mentioned nursey were used in the establishment of new hedge gardens proving the successful ability of dissemination. In a preliminary histopathological study, there is visual impact in the xylem tissue of the affected eucalypt plants as vessel elements are obliterated and cambium activity is hampered. These histological features of host plantlets can potentially compromise the growth and productivity of the eucalypt mini-hedges. Eradication control methods were implemented through manual removal of colonized and adjacent mini-hedges with subsequent incineration of cultural remains. This control practice was successful in removing the parasitic plant from the hedge gardens that were free of the Cuscuta sp. at 90 days after mechanical cultural control.

Keywords: *Cuscuta* sp, *Eucalyptus*, Nurse

Session 4 Pine wilt disease

Oral communications

Pine wilt nematode prevalence and phenology in native pines and insect vectors of the Rocky Mountains (Colorado, USA)

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Abstract

Native forest tree mortality associated with PWN is newly reported from the Front Range of Colorado, but there is no regional information on PWN frequency or biology of local insect vectors, limiting management options. A sampling array was established to survey PWN in native pines (*Pinus ponderosa*) and longhorn beetles (*Monochamus clamator* and *M. scutellatus*) over multiple years and across natural and urban forest landscapes. We developed flight phenology models and evaluated effects of landscape factors on vector abundance and probability of infection. Flight phenology was similar for vectors; Monochamus flight initiated in mid-July and continued into October for both species. We report the first *M. clamator*–PWN association in the United States. PWN was found in 3.6 and 4.2% of sampled pines and beetles, respectively. Most infected host trees were outwardly asymptomatic; infection frequency in tree populations varied considerably and four epicentres of vector infectivity were identified. Epicentres varied in timing of anomalous infective vector frequency – some epicentres had high abundances of infected beetles early in the growing season whereas others had high abundances of infected beetles late in the growing season. *Monochamus* populations were found primarily in natural forest stands but migrated to urban areas late in the growing season. We conclude that PWN epicentres in the Rocky Mountains exhibit specific temporal windows of vector activity that differ from proximal sites. Urban forests, where the disease was initially observed in the region, do not support vector populations. Our results suggest that natural forest landscapes in the region are important reservoirs of PWN, and vector populations are especially abundant near burned stands.

Keywords: *Monochamus*, pine wilt nematode, ponderosa pine, vector ecology

Pine Wilt Disease (PWD) risk and expression of symptoms in Portugal

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Abstract

Pine Wood Nematode (*Bursaphelenchus xylophilus* - PWN) was first detected in 1999, in continental Portugal and Europe. Since then, despite control measures implemented, the pest has steadily spread within mainly the central part of the Portuguese territory (since 2008).

Until very recently it was assumed that under Portuguese climatic conditions, trees would show symptoms in the same year of nematodes' inoculation by the vector. However, some uncertainty remains related to the likelihood of latency of expression of pine wilt arising from nematode transmission as predicted by the wilt process ETpN model applied to Portugal during the PHRAME and the REPHRAME projects. Such latency in development of symptoms is very poorly understood and such trees could be missed in winter surveys.

To assess the delayed expression of pine wilt (latency) a detailed climatic database was created and the ETpN model determined 3 sub-regions: "No wilt expected", "Latency (wilt expected in the year following the infestation)" or "Wilt expression in the year of inoculation".

For field experiments (2017- 2022), stands in diferents regions of Portugal were selected: "No wilt expected" (Sintra), "Latency" (Marinha Grande and Sines), "Wilt expression" (Mata de Valverde, Azambuja and Lousã). Field plots were implemented in locations with presence of the PWN, but with low natural incidence of the disease and with maritime pine trees similar in size and density.

Each month, from June to November, 5 healthy trees were inoculated at each selected location, with 10.000 Bx, using Arbojet Quick-Jet kit, in the lower part of the canopy.

Observation of visual symptoms was done every 2 weeks during the growth period. Two weeks after the first visual wilting symptoms, the tree was felled and sampled for the presence of PWN. At the end of the experimental period, all remaining trees were also felled and sampled for PWN.

The results obtained with the PWN inoculations, did not allow differentiation between the three regions. In Marinha Grande predicted as "Latency", wilt was observed in the same year of inoculation, but in Sines several trees show latency. In Sintra expected as "No wilt" the majority of trees showed symptoms in the same year of inoculation. However, the climatic plot conditions during the experimental period were not compatible with the no wilt situation according to the model.

Keywords: *Monochamus galloprovincialis*; *Pinus pinaster*

Funding: This work was funded by the Project SI2.728274 & SI2.734897 – "Support to scientific activities to enhance the efficiency and reliability of surveillance for Pine wood nematode in particular in the buffer zone in Portugal.

Pinewood nematode spread in *Pinus pinaster* stands in a central region of continental Portugal

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Abstract

The Pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is present in Portugal since 1999. Despite control measures implementation, it continues spreading to non-infected maritime pine (*Pinus pinaster*) stands in a central region of continental Portugal. For the PWN to reach healthy trees needs to be carried by insect-vectors (*Monochamus* spp.). Therefore, its spread depends on the insect-vector flight capacity, which is considered to be 4-5 km, in average, with some reports of insects dispersing longer distances such as 11 km, although these assays were performed in areas with traffic that could accidentally hitchhiked the beetles. To assess the PWN spread, a 6 km long and 100 m wide transect was established on a continuous P. pinaster stand, starting at one PWN infected tree detected in 2018, in a previously not-affected parish. From May to October 2019, 30 multi-funnel traps with Galloprotect 2D-plus lures were placed in 15 pairs with 50 m between and in progressive distance along the transect, from 100 m to 6 km. All insect-vectors (*M. galloprovincialis*) caught were assessed for PWN presence and quantification. In January 2020, the transect was also surveyed and all symptomatic trees were felled and sampled. From the 65 trees sampled, PWN was detected only in two, and these were in the surroundings of the initial infection spot (less than 100 m). Moreover, 35 out of 510 insect-vectors were found carrying PWN (out of the 510 caught in the traps). Insect vectors were caught in 19 different traps, being four of them recovered on a trap of the pair placed further away from the initial infection. This result had consequences in the following year survey (winter 2020-2021), since the number of dead or symptomatic pines increased to 104. From these, ten were infected with PWN and were found scattered until the end of the transect. As conclusion, within one year, the insect-vectors dispersed rapidly along the 6 km of the transect, some of them carrying PWN (6.4%) and consequently after two years, PWN infected pines were found through all the area. This work was funded by Project PDR2020-101-032086, GI(PiN): Gestão Integrada do Pinhal/Nemátode da Madeira do Pinheiro.

Keywords: Pine Wilt Disease, *Monochamus galloprovincialis*, insect dispersal, multi-funnel traps

Understanding the origins and diversity of the invasive pinewood nematode in Korea via a population-scale whole genome sequencing

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Abstract

The introduction of a pathogen species into a novel range with naïve hosts often result in an explosive growth and a rapid range expansion of the invading pathogen. Evolutionary biologists have long been investigating the evolutionary history of invasive pathogens, e.g. tracking their origins and routes of dispersal, and understanding the role of natural selection in the process. The pinewood nematode, Bursaphelenchus xylophilus, provides an excellent system to study the evolutionary dynamics of invasive pathogens, since they spread into multiple regions of the globe, including East Asia and Europe, out of its native range in North America. To investigate the genetic origins and diversity of this invasive species in South Korea since its first recorded introduction in late 1980's, we created chromosome-scale genome assemblies of two inbred lines derived from samples within Korea and whole genome sequenced 359 individuals from 173 infected trees across the country using a novel protocol using whole genome amplification. Co-analyzing these genomes with eight genomes of inbred lines from Japan and Portugal, we first show that the Korean pinewood nematodes show an extremely stratified population structure, mainly composed of two major lineages within which the genetic diversity is extremely limited. These two lineages are differentially related to strains outside Korea. While one lineage distributes across the entire country, the other is limited to the southeastern region and is closer to some lineages in Japan and Portugal, suggesting a later independent introduction. We also observe the admixture between the two lineages where the two lineages collocate. This study illuminates a unique population dynamics of the highly pathogenic invasive species and calls for whole genome sequence-based study of pinewood nematode global diversity.

Keywords: Pinewood nematode; genetic diversity; population structure; whole genome sequencing

Dispersal behaviour of *Monochamus galloprovincialis* in heterogeneous pine landscapes: a genetic evidence

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Abstract

Movements in fragmented landscapes include both within and between habitat movements. *Monochamus galloprovincialis* (Coleoptera, Cerambycidae) is the main factor involved in the natural spread of the Pine Wood Nematode (PWN) in Europe. Since its introduction in Portugal, the PWN has rapidly expanded its range to a large part of the country. This xylophagous species is a secondary pest on weakened or dying trees, with a cosmopolitan taste in conifers, especially of the genus *Pinus*. Previous studies showed that high pine densities are negatively correlated with beetle dispersal and that landscape composition influences dispersal behaviour.

In this study, we aimed to determine the effect of landscape composition on genetic structure. Highly polymorphic markers (microsatellites) provide high-spatial resolution neutral maps of population genetic connectivity to quantify contemporary dispersal in parentage. The study was carried out in five geographical sectors in the south-west of France, each composed of two landscape types, a homogeneous landscape of maritime pines and a heterogeneous landscape of (mixed) broadleaved and pine stands. Beetles were collected with three baited traps deployed within each of the two landscapes selected in the five sectors.

The genotyping of 135 individuals for 15 loci showed very low genetic differentiation between landscapes (pines and mixed), indicating high gene flow between populations. A total of 36 first generation migrants were detected between the 10 populations studied. Almost all of the displacement occurred between geographical sectors, suggesting long distance dispersal. Interestingly, about 70% of the migrants were moving towards pine dominated landscapes, whether originating from heterogeneous (14 migrants) or pure pine landscapes (10 migrants).

Keywords: PWN vector; dispersal; heterogeneous landscape; microsatellites; first generation migrants

Which strategy is the most cost effective: clear-cutting strategy or tree-by-tree selective cutting strategy?

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Abstract

The pine wood nematode (PWN) is a great threat to the pine and spruce forestry industry. In Europe, PWN can be transmitted via *Monochamus galloprovincialis* from infected trees to healthy trees. To mitigate the impact and decrease the spread of PWN, the European Union (EU) has specified that a 500 m clear-cutting zone shall be delimited around individual infected trees. Modelling has proved that the width was not sufficient. An alternative control strategy would be to do selective cutting on a tree by tree basis, thus without a clear-cut zone, combined with increased surveillance. The aim of this study is to compare the cost-effectiveness of two strategies: 1) 500 m radius clear-cutting strategy (S1); 2)Tree by tree selective cutting strategy (S2). We modified an individual-based model that describes the dispersal of *M. galloprovincialis* and transmission of PWN in a 20 km * 20 km homogenous forest, combined with sub-models for assessing the cost and effectiveness of alternative strategies. Effectiveness was defined as the number of total cut infected trees. Total cost incorporates surveillance cost, wood sampling cost and control cost. We determined three levels of surveillance: visual surveillance, aircraft surveillance, and trapping networks together with visual surveillance. For visual surveillance, mean cost per cut infected tree was 44,316 € for S1, in comparison to 4,742 € for S2. For trapping in combination of visual surveillance, mean cost per cut infected tree was 49,213 € for S1, in contrast to 5,517 € for S2. For aircraft surveillance, mean cost per cut infected tree was 98,372 € for S1, compared with 5,459 € for S2. Our results showed that S2 was more cost effective than S1 independent of surveillance methods. Therefore, S2 is a better choice to control PWN in case that forest managers want to achieve the goal of most cost-effectiveness of strategy.

Keywords: control, cost-effectiveness, individual-based model, pine wood nematode, surveillance

Current status of pine wilt disease damage in Japan and its countermeasures

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Abstract

More than 100 years have passed since pine wilt disease (PWD) invaded Japan. Pine-wilt damage, first occurred in the south-western region, has spread throughout the country and reached to the cool climate high-latitude and high-elevation regions. Pine trees are almost exterminated in some of the old-damaged areas, but a vast area of pine forest still remains in cool climate regions.

The impact of PWD on Japanese forests and forest industries is considered to be less than devastating, for forests relatively easily regenerate after the devastation of pine trees and pine trees are not important timber wood in Japan. However, there are locations where forests cannot regenerate depending on site conditions, and where pine forests are economically important for the timber industry or mushroom production. Moreover, pine trees are an essential component in wind- and sand-break forests in the coastal area or the traditional scenery of Japan. PWD entails an enormous cost for employing countermeasures to protect the important pine forests and trees.

Control methods of PWD disease in Japan mainly consist of the disposal of damaged trees, preventive spray of insecticide onto live trees and trunk injection of nematocide. Seedlings of resistant clones have commonly used in pine plantations. Although controlling PWD can be accomplished with such powerful items, it has ended unsuccessfully in many cases because of the constraint of budget and workforce, restricted use of pesticides, and so on. As it is difficult to protect all under Japanese situations, we alternatively propose to implement priorities in protecting the pine forests and concentrate the control effort in a top-priority location(s). On the other hand, the development of control methods by physical containment of vector beetles emerged from the damaged trees and the construction of a system for using damaged trees as biomass fuel for power generation are promoted as the pesticide-free control methods against PWD.

Keywords: affected area; control methods; impacts; pesticide-free alternatives; pine wilt disease

Pinewood nematode diagnostic services In North Carolina Department of Agriculture & Consumer Services

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Abstract

Bursaphelenchus xylophilus, the pinewood nematode (PWN), is the causal agent for pine wilt disease. The nematode was first discovered in the timber of longleaf pine (*Pinus palustris*) in Louisiana, USA. PWN is native to North America where it causes relatively minor damage to native conifers but is labeled as an EPPO-A2 pest and a quarantine nematode for many countries because of its potential for destruction of their native conifers. The Agronomic Division of the North Carolina Department of Agriculture & Consumer Services has a publicly-operated nematode assay lab providing nematode diagnostic services and control recommendations. In the past ten years, due to stricter regulations on PWN, a large number of pine wood samples have been submitted to the lab. From fiscal year 2012 to 2022, 67,135 pine wood samples were analyzed, and 7,462 reports were generated in connection with the issuance of phytosanitary certificates by USDA/APHIS/PPQ for export of pine wood logs. A large industrial funnel (30-cm diameter, 32-cm high, 6800-ml water capacity) and a movable rack (2.2-m x 1-m x 1.3-m H, 18 samples in total per rack) were designed to extract PWN. The required sample size is a minimum of 200 grams of wood-drilled shavings shipped overnight to the lab. Identification of PWN is accomplished by using traditional morphology, primarily by female tail shape and vulva flap and male spicule and bursa. The identification can also be confirmed by DNA sequencing and real-time-PCR if necessary to comply with the zero-tolerance export regulations. Overall, PWN prevalence in submitted samples has been 2.15%. These results indicate the low presence of PWN in exported pine wood logs and the importance of regulatory measures and laboratory testing.

Keywords: Diagnosis, quarantine, pinewood log export

Chemical adaptive interaction among pinewood nematodes, its vector beetle, associate microbes and pine trees

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Abstract

The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is the causative agent of PWD while the nematode forms the symbiotic interaction with its insect vectors *Monochamus* spp., associated bacteria and ophiostomatoid fungi in order to successfully infect and kill its pine host tree. This review focuses on the interspecific communication between the nematode and its symbiotic partners, and the potential role of this communication in promoting pathogenicity and invasiveness of the PWN. We describe the chemical and molecular signals positively influencing the survival, reproduction and spread of the PWN. Knowledge and understanding of these signals could potentially be used to interfere with the propagation and dispersal of this economically important plant-parasitic nematode.

Keywords: Chemical adaptive interaction; Pinewood nematodes; Vector beetle; Microbes; Pine trees

Linking pine cell wall composition and structure to pinewood nematode resistance

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Abstract

The pinewood nematode (PWN), *Bursaphelenchus xylophilus* uses its stylet to perforate cell walls (CW) and to release hydrolytic enzymes against CW glycans. Thus, understanding CW chemistry and structure is essential to understand how it may influence host susceptibility to PWN infection. Moreover, it is important to better understand how environmental conditions affect host resistance/susceptibility. To achieve this objective, an innovative transdisciplinary project PineWALL (FCT-PTDC/ASPSIL/3142/2020) was established. *Pinus* spp. (*P. pinaster*, high susceptible species and *P. pinea*, less susceptible) plants have been inoculated with PWN and disease progression is being monitored under controlled temperatures and soil water contents. Non-inoculated plants are used as controls. At the end of the assays, differences in the nematode reproduction factor on both species will be determined. High-throughput vibrational spectroscopy techniques (Raman and Fourier transform infrared) are being applied for a global CW characterisation. Preliminary data revealed that there are significantly different CW compositional properties between PWN-inoculated and control *P. pinaster* samples. Namely, phenolic compounds are upregulated in PWN-inoculated plants, presumably as a response to defence mechanisms in the plants. Furthermore, Pinus spp. morphology/anatomy will be compared, to understand whether anatomical features have implications on disease progression and chemical analytical methods will be employed to characterise different CW fractions. Immunological approaches will unveil the CW glycome profile and will reveal in muro glycan distributions by in situ immunolabelling. All data will be integrated to identify structural CW biomarkers that may be applied to predict pine susceptibility/resistance to PWN infection.

Keywords: *Bursaphelenchus xylophilus*; cell wall; climate change; *Pinus* spp.; spectroscopy techniques

Susceptibility in Pinus pinaster provenances against *Bursaphelenchus xylophilus*

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Abstract

Pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is considered a quarantine pest by the European Union; it threats European pine forests since it was reported in Portugal in 1999. The spread from Portugal to Europe and its establishment in Spain has been already predicted. According to previous studies, *Pinus pinaster*, an important pine distributed in the Mediterranean basin, shows a moderate susceptibility to PWN. This study aims to test the susceptibility of all Spanish provenances, one from Portugal, one from France and two from Morocco to PWN, and compare their behavior to that of two seed orchards, one belonging to the Galician *P. pinaster* breeding program in Spain and the other to the Landas one in France). The experiment was performed in a greenhouse, where temperature and watering conditions were controlled. We measured the morphological traits, wilting symptoms, disease evolution variables, survival and density of nematode population. Moreover, some constitutive chemical compounds were analyzed. Significant differences in survival, wilting symptoms, morphological traits and density of nematode populations were found among provenances and seed orchards. Clustering provenances into genetic groups, we also found significant differences among genetic groups. On the contrary, no differences were found for disease evolution variables. We also observed that the most resistant provenances showed lower values of constitutive condensed tannins, total polyphenolics and lipid-soluble substances than the most susceptible ones. A geographic clinal pattern was found for this disease, provenances from the south-east of the Iberian Peninsula having the highest resistance to the disease. Moreover, those provenances coming from arid areas, with higher temperatures and lower precipitations are less susceptible to PWN than those from humid and temperate climates.

Keywords: Maritime pine, Pinewood nematode, Genetic groups, Constitutive defenses, Geographic cline

PineEnemy – Exploring the NEmatode-MYcobiota interactions in Pine Wilt Disease

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Abstract

Pine wilt disease (PWD) is one of the most important threats to conifer forests in Europe and worldwide, causing severe economic and environmental damages. This complex disease results from the interaction between three biological elements: the plant-parasitic nematode *Bursaphelenchus xylophilus*, known as pinewood nematode (PWN); the insect-vector (cerambycid beetles of the genus *Monochamus*), and the host tree, mostly *Pinus* spp. In 1999, *B. xylophilus* was firstly identified in maritime pines (*Pinus pinaster*) in Portugal and in Europe. Despite a nationwide monitoring system, enforcement of strict phytosanitary measures and constraints implemented after PWN detection, this devastating disease has spread quickly in Portuguese mainland, Madeira Island and Northern Spain. As a migratory endoparasite, once inside a susceptible tree, PWN has the ability to feed on the living parenchyma of the epithelial cells in the resin canals, causing a reduction in water flux and ultimately cessation of resin flow. In the later stages of the disease, PWN assumes an obligatory mycetophagous phase. The development of the PWN population appears to be strongly associated with fungi that colonize the declining trees. Fungi harboured in weakened pines are seen as essential for the ongoing development and completion of the PWN life-cycle, affecting not only PWN reproduction but also the number of individuals carried by the insect-vector. Through a spatio-temporal analysis, PineEnemy focused on the characterization of the structure and dynamics of the nematode-fungi interactions using culturable and non-culturable approaches with special emphasis in metagenomics analyses. Our aim is to understand if PWN-associated mycobiota plays a key-role in the development of PWD, in interaction with PWN and insect-vector, and into which extend can be targeted to disrupt the disease cycle.

Keywords: *Bursaphelenchus xylophilus*, Metagenomics, *Monochamus* spp., Mycobiota, Pine Wilt Disease.

PineEnemy – Sustainable chemical management of pinewood nematode: Lessons learned from assaying volatiles

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Abstract

Bursaphelenchus xylophilus, commonly known as the pinewood nematode (PWN), parasitizes susceptible pines and originates pine wilt disease (PWD). While its expression is relatively low at the PWN's native ranges in the US, an increasing incidence of PWD has been reported in Japan, China, Korea and more recently in Portugal. These epidemic events have challenged national authorities to enforce strong containment strategies leading to heavy economic losses in countries where the wood industry is very important. Current control measures for the PWD rely on regulating wood transportation, treating wood products, clearing forests from infested wood, enforcing pine tree free buffer zones and chemical control of the PWN and its insect vector. Although highly effective, chemical control commonly resorts to the use of highly damaging synthetic chemicals with adverse effects on forest biosystems and human health. In an effort to develop a more sustainable chemical management of the PWN, volatiles and volatile extracts from natural sources, mainly plants and fungi, have been actively researched. Essential oils (EOs) are complex mixtures of plant volatiles, mainly extracted through hydrodistillation from medicinal and aromatic plant species. Against the PWN, several EOs and their volatiles have shown an impressive biocidal activity. The Lamiaceae, Myrtaceae and Apiaceae families are generally chosen as sources for plants with highly active EOs, however, other families can produce EOs that cause extensive PWN mortality, such as those of onion (*Allium cepa*) or cinnamon (*Cinnamomum* spp.). The activity of EOs is intrinsically linked to their richness in volatiles with highly electronegative elements, generally O or S, and can vary substantially with compound isomerism, carbon chain length, and number and position of double bonds. Understanding the chemical guidelines that favor volatile toxicity to the PWN and safety to the pine host is paramount to devise effective biopesticides with low environmental impact.

Keywords: biopesticides; Bursaphelenchus xylophilus; essential oils; pest management; volatiles

Funding: This work was partly supported by project PineEnemy: Exploring the NEmatode-MYcobiota interactions in Pine Wilt Disease (LISBOA-01-0145-FEDER-028724).

PineEnemy – Biological control of pinewood nematode using the fungal antagonists *Esteya* spp.

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Abstract

The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is the causal agent of pine wilt disease (PWD) and a quarantine organism in many countries. Due to its economic importance and widespread distribution, an enormous effort is devoted to study the virulence of B. xylophilus and the epidemiology of PWD. Understandably, the non-specificity of synthetic chemicals and lack of environmental-friendly options to control this parasite has led to an increasing focus on antagonists capable of suppressing the PWN. *Esteya* spp. are nematophagous fungi and promising biocontrol agents against the PWN. Two species are described: *E. vermicola* and *E. floridanum*. In a quest to explore compatibility for a potential combined application of these PWN antagonists in maritime pine, *Pinus pinaster*, the main and most affected species in Portuguese forests, we carried out fungus-fungus interaction assays on agar plates. These results should set the foundations for future research on exploring a synergistic effect and combined application of these PWN antagonists in maritime pine, allowing us to devise new strategies for the management of PWD.

Keywords: Biological control; Nematophagous fungi; Pinewood nematode; Pine wilt disease; *Pinus pinaster*

Funding: this work is funded by the Portuguese Foundation for Science and Technology (Fundação para a Ciência e a Tecnologia – FCT), under the project “PineEnemy – Exploring the NEmatode-MYcobiota interactions in Pine Wilt Disease” (PTDC/ASP-PLA/28724/2017), and FEDER (Fundo Europeu de Desenvolvimento Regional) (LISBOA-01-0145-FEDER-028724), through Lisbon and Alentejo Regional Operational Programmes. David Pires is also supported by the FCT PhD Fellowship 2021.08030.BD.

Using Nematophagous *Esteya vermicola coreana* (G810) to Control the Disastrous Pine Wilt Disease

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Abstract

Pine wilt disease induced by the pinewood nematode, is a great threat to pine tree in many country. Once this nematode transferred from the vector, to twig of pine tree, pine tree used to start wilting very quickly within 3 month.

We have been working this project more than 10 years to seek natural enemy in order to save pine tree. This study evaluated the protective effects of the nematophagous fungus *Esteya vermicola coreana* (G810) on the large pine trees of Mt. Wora, South Korea for six years. When pine trees were treated with G810 110 days before artificial pine wood nematode (PWN) infection(prevention study), ~50% of the trees survived for six years.

In contrast, all of the control trees were killed by pine wilt disease in the first year. Although it has been more than six years since the beginning of this experiment, the existence of G810 inside the treated pine trees was successfully detected using a PCR with two pairs of specific primers .

In addition, one of the province Hawon, Kwangdong province in China invited us to demonstrate this performance with more than 2,000 trees in the mountain. We have saved more than ~60% for prevention study.

Also we did same work for prevention study two different provinces guided by government manual. They have showed 80, and 92% survival rates respectively.

Still more than 90% pine tree are alive since completed this project three years ago.

These results suggest that G810 possesses great potential as a biocontrol agent to combat the disastrous pine wilt disease. This is the first report of using nematophagous fungi to control pine wilt disease in the field for a duration of over five years.

Keywords: pine wilt disease, biocontrol, *Esteya vormicola coreana*

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

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Abstract

Bursaphelenchus xylophilus, commonly known as Pine Wood Nematode (PWN), is one of the most damaging forest pathogens to conifer forests worldwide. This nematode causes the so-called Pine Wilt Disease (PWD), whose symptoms are discolouration of upper needles, sudden decay and finally the death of the trees. The pathogen requires an insect vector (sawyer beetles of the genus *Monochamus*) to be transported from affected trees to healthy ones and, once inside them, it lodges in the resin canals and feeds on their cells. So far, disease control has been mainly focused on vectors. 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, controlling the nematode as well is vital for an effective integrated management. To this end, the nematophagous potential of eight fungal species was in vitro tested, among them two *Beauveria* species, seeking to test if the reproduction rate of the nematode was reduced in their presence. The assay was conducted in glass vials with a base culture medium containing hulled barley. Inside the vials, each fungus was in contact with 270 nematodes during 13 days at 25ºC. Final counts were made to determine the progress of the populations. This experiment has attained encouraging results, since several species showed a strong antagonist effect on the nematode reproduction. In fact, *Beauveria* species (*B. bassiana* and *B. pseudobassiana*) and *Trichoderma citrinoviridae* not only annulled the reproduction, but also showed a nematicidal effect. In view of these initial results, it can be hypothesised that a future integrated biological control could be based on the use of *Beauveria* species. Nevertheless, further in vivo trials are needed to corroborate the effect of these fungal species on PWN populations.

Keywords: Pine Wilt Disease, *Beauveria* spp., nematicidal effect, biological control

Posters

The Pine Wilt Disease Containment in Europe: More than 23 years in Portugal and almost 14 years in Spain

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Abstract

The Pine Wilt Disease (PWD), caused by the pinewood nematode (PWN) *Bursaphelenchus xylophilus*, killed millions of pine trees after being accidentally introduced into Japan, China, Korea and Taiwan through the international trade of infected wood. In 1999 it was detected in Portugal on maritime pines (*Pinus pinaster*) and on the following 20 years, thousands of maritime pine trees were killed every year, in spite of the efforts to contain its spread. The specific action plan, implemented by the Portuguese Forest Authoritiy (ICNF), follows the guidelines of the Commission Implementing Decision 2012/535/EU. The PWN control actions are based on the survey, identification and elimination of symptomatic, wilted and declining trees during the autumn and winter months, and on the trapping of the insect-vector (*Monochamus galloprovincialis*) during its spring-summer flight period. Other complementary actions include the legislative regulation on host trees (e.g., wood transport), and inspection activities to economic operators and public awareness. Over the last 11 years more than 83,574 wood samples were analysed with an average of 8% PWN positive results, with none in the Buffer Zone which comprises 20 km-stretch along the Spanish border. In result of the containment actions, close to 18 million declining trees were felled in the mainland territory, especially in the Buffer Zone (2.9 million), and around 43 000 insect-vectors were caught on the traps placed every year. Despite of the Portuguese authorities' effort, the infested area has been increasing and approaching the Buffer Zone in the mainland, and a new focus was detected in Madeira Island in 2009. In Spain, the PWD was first detected in October 2008 and declared eradicated in 2013, although subsequently, five other outbreaks were reported from Caceres, As Neves, Valverde del Fresno, Sancti-Spiritus and Lagunilla (the furthest from the Portuguese border at about 70 km). As in Portugal demarcated areas were established and eradication measures implemented and still ongoing, in an attempt to prevent the dissemination of this pathogenic organism in the Iberian Peninsula.

Keywords: Pine wood nematode control; Portugal; Spain

Nematoxicity of medium-chain length saturated aliphatic fatty acids on the pinewood nematode *Bursaphelenchus xylophilus*

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Abstract

The pinewood nematode (PWN) *Bursaphelenchus xylophilus* is responsible for causing pine wilt disease (PWD) in susceptible pines. The pest management strategies adopted in Portugal and Spain were able to decrease PWN spread rate, yet the complete containment and eradication of this invasive phytoparasite are still not yet attained. Currently, research is focusing on sustainable cultural practices and the development of biopesticides or biocontrol agents. Phytochemicals or components of plant and fungal extracts have been studied with considerable success. Medium-chain length fatty acids extracted from aromatic plants have shown high nematicidal activity against other plant parasitic nematodes, e.g., the root-knot nematodes.

In the present work, decanoic and undecanoic acids, C10 and C11 fatty acids, respectively, were tested against the PWN, in direct contact bioassays, for 24 h. These compounds were added to aqueous suspensions of the PWN, in concentrations that ranged from 2 to 0.03 µg/mL. Dead and live PWNs were counted and compared to control assays. Undecanoic acid showed a stronger activity reaching a half maximal effective concentration (EC50) of 0.06 µg/mL, while decanoic acid showed a higher EC50, namely 0.13 µg/mL.

Future research will determine the activity of other medium chain fatty acids as potential biopesticidal molecules to be used in sustainable pest management strategies.

Keywords: Direct contact bioassays; Fatty acids; Nematicides; Pine Wilt Disease; Sustainable pest management

Bursaphelenchus species diversity associated with a declining *Pinus pinea*

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Abstract

A centennial *Pinus pinea* displaying severe wilting symptoms was identified and felled at Coimbra, Portugal. Thus, a survey for the presence of *Bursaphelenchus xylophilus*, the pinewood nematode, and other *Bursaphelenchus* species was carried out. Nematodes were extracted from wood samples from the trunk and branches using the Whitehead and Hemming tray method. Co-occurring *Bursaphelenchus* spp. specimens were isolated and four *Bursaphelenchus* spp. isolates were established and maintained in *Botrytis cinerea* cultures. Morphological analyses of these isolates are being carried out based on species-specific morphological characters and the molecular identification is being performed based on the amplification, restriction analysis and/or sequencing of the rDNA segment containing the 5.8S gene, ITS1 and ITS2 regions and partial regions of 18S and 28S genes. *Bursaphelenchus xylophilus* was not detected. Two *Bursaphelenchus* species were already characterised and identified as *B. fungivorus* and *B. sexdentati* (Type II). This study demonstrates the wide dissemination of *Bursaphelenchus* species, and their high plasticity, allowing the establishment in different hosts and highlights that wilted pine trees can act as nematode diversity hotspots.

Keywords: *Bursaphelenchus* spp.; diversity; *Pinus pinea*

Mycobiome diversity and structure of the Pine Wilt Disease complex in Portugal

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Abstract

Fungi are major ecological players in Pine Wilt Disease (PWD) complex. In the late stages of the disease, the pinewood nematode (PWN) *Bursaphelenchus xylophilus* feeds on the fungal flora available in the pine tree for survival and multiplication. Not all fungi are suitable PWN food source. Previous studies have already proved a close relation between the PWN and the blue-stain fungi (order Ophiostomatales), necrotrophic pathogens associated with bark beetles (Coleoptera: Scolytidae). The PWN is able to grow densely in the presence of these fungi, which results in a higher number of nematodes transferred to the insect-vector *Monochamus* spp. Despite the significant progresses in understanding the mycoflora diversity in the PWD complex using high-throughput sequencing, few references have addressed to the diversity and abundance of blue-stain fungi communities. To understand the spatio-temporal diversity and structure of *Pinus pinaster* mycobiome, stem samples from PWN-infected and non-infected pine trees were collected in three locations of Continental Portugal with different PWD histories, during the maturation phase of the insect-vector *M. galloprovinciallis* (winter 2019-spring 2020). The PWN-mycoflora from the PWN-infected *P. pinaster* was also characterized. A total of 30 samples of P. pinaster and 15 samples of PWN from PWN-infected trees were characterized using ITS2 amplicon sequencing. The diversity and structure of the fungal communities in *P. pinaster* varied by disease status suggesting that the PWN presence affects the endophytic fungal communities. For both *P. pinaster* and PWN fungal communities, differences were also seen in terms between locations (recent PWD loci Seia, and long-term PWN locus Companhia das Lezírias and Tróia). Ophiostomatales were only detected in PWN-infected *P. pinaster*, corroborating our culturomics results previously obtained. This research contributes to fill the knowledge-gap on the ecology of the fungal communities in PWD complex.

Keywords: Mycobiome; Metagenomics; Ophiostomatales; Pine Wilt Disease

A motif to search for parasitism genes regulators in *Bursaphelenchus xylophilus*.

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Abstract

From the previous transcriptomic data derived from the pharyngeal gland cells (considered a specialized tissue potentially related to parasitism) we have identified a non-coding DNA motif - STATAWAARS - associated in the promotor region of highly abundant and secreted expressed genes. Given that this non-coding genetic signature unifies many sequences of unrelated parasitism genes, it implies the existence of a potential major regulator(s), that binds to this sequence to control the expression of downstream genes.

The project NemaWAARS focuses on how parasitism in *B. xylophilus* is regulated and which mechanisms may control the gene expression of the parasitism-related genes. We hypothesize that by disrupting this regulator(s), it would be possible to simultaneously disrupt the expression of many associated parasitism-related genes. To test the hypothesis the project aims to identify proteins (or complex of proteins) that bind in the promoter regions of parasitism-related genes (in vivo), or identify other regulatory candidates for master regulators of parasitism-related genes expression that are enriched in the pharyngeal gland cell tissues. For the best candidate regulatory proteins, an RNAi approach will target the selected gene candidates and evaluate the regulatory role in effector genes expression and in interaction with the host (in planta). Under an ongoing national and international collaborative network, the strategy in NemaWAARS will include innovative approaches to explore the regulators that govern effector gene expression applied in *B. xylophilus* research.

Keywords: Molecular plant-nematode interaction, effector gene expression, pharyngeal gland cells, STATAWAARS; transcription factors

About the optimal time and temperature parameters for the extraction of *Bursaphelenchus xylophilus* nematodes by the Baermann method

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Abstract

The origin of the pinewood nematode (PWN) *Bursaphelenchus xylophilus* (Steiner & Buhrer 1934) Nickle 1970 is North America. At the beginning of the 20th century, the pathogen was introduced into Asia and the PWN range continues to expand. Therefore, all conifer commodities imported into the country, including wood packaging materials, are inspected for the PWN presence by NPPO personnel. To the PWN extraction from wood samples, the Baermann method (funnel or modified) 24°C or 48°C hours is generally recommended (Schroder et al., 2009; ISPM 27, 2016). The aim of our research was to determine the optimal exposure time and temperature for the B. xylophilus extraction the Baermann funnel method. The study included an experiment on the PWN (US1-Bx isolate) extraction from sawdust obtained by drilling with a battery-powered drill of PWN-infected logs of Scots pine (*Pinus sylvestris*). Exposure times for nematode extraction were 6 h, 24 h, and 48 h from the start of extraction in climate rooms at 18°C and 25°C. A total of 200 grams of sawdust were placed in a Berman funnel on a metal sieve with 105 mm of diameter and a mesh diagonal of 0.16 mm. The data were compared by Monte Carlo Randomization (MCR-test) with B = 1000 iterations in the environment of the statistical package PAST 4.06.

The average number of extracted nematodes over two days was 48.8 nematode/sample for 18°C and 68.5 for 25°C. In the first 6 hours at 18°C and 25°C, were extracted in average 32% and 17% and by the end of the first day (next +18 h), 45% and 62%, respectively, of the total nematode numbers (in 48 h). All these differences between temperature regimes were significant ($p < 0.028$). During the second day, no significant differentiation in the number of extracted nematodes was observed under the temperature regimes researches. The average increase in nematode numbers was 21 to 22% ($p = 0.730$). At both temperature regimes, the majority of nematodes, about 80%, were extracted during the first day, which was significantly higher than during the second day ($p < 0.036$).

Keywords: Pinewood nematode, *Bursaphelenchus xylophilus*, Baermann technique, nematode extraction

Using nematophagous *Esteya* sp to control the disastrous pine wilting disease

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Abstract

Pine wilting disease induced by pine wood nematode, Bursaphelenchus xylophilus is a great threat to pine tree in many country. Once this nematode transferred from the vector to twig of pine tree used to start wilting very quickly within 3 month.

We have been working more than 15 years to study how to use natural enemy to save pine tree from naturally occurring mycoflora. This is a lot advantages compare to chemical pesticide in earth ecosystem. Here disclose valuable several facts that we have been collected in lab and field investigation. These are included step wise process for natural enemy isolation, host cell killing mechanism in vivo and in vitro, curing output in field test, etc.

Keywords: Pine wilting disease, natural enemy, Esteya coreanus, bio control

An efficient protocol for sequencing the whole genome from a single nematode individual

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Abstract

The pinewood nematode, *Bursaphelenchus xylophilus*, has been spreading in Korea since its first introduction in late 1980's but its source(s) and the patterns of human-mediated long-distance dispersal remain elusive due to lack of fine-resolution markers for tracing their movements within and between countries. Population genetics approach has long promised information for such a fine-scaled tracing, but this genetic epidemiological approach so far has been poorly implemented due to difficulties in obtaining high-throughput genetic information at the individual level. In this study, we present a novel laboratory protocol that can directly retrieve the whole genome sequence of individual nematodes without time-consuming and labor intensive efforts for producing inbred lines prior to whole genome sequencing. In short, we cleanse and flash-freeze individual nematodes, amplify genomic DNA via whole genome amplification (WGA), and produce Illumina sequencing library from WGA product. The entire protocol requires 10< hours of hands-on time and per-individual cost of < $100 USD. Applying this protocol, we produce whole genome sequences of 359 pinewood nematode individuals collected across 170 infected sites in South Korea and discover over 1.1 million high-quality variants across the pinewood nematode genome.

Keywords: pinewood nematode; whole genome sequencing; whole genome amplification

Changes in leaf color and mortality of tree over time after artificial inoculation with pine wilt nematode in pine trees

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Abstract

Among the methods for controlling pine wood nematode (PWN) disease, tree injection is the most commonly used method for preventing PWN infection. In order to evaluate the effectiveness of preventive tree injection, the efficacy is evaluated by inoculating the PWN after tree injection, and then examining the death of the tree and the proliferation density of the PWN. This study investigated the tree mortality rate and change over time of coniferous discoloration according to the inoculation density of PWNs for two main nematode-host pine trees. Conifer discoloration was faster in *Pinus thunbergii* than in *P. densiflora*. Also, conifer discoloration in small-diameter trees was faster. The density of PWNs was higher in branches than in the trunk, and the average density was higher in *P. densiflora* than in *P. thunbergii*. The proportion of 100% dead trees 6 months after pine wood nematode inoculation was 30~56.7% in *P. densiflora* and 63.3~86.7% in *P. thunbergii*. The rate of needle discoloration and nematode reproduction according to the PWN inoculation density did not show significant differences between 10,000 and 30,000 PWN inoculations. For appropriate monitoring of the effect of tree injection on the PWN, investigations in time and space should take into consideration the independent response of individual tree species.

Keywords: *Bursaphelenchus xylophilus*, *Pinus densiflora*, *Pinus thunbergii*, reproduction, trunk injection.

Comparative bioactivity of emamectin benzoate formulations against the pine wood nematode, *Bursaphelenchus xylophilus*

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Abstract

The pine wood nematode (PWN), *Bursaphelenchus xylophilus* is a well-known devastating pathogen of economic importance in Korea and other countries. In Korea, trunk injection of nematicides is the preferred method of PWN control. In this study, the efficacy of sixteen locally produced formulations of emamectin benzoate (EB) against the PWN are compared. The nematodes were exposed to varying concentrations of the studied formulations in vitro. Additionally, nematode populations were pre-exposed to 0.0258 and 0.258 µg/ml concentration of the tested formulations for 24 hours before being inoculated in tree branches. Despite the uniformity in the concentration of the active ingredient (2.15%), efficacy was contrastingly different depending on the producer. EB formulations evidently conformed to three distinct categories based on similarities in sublethal activity in vitro. Formulations in group 1 were highly effective, with LC95 of as low as 0.095 mg/ml a.i. Significant variations in sublethal toxicities were more evident in group 2 (LC95 = 0.1732-0.868 mg/ml a.i.). Group 3 constituted the least effective formulations, with LC95 values of as high as 3.953-4.897 mg/ml a.i. A relatively similar trend was evident in the branch experiment albeit with significant variations. At high concentrations of 0.258 µg/ml, nematode reproduction was highly suppressed in all but three formulations. Group 1 formulations significantly reduced nematode reproduction in pine branches (Rf = 0.8-1.5) even at lower concentration of 0.0258 µg/ml. Reproduction of populations exposed to formulations from the two groups varied significantly irrespective of the sublethal toxicities. These variations may be attributed to differences in inert ingredients used by the producers.

Keywords: Efficacy, nematicide, trunk injection

Mode of pine wood nematode infection by GFP-labeled *Esteya vermicola coreana* and the fungus growth in living pine tree

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Abstract

Esteya vermicola coreana (EVC)is an endophytic fungus of pine wood nematode (PWN) with demonstrated biocontrol efficacy. To better understand of molecular and cellular mechanisms of fungal parasitism of nematodes infection process and mechanisms of controlling to PWD, *Agrobacterium tumefaciens*-mediated transformation (ATMT) conditions were established for EVC. The resulting transformants were similar to the corresponding wild-type strains on PWN infection rates, pine tree pathogenicity, and also control effect to Pine Wilt Disease (PWD).

The infection processes of PWN by eGFP-labelled EVC were microscopically analyzed. The hypha started to grew from nematode. Moreover, the mode of this fungus growth at inside of living pine tree was explored by fluorescence microscope observation with sliced wood section.

The result showed that the three different types (lunate, bacilloid, and blasto) of EVC spore injected into pine tree may be so important. But the critical function of these are not clear yet. We postulated that They may follow germination, hyphae production to mycelium, and lunate conidium formation for killing sequentially.

Keywords: Biocontrol, natural enemy, *Esteya* sp

The status of pine wilt disease in South Korea, 2022

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Abstract

The Pine Wilt Disease (PWD), caused by the pinewood nematode (PWN) *Bursaphelenchus xylophilus*, is most destructive forest disease and South Korea is one of the most severely damaged countries in the world. In Korea, more than thirty years have passed since PWD was first found in Busan in 1988. Now the PWD was reported from 135 districts, counties, and cities in 2022 although PWD started with only few numbers of infected pine trees in a small mountain in Busan. The number of infected pine trees has been fluctuated during 30 years and it peaked up to 2,180,000 in 2014. Recently it had declined down at 390,000 in 2021, but in the last season of the control (2021.5~2022.4), the number of infected pine trees increased slightly by 22.6% compared to the previous year. The system of PWD investigation and the diagnostic techniques have been dramatically changed and developed. NFC electronic tags are in use to prevent the monitoring blind spots, and a record management system using QR code from the monitoring and diagnosis of dead pine trees to the control has been established. It allows that information entered in the field can be checked in real time, and record on the work process can be managed. In addition, for rapid diagnosis of PWD, it is promoting practical use of field and early diagnosis method using a recombinase polymerase amplification (RPA) assay with a novel extraction buffer.

Keywords: Diagnosis, Monitoring, Pine wilt disease, Pine wood nematode, South Korea

In vitro pine-nematode co-cultures as biotechnological tools to analyze regulators of parasitism of the pinewood nematode

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Abstract

The increase in global trade activities has hastened the spread of pests and pathogens to new ecosystems. The pinewood nematode (PWN) *Bursaphelenchus xylophilus* is a phytoparasitic nematode, native to North America and introduced to Japan in the beginning of the 20th century, that quickly spread to neighbouring countries. In 1999, the PWN was identified for the first time in Europe, in Portugal, and has since caused great ecological damages to the pine forests and economic costs to related wood industries. Research for effective control strategies is hindered by biological constraints, environmental variations and the resources necessary in terms of space, time and specialized technicians. The use of in vitro cultures offers an innovative alternative to analyze the fine biochemical interplay between the pine host and its PWN parasite. The ongoing project NemaWAARS aims to understand how parasitism is regulated and which mechanisms control the gene expression of parasitism-related genes (effectors). We will provide new PWN control targets and explore novel strategies for sustainable forestry pest management. Pine-PWN co-cultures will be used for a faster in vitro functional validation technique, under controlled conditions, to evaluate the effects of silenced or overexpressed parasitism regulators, using in planta assays. In vitro shoot cultures will be established for Pinus pinaster, the main affected species in Portugal, using selected tree genotypes with susceptibility to Pine Wilt Disease (PWD). Ultimately, functionally validated targets for PWN control will be identified in the framework of an environmentally friendlier PWD management.

Keywords: Parasitism; Pine Wilt Disease; plant biotechnology; sustainable pest management; transcription factors

Flight performance of two insect vectors of pine wood nematode, *Monochamus saltuarius* and *M. alternatus* (Coleoptera: Cerambycidae)

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Abstract

The pine wilt disease caused by the pine wood nematode (PWN, *Bursaphelenchus xylophilus*), a major forest pest worldwide. The PWN is transmitted by Monochamus species to healthy pine trees (*Pinus densiflora* and *P. koraiensis* in Korea). Because flight capacity of the vector insects is important to protect pine forests, we tested flight distance, time, and velocity of *M. saltuarius* and *M. alternatus* adults, two vector species in Korea, using flight mills. In addition, flight performance of *M. saltuarius* and *M. alternatus* adults was compared between species and between male and female. In *M. saltuarius*, the average flight distance of females in a single flight session was 0.43 km, while males flew about 0.50 km. The average cumulated flight distances for entire life span in females and males were 1.93 km (maximum flight distance: 5.21 km) and 2.71 km (maximum flight distance: 5.73 km), respectively. In *M. alternatus*, the average flight distance of females in a single flight session was 1.53 km, while males flew about 2.05 km. The average cumulated flight distances for entire life span in females and males were 6.64 km (maximum flight distance: 15.35 km) and 9.89 km (maximum flight distance: 29.01 km), respectively.

Keywords: Flight, *Monochamus saltuarius*, *Monochamus alternatus*, pine wood nematode, vector insects

Deladenus sp. associated with *Pinus pinaster* wood packaging material in Portugal

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Abstract

In sawn wood samples collected from maritime pine (*Pinus pinaster*) wood packaging material for the detection of the pinewood nematode, *Bursaphelenchus xylophilus*, and other nematodes, specimens from the genus *Deladenus* were recovered. The nematodes were then observed, photographed under a stereomicroscope, and further used for DNA extraction. Afterwards, nematodes were characterised morphologically and molecularly by sequencing several molecular markers: small subunit (SSU), D2-D3 expansion segments of large subunit (LSU) and internal transcribed spacer region (ITS) of ribosomal RNA genes, and mitochondrial cytochrome oxidase subunit I (COI) gene. Nematodes have long body length, low cephalic region, small stylet, pharynx lacking a median bulb, a posterior vulva and no post-vulval uterine sac. The phylogenetic analyses, from the multiple sequence alignments of sequenced genomic regions with the homologous sequences available for Deladenus species in GenBank, revealed that these nematodes form a separate phylogenetic branch within the Deladenus genus opening the possibility to be a new species. Attempts are being made to maintain these nematodes in fungi laboratory cultures. Further morphological (scanning electron microscopy) and morphometric studies will be performed to better characterise/identify the species.

Keywords: *Deladenus*; phylogenetic analysis; *Pinus pinaster*; wood packaging materials

Session 5 The ecology and pathology of bark and ambrosia beetle-microbial symbioses

Oral communications

Illuminating the nutritional contributions of bark beetle fungal symbionts

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Abstract

Numerous studies have confirmed that symbiotic fungi can play crucial roles in bark beetle nutritional ecology. However, the difficulty of conducting traditional feeding experiments has hindered research in this area. Thus, alternate approaches are needed that can allow us to determine, whether and how much, particular symbiotic fungi influence nutrient availability to their host beetles. In this talk, I will describe how to apply ecological stoichiometry to the study of bark beetles with varying life history strategies (aggressive tree-killing beetles to non-aggressive beetles) and associations with fungi (obligate mutualists to commensals) in naturally infested trees. I will incorporate examples from recent studies using ecological stoichiometry with primary and secondary bark beetles and present a framework for using ecological stoichiometry to detect and fill gaps in our knowledge of bark beetle nutritional ecology and the evolution of mycophagy in Scolytinae-fungus symbioses.

Keywords: Scolytinae, bark beetle, symbiosis, mutualism, ecological stoichiometry

Chemistry of symbiosis: Volatiles mediate interactions between conifer bark beetles and their fungal symbionts

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Abstract

Many insects exhibit symbioses with other organisms through signaling compounds, which drive the evolutionary maintenance of mutualistic associations. Bark beetles are associated with a diverse species of symbiotic fungi that are vital for the successful colonization and development of these insects in their host trees. Fungi are able to mobilize/supplement nutrients for beetles by growing in the bark and wood, and also be able to detoxify the defense chemicals of trees which are toxic to beetles. We investigated if volatile organic compounds emitted from different fungal symbionts could act as cues for bark beetles to recognize and distinguish their microbial community. Fungi associated with bark beetles emit a variety of volatiles comprised of simple aliphatic, aromatic alcohols and its esters, benzenoids, mono-, and sesquiterpenes. Using the Eurasian spruce bark beetle, Ips typographus and its fungal symbionts, we showed that bark beetles were attracted to fungi in olfactometer assays and beetles preferred to tunnel on diet colonized by beneficial symbionts but not to commensals and pathogens. Testing these fungal compounds on beetle antennae using single sensillum recording revealed that beetles could detect many fungal volatiles and possess several new olfactory sensory neuron classes specialized in detecting the fungal volatiles. Finally, we showed that synthetic blend of fungal volatiles attracted bark beetles in olfactometer assays. These findings indicate that volatile compounds produced by fungi may act as recognition cues for bark beetles to maintain specific microbial communities that might have an impact on their fitness.

Keywords: Conifer bark beetles, Symbiotic fungi, Volatiles, Attraction

Ophiostomatoid fungi synergise attraction of *Ips typographus* to its aggregation pheromone in the field experiment

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Abstract

The Eurasian spruce bark beetle, *Ips typographus* is a devastating pest for Norway spruces in Europe. Bark beetles interact with complex olfactory cues originating from their environment, where the most powerful is the aggregation pheromone. *I. typographus* perceiving VOC signals produced by multiple other organisms during their life cycle. Besides the host trees, beetles can smell their symbiotic ophiostomatoid fungi supporting their successful colonisation and development. Recently was shown that *I. typographus* were attracted to VOCs of *Grosmannia penicillata*, *Endoconidiophora polonica* and *Leptographium europhioides* in a short distance assay. In the fungal VOC emissions were the most active fusel alcohols and their acetates. In this study, we tested the relevance of fungal VOCs to dispersing I. typographus using a long-distance field trapping experiment. Live cultures of fungi grown on potato dextrose agar or the dispensers containing synthetic fusel alcohols and acetates were combined with beetle pheromone in traps, and catches were compared. Consequently, the composition of VOCs emitted by tested fungal dispensers was analysed. Our results showed that fungi culture synergistically increased the attraction of beetles to pheromones in traps and the dose range of active fungal VOCs depended on fungal species. A low dose of E. polonica combined with the pheromone increased significantly trapping efficiency, while L. europhioides in a high dose caused the same effect. The synthetic mix of fusel alcohols and acetates in equal ratio improved the catches to pheromone only at a low dose (1 mg/day). VOC analysis of tested fungi dispensers revealed that three fungi have distinct volatile profiles and differ quantitatively and qualitatively. This is the first study comprehensively showing that VOCs from bark beetle symbiotic fungi can increase the attraction of dispersing beetles to their pheromones in a long-distance field trapping assay.

Keywords: Eurasian spruce bark beetle, *Grosmannia penicillate*, *Endoconidiophora polonica*, *Leptographium europhioides*, pheromone traps

The role of mutualistic fungi in the pheromonal communication of the european spruce bark beetle

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Abstract

The importance of fungal symbionts for bark and ambrosia beetles is reflected in the existence and repeated evolutionary origin of mycetangia – cuticular organs that evolved specifically for the transport of fungal spores. However, despite being associated with multiple ophiostomatoid fungi, the European spruce bark beetle (*Ips typographus*; ESBB) lack mycetangia even though some of its fungal symbionts are highly attractive for adult beetles in vitro.

In this study we tested for the first time if the attractivity of fungal symbionts for ESBB is also present under semi-field conditions with fungus-inoculated logs in flying cages. Moreover, in further lab and field experiments, we tested whether this attractivity is mediated by possible inhibitory effects of verbenone, the anti-aggregation pheromone of the ESBB.

While preliminary results suggest that ESBB do select breeding sites in the vicinity of associated fungi, we also saw that verbenone influences the readiness for site selection. Here, we present first results on the potential inhibitory effects of verbenone on the attractivity of fungal VOCs.

These results will shed new light on the tritrophic interactions between beetle, fungus and host tree. Possibly, our findings will help to explain the inconsistent effects of verbenone we find in the field.

Keywords: *Ips typographus*, verbenone, ophiostomatoid fungi, bioassay, symbiosis

Spatial genetic structure and coevolution patterns of a beetle-fungal system in thousand cankers disease complex

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Abstract

Thousand Cankers Disease (TCD) affects several species of walnut (*Juglans*) and wingnut (*Pterocarya*) trees and has now been reported in fifteen U.S. states and northern Italy. TCD represents not only a major threat to the nut and lumber industry but also to natural stands and to the ecosystem services they provide. Galleries created by the primary insect vector, *Pityophthorus juglandis* (walnut twig beetle), are colonized by the fungal pathogen *Geosmithia morbida*, the actual causal agent that creates dark brown- to- black cankers. High beetle infestation results in multiple cankers which coalesce causing wilting and yellowing foliage, girdled twigs and branches, and severe infections can cause tree mortality within 3–4 years. Although some progress has been made to understand the genetic diversity and spatial distribution of the pathogen and its vector, dispersal dynamics and co-evolutionary history of the pathogen-vector system in the native, Western U.S., range of distribution have not been studied. Here we generated data for *P. juglandis* (15 microsatellite loci, n=1,118 individual beetles) and *G. morbida* (16 microsatellite loci, n=501 axenic isolates) populations collected from Arizona, California, New Mexico, Oregon, Utah, and Washington. The objectives of this study were to investigate genetic diversity, spatial structure, gene flow, and co-evolutionary patterns between the vector beetles and the fungal pathogen populations across the Western U.S. Our results, for both beetle and the pathogen, indicate high genetic diversity, high population differentiation, high allelic richness, and the presence of distinct genetic clusters. The Mantel test indicated a weak correlation between the two datasets, suggesting that the TCD complex members evolved independently. These findings support the hypothesis that G. morbida and *P. juglandis* have spread into new areas multiple times from multiple sources and that both complex members are more likely native to the U.S. or have been present but undetected for a long time. The overarching goal of this study is to contribute to a better understanding of the genetic dynamics involved in the range expansion of native pathosystems and their dispersal pathways.

Keywords: beetle-fungal pathosystem, Thousand Cankers Disease, genetic diversity, spatial structure, coevolution

Tri-trophic interactions between EBB, *Geosmithia* spp. and *Ophiostoma* novo-*ulmi* in the DED pathosystem

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Abstract

Ophiostoma novo-ulmi is a pathogenic fungi that causes Dutch elm disease (DED), a destructive tracheomycosis that has devastated elm populations throughout Europe. DED is mainly spread by various species of elm bark beetles (EBB) in the genus *Scolytus*. In the DED pathosystem is also present *Geomithia* spp., a monophyletic morphogenus of anamorphic ascomycetes mainly associated to phloem-feeding bark beetles. The existence of a complicated relationship between

O. novo-ulmi and *Geosmithia* than just occupying the same habitat and having the same vectors has recently been suggested by the discovery of widespread horizontal gene transfer (HGT) of a genomic fragment comprising the cerato-ulmin (cu) gene between the two fungi. We have supposedly that between *Ophiostoma* and *Geosmithia* exist a parasitic association in which *Geosmithia* acts as a mycoparasite.

In order to study the association of *Geosmithia* spp., *O. novo-ulmi* and EBBs, in sites characterized by different stages of the epidemic, to increase our knowledge about the life cycle of *Geosmithia* within DED dynamics, a new duplex real-time PCR assay was developed for simultaneous recognition of *Geosmithia* sp. and *O. novo-ulmi* in elm tissue and all development stages of elm bark beetles. EBB were collected in natural environments where native field elms (*Ulmus minor*) were present by funnel traps baited with a aggregation pheromone bait specific to *Scolytus* spp., in six locations in Italy, at different stages of DED epidemic.

The duplex qPCR technique developed, besides being extremely sensitive, allows to specifically identify and quantify the presence of both *O. novo-ulmi* and *Geosmithia* sp. in plants with different levels of DED attacks and on the body of insects, giving a better insight of the dynamics of this complex fungus-fungus association mediated by *S. multistriatus*.

This work strengthens our knowledge on the spread of the disease and the role of insect vectors, providing indispensable information on the life cycle of *Geosmithia* within the DED pathogen system, and provides relevant insights into hypothesizing the use of *Geosmithia* as a natural biocontrol of *O. novo-ulmi*.

Keywords: bark beetles, Dutch elm disease (DED), *Geosmithia*, microorganisms association, *Ophiostoma novo-ulmi*

Effect of the microbial community associated with *Platypus cylindrus* on the vitality of the host *Quercus suber* L.

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Abstract

The ambrosia beetle *Platypus cylindrus* Fab. (Coleoptera: Curculionidae) has been associated in Portugal with cork oak (*Quercus suber*) tree death since the 1980's. Although traditionally known as a secondary pest damaging stressed and dead trees, its aggressiveness changed over the last decades. How fungal and bacterial associates of this beetle impact cork oak tree health is not yet well known. The effect of the fungal associates was studied for three major symbiotic fungi of this beetle (*Raffaelea montetyi*, *R. quercina* and *Ceratocystiopsis* sp.) on the release of volatile organic compounds (VOCs) from 30 cork oak seedlings by means of GC/MS techniques. These VOCs might include volatile cues for the beetle and plant defenses. The analysis of the VOCs from seedlings wound-inoculated with the symbiotic fungi, revealed that 58 VOCs changed significantly compared to control seedlings. Among them, the monoterpenes were quantitatively reduced. Finally, all fungal strains affected the phenotype and behaved virulently on the seedlings.

The bacterial community present in the prothoracic mycangia, body of *P. cylindrus*, and in the wood galleries, was investigated for the first time (15 galleries and 90 beetles) with the goal of highlighting its potential roles in oak decline. From *P. cylindrus* body and wood tree bored galleries, culture dependent and independent methods led to the identification of a novel and heterogeneous bacterial community. Within this diversity, a group of distinct strains potentially belonging to a new Pectobacteriaceae species, were able to produce mild symptoms on cork oak seedlings (small stem cankers, and leaf necrosis and chlorosis). 16S metabarcoding confirmed the presence of these Pectobacteiaceae in the mycangia. Overall, our study suggests a microbial contribution to cork oak decline, showing a potential new pathogenic bacterium, bacterial strains possibly involved in plant metabolite degradation, and symbiotic fungi decreasing plant defenses.

Keywords: Ambrosia beetle, bacteriome, oak decline, Ophiostomatales, VOCs

Posters

Survey of Scolytinae-fungus relationships in Switzerland

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Abstract

Many ambrosia beetles and bark beetles are known to be associated with a suite of fungi and other microorganisms that are symbiotic or coincidental. Some of these may be plant pathogens that could cause diseases in affected trees. This issue has not been studied much in Switzerland. In this study, we are investigating which fungi and other organisms are present externally and internally on the non-native *Xylosandrus germanus* and several native Scolytinae. We collected Scolytinae in three different regions in Switzerland, north of the Alps, in the central Alps and south of the Alps, using flight interception traps baited with ethanol, log sections baited with ethanol and collecting from naturally infested wood. To determine which microorganisms are associated with the various beetle species, beetles were allowed to walk over selective agar media, pressed into agar and plated out on agar after maceration. Preliminary results will be presented along with a discussion of the main findings.

Keywords: Ambrosia beetles, bark beetles, insect-fungus associations, pathogen vectors, tree pathogens

Session 6 Defense priming in forest trees: Mechanisms and practical applications

Oral communications

Defense priming in conifers and how to study it

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Abstract

Defense priming is a form of inducible defense where a prior stimulus induces a tree to defend itself more rapidly and/or vigorously to a subsequent attack or infection. In defense priming there is no clear immediate upregulation of defenses (such as resin production) but the response is delayed and is triggered by a subsequent attack or infection. Defense priming is thought to have evolved as a more cost-efficient defense response than immediate upregulation of defenses. At NIBIO we have studied defense priming in Norway spruce for 20 years and in this presentation I will describe the nature of defense priming in this important conifer species and outline how defense priming can be studied experimentally.

Keywords: Conifer defense, chemical defenses, defense priming, *Ips typographus*, Norway spruce

Molecular underpinnings of Norway spruce defense priming

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Abstract

Defense priming, the sensitization of inducible defenses, has been extensively studied in annual angiosperms. However, we are just beginning to explore defense priming in woody, long-lived plants. The natural compound methyl jasmonate (MeJA) has been used for over 20 years to study spruce inducible defenses. Recently, it was discovered that MeJA not only directly induces defense, but also primes defense responses in spruce. Metabolite and transcriptional analyses of mature trees treated with MeJA and subsequently wounded showed that while terpenes accumulate at the wound site in a primed manner, terpene biosynthesis genes are directly induced by MeJA. Pathogen resistance (PR) genes, on the other hand, are primed. Sequencing of miRNAs suggests that miRNAs have a regulatory role in MeJA-induced defenses in spruce. Additionally, a detailed transcriptional time course of 2-year-old spruce treated with MeJA indicated that the RNA-directed DNA methylation (RdDM) pathway is involved in the establishment and maintenance of primed defenses. When comparing mechanisms of defense priming in spruce to those in Arabidopsis, it seems that many mechanisms are conserved. However, some aspects, such as jasmonic acid-salicylic acid crosstalk, may be different. Identifying these differences and how they affect forest species is important for practical application of defense priming in forest management.

Keywords: *Picea abies*; defense priminig; methyl jasmonate; transcriptome

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

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Abstract

Knowledge on tree systemic induced resistance (SIR) (whole plant-level immunity) and its underlying mechanisms remains limited, and it is non-existent for the systemic signaling systems that underlies such SIR. 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 non-wounded controls. Notably, lesions were progressively shorter with increasing time between induction and challenge, demonstrating induction of SIR in a time-dependent manner and suggesting a time-dependent propagation of a signal. 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, alpha-pinene and a group that includes beta-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.

Keywords: Austrian pine, *Diplodia*, host defense, necrotrophic, systemic induced resistance

Combining new methods for increased plant protection

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Abstract

In plant protection, utilizing plants with enhanced resistance traits is gaining interest. Recently, the plant defence-signalling hormone methyl jasmonate (MeJA) has been emerged as a promising plant protection tool within forestry. Exogenous MeJA application induces plant defences and effectively reduces damage to conifer seedlings by the pine weevil (*Hylobius abietis*), a major forest regeneration pest in Europe. Another recent finding showed that Norway spruce (*Picea abies*) seedlings (emblings hereafter) propagated through somatic embryogenesis (SE), an in vitro mass clonal propagation technique, displayed higher resistance against the pine weevil than regular spruce seedlings. Due to an increasing interest in producing SE plants for commercial use, it is timely to examine the plant protective effects of SE in combination with MeJA.

In this study, we examined the effects of MeJA treatment on seedling and embling resistance against pine weevil damage (and survival). We hypothesized that if emblings are primed or induced during SE-production, a second stimulus from MeJA could provide an even faster or greater onset of defenses. Alternatively, emblings could already be expressing greater levels of defense, and treatment with MeJA would elucidate no further response.

A field and lab experiment were set up consisting of MeJA-treated and non-MeJA treated Norway spruce seedlings (1.5-year-old containerized and 3-year-old bare-root seedlings) and emblings (1-year-old). Treated plants were sprayed with 10 mM MeJA prior to exposure to pine weevils. Pine weevil attack rate, stem feeding damage (area debarked and girdling) and mortality were scored in the lab and field.

In line with previous studies, we found that MeJA and SE separately reduced pine weevil damage by 53% and 32%, respectively, relative to non-treated seedlings. In addition, we found that MeJA-treated emblings experienced very large reductions in pine weevil damage and experienced greater survival than both the non-treated emblings and MeJA-treated seedlings. MeJA-treated emblings experienced an 86% and 48% reduction in damage in the field and lab respectively, and a 98% decrease in girdling rate and mortality in the field compared to untreated seedlings.

We conclude that the effect of MeJA on seedling resistance and survival differed between SE and non-SE plants. SE and MeJA together appear to have a synergistic effect on resistance, they can enhance resistance to an even greater level than each alone. From a practical perspective, these results suggest that plant production through SE can be combined with (other) plant defense inducing strategies to achieve sustainable forest regeneration.

Keywords: Emblings; *Hylobius abietis*; Methyl jasmonate; *Picea abies*; Somatic embryogenesis

Exploiting metabolomics to unravel priming of defence in oak seedlings against powdery mildew

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Abstract

Oaks are predominant species in European forests and show high vulnerability to *Erysiphe alphitoides*, the causal agent of powdery mildew (PM) at seedling stage. Alternative solutions to the use of fungicides are required. One potential strategy is priming of defence, which consists in the sensitisation of defence mechanisms for a faster and stronger activation upon subsequent attack. Priming has been described in many plant species, however, studies in oak are lacking. Here, we determine whether oak seedlings can express chemical-induced priming. Seedlings were treated with salicylic (SA), jasmonic (JA) and β-aminobutyric (BABA) acids 7 days before infection with *E. alphitoides* conidia (PM). Treatments with SA and BABA resulted in enhanced resistance to PM and JA in enhanced susceptibility. Resistance by SA and BABA was based on priming of PR1 gene expression and callose deposition, respectively. To investigate the mechanisms behind specific SA and BABA priming, green leaves were subjected to LC-MS/MS. Spectra pre-filter (using XCMS R script) resolved 17865 and 9408 putative masses in positive and negative modes, respectively. Subsequently, MANOVA analysis showed 3837 significant global differences ($p<0.05$). Fold changes versus water treatment were applied to isolate the primed metabolites, showing a total of 272 for SA and 313 for BABA primed masses. Pathway enrichment analysis determined substantial differences between BABA (mostly alkaloids, flavonols and lignans biosynthesis), and SA priming mechanisms (did not target any specific pathways). The next step in the project is to identify the specific metabolites responsible for priming by BABA and SA.

Keywords: Priming of defence, oak powdery mildew, elicitors treatments, untargeted metabolomics, defence-response compounds

Ulmus minor fungal endophytes induce plant defense priming against the vascular pathogen *Ophiostoma* novo-*ulmi*

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Abstract

Some fungal endophytes of forest trees are recognized as beneficial symbionts against stresses. In elm (*Ulmus minor*) trees, two fungal endophytes from classes *Cystobasidiomycetes* and E*urotiomycetes* promoted host resistance to abiotic stress, and another endophyte from Dothideomycetes enhanced host resistance to Dutch Elm Disease (DED). In this work, we hypothesize that the combined inoculation of these three endophytes will activate the plant immune or the antioxidant system, leading to a defense primed state when exposed to the DED pathogen *Ophiostoma novo-ulmi*. Furthermore, we hypothesize that different microbial assemblages in DED-resistant (MDV2.3 and VAD2) and DED-susceptible (MDV1) elm genotypes will have an influence on the plant response to the pathogen. To test these hypotheses, we evaluated the short-term defense gene activation, and tracked the associated microbial changes in DED-susceptible and DED-resistant *U. minor* genotypes inoculated with *O. novo-ulmi* alone and with a mixture of the above-mentioned endophytes two weeks earlier than the pathogen inoculation. Endophyte inoculation induced a generalized transient defense activation mediated primarily by salicylic acid (SA) and altered the presence of some of the members of the mycobiome. Subsequent pathogen inoculation resulted in a primed defense response of variable intensity among genotypes. Genotypes MDV1 and VAD2 displayed a clear defense priming driven by SA, jasmonic acid (JA), and ethylene (ET), with a higher intensity in MDV1 than in VAD2. Meanwhile, the genotype MDV2.3 showed a lower defense priming but a stronger and earlier antioxidant response. We also observed that the higher defense activation upon *O. novo-ulmi* inoculation in the susceptible genotype (MDV1) might be attributed to a different defense system modulated by an enhanced presence of other pathogens in its mycobiome. The defense priming stimulated by elm fungal endophytes and the genotype-specific changes on plant mycobiome broadens our current knowledge of the ecological functions of endophytic fungi in forest trees and opens new prospects for their use in the biocontrol of plant diseases.

Keywords: Fungal endophytes, mycobiome, elm, priming, fungal pathogen.

Posters

Phenolic metabolites in the resistance of *Larix* sp. to *Lachnellula willkommii*

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Abstract

Future climate change demands new forest management strategies that would be suitable for the new climate, such as diversifying forests with native and alternative tree species, including exotics. In Sweden, stakeholders are increasingly interested in using larch (*Larix*) species, due to its good production potential, and the increased threat to spruce and pine forests due to bark beetles, windstorms and other fungal diseases. However, larch cultivation is currently limited for many reasons, including its susceptibility to the larch canker pathogen *Lachnellula willkommii*. However, noticeably resistant material from Poland and Czech Republic of L. decidua brings about a renewed possibility, but its potential needs to be verified under Swedish conditions. In addition, interspecific variation in resistance among the three tree species to *L. willkommii* is not well understood. A greater understanding of the mechanism of host resistance to *L. willkommii* would allow us to improve our knowledge about this host-fungus relationship and what defense traits are important in regulating responses to contain infections.

The aim of present study was to assess the resistance level of this European larch, hybrid larch and Siberian larch to *L. willkommii* and to investigate the role of plant secondary metabolites in their defense. A study was carried out in a progeny trial in Tagel, southern Sweden, with 10 select half-sib families of European larch, 4 families of hybrid larch and 2 families of Siberian larch. Lesion development was assessed following artificial inoculation with *L. willkommii* after 90 days. The results showed that lesion development among Larix species and three families inoculated by *L. willkommii* were significantly different ($P<0.05$). To investigate the constitutive and induced chemical defenses of Larix species, phloem from branches were collected before and after inoculation with *L. willkommii* for analysis of secondary metabolites associated to resistance phenotypes.

Keywords: Exotic species; Tree resistance; European larch canker; *Larix* sp.

Examining the transcriptional response to multiple attacks by *Hylobius abietis* on *Picea sitchensis*

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Abstract

Some conifer species have been shown to transcriptionally respond to *Hylobius abietis* feeding. The objective of this study is to examine the varying transcriptional responses of *Picea sitchensis* (Sitka Spruce) when exposed to different numbers of *H. abietis* exposure.

In order to determine:

1. If *H. abietis* does elicit a transcriptional response in Sitka spruce,
2. How that response varies over a time course,
3. Examine if previous exposure to the insect primes the trees inducible defences.

Clonal cuttings were taken from 6 individuals each from a different family in the UK breeding population of Sitka spruce. The seedlings received one of three different treatments, no exposure, a single period of three-day exposure to *H. abietis*, or two three-day periods of exposure a month apart. Samples of needle and bark tissue were taken at three time points, directly after exposure, 5 days later and a month later. The RNA is being extracted to be sequenced and will be analysed to look for differential gene expression. There are each seeding is one of 4 clonal replicates. Debarked area and number of scars will be measured. This experiment is currently underway.

This study builds on other work in conifers in that it looks at transcriptional response over a time course which will allow us to see how long a response lasts for and if it varies. It also allows us to see if any inducible defences are primed by previous wounding from *H. abietis*.

Keywords: Defence response; *Hylobius abietis*; *Picea sitchensis*; Priming; Transcriptional response

Session 7 Gender issues in forest entomology and pathology (GIFEP)

Oral communications

Gender issues in forest management

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Abstract

Globally, forests play an important role in food security and the mitigation of climate change and contribute to local economies by providing forest products and livelihoods to 1.6 billion people. However, these benefits have been severely impacted by forest degradation and deforestation. Among the most impacted populations, women are often part of the most affected demographic while being given less opportunities to contribute to the sustainable management of the forests on which they rely. For example, within communities living near forested areas, deforestation has forced women to walk longer distances to source forest products, impacting their safety, time availability and income. Access and control over high market value forest products such as timber is also gender-biased due to land ownership laws that limit ownership by women. This reduces the capacity of women to create income. Moreover, as forest exploitation becomes industrialized, studies have shown that women become less involved due to cultural norms and increased commuting time. Finally, because of their greater involvement in the household, women tend to prioritise the planting of trees that can provide food and firewood to their communities or that have medicinal properties or can promote soil fertility. Despite their knowledge and needs, women are less involved in forest management and decision and policy-making due to legislation and socio-economic norms. To solve these inequality and gender-related issues in forest management, solutions can focus on policy, cultural change through education and on the creation of equity plans within decision-making bodies.

Keywords: Gender; Equality; Forest Sustainable Management

Gender issues in forest entomology and pathology (GIFEP)

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Abstract

Inclusion of women in the forest entomology and forest pathology workforce has been a difficult journey. While resources exist for departments and organizations to increase diversity and retain women, there still exists large gaps in gender parity at forestry institutions globally. It is imperative that we better understand the barriers that exist for women in forest entomology and pathology so that we can create more inclusive environments that are more welcoming towards women and other underrepresented groups. To assess these barriers, and subsequent opportunities for improvement, we surveyed forest entomology and pathology professionals globally to ask about their experiences in the workforce. Barriers to success for women and men were very different; women experienced more barriers related to family caretaking while men experienced more barriers related to funding opportunities. These differences show where opportunities lie to better attract, support, and retain women in forest entomology and forest pathology.

Keywords: diversity, equity, gender, inclusion

Addressing gender issues in forest entomology and pathology

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Abstract

Addressing gender issues in forest entomology and pathology. Summing up from the Gender Issues in Forest Entomology and Pathology Network talks, the future directions, including the short- and long-term goals for the network will be discussed.

Keywords: diversity, equality, Forest Entomology, Forest Pathology, gender

Session 8 Invasive forest pests and pathogens – pathways, impacts and mitigation measures

Oral communications

Expansion of *Ips typographus* in Great-Britain by flight over the English Channel, possibly helped by pre-emergence mating

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Abstract

In 2018 the European spruce beetle, *Ips typographus*, has been detected for the first time in the United Kingdom. And this, despite all the controls carried out on the timber import trade which is considered as the main way of propagation for many scolitids. The area where these insects were found (Kent) is the one where the English Channel is the narrowest between France and England. We therefore tested the hypothesis that these beetles were arriving from France by flying, on their own.

In 2021 a transect of pheromones traps was conducted from the nearest stronghold of Ips typographus in France (the Champagne-Ardenne) to the French coast and on the other side of the English Channel in Kent. This survey showed that beetles were present on both coasts and all the way from the Champagne-Ardenne. This year, another survey is being conducted adding a larger set of trapping sites including another transect starting in an outbreak hotspot in Belgium and ending on the Belgian coast. The first 2022 results show that beetles are also present all the way to the Belgian coast.

In addition, recent observations showed that part of the emerging females of *Ips typographus* were already mated. A part of the captured females in each station was dissected to assess the presence or the absence of sperm in their spermatheca. At least, half of the females observed in every station (67,1% in England) were fertilized. It has been tested in the lab that those pre-emerging mated female can colonise a log and consequently start a new gallery on their own. This could facilitate the colonization of new territories, such as the United Kingdom.

Finally, a genetical study based on 15-20 microsatellite markers is under way to unravel the population structure, the genetic proximity of the insects caught along the transect and retrace the invasion route through a Bayesian analysis.

A better understanding of the movements of *Ips typographus* and of its mating strategies could help understanding and, possibly preventing, new incursions into Great-Britain.

Keywords: Insect invasions; *Ips typographus*; mating strategies

Assessing the impact of *Neoleucopis kartliana* (Diptera: Chamaemyiidae) on *Marchalina hellenica* (Hemiptera: Margarodidae)

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Abstract

Assessing the impact of *Neoleucopis kartliana* (Diptera: Chamaemyiidae) on *Marchalina hellenica* (Hemiptera: Margarodidae)

Giant pine scale (GPS) *Marchalina hellenica* (Hemiptera, Margarodidae) is endemic in Greece and Turkey where it feeds mostly on pine trees (*Pinus* spp.). GPS has long been considered a beneficial species that supports honey production in those countries. However, shortly after its detection in Australia (Melbourne and Adelaide) in 2014, significant feeding damage was observed on *P. radiata* growing in urban and peri-urban settings. *Pinus radiata* is a commercially important species in Australia and spread of GPS into *P. radiata* plantations could seriously impact tree health and timber production. A preliminary study in Greece highlighted the silver fly *Neoleucopis kartliana* (Diptera, Chamaemyiidae) as the most abundant and widespread predator of GPS. Studies of *N. kartliana* were initiated to assess its potential as a classical biological control agent against *M. hellenica* in Australia. We assessed (1) the abundance of *Neocleucopis kartliana* under different GPS infestation levels across Greece, and (2) prey preferences of GPS in replicated choice and no-choice prey-specificity experiments with the Australian scale insect Icerya purchasi. Our results to date indicate that *N. kartliana* has considerable potential as an effective and prey-specific biological control agent. Nevertheless, quarantine laboratory experiments in Australia are now required to confirm the suitability of this silver fly for introduction against giant pine scale; something that will be attempted in the coming months.

Keywords: Giant Pine Scale; invasive species; classical biological control; silver fly

Epidemiology of *Contarinia pseudotsugae* s.l. a new invasive gall midge for Europe attacking douglas firs

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Abstract

Contarinia pseudotsugae s.l. has been observed for the first time in Europe in 2015 in Belgium and The Netherlands and continues to expand its range in the invaded area. The larvae of this North American gall midge (Diptera: Cecidomyiidae) develop into galls on the needles of douglas firs (*Pseudotsuga menziesii*) and can cause severe defoliations when the population levels are high. Its arrival has therefore raised concern about its potential impact on douglas firs stands, a species of major economic importance in Europe.

We have monitored the sanitary state of douglas firs in more than 150 locations in Belgium since 2016, including several pathogens that could contribute to the poor sanitary state observed in the area for several years. At the start of the monitoring, *C. pseudotsugae* s.l. was already present at all sites – indicating an older undetected presence – but generally with very low levels of attack. Higher levels of attack were restricted to a few locations. However, over the years, the % of needles attacked has strongly increased in all areas. The level of attacks was much higher for young trees but the difference in attack levels between young and old trees has narrowed over the years. The expansion and rise of the population levels of *C. pseudotsugae* is now clearly documented, however its actual impact on the growth or survival of douglas firs in the invaded area remains uncertain. The populations dynamic in the native area is known to exhibit cyclic patterns and it is still unclear if the population levels in the invaded area will remain at a high level or will naturally decrease at some point.

Keywords: Cecidogenous insect; Coniferous trees; Defoliation; Insect invasions; Pest

Malice at the Gates of Eden: on-going threat and management of *Agrilus mali* in wild and domestic apples

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Abstract

A great deal of attention has focused on the emerald ash borer, *Agrilus planipennis* because of it impacts on host trees in its invaded range in north American and Europe. But another member of this group of beetles, the apple buprestid, *Agrilus mali*, that was widespread in domestic apples in north-eastern China, has invaded the wild apple forest ecosystem in mountainous areas of Xinjiang, with devastating effects. This invasive beetle feeds on domesticated apples and many species of Malus and is a clear and present danger to ancestral apple germplasm sources and apple production worldwide. Using a CLIMEX model we estimate the potential area at risk of colonization by *A. mali*. Under current climate, *A. mali* could potentially invade neighbouring central Asia and eventually the mid-latitude temperate zone. This potential distribution encompasses all wild apples species, the ancestral germplasm for domesticated apples. With global warming, the potential distribution shifts to higher latitudes. Strategies to prevent the spread of this pest, managing the threats to wild apples in Tian Shan Mountain forests in Central Asia, the garden of Eden for apples, and commercial apple production globally need to be assiduously implemented, before not after the species arrives. We discuss practical management tactics to reduce the spread of this pest and mitigate its impacts.

Keywords: Apple buprestid; biosecurity; CLIMEX; niche modelling; wild apple

Using chemical ecology to predict establishment risk of non-native bark beetles

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Abstract

Invasive species are a rising global concern, with the potential to cause serious economic and ecological damage. Preventing or mitigating future establishments of non-native and potentially invasive species is best done by allocating the limited resources available towards the species that pose the greatest risk. Among the many determinants of risk are the abilities of an introduced species to establish, which is influenced in part by ecological interactions with native species, e.g., predation and facilitation. Predicting these interactions may be very difficult, however, in cases where native and non-native species have yet to come into contact. Our research focused on two species of spruce bark beetles, one native to North America (*Dendroctonus rufipennis*), and another to Europe (*Ips typographus*), which may become invasive in the range of the other. By using beetle pheromones as a proxy for the presence of a non-native species, we were able to assess the responses of predators and competitors and use them as a basis for predicting ecological interactions between live insects. We found that *D. rufipennis* may avoid a degree of competition and predation by being semiochemically inconspicuous in Europe, and that both species may facilitate each other by cooperating in mass attacks. We propose that similar methods can be used for other species that rely heavily on chemical communication, and that the results may be of considerable value for invasive species risk assessments.

Keywords: Bark beetles; Biotic resistance; Facilitation; Insect invasions; Semiochemicals

Host preference of the invasive Eucalyptus psyllid, *Spondyliaspis* cf. *plicatuloides* (Froggatt) (Hemiptera: Aphalaridae)

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Abstract

Spondyliaspis cf. *plicatuloides* (Hemiptera: Aphalaridae) is an invasive insect native to Australia, and first reported in South Africa in 2014. It feed on *Eucalyptus* sap, and there are clear differences between the psyllid's preferences for different species and hybrids of *Eucalyptus*. The objective of this study was to identify the constitutive morphological and phytochemical features underlying these host preferences. A total of six preferred and six non-preferred eucalypt species from a common garden with natural infestation of the insect were selected for evaluation. Thirteen morphological features of the leaves were analyzed. The essential oils and polar metabolites in and on the surface of leaves of each eucalypt species were extracted and their chemical composition was determined using gas chromatography coupled with mass spectrometry. All the physical parameters assessed in relation to level of infestation did not show any correlation with either preference or non-preference. The abundance of three volatile compounds, α-pinene, eucalyptol and neophytadiene significantly correlated with the preferences of the host. Among several constituents, four polar secondary metabolites: 2-O-(2-(4-hydroxyphenyl)-ethyl)-d-β-glucopyranose, glyceryl-glycoside, scyllo-inositol, and stearic acid, were absent in the two most preferred hosts. Moreover, eight other polar metabolites were present in high amounts in non-preferred eucalypts. These compounds include organic acids, phenylpropanoid, fatty acids, plant sugar alcohols and sugar derivates. In addition, three epicuticular wax compounds, 28-norolean-17-en-3-one, 1-heneicosanol and tetracosane abundance correlated to host preference. We therefore conclude that, phytochemical features influence host preference of the *Eucalyptus*-feeding psyllid. These results provide important information for developing management strategies based on semiochemicals for mass trapping and planting of resistant or non-preferred hosts.

Keywords: GC/MS, invasive, psyllid, Spondyliaspis

Experimental trapping program in the Pacific Northwest of North America reveals four new exotic wood-boring insects

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Abstract

Despite safeguarding measures, new exotic forest insects are escaping detection and becoming established in the U.S. at the rate of 2.5 species per year. Technology that enables land managers and scientists to detect early the exotic invasive wood-boring insect species in the taxa Buprestidae, Cerambycidae, Scolytinae and Hymenoptera (i.e. "wood-boring insects") is critical in providing successful rapid response and eradication. In the Pacific Northwest of North America, the states of Oregon and Washington are under increasing threat from exotic wood-boring species arriving through global trade at international ports on the Columbia River. For three years (2016-2018) we conducted a survey at 16 sites along approximately 150 km of the Columbia River shipping corridor, representing a variety of forest types including typical hemlock/Douglas-fir forests under both private and public ownership; urban forests of the Portland-metro area; and semi-arid ponderosa pine forests at the eastern survey extent. At each site, eight Lindgren funnel traps with unique baits and lures, some of which were new to the market, were established April thru September. These eight trap types were comprised of (1) (-)alpha-pinene/UHR ethanol, (2) UHR ethanol, (3) *Monochamus* lure set (alpha-pinene, Ipsenol, Ipsdienol, Monochamol, Ethanol), (4) exotic *Ips* lure set (2-methy-3-buten-2-ol, cis-verbenol, Ipsdienol), (5) oak pinhole borer lure set (leaf alchol, ethyl alcohol, sulcatol, sulcatone), (6) pine shoot beetle lure set ((-)alpha-pinene, trans-verbenol, myrtenol), (7) Control (no lure) and (8) emerald ash borer lure set (Z-3-hexenol, manuka oil). In 2018, we adjusted the experimental design by testing funnel traps in tree canopies vs ground-based traps alone. Across all years and all traps, we collected a total of 2,225 samples, over 400,000 individual specimens and over 200 species of native and exotic wood-boring insects. We detected four new first detections in Oregon, one of which a the time was a new record for North America, the Mediterranean oak borer, *Xyleborus monographus* (Fabr.). X. monographus is currently causing significant mortality to native North American oaks in central California. Over 90% of the total trap catch abundance was a single exotic species, Xyleborinus saxesenii (Ratzburg). The four traps placed in the tree canopies in 2018 caught over two times the species richness and total abundance per trap that their counterparts on the ground captured. This pattern was particularly apparent for Scolytinae captured in a-pinene/EtOH traps and Cerambycidae captured in monochamol traps. Exceptions to this pattern included *Phaenops drummondi*, *Scolytus* sp. and *Monochamus* sp, both of which were captured in higher numbers in the ground-based traps. We observed temporal and spatial patterns in trap captures as well. For instance, *Monochamus* sp peaked in the late summer in the eastern portion of the sample area. Early detection is essential to stopping an invasion before it becomes established and too widespread to eradicate. Our project provides information on when and how to run surveillance programs for a wide taxonomic diversity of exotic invasive wood-boring insect species with the goal of protecting the Pacific Northwest forests of North America from exotic invasive wood-boring insect species.

Keywords: Exotic species, invasive insects, surveillance trapping program, woodborers, *Xyleborus monographus*

Efficacy of semiochemical lures vs. unbaited traps for surveillance of *Agrilus* spp.

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Abstract

There are more than 3000 Agrilus species worldwide, many of which breed in tree genera common to forests in North America, Europe and Asia. Phytosanitary treatment of wood packaging has reduced but not eliminated the risk of live insects in shipments and the sheer volume of global trade ensures continued arrival of live woodborers in new habitats. Some of these arrivals may become damaging pests, e.g., emerald ash borer, Agrilus planipennis, (EAB) in North America and Eastern Europe. Thus, many countries conduct annual trapping surveys to detect potentially invasive insects as early as possible, when management costs are lower and the likelihood of eradication or containment is greater. Black funnel/intercept traps baited with semiochemicals like ethanol and longhorn beetle pheromones are very effective at detecting many species of Scolytinae and Cerambycidae but detect very few *Agrilus* species. Green or purple traps in sun-exposed areas of the tree canopy are effective at detecting Agrilus species, but except for EAB, we know few semiochemicals that enhance *Agrilus* detection. Therefore, for *Agrilus* surveillance, it may be more cost-effective to use unbaited traps and redirect cost savings towards more traps or survey sites per year. In 2021, we compared the efficacy of four lure treatments [1) ethanol; 2) ethanol, E,Z-fuscumol, E,Z-fuscumol acetate; 3) ethanol, racemic 3-hydroxyhexan-2-one, racemic 3-hydroxyoctan-2-one, syn, 2,3-hexanediols; and 4) unbaited] for detection of *Agrilus* spp. and other target taxa (Buprestidae, Cerambycidae, Scolytinae). We replicated treatments 6–8 times per site in green Lindgren funnel traps set in the canopy of oak-dominated forests in Canada, China, England, Italy, Poland, and the USA. We captured 84 species and > 12500 specimens of buprestids including 48 species and 7929 specimens of *Agrilus*. Buprestid species richness and abundance were greatest in unbaited traps; baiting traps with the hydroxyketone multi-lure did not affect species richness but significantly decreased buprestid abundance. However, the hydroxyketone multi-lure significantly increased abundance of total target taxa (Buprestidae, Cerambycidae, Scolytinae) in traps. These results may be useful for optimizing trapping surveys, e.g., by targeting different taxa in alternate years.

Keywords: woodborers, surveillance, *Agrilus*, traps, multi-lures

Sentinel meta-forests: Fine-scale tracking of emergent forest pests by leveraging databases, experts, and urban forests

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Abstract

Emergent forest pest (EFP) epidemics caused by globalization and climate change imperil human wellbeing and the biosphere. As forest health specialists aim to prevent, delay, and prepare for the next EFP, many are turning to sentinel trees that provide host, distribution, and genetic information about potential threats. Sentinels may be considered broadly as "meta-forests" because pest emergence in the Anthropocene is driven by ever-changing biogeography and diversity of plants and pests in novel environments. In particular, nature-based climate solutions (e.g., urban forests and commercial afforestation) are breeding grounds for EFPs. Urban forests harbor exotic hosts and are connected to trade corridors, and commercial afforestation entails high volume monocultures of exotics in novel bioregions. Both are vulnerable to EFP outbreaks but also valuable as sentinel meta-forests. We identified objectives to maximize utility of sentinel meta-forests to improve forest biosecurity: a pipeline to reproducibly compile data on pest-host associations and distributions from databases; expert knowledge of pest impacts to assess risk to native host populations and afforestation schemes; finer taxonomic and genetic resolution of information for risk assessment and improvement of resistance; and means for predicting where EFPs will emerge. As proof of concept, we summarize the annotation of information compiled by the data pipeline with expert knowledge of pests of *Pinus contorta* in Argentina, threats they may pose to North America, and outside threats to Argentinian pine resources. We present available genetic-level information on non-established exotic pest populations for key North American conifers with adventive global distributions. This highlights the potential of incorporating fine-scale genetic information into the database. Finally, we test hypotheses on the relationship of relative abundance or relatedness to sympatric hosts and the likelihood of pest emergence using richness of pests on North American species in Swiss urban forests as a proxy. Integrating association, distribution, and genetic data with expert local knowledge and urban tree inventories holds promise for improving understanding and preparedness for EFPs.

Keywords: insect invasion, emergent disease, exotic tree, risk assessment, biogeography

The box tree moth severely threatening *Buxus* natural formation in NW Italy

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Abstract

The box tree moth (BTM), *Cydalima perspectalis* Walker, represents one of the recent examples of exotic insect pests native to Asia accidentally introduced into Europe by the nursery trade. In the area of introduction, it develops mainly on *Buxus sempervirens*, typically used for hedges, borders, and plant sculptures as ornamental plant. Box trees are also found in beech wood and on Alpine rocky slopes forming the Habitat 5110 "Stable xero-thermophilous formations with *Buxus sempervirens* on rock slopes (Berberidion p.p.)" according to the 92/43/EEC Habitats Directive.

A 3-year study was carried out in NW Italy to investigate: (i) the seasonal flight activity with sex pheromone-baited traps, (ii) the effectiveness of microbiological treatments with *Bacillus thuringiensis* var. *kurstaki* (Btk) performed in laboratory and field conditions; iii) the butterfly community through semi-quantitative transects in the areas subjected to microbiological treatment and in control areas to assess the potential impact on non-target butterflies. In all years, two main flight periods were identified annually, highlighting two clearly separated generations per year. Funnel traps were found to be more suitable for monitoring flight activity, when compared to pheromone-baited sticky traps. The microbiological treatments proved to be effective showing a high mortality on early instar larvae.

These results will be crucial to timely planning management strategies to preserve *Buxus* natural formations. Even if no impact has been observed on protected or endangered butterflies, a rigorous protocol for the application and surveillance of the effects on non-target organisms needs to be applied in every natural context where the use of Btk may help control the pest.

Keywords: *Cydalima perspectalis*; *Buxus sempervirens*; invasive alien species; forest insect pest; Habitat 5110

Review of eradication attempts against insect pests of woody plants in Europe

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Abstract

Preventive measures are the first line against the arrival and establishment of invading populations of non-native harmful arthropods in new habitats. However, when such measures fail, suppressing the invaders through prompt eradication should be considered. Eradication can be very cost-effective, preventing the indefinite accumulation of deleterious effects and economic impacts often associated to the invader establishment.

In this work, we aimed at reviewing the eradication attempts of woody-plant insect pests in Europe, and identifying which factors affect the success of an eradication program and the probability of a population establishing in a new habitat before its first detection.

Information on eradication campaigns targeting invasive pests of woody plants in Europe was collected from 1980 until 2021. Data was obtained from online databases, scientific and grey literature, including National and Regional Plant Protection Organization's reports, pest alerts, and press releases. LASSO regression and regression trees were used to assess factors influencing eradication and establishment success.

A total of 314 eradication attempts were documented. In about half of these cases, the target species was still restricted to the primary plant material with which it had been introduced. In these cases, the eradication success was 100%. In cases where establishment had already occurred, eradication was successful in 50% of the programs. Three species rank highest in the number of eradication attempts: *Anoplophora glabripennis*, *Anoplophora chinensis* and *Rhynchophorus ferrugineus*. The spatial extent of the outbreak was the factor that most affected the outcome of eradication campaigns. Other variables, such as the implementation of quarantine restriction, and the type of environment, also affected eradication success. The highest success rate was found in relatively confined environments, such as nurseries and glasshouses. Establishment probability was also linked to climate and to species' traits. Although the word eradication is still "frowned upon" by many in the scientific community, both the number of eradication programs and their success rate have increased dramatically over the past two decades.

Keywords: alien invasive insect species, biological invasions, pest supression

Exploiting species-habitat networks to improve wood-boring beetle surveillance in areas surrounding entry-points

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Abstract

The ever-growing international trade along with ongoing changes in trade networks lead to an alarming number of forest insect pest introductions. This trend is particularly evident for wood-boring Coleoptera, especially bark and ambrosia beetles, longhorn beetles, and jewel beetles. These insects may be present in wood-packaging materials, timber, and plants, and are difficult to detect during routine inspections as infested materials may show little or no sign of infestation. Traps baited with attractive lures placed in and around entry points are commonly used to complement visual inspections and improve chances of intercepting incoming species soon after their arrival. However, it is still unclear at which locations these traps can be optimally placed and how composition of the landscape around entry points can affect the chances of intercepting incoming species. In order to investigate these mechanisms, we used multi-funnel traps baited with a multi-lure blend in areas surrounding 13 entry-points located in a number of countries (Canada, Czech Republic, France, Italy, Portugal, Sweden, Switzerland, USA). Sixteen traps were deployed at each selected site based on a grid of 2 km x 2 km defined a priori, with one trap per each cell of the grid. Selected sites covered a gradient of forest cover (from urban-dominated areas to forest-dominated areas). Traps were left in the field for 5 months and all Buprestidae, Cerambycidae, and Scolytinae were identified to species. A total of 412 species and 91711 individuals were collected. Among them, 24 were exotic species. Linking the structure of native and exotic beetle communities to landscape composition at different spatial scales (i.e., within-site and among-sites) will allow us to i) identify the locations where exotic species are more likely to establish once they arrive in a new environment, and thus determine the optimal locations for baited trap placement, and ii) understand what number of traps is optimal depending on the landscape composition surrounding entry sites.

Keywords: Early-detection, Exotic species, Surveillance, Wood-boring beetles

Tracing lineages of the sudden oak death pathogen across time and space using genomic biosurveillance

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Abstract

The invasive plant pathogen *Phytophthora ramorum* (Oomycota) has been spreading in North America and Europe since the mid-1990's. It attacks over 130 woody plant species and is responsible for the Sudden Oak Death epidemics in the Western United States and the Sudden Larch Death epidemics in the United Kingdom. In Canada, *P. ramorum* was detected the first time in 2003 in a British Columbian nursery. Nursery inspections and aggressive eradication measures conducted during the past 19 years have helped containing the pathogen to nurseries in British Columbia and prevent further spread within Canada. However, complete eradication has not been achieved. To get a deeper understanding of the dynamics of the P. ramorum outbreak in British Columbia, we analyzed the genomes of 353 isolates collected during nursery inspections between 2003 and 2018, as well as of 160 isolates from the USA and Europe. The genomes were assigned to three distinct clonal lineages of P. ramorum: EU1 (n = 140 isolates), NA1 (n = 119 isolates) and NA2 (n = 254 isolates). Within each clonal lineage, we looked for mutations (SNPs, runs of homozygosity) shared among multiple isolates and potentially accumulated in a hierarchical manner over time. For each clonal lineage we identified variants which often where geographically and/or chronologically restricted. For EU1 and NA2, the most abundant clonal lineages in British Columbia, we observe a shift in variants over the 15-year study period, with only a few dominant variants remaining, indicating partial success of the eradication measures taken. Finally, we developed a targeted resequencing assay for variant detection within clonal lineages. The assay is currently evaluated with more than 600 P. ramorum DNA samples from Europe, the USA and Canada collected since the mid-1990's. The first results are promising; for example, the EU1 populations currently present in British Columbia, Oregon (USA) and Europe are distinct. This may allow to narrow down the potential source of a new EU1 outbreak as well as to discriminate local from global spread. We believe our work has the potential to inform pest management authorities and may lead to genomic biosurveillance becoming a standard practice in *P. ramorum* management.

Keywords: genome sequencing; invasive tree pathogen; outbreak dynamics; *Phytophthora ramorum*

Characterization of *Cryphonectria parasitica* and *Cryphonectria hypovirus* 1 present in South Tyrol (Northern Italy)

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Abstract

Characterization of *Cryphonectria parasitica* and *Cryphonectria hypovirus 1* present in South Tyrol (Northern Italy) *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 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 some hypovirulent strains of potential interest to biocontrol. The results obtained in the study represent a significant contribution to the implementation and improvement of biocontrol measures against the pathogen and advance our understanding of the invasive pathogen.

Keywords: Biological control, chestnut blight, invasive pathogens, phylogeography, Population genetics,

New methodologies to identify *Xylella fastidiosa* insect vectors: the case of *Philaenus italosignus*

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Abstract

Following the 2019 discovery of a population of the spittle bug *Philaenus italosignus* in Tuscany, new diagnostic studies have been carried out regarding this *Xylella fastidiosa* vector. Indeed, the availability of molecular assays to discriminate similar *Philaenus* species is central to facilitate the identification of *X. fastidiosa* vectors. Based on the distribution of the lily Asphodelus ramosus, the only known host plant for *P. italosignus*' nymphs, the presence of the spittle bug was investigated in 2020-2022 along the coast of Tuscany and Latium. This led to the finding of a second population in the municipality of Orbetello (Tuscany), about 30 km south of the 2019 population. Spittle bug adults were collected on Mediterranean trees, shrubs, and herbaceous vegetation, by using sweeping nets and aspirators, in November 2021 and June 2022. All specimens were initially measured and examined morphologically (e.g. male genitalia). Adult size was confirmed to be a reliable trait when discriminating between *P. italosignus* and *P. spumarius* adult specimens since, even before genitalia could be examined in the laboratory, P. italosignus adults were correctly identified by measuring their total length, being larger than P. spumarius. Adult size is key to *P. italosignus*/*P. spumarius* discrimination, and it can be most valuable especially when males cannot be found. We then used a sub-sample of the spittlebugs in a comparative test to validate a novel SYBR Green-based real-time PCR assay which is specific for *P. italosignus*. What we found is that, regardless of sex, locality, plant-host, and sampling time, *P. italosignus* adults were always unambiguously identified by the real-time approach. This novel molecular assay provides easily interpretable, highly specific, and reproducible results for the identification of this X. fastidiosa vector; thus, it will allow us to better monitor the ongoing phytosanitary emergence in Europe, both in agriculture and natural environments.

Keywords: molecular diagnostics, spittle bugs, Aphrophoridae, insect vectors

A global framework to enhance national forest biosecurity strategies

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Abstract

Healthy forests are essential for a healthy planet, yet, like many other ecosystems in the current climate, they face a number of threats. One major threat is the emergence and spread of invasive species. Increased global travel and trade have facilitated the movement of invasive species around the world. Some of these invasive species become pests in their new environments, causing significant environmental and economic damage, including damage to forests and the forest sector. These impacts are further exacerbated by climate change.

Invasive species are an issue that requires national, regional and global cooperation and action. Today, protecting forests from pests and diseases through biosecurity has become a crucial national and global task. There are many voluntary guidelines and international and regional agreements that address invasive species; however, the capacity to implement them varies widely around the world.

Biosecurity has been defined by FAO as “a strategic and integrated approach to analyzing and managing relevant risks to human, animal and plant life and health and associated risks to the environment"". A biosecurity failure in one country will often cause problems in neighboring countries, particularly where shared terrestrial boundaries are not protected by natural ecological barriers. No country can afford to ignore transboundary risks, which can have catastrophic implications for human well-being, the environment and the economy.

Biosecurity is emerging as one of the most pressing issues facing developed, developing and transition countries. FAO’s work on biosecurity is part of the wider One Health approach, which aims to sustainably balance and optimize the health of people, animals and ecosystems. The COVID-19 pandemic demonstrated that the responsibility for a healthy planet lies with all of us.

Countries like Australia and New Zealand have implemented national biosecurity strategies. They ensure all stakeholders are aware of biosecurity issues and are motivated to act, encourage the active participation of indigenous peoples and boost collaboration and partnerships. Similarly, the USA established the Crowdsourcing and Citizen Science Act of 2016 and the Forest Health Citizen Science Community to widen public participation in biosecurity.

Based on national and organisational best practices, FAO in collaboration with partners will initiate a multi-stakeholder, multidisciplinary approach to develop a guide to inform a biosecurity framework for Forestry. This guide will help member countries to increase awareness, knowledge and action about forest biosecurity. Targeting policy makers, forest management decision-makers, educators and forest practitioners the guide will describe the roadmap for the development and implementation of national forest biosecurity strategies as a shared responsibility.

Keywords: Forest Biosecurity, forest health, Invasive species, one health, pests and diseases

Factors driving historic intercontinental invasions of European pine bark beetles

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Abstract

Largely assisted by global trade, alien insect species are being introduced into new territories at unprecedented rates. Among forest insects, pine bark beetles (Coleoptera: Curculionidae, Scolytinae) are a large and diverse group commonly recognized as successful invaders and important tree mortality agents in pine forests and commercial plantations. In this study, we collected information on the native and invaded distribution of 51 European bark beetles developing in *Pinus* species. We analyzed their invasion history in the Southern Hemisphere and the Americas and explored several factors that can help explain their invasion success: (1) propagule pressure: interception frequency in the non-native range(2) invasibility: potential establishment area based on climatic matching and host availability and(3) invasiveness: biological traits of the bark beetles (i.e., feeding habit, host range, body size, mating system, colonization behavior). We found that most (87%) of the introductions of the species to new regions occurred in the period 1960-2013, and that variables related with the three main factors were relevant in explaining invasion success. Propagule pressure was the factor that best explained bark beetle invasion probability, followed by invasibility of the novel area. In turn, biological attributes like mating system, body size and host range were also relevant, but showed a lower relative importance. Our study contributes to understand the main factors that explain forest insect invasion success. This information is critical for predicting future invasions to new regions and optimizing early-detection and biosecurity policies.

Keywords: biological invasions; biological traits; forest insects; pine forests; Scolytinae; species distribution models

Early detection of exotic xylophagous species using generic lures at potential ports-of-entry in Europe

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Abstract

Due to globalization and renewed trade routes, exotic insect invaders are increasingly consisting of emergent species, which have never been observed before in areas other than their native ones. A large number of these recent invaders were thus “unknown” species, not included in the quarantine lists. Their detection at arrival could not rely on specific attractants but on compounds or blends expected to allow a rather generic attractiveness at genus or family level in some insect groups. Significant progresses in the chemistry of cerambycid pheromones allowed to define such a blend mixing 8 of these pheromones. Traps baited with this blend were deployed in 2020 and 2021 in 27 ports of entry in France, Portugal, Spain Italy and Switzerland during the course of the EU HOMED project. The addition of plant volatiles known to have a generic attractiveness for bark and ambrosia beetles, such as ethanol and (-) α-pinene, was also expected to enhance trapping scope. The trapping design consisted in a pair of black and green traps installed in the port and a similar pair placed in nearby forests within a radius of 1 km from the port in order to detect possible expansion and establishment of the non-native species. Green traps were used because of their attractiveness for most jewel beetles (especially *Agrilus* spp.) although no lure was designed for such species.

No exotic species was trapped in the Portuguese ports nor in the Swiss ones but a number of them were caught in France, Italy and Spain. Actually, 5 non-native species of cerambycids and 7 non-native species of scolytids were trapped in 2020 whereas these numbers were 5 and 8, respectively in 2021. The Asian cerambycid Xylotrechus stebbingi was trapped all over the Mediterranean but also in more northern locations, e.g. in Paris area, having probably been moved with ornamental trade. The trapping design also allowed to show that 3 species only captured within the ports in 2020, namely the Asian cerambycid Trichoferus campestris in Huningue and the scolytids (platypodids) *Euplatypus hintzi* and *E. parallelus* in La Rochelle, were trapped in the surroundings of the same ports in 2021, probably indicating a dispersal from the point of entry. Some other species were still arriving but did not move apparently from the port (e.g. the African cerambycid *Cordylomera spinicornis*) whereas others were already present in ports as well as in the surroundings (e.g. the ambrosia beetles *Ambrosiophilus atratus*, *Cyrtogenius luteus*, *Amasa nr. truncata* and *Xylosandrus crassiusculus*). Thus, the trapping design with generic lures first allowed to detect the arrival of unpredicted non-native species but also the dispersal of some of them. Obviously, only the insects arriving at the adult stage and capable of flying are susceptible to be detected by such traps. It is therefore suggested a priority installation of traps in places where wooden pallets are stored or in garbage places existing in the ports, in which the insects arriving as larvae can reach the adult stage.

Keywords: Generic lures; Insect Invasions; Ports; attractive Traps; Xylophages

Early detection of forestry pests in New Zealand – the Forest Biosecurity Surveillance system

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Abstract

Invasion pathways for forest insects include the trade of live plants, timber and other wood items including wood packaging material. Certain species can also “hitchhike” on inanimate objects traded or carried by travelers, or in the conveyances used for transport.

New Zealand’s commercial forestry plantations are comparatively well preserved from pest damage, benefiting from the country’s geographic isolation as well as stringent biosecurity procedures applied pre-border and at the border. Still, the system is not fully effective and new invasive forest insects and plant pathogens are discovered almost every year.

The New Zealand Forest Biosecurity Surveillance system aims for the early detection of new invasive forest pests before they spread to major commercial plantation forests. The system utilises a dedicated pest entry probability model, addressing a range of potentially pest-carrying commodities and tracking their itineraries across the country. This information is then used to generate nationwide entry risk maps for key priority pests, hence inform a dedicated surveillance network for New Zealand. The redesigned surveillance system is now in its fifth year of operation. Areas that are surveyed in priority include seaports and airports, transit locations for imported cars and containers, and more generally urban and peri-urban areas.

Keywords: biosecurity, early detection, pest risk assessment, pathway management, *Pinus radiata*

Seed-borne fungi of *Pinus sylvestris* and *Fagus sylvatica* are frequently transmitted into seedlings

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Abstract

Forest tree seeds have been considered as a minor introduction pathway for plant pathogenic fungi, thus few restrictions target seed exchange in Europe. This is despite recent studies which showed that tree seeds harbor highly diverse fungi, including potential plant pathogens that may be introduced with seeds and cause damage in the new areas. Risk assessment of seed trade as a pathway for harmful fungi requires additional information about the transmission of seed-borne fungi into seedlings, but this knowledge is currently lacking. We characterized fungi associated with seeds and seedlings of two commonly traded and ecologically and economically important European tree species, *Pinus sylvestris* and *Fagus sylvatica* to identify seed-borne fungi which are transmitted into seedlings. Seeds of each tree species were obtained from five locations in Europe from commercial seed suppliers. Seedlings were grown sterile on artificial Murashige and Skoog medium in Falcon tubes and/or semi-sterile in pots with autoclaved soil in the greenhouse and harvested two weeks after the first true leaves appeared. Fungi were assessed directly from seeds and seedlings using Illumina amplicon sequencing. The results show that seeds and seedlings of *F. sylvatica* contained similar number of fungal taxa, while higher number of fungal taxa appeared in seeds than in seedlings of *P. sylvestris*. Fungal communities were similar between seeds and seedlings of both tree species, with around 70% of fungal taxa associated with seeds also being found in seedlings. Total of 13 out of 110 (12%) and 12 out of 70 (17%) potentially pathogenic fungal genera were identified from seeds and seedlings of *P. sylvestris* and *F. sylvatica*, respectively. Our results suggest that large fraction of seed-borne fungi, including plant pathogens, is frequently transmitted into seedlings which emphasizes the need for studies assessing the damage of identified fungi, and possibly stricter regulation of seed trade.

Keywords: Non-native pathogens; Pathogenic fungi; Seed trade; Vertical transmission

Colonization success for two non-native beetle species with different reproductive strategies

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Abstract

Invasive forest insects are one of the biggest threats to forestry and biodiversity. The larch spruce bark beetle, *Ips cembrae* and the larch longhorn beetle *Tetropium gabrieli*, are two examples of insects spreading to new areas. A minimum population density is required for establishment in new localities and this threshold value is influenced by species reproductive strategies at different population densities. There is also a positive relationship for non-native species between habitat patch size and probability of establishment. In this context, we ask the following questions: (i) what is the minimum local population density needed for colonization and how does that differ between the two species? (ii) Does the habitat patch size influence the probability of colonization? The study was conducted in Denmark and Southern Sweden, during 2020. Forty-two stands of larch, were selected for the experiment. In each stand local population density of *I. cembrae* and *T. gabrieli* was estimated with pheromone-baited traps. In addition, in 34 of the stands the species colonization ability was determined by inspecting newly felled larch trees for attacks. Five larch trees were cut in each of those stands. Stands with cut trees had a minimum size of 1.8 ha. The felled trees were inspected four times during the summer at the same time as the traps were emptied. Two buffer zones with a radius of 500 and 1000 m respectively, were used to assess if the habitat patch size influences the probability of colonization. The results show that in Sweden the larch bark beetle was still found only within its known distribution area. The larch longhorn beetle was found in few new localities outside its known distribution area and seems to be established in certain areas in Southern Sweden.

Keywords: colonization success, invasive species, *Ips cembrae*, *Tetropium gabrieli*

Early in-field detection of beech leaf disease

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Abstract

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.

Keywords: Beech leaf disease, early detection, near-infrared spectroscopy, machine learning

Posters

Biosurveillance for invasive xylophagous beetles using a DNA metabarcoding approach

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Abstract

Invasive xylophagous beetles represent a threat to forests worldwide. There is an urgent need to develop a biosurveillance system for early detection of invasive species. New approaches such as DNA metabarcoding allow rapid and accurate identification of species. However, many insects are not detectable with these approaches because they are not referenced in barcode databases. Moreover, these approaches are often expensive, time consuming and based on short DNA sequences, thus limiting taxonomic information. The aim of this study is to develop an approach based on the MinION long-read sequencer which provides a cheap, direct and fast analysis within the framework of a biomonitoring study of invasive xylophagous insects. We have used multi component blends for mass trapping both native and exotic longhorn beetles at several European ports of entry. With this biological material at our disposal, we are developing reference databases of Cerambycides to improve the number of species detectable in metabarcoding analyses. We are also working on the design of beetle-specific primers to amplify short overlapping amplicons and re-construct the entire barcode fragment (658 bp), which would improve the detectability rate of these species using Oxford Nanopore Technology's MinION portable sequencing platform. Finally, we tested the efficiency of MinION sequencing for a biosurveillance application of wood-boring insects.

Keywords: Biomonitoring; DNA barcoding; Forest pests, Metabarcoding; MinION

Accumulation of insect herbivores on invasive black locust worldwide

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Abstract

With increasing globalization there is a trend of global homogenization of plant communities following species invasions. When plants invade new regions, they often benefit from the absence of insect herbivores but over time specialist plant-feeding insects may also invade and re-establish host associations. Native to a relatively small part of North America, black locust (*Robinia pseudoacacia*) is a good example of a tree species that has invaded large areas in virtually every temperate region of the world. Here we explore i) spatial and taxonomic patterns of native and alien insect species utilizing R. pseudoacacia as a host worldwide, ii) evaluate environmental drivers of these insect invasions and iii) observe possible enemy release from herbivory. Occurrence records for herbivorous insect species associated with R. pseudoacacia in all world regions were compiled from various sources. From this list we identified species that specialize on the genus *Robinia* and investigated statistical associations between environmental features and the number of *R. pseudoacacia* of specialists over North America. A total of 454 herbivorous species were recorded feeding on *R. pseudoacacia* across the world, with 23 of these being specialized on *Robinia*. From this group, seven species have successfully expanded their range beyond North America. Within North America, richness of specialists was explained by a combination of *R. pseudoacacia* density, distance from the native range, transportation corridors, human population, and climate. We document the accumulation of non-native herbivore species on invasive *R. pseudoacacia* in both North America and in other continents. The steady accumulation of invasions has diminished the enemy release that this invasive tree species has benefited from – a trend that will likely continue in the future.

Keywords: Biological invasion, black locust, enemy release hypothesis, herbivore community, *Robinia pseudoacacia*

Remote sensing in invasive pest monitoring in Croatia

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Abstract

Monitoring of pests, native or alien invasive is a key activity in prevention of damages in forests. Conventional monitoring methods (pheromone traps, visual transects, glue rings etc) are time consuming and require significant financial inputs. Development of remote sensing sensors, GPS technology and geographic information systems (GIS) support novel approach towards detecting pests, which has the potential of accurately measuring pest damage and populations on a whole area basis. Accurate and efficient monitoring of insect populations is a key point to improve pest control and mitigate damages. Technical limitations of remote scouting, especially resolution of satellite and aerial images, have until recently precluded wide scale adoption. Recent technological advancements have increased resolution of visible and NIR sensors while also decreasing size and cost providing more affordable ways to assess forest health. Therefore, usage of small aerial vehicles or drones equipped with multispectral sensors could provide a better insight into forest stand health and damages caused by pests. With relatively low-cost platform, we have insight on the area with better resolution than with those from satellite. Surveys of quarantine pests in forests are being conducted in Croatia, and those are co-financed by European commission. European food safety authority (EFSA) is recommending usage of unmanned aerial vehicles (UAVs) as a versatile remote sensing-based toolkit for monitoring forest health and occurrence of forest pests. Use of UAVs for monitoring of *Bursaphelenchus xylophilus* and *Polygraphus proximus* has been applied in Croatia.

Keywords: Alien invasive forest pest, remote sensing, *Bursaphelenchus xylophilus*, *Polygraphus proximus*

Using REINFFORCE network as sentinel to monitor emerging pest and diseases invasion in Europe

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Abstract

Forest is one of the major terrestrial ecosystems in term of size, diversity and importance in land use. During the last few decades, the presence of emerging pest and diseases in forest has raised a lot causing huge damage to trees. The combination of globalization and trades around the world along with the climate change has led to a strong increased of the risk of invasions by exotic pathogen affecting both the biodiversity and the bioeconomy. The will of the 23 HOMED partners to understand the establishment of pathogens and to predict the areas at risk with several models lead to analyse if emerging pest and diseases can be monitored from sentinel plantations such as REINFFORCE arboreta network to monitor health status of French forest maximising the number of species monitored while visiting a limited number of sites. So, this study was designed to assess the potential sentinel role of REINFFORCE arboretum (https://reinfforce.iefc.net/en) comparing the presence of well-known emerging pests and diseases with national reports in the areas where the arboreta are located. To address this topic an exhaustive monitoring was performed during summer 2019 and 2021 in every REINFFORCE arboretum (with 38 sites and 35 species each) to identify the presence of 4 main pests and diseases: Contarinia pseudotsugae - Gonipterus scutellatus - Dryocosmus kuriphilus -Corythucha arcuate, affecting four different tree species: Douglas fir, eucalyptus, chestnut and oaks. Moreover, we gathered existing data from mainly EPPO (European and Mediterranean Plant Protection Organization), DSF (The forest health department) and other open sources database about the presence of these 4 pathogens in Europe in order to be able to compare and to have a better overview of the spread inside forest. After compiling all the date from various sources, we prepared 4 maps with QGIS to visualize the presence across the entire REINFFORCE network. The results shows that REINFFORCE arboretum could be an efficient tool providing more information as sentinel site to confirm the presence of species (C.arcuata and G. scutellatus) already present in European forest. But for C.pseudotsugae and D.Kuriphilus the results were less convincing because the network only cover the West part of Europe and also because chestnuts suffer a huge mortality in our arboretum. Finally, with this study we show that detecting the presence of pathogen is not an easy task and yet it is a crucial information for current and future plantation. We hope that the use of network such as REINFFORCE will be more common in order to have a better mapping of the pathogen presence, because we plan a surge of invasive species due to the climate change.

Keywords: Detecting; Forest Pathogens; invasive species; REINFFORCE network; sentinel

Physiological, metabolic and hormonal responses of *Pinus* spp. to brown-spot needle blight disease

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Abstract

Pine needle blights are serious fungal diseases affecting European natural and planted Pine forests. Brown-spot needle blight (BSNB), caused by *Lecanosticta acicola* causes canopy defoliation and severe productivity losses with consequences depending on host susceptibility. Furthermore, BSNB plant-pathogen interactions are complex and still in infancy. Our experi-mental system was designed to fill the knowledge gap behind pine defense mechanisms against BSNB by exploring constitutive and induced traits in two hosts with differential disease behavior. Six-months old *P. radiata* (susceptible) and *P. pinea* (relatively resistant) seedlings were inoculated with *L. acicola* under controlled conditions. Healthy-looking leaves from symptomatic plants where sampled when 50% of both species presented BSNB symptoms. Disease severity, morphological, physiological (leaf-gas exchange) and key biochemical parameters including primary and secondary metabolisms and phytohormones were assessed. Our results showed that constitutively, *P. pinea* leaves presented higher stomatal conductance and transpiration rates as well as higher amino- and organic acids and abscisic acid content in comparison with *P. radiata*. Moreover, on 80th day after inoculation, 54% and 45% of *P. radiata* and *P. pinea* seedlings showed BSNB symptoms, respectively, being more pronounced in *P. radiata* in comparison to *P. pinea*. For both species, plant height and CO2 assimilation were negatively impacted by infection, particularly in *P. radiata*, where an increase in total soluble sugars and starch was observed. Although no differences in hormonal dynamics were noticed, secondary metabolism was trigger in *P. pinea*, through phenolic and flavonoids accumulation and a differential modulation of carbohydrates and nitrogen metabolism was observed. This is the first study in species-specific defense strategies in the Pinus-Lecanosticta pa-thosysthem, opening new perspectives for genotype selection tools that aim to carry out de-sign innovative disease mitigation approaches.

Keywords: *Lecanosticta acicola*, physiological performance, emergent diseases, forest pathogens, primary metabolism

Invasion dynamics of the African citrus psyllid in Portugal

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Abstract

The African citrus psyllid*, Trioza erytreae* (Del Guercio) (Hemiptera, Triozidae) is a vector of the huanglongbing (HLB), considered the most damaging citrus disease in the world, caused by *Candidatus Liberibacter* spp. Recently, the psyllid was detected in the Northwest region of the Iberian Peninsula, both in Spain in 2014, and Portugal in 2015, and since then has been spreading, despite the control measures implemented by the National Plant Protection Authorities. The objective of the present work is to describe the spatio-temporal dynamics of the invasion in Portugal. Between 2015 and 2021, *T. erytreae* spread mostly southward, along the coastal area and reached the region of Algarve, more than 450 Km far from Porto region, where it was first detected. The observed spread pattern of *T. erytreae* corresponds to a "stratified dispersal", combining short-range and long-range dispersal.

Key-words: non-native species, *Trioza erytreae,* citrus

Invasion frameworks: a forest pathogen perspective

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Abstract

Within the discipline of invasion science, researchers studying different taxonomic groups have developed separate ways of investigating the phenomenon of biological invasions. While there have been efforts to reconcile these differences, a lack of knowledge of diversity, biogeography and ecology hampers researchers seeking to understand invasive microorganisms, including invasive forest pathogens. However, advances in molecular technologies such as gene and genome sequencing and metagenomics studies have increased the “visibility” of microorganisms, providing opportunities to better integrate forest pathology and invasion science. The two fields have much to gain from closer collaboration. Following this view, we have proposed a modified version of the Unified Framework for Biological Invasions to accommodate IFPs. This recognizes the challenges and limitations of more effectively integrating forest pathology and invasion science, and options to tackle these issues are suggested. With a clearer understanding of how microorganisms move around and the stages through which they pass to become invasive, we hope that forest pathologists will better understand how and why invasions occur. And more importantly, where, when and how invasions can be stopped or at least mitigated.

Keywords: Coevolution; emerging diseases; invasion science, invasive forest pathogens; microbial invasions; tree disease

An update on the invasive oak lace bug spread in urban and peri-urban areas of Central Italy

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Abstract

The invasive oak lace bug (OLB), *Corythucha arcuata* Say (Hemiptera: Tingidae), is a sap-sucking insect mainly associated with oaks. From its native range (North America) it was accidentally introduced to Europe, being recorded for the first time in northern Italy in 2000. Only after more than a decade, starting in 2013, was the OLB reported in other 20 European countries. In Italy, the insect was reported for the first time in Lombardy; however, at that time it had already invaded a vast area between Lombardy and Piedmont. In subsequent years the insect was found also in other northern Italian regions: Veneto, Friuli-Venezia-Giulia, and Emilia Romagna. The aim of the present work was to ascertain, after two decades from its first recorded sighting in Italy, the presence of the OLB in central Italy, namely in Tuscany. Our survey of Tuscany was carried out from late summer to early autumn 2021. All Tuscan provinces were included, as well as the province of Viterbo (Latium), for a total of 50 survey points (SPs) located along roads in urban and peri-urban areas. At each SP, the number of attacked trees, as well as the infestation level of each oak species, were recorded. A sample of OLB specimens was collected for morphological and molecular identification. OLB presence was confirmed in 29 out of 50 SPs (28 in Tuscany and one in Latium). Urban sites were more infested than peri-urban sites: about 79% of the urban SPs investigated had been invaded, as opposed to only 32% of peri-urban SPs. *Quercus robur* and *Q. cerris* had the highest percentage of infested trees (72% and 69% respectively), while only 8% of *Q. pubescens* trees showed signs of attack. Although first sightings of OLB in European countries often occur in urban environments, in eastern Europe, instead, infested forest areas exceeded 1.7 million hectares. OLB causes great concern for oak forest health, particularly in light of the current European oak decline.

Keywords: *Quercus*, *Corythucha arcuata*, sap-supcking insect, alien species

Rapid spread of the oak lace bug, *Corythucha arcuata*, in Austria.

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Abstract

The oak lace bug, *Corythucha arcuata*, was first recorded in Austria in late summer 2019. A quick survey for delimitation of the infested area showed the presence of the pest in the southeast of the country, adjacent to borders to Hungary and Slovenia. Detailed surveys based on visual inspections of oak trees with binoculars were performed in late September 2020 and 2021. Oak trees in urban areas, along traffic infrastructure as well as in forests were included. The two surveys indicate a rapid spread of *C. arcuata* to the north. In 2021, the pest was confirmed in the Federal States of Styria, Burgenland, Lower Austria and Vienna. *Quercus robur*, *Q. petraea* and *Q. cerris* were found to be infested. Locations with high infestation density were detected in three regions near the southeast border of Austria; *C. arcuata* appeared to have invaded along several routes from Slovenia and Hungary. Findings on oak trees near traffic infrastructure, such as rest stops or gas stations as well as commercial places, at the front of the expanding range indicate hitchhiking of adult lace bugs as the main means for long distance dispersal. On the other hand, detection of infested oak trees in forests distant from settlements and in mixed forest dominated by non-host trees in the generally infested area in 2021 shows the ability to find host trees and naturally spread once established. Comparison of infestation of oak trees along a transect in 2019 and 2021 showed this natural spread as well as the buildup of populations.

Keywords: Oak lace bug, invasion, range expansion, hitchhiking

Session 9 Biological control of forest pests: Challenges and opportunities

Oral communications

Opportunities and constraints of biological control in forest ecosystems

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Abstract

Opinions about biological control in planted and natural forests are divided. While some hail the advantages of this pest control method, others have claimed that it is generally unsuccessful in natural forests and too risky. Certainly, both successes and failures have been recorded for biological control in forests. However, empirical data suggest that classical biological control programs are more successful against insect pests of trees, when compared to pests of herbaceous plants (e.g., annual plants or crops). Here, we discuss why and under which circumstances biological control may be more successful in different types of forests, when compared to other contexts. Considering the unique ecosystems of forests as well as the economic and ecologic interests and management goals, we weigh the pros and cons of different biological control methods and their chances of success. We contemplate the different definitions of success in relation to forests and identify challenges and opportunities for biological control that may be more or less unique to forests. In many countries, the missing or restrictive regulatory basis for biological control still clearly represents a constraint. In part, this can be attributed to a misplaced sense of risk by decision makers and the general public. Limitations in our ability to successfully identify and release biological control agents continue to be a challenge that needs to be overcome. In contrast, advances have been made recently in the understanding of functional relationships in forest ecosystems and in the development of new methods and technologies, which clearly represent opportunities for biological control in general and for its application in forest ecosystems. The worldwide need to develop sustainable and environmentally-friendly but yet efficient pest management methods clearly presents an opportunity for biological control, which should be seized by forest stakeholders.

Keywords: Biological control; forest ecosystems; insects; invasive species; success

Forest health improvement and pest/pathogen reduction by use of natural myco-control products and native fungal species

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Abstract

Mediterranean forests are filled with microorganisms that can affect the overall health of the ecosystem. The balance of pests and pathogens with other mycorrhizae or biocontrol microorganisms may either weaken and kill forest flora or contribute on helping the forests thrive. For this reason, we are interested in searching for natural myco-control products and native fungal species that may be found in our local ecosystems. These microorganisms could be cultured and reapplied in our local forests 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 diversity in the field. For the purpose of fulfilling our objectives, four sites were selected in different areas of the Salamanca region. In each selected site the predominant tree species were different, being *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 is ongoing.

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

Criteria for selecting natural enemies for classical biological control of tree pests

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Abstract

Selecting the most efficient and safest natural enemy species is a key element of a classical biological control programme. We reviewed the criteria that are used to select parasitoids and predators for introduction against an invasive arthropod pest, with a specific focus on tree pests. These criteria have changed with time, but presently natural enemies are mainly chosen for introduction based on their ability to control the pest in the area of origin or in other areas of introduction and on their specificity for their host or prey to prevent non-target effects. Natural enemies are also often selected for their availability in laboratory cultures elsewhere, but this option may lead to the selection of natural enemies that are poorly adapted to the area of introduction. More consideration is needed for other criteria, such as climate suitability, the composition of the natural enemy complex in the region of introduction, and the occurrence of sibling species or biotypes with specific attributes. Many biological traits of natural enemies favouring establishment and control have been mentioned, but analyses tend to show that few of these traits influence the outcome of classical biological control and, therefore, the criteria should also focus on target traits, abiotic factors and biological control management strategies.

Keywords: Classical biological control; parasitoids, predator; species traits

The challenge of managing an alien invasive forest insect pest-a successful case of classical biological control in Croatia

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Abstract

Classical biological control against invasive forest pests is sustainable pest management solution in large natural forests and protected forest ecosystems. Introduction and use of classical biocontrol agent *Torymus sinensis* (Hymenoptera; Torymidae) for control of invasive sweet chestnut gall wasp *Dryocosmus kuriphillus* (hymenoptera; Cynipidae) is one of recent examples of effective strategy to control a highly invasive forest pest in Europe. *T. sinensis* is a univoltine, host specific parasitoid, phenologically synchronized and morphologically adapted to *D. kuriphilus*. *T. sinensis* has good dispersal ability, builds up populations quickly and effectively controls the pest already few years after the release. Seven years after first releases and natural dispersal in Croatia, we have evaluated the impact of this classical biocontrol agent on invasive population of *D. kuriphilus*. *T. sinensis* has shown traits of very effective biocontrol agent: the methods for raising and release are not difficult to apply, it disperses very quickly when released, it lowers the population of its host for more than 90% in only few years, suffers no bottleneck -induced founder effect phenomenon and leads to recovery of chestnut vigour and yield in natural sweet chestnut forests. Seven years after first releases, an ecological equilibrium between the pest and classical biocontrol agent has been reached.

Keywords: alien invasive forest pest, *Dryocosmus kuriphilus*, classical biological control, *Torymus sinensis*

Natural enemy hypothesis; Time for a herbivore-trait based approach?

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Abstract

For many reasons, forest management has been evaluated in the recent years. How do monocultures compare to forest stands with more than one tree species with respect to risk of biotic and abiotic disturbances? Accumulated research appears to underline the decreased vulnerability of more diverse forests (Jactel et al. 2017) and the provision of ecosystem services appears to be stronger as well (Brockerhoff et al. 2017). However, the relationship between tree diversity and herbivore performance appears to be species specific. This led to the question if host trait might affect the associated natural enemy community, which in turn will then have consequences for the sensitivity of effective community composition to tree diversity.

In this study, we set out to compare predation rates for *Neodiprion sertifer* in mixed and monoculture stands in Sweden as a gregarious and defended prey. We contrasted the results to plasticine models, individually and in groups, to assess general predation rates, to represent gregarious and solitarous undefended prey. We measured forest heterogeneity as understory diversity and tree diversity and divided the stands into categories with respect to the presence of the focal tree species, *Pinus sylvestris*. We found that predation rates appear to be less variable in monocultures compared to mixed forests for both gregarious defended larvae as well as the non-defended solitarous and gregarious plasticine models.

Keywords: predator-prey interactions; European pine sawfly; herbivory; predation rate

Biological control of emerging forest diseases: How can we move from dreams to reality?

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Abstract

Biological control (BC) is defined as the use of living natural enemies, antagonists, or competitors (biological control agents) to control other living organisms. In the second half of the last century, the general interest in BC has increased, but BC is still only rarely used for pathogens (fungi, bacteria, viruses, nematodes, and phytoplasmas) of forest trees. Here, we present and discuss the biological specificities of both the hosts and the fungal pathogens which may account for this situation. To increase the likelihood of BC success, we suggest a holistic approach involving the use of top-down regulators, competitors and amensalists, all exerting pressure on the pathogen, as well as bottom-up forces helping the host (e.g., endophytes, mycorrhiza). Moreover, BC to mitigate emerging forest diseases should be fully integrated into other sustainable management strategies.

Keywords: biological control agent, native vs. exotic pathogen and host, resistance, virulence

Cryptic species and biological control: four case studies of eucalypt plantation pests

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Abstract

The increasingly widespread use of molecular techniques to differentiate species has been critical in elucidating the occurrence of cryptic species complexes. Among the most significant pests of eucalypt plantations, cryptic species complexes have been revealed for Leptocybe spp. gall wasps, *Ophelimus* spp. gall wasps and *Gonipterus* spp. weevils. Recently, cryptic species have also been resolved for a chrysomelid leaf beetle *Paropsisterna cloelia* (= *P. variicollis*), a pest of eucalypt plantations in Australia and recently invasive to New Zealand. Understanding species delimitations for pests is of crucial importance to the effective development and deployment of biological control programs. Leptocybe invasa, first reported as invasive in Israel in 2000, has spread to every continent of the world that grows eucalypts and has since been followed by a cryptic species that is currently expanding its range and which now overlaps with *L. invasa* in several regions. Until 2012, *Gonipterus scutellatus* was thought to be the major invasive *Gonipterus* pest internationally but is now recognised as a complex of at least 8 species, with three species (*G. platensis*, *G. sp.2* and *G. pulverulentus*) invasive. A number of *Ophelimus* species have now been identified as pests on eucalypts outside Australia but the relationships among them and the Australian fauna and their associated natural enemies is currently poorly understood. For each of these four case studies we discuss the implications of cryptic species complexes on the success of current and future biological control programs, as well as directions for future research to further elucidate these relationships to help improve biological control.

Keywords: Biological control, *Eucalyptus*, Cryptic species, *Gonipterus*, gall wasps, leaf beetle

Diversification of the biological control of *Gonipterus* sp. n. 2 in South Africa

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Abstract

Three invasive *Gonipterus* species are important pests of *Eucalyptu*s spp. worldwide. A single species, Anaphes nitens, an egg parasitoid wasp, is utilised in many countries for the biological control of these pests. Despite the initial successes of *Gonipterus* biological control programmes, frequent outbreaks have been reported. For this reason, diversification of biological control by releasing additional species or populations of parasitoid wasps as well as the use of entomopathogens have been used to improve the biological control of *Eucalyptus* pests. The aim of this study was to consider options to enhance the biological control of *Gonipterus sp. n. 2* in South Africa (SA). This included understanding the species composition and distribution of *Gonipterus* spp. and their egg parasitoids in Australia and to identify entomopathogens associated with *Gonipterus sp. n. 2* in SA. Adults of *Gonipterus* spp. were collected in south-eastern Australia and Tasmania and identified using morphology and DNA barcoding. Parasitoids were reared and identified from egg capsules of these *Gonipterus* spp.. Entomopathogens were isolated from adult *Gonipterus* sp. n. 2 collected from *Eucalyptus* plantations in SA. These were identified using DNA barcoding. Twelve species of *Gonipterus* were collected on the Australian mainland and one in Tasmania. *Gonipterus* egg parasitoid species included *A. nitens*, *Centrodora damoni* and a *Euderus* sp. on the Australian mainland and *A. tasmaniae* and *A. inexpectatus* in Tasmania. These results led to the release of climatically matched populations of *A. nitens* in SA and a study on the biology of *C. damoni* as a potential biological control agent. Entomopathogen species included two species in the *Beauveria bassiana* cryptic species complex and *B. pseudobassiana*. These are promising first steps in the development of a more robust biological control programme for *Gonipterus sp. n. 2* in SA that should also inform similar programmes elsewhere in the world.

Keywords: *Anaphes nitens*, *Beauveria bassiana*, *Centrodora damoni*, cryptic species, climate matching

Using 3PG to compare the cost-effectiveness of augmentative biological control to reduce the wood loss caused by *Gonipteus platensis*

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Abstract

The weevil *Gonipterus platensis* is the most severe insect pest of *Eucalyptus* plantations in the Iberian region after its invasion in the late 90s. This defoliator causes yearly 50%-80% of wood loss in the most affected areas. A Classical Biological Control program was launched soon after its first detection, with the release of the egg parasitoid *Anaphes nitens* (Hym: Mymaridae). Although the program was successful, it did not guarantee complete success in colder areas. Currently, two main strategies are used to fight against the weevil, chemical control with a neonicotinoid, or yearly augmentative release of the parasitoid *A. nitens* to reinforce its local populations. Chemical control causes environmental concern and is expected to be banned in the near future according to the Forest Certification objectives. Augmentative biological control (ABC) with A. nitens is considered by many as cost-ineffective.

Using a modified version of the processed-based model 3-PG we simulated volume loss under different scenarios of the defoliation intensity and treatment strategies. Treatments were applied during different years of the rotation period. The host-parasitoid population dynamic under the studied conditions was simulated using field and laboratory data. This allowed estimating ABC efficiency and the number of wasps to be released in order to achieve control. Two ABC scenarios, one optimistic and one pessimist were then simulated. Finally, we compared the economic effectiveness of the two ABC scenarios and insecticide treatments.

Both chemical and ABC control treatments were cost-effective in comparison with the no treatment. Differences increased with defoliation levels. The optimistic ABC scenario provided almost similar cost-effectiveness to chemical control. The pessimistic ABC scenario was less effective. Still, even the pessimistic ABC, provided relevant reductions in the wood loss. ABC is an ecological alternative to the usage of insecticides being thus recommended although may be less effective. Our work further demonstrates that process-based growth models are a useful tool for decision-making on insect pest control strategies.

Keywords: Augmentative release, Biological control, defoliation, *Eucalyptus*, process-based model

Drivers of predation pressure of *Neodiprion sertifer* in boreal semi-natural forests

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Abstract

Homogenous semi-natural forests are subjected to large scale outbreaks of insect pests. Tree species diversity is suggested to favour natural enemies, by increasing predation pressure of insect pests. However, results concerning pest suppression by natural enemies are not unequivocal according to a recent review, and studies about the importance of mixing tree species in forestry for pest control show differing results. Further, there are few experimental studies in boreal semi-natural forests about the underlying mechanisms of natural regulation of insect pests.

Previous studies of the gregarious insect pest *Neodiprion sertifer* has shown that forest heterogeneity is a better determinant of predation pressure than tree species diversity.

To investigate the relationship between forest heterogeneity and predation pressure of *Neodiprion sertifer*, we measured a wide range of forest heterogeneity variables in six young monoculture and six young mixed, semi-natural forests in Sweden. Larvae of *Neodiprion sertifer* were placed out at five experimental trees per stand for three weeks. Around each tree, we measured tree diversity and structure, type of understory and structure, deadwood, ant-presence, and large stones.

We found that tree species diversity did not increase predation pressure when comparing monocultures with mixed forests at stand level. However, tree and vegetation diversity, dead wood and the presence of large stones did affect predation pressure directly or indirectly within the stands.

We conclude that local heterogeneity, at tree level, may be of more importance than tree diversity at stand level, and that the importance of large stones affecting the outcome of predator-prey interactions may be underemphasized. Future experimental studies are needed to identify the underlying mechanisms for natural regulation presumably linked to the factors identified in this study.

Keywords: Insect pests, Natural enemies, Forest heterogeneity, Tree diversity, *Neodiprion sertifer*

The use of entomopathogenic nematodes to control forest insect pests

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Abstract

Entomopathogenic nematodes (EPNs) offer an alternative control method to insecticides for a number of insect pests in plantation forests, especially those where one or more of the life stages inhabit the soil. Alternative options to chemical control are becoming increasingly relevant due to restrictions from certification bodies such as the Forest Stewardship Council (FSC), potential environmental impact of insecticides and the high costs associated with their use over large areas. Recent studies have investigated the efficacy of EPNs against two important forest insect pests, namely white grubs, the larvae of chafer beetles (Scarabaeidae, Coleoptera) and pupae of *Gonipterus sp. 2* (Curculionidae, Coleoptera). Locally isolated and commercially available EPNs were tested for their efficacy against different white grub species and *Gonipterus sp. 2* pupae, and lethal dosages determined. Of the EPNs tested, Heterorhabditis zealandica MJ2C caused the highest mortality of most white grub species, while Steinernema yirgalemense caused the highest mortality of *Gonipterus sp. 2*. The physiological defence mechanisms used by white grubs were investigated to understand the variation between white grub species. The results showed that the different species of the EPN symbiotic bacteria were able to grow in the haemolymph of all the white grub species tested, but that the ability of the nematodes to penetrate white grubs, the encapsulation rates and growth in the haemolymph varied between EPN as well as white grub species. Studies are ongoing to test different application methods of the EPNs and investigate their foraging strategy.

Keywords: white grubs, *Gonipterus*, biological control, physiological defence mechanisms

Suppressive effect of aqueous root extracts of Brassicaceae species on *Phytophthora cinnamomi* activity

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Abstract

Iberian oak decline is a serious ecological and economic problem associated with the *Phytophthora cinnamomi* presence. This work focuses on the development of new biocontrol tools to minimize the risk of root infection and control the dynamics of pathogen populations.

We explore and compare the efficacy of root aqueous extracts (ARE) of *Diplotaxis tenuifolia*, Eruca vesicaria and Raphanus raphanistrum in inhibiting *P. cinnamomi* development on non-sterile soil suspension. The volatiles released by the AREs were identified and quantified by GC-MS and also the specific bacteria genera involved in the reaction.

The results showed the *P. cinnamomi mycelium* in the presence of non-sterile soil suspension supplemented with Diplotaxis´ ARE had almost zero growth, with inhibition in the production and viability of sporangia. A release of mainly ITC volatiles and the presence of the bacteria genera *Acinetobacter* and *Pseudomonas* were observed. In the soil suspension non-supplemented with ARE, Pseudomonas was the predominant genera identified.

These results emphasize the importance of the surrounding microbiome in the plant-pathogen interaction and offer interesting allelopathic capacities based on the kinetics of volatile release, mainly of *D. tenuifolia*, which hinder the dispersion and viability of *P. cinnamomi*, and could be considered an interesting tool to include in the integrated control against *Quercus* decline disease.

Keywords: Volatiles, *Diplotaxis tenuifolia*, bacteria, soil suspension, *Phytophthora cinnamomi*

The potential of *Pseudomonas* spp. to control ink disease of chestnut

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Abstract

In Europe, sweet chestnut (*Castanea sativa*) is threatened by the ink disease, caused by *Phytophthora cinnamomi* and *P. cambivora*. In Switzerland, the extensive chestnut forests in Ticino are affected. Since the use of chemicals is prohibited in Swiss forests, alternative treatment methods are needed. One such method is biological control with antagonistic bacteria. Fluorescent pseudomonads are a group of bacteria which display antimicrobial traits against plant pathogens in agricultural systems, but their use in forests has hardly been explored.

Our aim was to isolate *Pseudomonas* spp. from the rhizosphere of chestnut trees in Ticino and then to test them for their antagonistic properties against the pathogens *P. cinnamomi* and *P. cambivora*. For this purpose, the rhizosphere of 30 chestnut trees at six sites showing symptoms of ink disease was used for the isolation of the bacteria.

The results showed that fluorescent pseudomonads are present in the chestnut forests of Ticino and that they form associations with the roots of these trees. Out of 25 tested isolates, five displayed in-vitro antagonistic properties against *P. cinnamomi* and *P. cambivora*. Split plate assays showed that volatile compounds play a minor role in the inhibition of pathogen growth. In a screening with specific PCRs, the strains did not possess any genes encoding the widely known metabolites involved in antifungal activity (such as hydrogen cyanide and phenazines). Phylogenetic characterization of the inhibitory strains revealed that they can be assigned to the *P. putida* and *P. fluorescens* groups. Greenhouse experiments are currently underway to test the biocontrol potential of the strains in-vivo.

Overall, fluorescent pseudomonads are promising biocontrol agents against *Phytophthora* species affecting sweet chestnut. Further research is needed to test for their efficacy in field conditions and to clarify the molecular mechanisms through which these strains inhibit the growth of *Phytophthora* sp.

Keywords: Chestnut, Ink disease, *Phytophthora*, *Pseudomonas*

Rhizobiome of *Pinus pinea*: a source of plant growth promoting bacteria with biocontrol potential against pine pitch canker

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Abstract

Pine pitch canker, caused by *Fusarium circinatum*, is a threatening forest disease that affects pine species differently. *Pinus radiata* is the most susceptible, whereas *Pinus pinea* trees are a generally resistant species. Previous work studying the resistant host, using data obtained from a culture-independent analysis of *P. pinea* rhizobiome, suggested the presence of plant-growth promoting (PGP) bacteria. Given that PGP rhizobacteria have been described to enhance plant defence, the rhizobiome of P. pinea could be a useful source of biocontrol agents against pine pitch canker. Therefore, the main goals of this work were to isolate these bacteria and to assess their PGP traits in vitro. Pine roots from five adult *P. pinea* trees were sampled in triplicate. Root processing underwent a periderm removal, followed by homogenization, gauze filtration and a low speed centrifugation to collect the bacterial supernatant. The supernatant was suspended in PBS and plated in both Tryptic soy agar and Reasoner's 2A agar for 7 days at 28ºC. Morphologically distinct colonies were selected and purified. The obtained isolates (n=69) were identified by 16S rRNA gene sequencing and tested for PGP traits, tolerance to oxidative stress, enzymatic and antimicrobial activities. In this bacterial collection, 15% of the isolates displayed phosphate solubilization traits; 94% displayed IAA production; 36% tested positive for siderophore production; 13% tested positive for ACC deaminase production; and 18% were found to be tolerant to oxidative stress. Twenty isolates were selected based on these characteristics for antagonism assays against *F. circinatum*. In total, two isolates showed in vitro inhibition of *F. circinatum*, with 32% and 42% inhibition of fungal growth, these isolates were identified as Paraburkholderia sediminicola and *Rhanella victoriana*, respectively. Of note, the *Rhanella victoriana* isolate was the one with the highest IAA production, producing 67 μg/mL of IAA. Overall, *P. pinea* root tissue proved to be a potential source of PGP bacteria, with isolates having promising characteristics to combat pine canker. Further studies will be carried out in planta to test the promotion of growth and resistance to the fungus.

Keywords: Biocontrol, Microbiome, *Pinus*, Plant-growth promoting bacteria, *Fusarium circinatum*

The virome of *Dothistroma septosporum*

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Abstract

With its worldwide distribution, *Dothistroma septosporum* is one of the major foliar pathogens of *Pinus* spp. It causes Dothistroma needle blight, which starts with chlorosis and then leads to severe necrosis, defoliation of needles, and even the death of pine trees in severe circumstances. Several biological approaches to control this pathogen have been suggested. Could mycoviruses be also one of them?

Screening of our collection of *D. septosporum* isolates from the Czech Republic, Slovenia and Ireland for the presence of dsRNA segments revealed that only five were positive. Together with the other 16 randomly selected, those five were prepared for total RNA extractions. RNA samples were then pooled, and two separated RNA libraries were prepared for stranded total RNA sequencing. De novo assembly of RNA-seq data revealed the occurrence of 13 new putative mycovirus species with different genome types: seven (-)ssRNA viruses, including six bunya-like viruses, and one new member of the order Mononegavirales; three (+)ssRNA viruses, two resemble those of the family Narnaviridae, while the genome of the third one is close to those of the family Gammaflexiviridae; and finally, three dsRNA viruses, two novel members of the family Chrysoviridae, and a potentially new species of gammapartitivirus.

These results suggested that *D. septosporum* isolates are infected with different mycoviruses and could be potentially used to control this pathogen.

Keywords: Biological control; DNB; mycoviruses

Transmissibility and impact of fusagraviruses in different *Cryphonectria* species

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Abstract

Cryphonectria is a fungal genus known to infest important broadleaf tree genera of Fagaceae and Betulaceae in forests, orchards, and urban areas, with great flexibility as pathogenic, weakly parasitic, or saprophytic. Our interest is in the influence of viruses on this behavioral spectrum in the recently discovered species *Cryphonectria carpinicola* and *Cryphonectria naterciae*, which are poorly known as virus host. Co-cultivation of virus-free and virus-positive field samples naturally infected with two previously characterized dsRNA fusagraviruses, CnFGV1 and CcFGV2, resulted in within- and between-species transmission that crosses the boundaries of vegetative incompatibility within individuals of the same species and between the closely related *C. carpinicola*, *C. naterciae*, and *C. radicalis*. Various phenotypic changes due to experimental mycovirus infection were recorded, ranging from asymptomatic or only mild effects to more severe phenotypic changes, including the formation of abnormal colony sectors. The naturally and experimentally infected *Cryphonectria* spp. readily transmitted fusagraviruses through asexual spores to the next generation. But CnFGV1 was found to severely reduce conidia formation and, thus, affect the fitness of experimentally infected C. carpinicola, the causal agent of European hornbeam disease. The biological effects of these fusagraviruses on various *Cryphonectria* hosts and their potential for biological control of tree diseases will be discussed.

Keywords: dsRNA, mycovirus, phenotypic alteration, pathogen, tree disease

Posters

First detection of *Selitrichodes neseri* (Hymenoptera: Eulophidae) in Uruguay: natural and potential distribution

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Abstract

Leptocybe invasa, the blue gum chalcid, is one of the most important *Eucalyptus* plantation pest worldwide. This pest was first detected in Uruguay in 2011 and had spread throughout almost all the country. The biological control with the parasitoids Selitrichodes neseri and Quadrastichus mendelli (Hymenoptera: Eulophidae) is one of the best strategies for their management. These parasitoids have been used to reduce the damage caused by *L. invasa* in Argentina and Brazil. In February 2021, branches with galls of *L. invasa* were collected in an E. grandis plantation near Melo city, from which 5 adults of *S. neseri* emerged. In this sense, the aim of this work was to determine the actual dispersion of *S. neseri* in the north of Uruguay and their potential distribution. In order to evaluate the distribution of *S. neseri* collections of Eucalyptus branches with *L. invasa* galls were made in 21 sites in the north of the country during March 2021 to May 2022. The samples were kept in a climatic room at 23 C with photoperiod (12:12) and adult emergences were checked daily. The emerged exemplars were identified, sexed and placed in tubes with 70% alcohol. A potential distribution model was made with MaxEnt software, the WorldClim 2.5 climatic data, and the parasitoids emergence sites. 726 adults (292 M and 434 F) of *S. neseri* emerged at 6 sites (87.5% from a site near to Brazil). The model showed that Uruguay is climatically favorable for *S. neseri* with the exception of the border with Argentina and part of the east coast of the country (AUC: 0.98). Precipitation Seasonality (54%) and Isothermality (16%) were the variables that most contributed. Based on the fact that the site near to Brazil presented the highest abundance and supported by the potential distribution model, we estimate that *S. neseri* could have entered from Brazil and is slowly spreading.

Keywords: Biological control; *Eucalyptus* gall wasps; *Leptocybe invasa*; Parasitoids, Species distribution models

“Catch-and-release” of natural enemies to the management of *Glycaspis brimblecombei* (Hemiptera: Aphalaridae) and *Leptocybe invasa* (Hymenoptera: Eulophidae)

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Abstract

Brazil has one of the largest areas of forests planted with species of the genus *Eucalyptus* in the world, with about 7.47 million hectares. The Brazilian forest sector suffers significant losses from native and exotic pests. The damage and the difficulty of pest management, with emphasis on the *Glycaspis brimblecombei* (Hemiptera: Aphalaridae) and *Leptocybe invasa* (Hymenoptera: Eulophidae), present in several parts of the world, increase the importance of integrated pest management. Biological control with parasitoids *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae) to *G. brimblecombei* and *Quadrastichus mendeli* and *Selitrichodes neseri* (Hymenoptera, Eulophidae) to *L. invasa* contributes to the management of these pests. Mass releases of these parasitoids increase the rate of parasitism in the field, but the difficulty of rearing these pests and consequently their respective natural enemies is a challenge. A common practice within Suzano S.A. is the “catch-and-release” of insects, which consists of collecting natural enemies in the field with subsequent release in areas with low parasitism, thus favoring the population of areas for future collections. The objective of this work was to describe the technique “catch-and-release” natural enemies used in the management of *G. brimblecombei* and *L. invasa* at Suzano. Branches infested with *G. brimblecombei* nymphs or *L. invasa* galls parasitized are collected in Suzano plantations (for *L. invasa*, rearing sites with susceptible clones are kept in strategic locations). The branches are taken to “laboratório de sanidade e proteção florestal” of Suzano in Três Lagoas-MS and placed in pots with water in standard insect rearing cages. After emergence, only the parasitoids are collected in acrylic vials with a voile lid and counted for field release. Pure honey (100%) is offered in the acrylic vials to ensure the survival of the insects until release (if necessary, they are stored at 20°C). Data collection is done through an internally developed application (PowerApps) and data from natural enemy releases are made available directly in PowerBI to identify the parasitism rate of the areas and signal their potential for future collections. In 2021, a total of 17,040 adults of *P. bliteus*, 9,573 of S. neseri and 23,003 of *Q. mendeli* were released on 14,279.47 ha of Suzano. The technique of “catch-and-release” natural enemies is an effective strategy that complements the integrated management of *G. brimblecombei* and *L. invasa* while new techniques of mass production of the pests are not available.

Keywords: Biological control, *Quadrastichus mendeli*, *Selitrichodes neseri*, red gum lerp psyllid, *Psyllaephagus bliteus*

Biological activities of *Cleruchoides noackae* fed honey types with pollen as food supplement

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Abstract

Cleruchoides noackae (Hymenoptera: Mymaridae) is important in biological control parasitizing eggs of *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae). Adult parasitoids feed on nectar, honeydew and pollen in the field, being necessary research with these materials to improve the performance of these natural enemies. In this study it was evaluated the duration of the development period (egg to adult), proportion of females, parasitism and survival of *C. noackae* fed with Eucalyptus honey, multiflora honey, Apple (*Malus* sp.) and bracatinga honeydew honey (*Mimosa scabrella*) (melato) supplemented with multiflora pollen grains. The parasitism rate of 89.55 ± 12.65 in the first 24 hours of *C. noackae* life was higher with bracatinga honeydew honey + multiflora pollen grains. The average development period (egg to adult) and sex ratio of *C. noackae* were 16.03 ± 3.35 days and 63.36 ± 42.18 %, respectively, and were similar with the different diets. The use of an adequate diet can improve the biological activities of *C. noackae* in mass rearing in the laboratory.

Keywords: Biological control, *Eucalyptus*, Diet, Honey, Parasitism

Biological control of chestnut blight in Portugal: the S. Mamede National Park case study

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Abstract

The fungus *Cryphonectria parasitica* is the causal agent of chestnut blight. It has been considered one of the major decline factors of the sweet chestnut *Castanea sativa* across the world. In Portugal, the disease was detected in 1987 and it's now widespread across all chestnut regions. The control of chestnut blight, a difficult endeavor, must include the implementation of appropriate measures (such as chirurgical pruning and canker removal) and also involves the implementation of biological control with hypovirulent forms of the fungus. Hypovirulence is a natural phenomenon, in which fungal viruses significantly reduce virulence and sporulation of C. parasitica. The aim of the present study was the application of biological control of C. parasitica using hypovirulence in Portuguese chestnut stands, located at S. Mamede Natural Park (Marvão County) and contribute for evaluating the potential for extended field application of this treatment method over this chestnut area. Since the success of biocontrol depends of achieving the integration of many factors, prior to implementation, tree health status, canker extensions and pathogen population structure were assessed for each stand. Canker samples were collected for pathogen isolation and characterization of population structure (identifying vegetative compatibility (vc) types, mating types). After confirming the predominance of vc type EU-11 and the occurrence of asexual reproduction, biocontrol using a bioproduct prepared with the compatible hypovirulent strain was applied in spring 2021. One year later (spring 2022), tree health status was evaluated. Canker healing was observed in some treated trees and hypovirulent strains were recovered from healed lesions in fungal culture. These results suggest that biocontrol of chestnut blight using hypovirulence have good perspectives for a successful and more extensive field application in this important chestnut region in Portugal.

Keywords: *Castanea sativa*; Chestnut blight; CV types; hypovirus; invasive pathogens

Host specificity of *Psyllaephagus* (Hymenoptera: Encyrtidae) towards invasive eucalypt psyllids (Hemiptera: Aphalaridae)

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Abstract

Invasive eucalypt psyllids pose a threat to the long-term production of eucalypts worldwide. In order to reduce their economic impact and further dispersal, classical biological control using *Psyllaephagus* species has been used successfully. *Psyllaephagus* wasps are effective and highly specific in their parasitisation towards psyllids. They are usually sourced from the native range of eucalypts, Australia. However, in some incidents they are accidentally introduced alongside their psyllid hosts. The objective of the study was to determine the host specificity of *Psyllaephagus blastopsyllae*, *P. pilosus* and *P. bliteus* using eucalypt-feeding psyllids, *Blastopsylla occidentalis*, *Ctenarytaina eucalypti*, *Glycaspis brimblecombei* and *Spondyliaspis* cf. *plicatuloides*. To determine host specificity, the three different species of Psyllaephagus were collected from their respective hosts locally, and five-day-old parasitoids were exposed to non-host psyllid nymphal instars in a choice and non-choice setting. In both choice and non-choice settings, the parasitoids were exposed to various stages of the psyllid nymphal instars. *Glycaspis brimblecombei* was identified as a potential new host for *P. blastopsyllae*, and *S.* cf. *plicatuloides* was confirmed as an alternate host for *P. bliteus*. *Psyllaephagus pilosus* was the only parasitoid to show no interest in the other psyllids tested. All female parasitoids preferred specific nymphal stages that promote parasitoid larval development. Our findings indicate that some *Psyllaephagus* spp. attack hosts within an ecological niche, whereas others are highly host specific.

Keywords: biological control, choice test, eucalypt psyllids, invasive, no-choice test

Overview of Suzano's biological pest control program in 2021

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Abstract

Forest plantations have increased considerably in recent decades, with the genus *Eucalyptus* being the most planted, in about 95 countries around the world, with a planting area of approximately 22.57 million ha. Insects of different orders, such as *Gonipterus platensis* (Coleoptera: Curculionidae), *Glycaspis brimblecombei* (Hemiptera: Aphalaridae), *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae), *Leptocybe invasa* (Hymenoptera: Eulophidae) and several species of defoliating caterpillars of the order Lepidoptera, are pests and cause losses. Integrated pest management is the most adequate and effective alternative of the described species, with biological control with natural enemies being a great ally. The main natural enemies used in the management of these pests are *Anaphes nitens* (Hymenoptera, Mymaridae) and *Podisus nigrispinus* (Hemiptera: Pentatomidae) to *G. platensis*, *Atopozelus opsimus* (Hemiptera: Reduviidae) and *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae) to *G. brimblecombei*, *Cleruchoides noackae* (Hymenoptera: Mymaridae) to *T. peregrinus*, *Quadrastichus mendeli* and *Selitrichodes neseri* (Hymenoptera, Eulophidae) to *L. invasa* and *Palmistichus elaeisis*, *Tetrastichus howardi* and *Trichospilus diatraeae* (Hymenoptera: Eulophidae) to the management of defoliating caterpillars. Suzano S.A. is a pioneer in the use of biological control techniques with great investment in this theme. The objective was to describe the biological control program carried out at Suzano. Currently, the company has four laboratories to produce natural enemies, located in the cities of Alambari – SP (ALA), Aracruz – ES (ARA), Cidelândia – MA (CID) and Três Lagoas – MS (TLA). The laboratories rely on the production of *A. opsimus* (TLA), *C. noackae* (ALA), *P. bliteus* (TLA), *P. nigrispinus* (ALA), *Q. mendeli* (TLA), *S. neseri* (TLA), *T. howardi* (ARA and CID) and *T. diatraeae* (ARA and CID). All data collected from production is through an in-house developed application (PowerApps), and data from natural enemy releases are made available directly in dynamic reports in PowerBI. The disclosure panel follows the visual management model that aims to facilitate the organization and optimize the team's processes by sharing the main performance indicators, such as the production and release of natural enemies by year, species and area (ha) per laboratory of production. In 2021, Suzano released a total of 135.47 MM of natural enemies in 178.39 K ha, with 3.09 K *A. opsimus* released in 3.37 K ha (Production in TLA), 373 K *C. noackae* in 16.96 K ha (ALA), 17.04 K *P. bliteus* in 4.54 K ha (TLA), 111.15 K *P. nigrispinus* in 5.21 K ha (ALA), 23 K *Q. mendeli* in 5.01 K ha (TLA), 9.57 K *S. neseri* in 4.72 K ha (TLA), 99.98 MM *T. howardi* in 104.41 K ha (ARA and CID) and 34.95 MM *T. diatraeae* in 34.17 K ha (ARA and CID). These results demonstrate Suzano's engagement in biological control practices and in the digitalized model in its data management. The release of natural enemies in 178.39 K ha of Suzano, 80% preventively and 20% curatively, complements integrated pest management, reduces costs and ensures the rational use of insecticides.

Keywords: eucalypts pests, mass-rearing, natural enemies, parasitoids, predators

The behavior of Atopozelus opsimus preying on *Glycaspis brimblecombei* and *Thaumastocoris peregrinus*

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Abstract

The red gum lerp psyllid *Glycaspis brimblecombei* (Hemiptera: Aphalaridae) and the bronze bug *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae) are two of the main pests in *Eucalyptus* plantations in Brazil. The predatory bug *Atopozelus opsimus* (Hemiptera: Reduviidae), is a zoophytophagous predator from Brazil, capable of feeding on different prey during its life cycle. The objective of this study was to evaluate the predation behavior of A. opsimus with *G. brimblecombei* and *T. peregrinus* as prey. Adults of *A. opsimus* were fasted for 24 hours and individualized in containers with 20 adults of *G. brimblecombei* and *T. peregrinus* in each container. The prey was immediately replaced after consumption and the times used in predation, foraging, self-cleaning and other behaviors (locomotion and rest) were counted for 360 uninterrupted minutes. The completely randomized design was used in this study, with 15 repetitions and submitted to deviance analysis. The means were compared by the Tukey-Kramer test, at a 5% probability level. With *G. brimblecombei* as prey, the most time spent by *A. opsimus* was during predation and other behaviors, spending less time on foraging. With *T. peregrinus* as prey, the longest time spent was with other behaviors, the least with foraging, and the times used in self-cleaning and predation behaviors were similar. The observed results show that A. opsimus can be a potential agent to be used in biological control programs for the red gum lerp psyllid. The study collaborates with the theory of feed preference of *A. opsimus* for *G. brimblecombei*, rather than the other main pests of *Eucalyptus* in Brazil.

Keywords: *Eucalyptus* spp.; Forest pests; Natural enemy

Egg parasitoids of Thaumetopoea pityocampa in North-Western Italy

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Abstract

The pine processionary moth, *Thaumetopoea pityocampa* (Denis and Schiffermüller), is considered as one of the main insect defoliators of conifers over a wide area of the Mediterranean basin and southern parts of Europe. In the last 50 years a latitudinal and altitudinal expansion has been recorded, mainly related to global warming and to an increase in the winter temperature.

Several parasitoids play an important role as biological control agents, and parasitism by hymenopteran wasps is considered to be the largest cause of mortality during the egg stage of the pest.

Within the Interreg V-A Italy-Switzerland 2014/2020 cooperation program “Linee Guida per il monitoraggio e la gestione delle emergenze fitosanitarie nelle foreste delle Alpi centro-occidentali (MONGEFITOFOR)”, surveys were carried out over the 2-year period 2020-2021 in 8 sites located in Aosta Valley (NW Italy). Egg batches were collected on Pinus nigra and P. sylvestris. In the laboratory, eggs were counted and kept in outdoor conditions. Egg batches were constantly monitored for larvae and parasitoids emergence from the collection to the end of the summer period of the following year. After emergence, larvae were discarded, and parasitoids were counted and stored in 1,5 ml Eppendorf tubes with 99% ethanol.

The mean fecundity varied around 180 eggs/batch. In total, 7330 parasitoid specimens emerged, belonging to four different families, namely Eulophidae, Eupelmidae, Encyrtidae, and Trichogrammatidae. In particular, the most frequent species proved to be *Ooencyrtus pityocampae* (Mercet), as already reported in several other geographical areas. Less frequent parasitoids were identified as *Baryscapus servadeii* (Domenichini), *Anastatus bifasciatus* (Fonscolombe) and *Trichogramma* sp. Further investigations are needed to assess the role of these species for the pine processionary moth management.

Keywords: Chalcidoidea, parasitoid wasps, biological control, pine processionary moth, management

Session 10 Forest Health in southern hemisphere commercial plantations

Oral communications

Outbreak of eucalyptus green looper *Iridopsis panopla* (Lepidoptera: Geometridae) in *Eucalyptus* plantations in Central Brazil

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Abstract

Eucalyptus plantations in Brazil are affected by invasive and native insect pests. Among native pests, lepidopterous defoliators are the most important along with leaf-cutting ants. In Brazil occurs nine caterpillar species in *Eucalyptus* and *Thyrinteina arnobia* (Lepidoptera: Geometridae) is the most harmful, defoliated about 400,000 ha in Minas Gerais state in 1980's, 60,000 in Sao Paulo state in 1990's and 180,000 ha in Bahia state in 2018. In 2021 a new lepidopterous species occurred in Mato Grosso do Sul and Sao Paulo states, called Eucalyptus Green Looper *Iridopsis panopla* (Lepidoptera: Geometridae). It is a native species that infested sporadically eucalyptus without economic impact. The identification was confirmed used molecular tools and this species occurs in Argentina and Brazil. Iridopsis panopla has become a pest, infesting 570,000 ha in 2021 and causing partial to total defoliation in eucalyptus in both states. The main control methods used to this pest has been biological control using egg parasitoid Trichogramma pretiosum, pupal parasitoids Palmistichus elaeisis and Thichospilus diatraeae, predatory bug Podisus nigrispinus and Bt aerial sprayings. The possible cause to this outbreak is related with expansion of *Eucalyptus* plantations in MS and with climatic changes, considering La Niña phenomenon in last three years.

Keywords: Lepidoterous defoliator, Eucalypt, biological control

Eucalyptus scab and shoot malformation: An unexpected new threat to plantation forestry globally

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Abstract

A new and serious leaf and shoot disease of *Eucalyptus* of unknown aetiology was detected in North Sumatra, Indonesia in 2014. The disease is distinguished by water-soaked necrotic spots that appear initially on young leaves and petioles and then become scab-like as the lesions age. The scabs commonly dry and drop from the leaves resulting in a shot-hole symptom. In severely affected trees, lesions can lead to girdled and distorted shoots and leaves. Once the leaves are infected, the damage to the tissues is permanent and the infected tissues do not recover. In the most susceptible *Eucalyptus* genotypes, secondary symptoms include a "feathering" effect, resulting in loss of apical dominance and abnormal elongated branches. Severely infected *Eucalyptus* genotypes typically die after several successive infection cycles, usually over a two to three year period. During early investigations seeking to determine the cause of the disease, fungal fruiting structures typical of known *Eucalyptus* pathogens were not found and the symptoms were unlike any other foliar or shoot disease seen on these trees elsewhere in the world. A slow-growing fungus was isolated from the disease symptoms and this was identified as a species of Elsinoe. Phylogenetic analyses of DNA sequence data showed that this was of a novel species that was recently described as *Elsinoe necatrix*. Pathogenicity trials provided unequivocal evidence that *E. necatrix* is the cause of the disease in North Sumatra, which is now referred to as *Eucalyptus* scab and shoot malformation. This is the first disease of *Eucalyptus* known to be caused by a species of Elsinoe and it is amongst the most serious to have been encountered in plantations of these trees globally. Studies are currently underway to better understand the biology of the pathogen, its likely origin and to develop management strategies to reduce its impact.

Keywords: Elsinoe, eucalypts, fungal pathogens, plantation forestry, scab disease

Monitoring the Eucalyptus gall wasp *Leptocybe invasa* and its parasitoids in the commercial plantation of South Africa

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Abstract

Since its arrival in 2007, the blue gum chalcid wasp *Leptocybe invasa* has been a cause of serious concern for *Eucalyptus* growers in South Africa. The management of this insect pest has been primarily based on classical biological control consisting in the introduction and repeated releases of Selitrichodes neseri, a parasitoid of *L. invasa*, and the deployment of tolerant *Eucalyptus* clones. Since 2016, as part of this management strategy, the populations of *L. invasa*, the damage type and intensity, and its biological control agent are monitored annually across the Eucalyptus plantations landscape by means of field surveys and insect emergencies from galled branches collected at the survey sites. During the 2016-2021 period, the presence of *L. invasa* fluctuated across the landscape, with peaks in 2017 and 2019. Eucalytpus grandis and its hybrids showed a higher susceptibility to *L. invasa* in comparison with other *Eucalyptus* species commercially grown in South Africa, and clone x climate interactions affecting the susceptibility to this gall wasp were detected in *E. grandis* x *E. urophylla* and *E. grandis* x *E. nitens* clones. Although S. neseri was the only biological control agent intentionally introduced in South Africa, gall emergencies revealed the presence of three other *L. invasa* parasitoids: Megastigmus pretorianensis, *Megastigmus zebrinus* and *Quadrastichus mendeli*. Regional differences in the presence and prevalence of these parasitoids were detected particularly for the two Megastigmus species and *Q. mendeli*; the more uniform occurrence of *S. neseri* is likely due to its systematic releases across the landscape. The spatio-temporal analysis of the distribution of *L. invasa* and its parasitoids highlighted areas where severe *L. invasa* damage is associated with limited presence of parasitoids, rendering the possibility of *L. invasa* outbreaks higher. Both L. invasa lineages were detected in South Africa, and their spatio-temporal distribution and host susceptibiliyy evaluated. Industrial and small-scale *Eucalyptus* plantations are important contributors to the economy and to rural development in South Africa; the systematic monitoring of *L. invasa* and its parasitoids is an integral part of the management of *Eucalyptus* plantations to curb the potentially severe economic losses caused by this insect pest.

Keywords: gall wasp, Leptocybe, parasitoid, Selitrichodes

Long-term monitoring of red needle cast of radiata pine in New Zealand. What drives episodic outbreaks?

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Abstract

The severity of plant disease epidemics is strongly influenced by temperature and precipitation and is thus highly sensitive to annual variation in weather patterns. Red needle cast (RNC) of *Pinus radiata*, caused by *Phytophthora pluvialis*, has caused damage in New Zealand since at least 2008. Epidemics tend to occur between autumn (April) and spring (October) and can develop quickly, with apparently unaffected trees becoming heavily defoliated within 4-5 months. Weather has been shown to be important in determining epidemic development within years, but no research had previously been undertaken on the drivers of between year variation in expression, which can be significant. In 2015, a series of 33 RNC monitoring transects was established in two regions of New Zealand, the central and eastern North Island. A disease severity score was estimated annually for each site based on the percentage of trees with symptoms and the percentage of foliage with symptoms on the worst affected tree. Average daily maximum temperature (Tmax), average daily relative humidity (RH), number of days with rainfall (DwR) and frequency of 3-day rain events (3daysE) were compiled into seasonal averages for the year preceding assessments for each site. In a preliminary analysis with data up to 2019, generalized additive mixed-effects models were created to identify the most important seasonal weather variables. Disease expressed more consistently in the eastern North Island, where a combination of spring maxT, autumn maxT and autumn DwR explained 82% of variance in disease. Severity increased with all three weather variables. The situation was more complex in the central North Island, where the interaction between summer maxT and 3daysE combined with autumn DwR, autumn maxT, and winter RH explained 68% of variance in disease. Severity was thus greater in years with wetter and cooler summer and autumns. These data will contribute to the development of management programmes for the disease.

Keywords: Aerial *Phytophthora*, Annual variation, Epidemiology, Pine needle disease

Determining the impact of canopy pests on growth of *Eucalyptus* spp. in South Africa

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Abstract

Eucalypt plantations in South Africa suffer from periodic infestation by several pests and diseases, most of which do not result in tree mortality. However, damage to the canopy resulting in leaf area reduction can reduce growth and mean annual increment of a plantation stand. Management intervention, using Integrated Pest Management (IPM) strategies, is an economic decision which requires estimates of probable growth losses for a damage event. In South Africa, the impact of pest-related defoliation events on Eucalyptus yield is not well quantified. Two insect exclusion trials in KwaZulu-Natal, South Africa, were implemented to measure the impact of pests by comparing the growth of trees with and without pesticide protection. The trials were established on sites with low and high pest incidence, each with 30 eucalypt species that included 26 hybrid clones (*E. grandis* x *E. nitens*, *E. grandis* x *E. urophylla*, *E. saligna* x *E. urophylla*) and four pure species (*E. benthamii*, *E. dunnii*, *E. grandis*, *E. saligna*). Exclusion plots were treated annually with imidacloprid (350g L-1). Measurements of tree height (m) and diameter at breast height (dbh in cm) were taken at five years and used to calculate basal area (m2ha-1) and volume (m3ha-1). At the high pest incidence site, treated trees showed superior height (7%), dbh (5%) and volume (15%). However, at the site with low pest incidence, no statistically significant difference between treated and untreated trees were found for all growth variables. Differences in impact attributed to genetics, site and their interaction will be discussed. Continuing growth measurements through to rotation-end will enable the determination of the true impact of pests on growth and yield. This information will be used in the development of economic thresholds in support of IPM strategies for eucalypt canopy pests.

Keywords: Eucalypt pests; exclusion trial; growth impact; imidacloprid

Development of standardized sporulation and molecular genetic tools enable new approaches in *D. sapinea* research

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Abstract

Diplodia sapinea is a globally distributed pathogen of conifers, damaging forests worldwide. Common symptoms of *D. sapinea* infections are shoot tip dieback, tree canker, discoloration, root disease, and tree death. The spread and impact of *D. sapinea* on forest health is expected to increase in the context of climate change. A better understanding of the biology of *D. sapinea* and its infection mechanisms is essential for the development of diagnosis and control strategies. Therefore, we developed methods for cultivation and genetic manipulation of *D. sapinea* in the laboratory. First, an efficient, standardized protocol for the production and storage of highly viable vegetative spores was developed. The spores were then used to infect healthy pine seedlings, comparing different inoculation methods. Most efficient laboratory infections were achieved when spores were inoculated on small wounds. This inoculation method allows highly reproducible large-scale spore infection and virulence assays.

Second, a protocol for the *Agrobacterium*-mediated transformation of *D. sapinea* was developed, allowing molecular genetic studies for the first time. Our method is easy to implement and leads to a high rate of homologous integration. This will allow the creation of gene knockout mutants and the expression of fluorescently labelled proteins for live cell imaging, in order to identify and characterize factors controlling pathogenicity. This new tool set will enable experimental verification of hypotheses obtained by previous studies, leading to a better understanding of the opportunistic pathogen and its interaction with the environment and its hosts.

Keywords: *Diplodia sapinea*, Diplodia tip blight, infection assay, sporulation, transformation

An artificial inoculation system for disease screening against the Eucalyptus leaf blight pathogen, *Teratosphaeria destructans*

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Abstract

Teratosphaeria leaf blight disease (TLB) is one of the most important foliar diseases of *Eucalyptus*. Little is known about the infection biology of pathogens causing TLB and a robust inoculation protocol has, until recently, not been available. We thus determined the conditions for disease devolpment of *Teratosphaeria destructans*, an aggressive pathogen causing TLB in the tropics and compared this with the less aggressive Teratosphaeria epicoccoides causing TLB in temperate and sub-tropical areas of the world. The optimal temperature for conidial germination for *T. destructans* ranged from 25 to 30 o C and 15 to 20 o C for *T. epicoccoides*. The germination was enhanced by light and high levels of relative humidity. Infection ocurred via stomata at 48h after inoculation and symptoms appeared three weeks post inoculation for both pathogens. To develop a reliable scoring method to screen *Eucalyptus* for resistance to TLB, six genotypes of *E. grandis* x *E. urophylla* (GU), and a susceptible *E. grandis* genotype were inoculated with *T. destructans*. The susceptibility of each genotype was calculated using two approaches; percentage of leaf area covered by lesions (PLACL) and a disease susceptibility index (SI) based on symptom severity. PLACL and SI allowed the differentiation of six categories of susceptibity ranging from highly resistant (SI= 0), to highly susceptible (SI= 1.5-2). Using these approaches, moderately resistant GU genotypes scored an SI between 0.49 and 0.54 and a PLACL ranging between 6.5 and 9%, while the most susceptible GU genotype scored an SI of 1.52 and a PLACL of 48%. The artificial inoculation protocol and disease scoring system developed in this study provides the means to select resistant *Eucalyptus* varieties against TLB. This will, in turn, contribute towards effective disease management strategies.

Keywords: Teratosphaeria leaf blight; Screening; Resistance; *Eucalyptus*

Posters

Initial survey of the causal agent of black crust on rubber trees in Brazil

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Abstract

The rubber tree (*Hevea brasiliensis*) is a species from the Amazon region, widely cultivated in Brazil. In recent years, with the expansion of rubber plantations to different locations with different environmental conditions, the occurrence of black crust disease has been increasing and causing concerns, as it causes early leaf fall. This disease has been associated with the fungi *Phyllachora huberi* and *Rosenscheldiella heveae*, which can occur together in isolation. Thus, this work aims to identify the species of fungi associated with the black crust of rubber trees in different Brazilian states. For this, until the present moment, phylogenetic analysis was carried out, using partial sequences from the region of the ITS-5.8S rDNA, the isolates were also pathologically characterized by inoculation in the RRIM600 clone. So far, we have been able to collect 66 samples from different locations in the State of São Paulo, Mato Grosso do Sul, Minas Gerais and Bahia (totaling 12 cities). The predominance of the fungus *Cladosporum* was observed, which, according to the literature, is the asexual form of *R. heveae*. Thus, the following species were found: *C. pseudocladosporioides*, *C. tenuissimum*, *C. cladosporioides*, *C. perangustum*, *C. anthropophilum*, *C. delicatulum*, *C. uredinicola*, *C. ramotenellum*, *C. kenpeggii*. Since 15 isolates were identified only as *Cladosporum sp.* Another fungus isolated and associated with the black crust was *Phyllosticta capitalensis*. In the pathogenicity tests carried out in a greenhouse with the asexual phase of the fungus, it was found that the symptoms appear quickly, since most of the isolates already showed apical death, stem damage and, consequently, intense defoliation, but some isolates did not present present present showed pathogens. This information contributes to the understanding of the epidemiology of the disease and can help in its management in the field.

Keywords: *Hevea brasiliensis*, *Cladosporum sp.* and *Rosencheldiella heveae*

In vitro antifungal potential of *Trichoderma* spp. against black crust from rubber tree

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Abstract

The rubber tree (*Hevea brasiliensis*) is a species from the Amazon region, cultivated in Brazil. Over the years, a disease that has always been treated as a secondary disease, black crust, has become worrying, due to the premature fall of the leaves of the trees. This disease has been associated with the fungi *Phyllachora huberi* and *Rosenscheldiella heveae*. Knowing that there are no registered fungicides for the control of this disease or the use of antagonistic organisms is an alternative. Therefore, this work aimed to evaluate the in vitro antagonism of *Trichoderma* sp. against *Cladosporum* spp. (asexual form of *R. heveae*). Three in vitro experiments were carried out using two isolates of *Trichoderma* (TC1 and AP1) and six of *Cladosporum* spp. Three types of tests were carried out to verify the control capacity of Trichodermas. A production of non-volatile metabolites by the bilayer technique, a production of volatiles by the overlapping plates technique and the pairing technique between fungi were available to control the parasitism capacity of the TC1 and AP1 isoalds. The analysis of the production of volatiles was carried out in an accurate way and of parasitism of non-volatiles in a qualitative way. The AP1 isolates were tested in the evaluation method of all *Cladosporum* isolates tested through the production of volatile metab, the opposite was observed and no method of evaluation of recognized non-volatiles was observed. In this regard, Trichodermas had this behavior, most used - if both are more aggressive in this aspect. The results show a potential for testing these isolates, which could be an alternative in the control of the black crust of the rubber tree.

Keywords: Biologic control, *Hevea brasiliensis*, Leaf spot, *Rosenscheldiella heveae*

Adequation of mineral fertilization reduces defoliation caused by *Calonectria* in *Eucalyptus*

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Abstract

Defoliation caused by *Calonectria* spp. is a major problem in eucalyptus commercial stands in north Brazil. Some elite high-productive clones are, however, susceptible to such pathogen, thus alternative control methods are needed. Plant physiological and nutritional status are key factors for plant defense against pathogens. Mineral fertilization is a cultural disease control method, that can be easily incorporated into the eucalyptus management operations. The objective of this work was to verify the effect of calcium or nitrogen supplementation on eucalyptus resistance to defoliation caused by *Calonectria* spp.. A field trial was planted in May 2020 with one susceptible clone in a 3x3m planting density, in a completely randomized block design, 25 replications. Treatments: 1) Operational limestone fertilization in the ripper line before planting (1,050 kg/ha); 2) Limestone fertilization in the ripper line (600 kg/ha) and above ground (450 kg/ha); 3) Applications of limestone in the ripper line (600 kg/ha) and above ground (2,025 kg/ha); 4) Nitrogen application (138 kg/ha) before the defoliation season (Nov/2020); 5) Nitrogen application (138 kg/ha) after the defoliation season (Apr/2021); 6) Chemical control (Comet®) applied in the canopy before the defoliation season (Apr/2021). Tree canopy depth (length from plant tip to the lowest branch) and shoot biomass were evaluated 13 months after planting. Application of limestone below ground, in the ripper line, and above ground (T2) resulted in the conservation of 36% of the canopy and 81% more biomass than the operational treatment, with limestone only in the ripper line (T1). Application of nitrogen before the defoliation season (T4) reduced defoliation by 32% when compared to T1, resulting in a 70% gain in biomass. Mineral supplementation with calcium and nitrogen before the defoliation season increases plant resistance and/or reduces the initial pathogen inoculum, resulting in less defoliation caused by the fungus.

Keywords: Cultural control, eucalyptus defoliation, mineral fertilization

Epidemiological factors associated with *Calonectria* spores dispersal in *Eucalyptus*

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Abstract

Calonectria spp. is an important pathogen on commercial eucalyptus stands, mainly in north Brazil, causing severe defoliation, impairing productivity. However, the most efficient means for pathogen dispersion still not fully understood, the main goal of this study. Spore traps were installed in a commercial stand planted with a susceptible eucalyptus clone. The traps contained glass microscope slides with double-sided tape and were distributed at five heights (15, 30, 60, 100 and 150 cm) on each treatment. The litter around the traps was completely removed in one treatment (weekly cleaning), leaving the soil surface exposed, litter was left in the area on the other treatment. Microscope slides were evaluated weekly, by counting the number of spores in an area of 324 mm². The trial was evaluated along one full year. Results were correlated with climate data from a meteorological station located in the area. As expected spore concentration fluctuated along the year, with more spores during the raining season (between May and June). Throughout the evaluated period, More *Calonectria* spores were always observed on traps kept in the area with litter covering the soil. More spores were observed closer to the ground, on traps located at the heights of 15 and 30 cm, regardless of the litter. Spore concentration at 100 and 150 cm heights were low at any season, in both treatments. The results indicate that the main source of *Calonectria* inoculum is litter. Fungal sporulation on fallen leaves is more easily dispersed. Fungus dispersion by wind is unlikely. *Calonectria* spores are dispersed by the impact of rain drops on litter containing spores of the fungus. Heavier rains are associated with higher incidence of the disease in eucalyptus commercial stands.

Keywords: *Calonectria*, litter, precipitation, spore trap

Early detection of *Ralstonia solanaceraum* on eucalyptus commercial nurseries, an efficient strategy for disease control

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Abstract

Ralstonia solanacearum is the most important plant pathogenic bacteria of eucalyptus. First detected in 2005 in a commercial eucalyptus nursery and in commercial stands in the field, it is currently widely distributed in Brazil. The main form of bacteria dispersion is through contaminated rooted cuttings transported among nurseries, to form new clonal hedges, or to the field for commercial planting. There is no curative treatment for such bacterial disease and the vast majority of the commercial clones are susceptible. The main strategy for disease control is the use of certified rooted cuttings, pathogen-free. A large-scale annual campaign was implemented, every single commercial nursery that supplies cuttings to Suzano is monitored, aiming to detect clonal-hedges infected with the bacteria. Clonal-hedges displaying stress symptoms such as leaf chlorosis, wilting, defoliation or death are sent to the Forest Protection Laboratory for pathogen detection analysis. Both serological testing and qPCR can be used for pathogen detection. If the bacteria is the detected in a given sample the whole sand-bed where the clonal-hedge came from is fully eradicated, eliminating all hedges in that same sand-bed as well as the sand used for planting. Since 2017, 7.790 clonal-hedges have been evaluated, from 18 commercial nurseries in Brazil. A total of 417 sand-beds have been fully eradicated, representing 5,4% of the inspected sand-beds. The large-scale annual campaign in commercial nurseries is essential to keep the production of healthy rooted cuttings and to avoid the planting of infected plants in the field, and the consequent spread of the pathogen to commercial eucalyptus stands.

Keywords: plant bacteria, dissemination, eradication

Behavior of *Ralstonia solanacearum* from eucalyptus in soil containing glyphosate

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Abstract

Ralstonia solanacearum is a bacterial of eucalyptus it causes wilt, a disease that has been a limiting factor in the culture. The bacterium is a soil pathogen with saprophytic habits and is able to interact with the microbiota and soil elements in which it lives. This study aimed to analyze the behavior of R. solanacearum from eucalyptus in soil containing doses diverts of glyphosate. To carry out the experiments, the bacterial solution was prepared and inoculated in sterile soil, in the presence of eucalyptus seedlings, treated with different doses of glyphosate. Seven treatments were used: soil only; soil + *R. solanacearum*; soil + *R. solanacearum* + recommended dose of glyphosate (0.36 mg e.a. L-1); and for the following treatments the doses were doubled until reaching a dose of 16 times (0.72 mg a.e. L-1, 1.44 mg a.e. L-1, 2.88 mg a.e. L-1 and 5.76 mg e.a. L-1, respectively). The experimental design used was completely randomized, with 10 replications per treatment, totaling 70 experimental parcels. The quantification of the bacterial population of soil samples from the treatments at different periods was carried out, in Kelman culture medium, in addition to the analysis of the activity of the enzymes peroxidase and phenylalanine ammonia-lyase, linked to plant defense. Morphophysiological analyzes of the plants and respirometric analysis of the soil were also carried out. There was an increase in the population of bacteria in the soil with increasing doses of the herbicide, with a decrease only in the highest doses. Treatments that contained overdoses of glyphosate treatments increased the peroxidase activity and decreased the phenylalanine ammonia-lyase activity. Glyphosate doses induced the highest biomass production. The presence of glyphosate showed an increase in soil respiration with the presence of the pathogen, especially in the treatment with 8 times the recommended dose.

Keywords: bacterial wilt; herbicide; biodegradation; biochemical response,

Black crust on rubber tree: initial selection of resistant clones

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Abstract

With climatic changes added to factors such as the expansion of rubber plantations to different locations, the occurrence of black crust, a disease that has always been treated as a secondary disease, has become worrying due to the early fall of rubber tree (Hevea brasiliensis) leaves. This disease has been associated with the fungi *Phyllachora huberi and Rosenscheldiella* heveae. Because this disease has been treated as of low importance in rubber plantations, studies related to it are scarce. Thus, the work sought to identify sources of resistance to black crust in 22 rubber tree clones in a clonal garden. For this, the severity of the disease was evaluated based on four collections (03/05/2021, 05/29/2021/12/08/2021 and 11/14/2021). Five leaflets were randomly collected from three different locations on the plant (lower part, middle third and crown), from three different plants of each clone. The leaflets collected in the clonal garden were taken to the laboratory, scaled and the percentage of injured area was measured with the computer program. The results were submitted to the Scott Knott Test at the 5% probability level. The clone most susceptible to this disease was the IAC300, as it was the one with the highest severity in all evaluations. Following it, clones IAC406, 410, 501 and PB311 can also be identified as susceptible materials. On the contrary, clones IAC507 and PC119 were resistant to this disease. This result is important, even when it comes to clone IAC507, as it is resistant to anthracnose. In this study, it was possible to observe the evolution of the disease during the collection period. Initially, it seems that its attack starts on the youngest leaves (top) in the period of March and then it will be severely expressed in the middle third of the plant, which would correspond to the youngest leaf that was previously infected in the crown. The data contribute to the management of this disease in the field based on the use of resistant materials.

Keywords: *Hevea brasiliensis; Phyllachora huberi; Rosenscheldiella heveae*; genetic control

Soil-borne *Calonectria* species associated with commercial *Eucalyptus* plantation in Colombia

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Abstract

Eucalyptus spp. have been widely deployed as an important component of a growing paper and pulp industry. As these plantations have grown, damage due to insect pests and microbial pathogens has become increasingly important. Leaf and shoot blight caused by *Calonectria* spp. was one of the first disease problems to emerge in Colombian *Eucalyptus* plantations. Extensive surveys were conducted across *Eucalyptus* plantations in four forestry regions of Colombia during 2016, resulting in a large number of *Calonectria* isolates obtained from soil samples collected in the understories of trees having symptoms of *Calonectria* leaf and shoot blight. The aim of this study was to identify and resolve the phylogenetic relationships for these isolates using DNA sequence comparisons of six gene regions as well as morphological characters. From a collection of 108 isolates, the study revealed seven *Calonectria* species residing in three species complexes. Two of these represented undescribed species. *Calonectria parvispora* and *Calonectria spathulata*, were the most commonly isolated fungi, each of which accounted for approximately 30% of the isolates. This is the most comprehensive study yet to consider the diversity of *Calonectria* species in *Colombia*. The results suggest that many more species of *Calonectria* await discovery in Colombia and that these fungi will challenge *Eucalyptus* plantation forestry in the future.

Keywords: Calonectria leaf blight, *Cylindrocladium*, multi-gene phylogeny, taxonomy, two new taxa.

A LAMP assay for in-field detection of *Elsinoë necatrix*, the causal agent of E*ucalyptus* scab and shoot malformation

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Abstract

Elsinoë necatrix is a newly described pathogen that causes a serious disease on plantation-grown *Eucalyptus* trees. The disease known as *Eucalyptus* scab and shoot malformation, was first observed in in North Sumatra, Indonesia in 2014 and causes very serious damage to susceptible trees where the environment is conducive to infection. The destructive nature of this pathogen and a need to monitor its occurrence has necessitated a simple and effective method for the early detection of *E. necatrix* in infected plant material. In this study, a rapid and sensitive Loop-mediated isothermal amplification assay (LAMP) was developed for the in-field detection of *E. necatrix*. The assay was designed to target a unique intron region of a gene known as Efhp1. This gene forms part of the elsinochrome toxin cluster, a unique cluster responsible for virulence and lesion formation in all *Elsinoë* species. Validation of the LAMP assay was confirmed by obtaining positive amplification on 28 strains of *E. necatrix*. A specificity test against 23 closely related Elsinoë species and three fungal species frequently found on Eucalyptus leaf tissues showed that the LAMP assay exclusively amplified the *E. necatrix* isolates. This assay also showed a high level of sensitivity, being able to detect the presence of E. necatrix in amounts as low as 0.001 ng of genomic DNA. Furthermore, the LAMP assay was able to detect *E. necatrix* in infected Eucalyptus leaves using a simple DNA extraction method. The high level of specificity, sensitivity and robustness of this assay suggests great potential for field applications, which will allow for early detection and reduce the risk of this destructive pathogen spreading to new areas.

Keywords: *Elsinoë necatrix*; *Eucalyptus* scab and shoot malformation; in-field detection; LAMP assay; Loop-mediated isothermal amplification

Genetic resistance to *Calonectria* rot on *Eucalyptus*

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Abstract

Calonectria spp. cause many different diseases on *Eucalyptus*, being root and collar rot the most important one in Uruguay. Preliminary studies have identified several species involved, however very little is known about the aggressiveness of these species and the genetic resistance of the host. The aim of this study was to explore the aggressiveness of certain isolates and to test genetic resistance of *Eucalyptus grandis* and *E. dunnii* clones against this pathogen. Thus, preliminary aggressiveness test was performed with all available isolates and the three most aggressive isolates (UY66-*C. seminaria*; UY100-*C. pauciramosa*, and UY119-*C. sulawesiensis*) were inoculated on three clones of *E. dunnii* and three clones of *E. grandis*. Inoculation was performed using rice grain as a vehicle, colonized by the pathogen, and located in contact with wounded roots in pots. Ten 4-month old plants per isolate were inoculated and incubated in greenhouse for 70 days. Pots were located in trays to keep the bottom of the pot flooded to enhance conducive conditions for disease. Wilted and dead plants were registered after that period. Symptomatic plants were examined to complete Koch's postulates. The design was complete randomized with 10 replicates, including a check control with similar conditions but without the pathogen. Our results indicate that significant differences on aggressiveness among *Calonectria* strains, and variation on genetic resistance inter and intra *Eucalyptus* species. These preliminary results highlight the importance to know the population structure of the pathogen, and the interaction with the host resistance for a better disease management. Further investigation is needed for a deeper knowledge of this pathosystem to minimize the effect of this important disease on Eucalyptus plantations.

Keywords: eucalypts root rot, collar rot, *Cyllindrocladium*

Insect pests and diseases in plantation forestry nurseries in South Africa

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Abstract

Diseases and pests associated with plantation nurseries affect plant supply for plantation establishment. This results in major economic impacts, both directly to the nursery as well as down-stream due to planting of second choice genotypes, resulting in reduced yields at harvesting. Conditions in plantation nurseries are often optimal for pest and pathogen development due to the warm, humid conditions required for enhanced plant growth. In recent years a number of previously unknown insect pests have been observed in plantation nurseries, affecting both Eucalypt and Pine seedlings and cuttings. This study reports on diseases and pests that were recorded on different eucalypt and pine genotypes in three plantation forestry nurseries in South Africa between 2020 and 2022. The most common diseases affecting eucalypt genotypes were powdery mildew and Quambalaria leaf and shoot blight on hedges and cutting death caused by species of *Botrytis*, *Calonectria* and *Rhizoctonia*. Other pathogens recorded were *Apoharknessia* sp., *Colletotrichum* sp., *Chaetomella raphigera*, *Pilidium* sp. and *Teratosphaeria destructans*. Diseases of pine were caused by *Fusarium circinatum*, *Botrytis* sp. and *Calonectria pauciramosa*. Insect pests on eucalypts included *Achaea catella*, *Agrotis* sp., *Heliothrips haemorrhoidalis*, *Scirtothrips aurantii*, *Spodoptera littoralis*, *Strepsicrates* sp. and insects belonging to the Aphidae, as well as mites. *Pinus* species were infested by *H. haemorrhoidalis* and insects belonging to the Aphidae. This study includes first reports for plantation forestry nurseries in South Africa and provides important information for the optimization of Integrated Pest Management (IPM) systems in the affected nurseries.

Keywords: *Eucalyptus*, fungal pathogens, leaf damage, *Pinus*

Lophodermium species diversity on needles of non-native Pinus spp. in the Southern Hemisphere

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Abstract

Species of *Lophodermium*, of which approximately 38 have been described, are fungi well known to infect conifer needles and cones. Other than *Lophodermium seditiosum*, that can cause a needle cast disease on *Pinus* spp., these fungi are considered to be endophytic. Morphological studies have in the past revealed cryptic taxonomic boundaries between *Lophodermium* spp., and these have recently been confirmed using phylogenetic analyses for available isolates, mostly from the Northern Hemisphere. Several of these species have been reported from plantations of non-native Pinus spp. in the Southern Hemisphere but most have been identified solely based on morphology. The aim of this study was to confirm the identity of *Lophodermium* spp. collected in Australia, Chile, Colombia, New Zealand and South Africa, where non-native Pinus spp. are intensively propagated in plantations. A multigene phylogenetic analysis of sequence data for more than 100 isolates revealed the presence of five distinct *Lophodermium* taxa. The majority of the isolates resided in the *L. conigenum*-australe complex, and these were found in all countries, except Chile. *Lophodermium indianum* and *L. molitoris* were confined to Colombia and New Zealand, respectively. Two distinct lineages were observed for *L. pinastri*. One of these represented isolates collected in Chile and New Zealand, and the other was for the isolates collected in Australia. Although distinct morphological characteristics were recorded for some of the identified taxa, within species variability was observed when studying these features on different pine hosts. Numerous new hosts were recorded for the identified *Lophodermium* spp. These new associations could imply relevant biological relationships or even future threats to non-native plantations of *Pinus* spp. in the Southern Hemisphere.

Keywords: needle diseases; pathogens; endophytic fungi

Infection and expression of secondary metabolite gene clusters in the *Eucalyptus* shoot and leaf pathogen, *Teratosphaeria destructans*

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Abstract

Teratosphaeria destructans is a Dothideomycete fungus that causes a destructive shoot and leaf blight disease on *Eucalyptus* trees. The pathogen thrives in tropical and subtropical areas and occurs in plantations across South East Asia. South Africa is the only country beyond this region to which it has spread. The aim of this study was to investigate the manner in which *T. destructans* infects and colonises a susceptible host and to consider whether phytotoxins could play a role in this the process. Leaves were sprayed with a *T. destructans* spore suspension and infection was studied using electron microscopy. We found that the pathogen infects *Eucalyptus* leaves through the stomata and grows in the apoplastic spaces. Fruiting structures developed in the sub-stomatal cavities and sporulation from these structures was observed four weeks after inoculation. The published genome sequences of *T. destructans* were used to computationally predict the presence of secondary metabolite biosynthesis clusters that may play a role in infection. Twenty-five clusters were identified and RNA sequencing confirmed that all predicted genes in these clusters were expressed in vitro. A putative product could be assigned to five of these clusters, based on similarity to previously characterised secondary metabolites. Based on these results, *T. destructans* most likely produces fujikurin, melanin, mellein and a betaenone phytotoxin, as well as 20 other unidentified metabolites that could also play a role during infection. The ease with which the expression of these clusters could be detected in vitro was surprising and indicates that *T. destructans* readily expresses secondary metabolite genes. This may be one of the reasons why *T. destructans* is such an aggressive pathogen, while its confinement to the apoplast could explain how it evades host defences in a susceptible host.

Keywords: *Teratosphaeria destructans*, colonization, expression, stomata

Session 11 Myrtle rust: biology, impacts and management opportunities

Oral communications

Elevating and recognising knowledge of indigenous peoples to improve forest biosecurity

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Abstract

Current forest biosecurity systems and processes employed in many countries are, in large, constructs of Western principles, values and science knowledge that have been introduced and integrated internationally. They are often devoid of knowledge, and of the values and principles, held by indigenous people, even those who have an intimate and enduring relationships with their forests. Indigenous people are also often overlooked in policy and decision making processes, yet are often most effected by biosecurity pests that impact native plants species that they may rely on for sustenance, cultural or spiritual purposes. By adopting an inclusive approach scientists and indigenous people can achieve more comprehensive and robust biosecurity outcomes through a shared diversity of knowledge and at the same time serves to elevate and recognise the importance of indigenous knowledge. A co-innovation approach can also result in more widespread adoption of tools or practices by end-users including indigenous people. Understanding New Zealand Māori and their unique knowledge base can help improve forest biosecurity systems and practices, as can discussions of barriers that can and have prevented adoption of inclusiveness. Here we outline key principles behind indigenous engagement, specifically the need to develop enduring relationships.

Keywords: Indigenous knowledge, community, indigenous people, environment, engagement

The molecular interaction between *Eucalyptus grandis* and myrtle rust, *Austropuccinia psidii*

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Abstract

Austropuccinia psidii, an obligate, biotrophic rust pathogen causes rust disease on a broad range of Myrtaceae species. The pathogen infects *Eucalyptus grandis*, a widely cultivated hardwood Myrtaceae species in its natural range and in forest plantations. This study aimed to investigate the molecular dialogue between *E. grandis* and *A. psidii* in resistant and susceptible E. grandis. Resistant and susceptible seedlings were artificially inoculated with the pandemic isolate of the pathogen and RNA extracted for RNA-seq from leaves harvested at 12-hours post inoculation (hpi), 1-, 2- and 5-days post inoculation (dpi). Control, non-inoculated leaf tissue were harvested at the same time points. A dual RNA-seq approach was undertaken to determine both host and pathogen responses with reads mapped to the *E. grandis* and *A. psidii* reference genomes respectively. Differential gene expression and gene ontology enrichment were indicative of a hypersensitive response in resistant seedlings. Brassinosteroid signalling was an enriched term in the resistant seedling data-set at 2-dpi. This was not observed in the susceptible interaction until 5-dpi. Further evidence implicates brassinosteroid signalling as an important role player in the interaction as brassinosteroid mediated signalling genes are among the candidate genes within two major disease resistance loci (*Puccinia psidii* resistance), Ppr3 and Ppr5. The pathogen responses included forty-three putative effector proteins. Malate metabolic and malate dehydrogenase activity processes were enriched among the susceptible host expressed genes, implicating oxalic acid in disease susceptibility. These results provide the first insight into the molecular dialogue between *A. psidii* in *E. grandis* over time and we suspect a brassinosteroid-oxalic acid interplay. This warrants further scrutiny to identify mechanisms to circumvent myrtle rust disease in Eucalyptus.

Keywords: brassinosteroids; dual RNA-seq; effectors; oxalic acid; phytohormones

Myrtle rust infection of an endemic rain forest tree across a forest edge gradient in New Zealand

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Abstract

Myrtle rust (*Austropuccinia psidii*) arrived in mainland New Zealand from Australia in May 2017, and New Zealand's 28 native species of Myrtaceae, 27 of which are endemic, may be vulnerable to it. Five years after its arrival, the small rain forest tree Lophomyrtus bullata is one of the most susceptible to myrtle rust and it is infected across most of its entire natural range which covers the northern half of New Zealand. Localised extinctions of this species due to myrtle rust infection are likely in the near-term, with death of adult trees occurring in highly-affected areas, and also reduced recruitment due to infection of fruit and flowers and death of highly-susceptible seedlings. Shortly after the arrival of myrtle rust, it was observed that *L. bullata* was infected more heavily at forest edges. It was also found that the disease was most successful in areas with higher humidity and temperature. To explore these observations, we established a set of transects running from forest edge to 60 m into the interior in areas where *L. bullata* was abundant in a lowland rain forest. Along these transects we measured abundance and density of *L. bullata* and their co-occurring species, canopy cover, and temperature and humidity. We also did a detailed survey of myrtle rust infection at the time of peak infection in early-2021 and early-2022 with almost 40,000 leaves inspected across 90 plants. Here we show that infection depends on an interaction among distance to edge, canopy cover, and forest structure. High canopy cover results in uniform infection, likely due to increased understorey humidity. Trends of infection are presented over two years of collected data.

Keywords: Humidity; *Lophomyrtus bullata*; Myrtaceae

Testing and validating molecular tools for detecting genetically distinct biotypes of *Austropuccinia psidii*, Myrtle Rust

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Abstract

Myrtle rust is a disease caused by a biotrophic fungal pathogen, *Austropuccinia psidii*, thought to have originated in Central/South America. Recently, *A. psidii* has appeared in all continents, except Antarctica. A recent study revealed at least three major genetic groups of *A. psidii*: (1) a eucalypt/rose apple biotype found in Brazil, (2) a guava biotype found in Brazil, and (3) a pandemic biotype that occurs on multiple hosts with a wide global distribution. A distinct biotype from South Africa has been identified. Bioclimatic modeling was used to predict areas with suitable climate for *A. psidii* biotypes 1, 2, and 3 that are at risk from invasion. The bioclimatic modeling found that the eucalypt/rose biotype poses a threat to Hawai'i, Australia, and New Zealand; while the pandemic biotype threatens regions in Brazil/Uruguay. Our research objectives are to: identify genetic markers and develop loop-mediated isothermal amplification (LAMP) assays to easily distinguish among the four *A. psidii* biotypes; use LAMP assays to further document the distribution and host associations of each A. psidii biotype, and provide stakeholder groups with LAMP assays and associated training. Preliminary LAMP assays

detect and distinguish the four known *A. psidii* biotypes, though improvement in sensitively of the assays is ongoing. Further testing is ongoing with more isolates from different regions and diverse hosts. The developed tools are critical to evaluate invasive threats by *A. psidii* biotypes, develop regulatory protocols for preventing spread into new areas, and improve management strategies for minimizing the damage caused by *A. psidii*.

Keywords: LAMP assay, Molecular Field testing, Myrtle Rust, Regulatory Measures

Predicting climate-change influences on potential distributions of three forest pathogens

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Abstract

Forest pathogens will likely have increasing impacts on forest health under future climate-change scenarios as rapidly changing climates alter pathogen distribution and predispose trees to climatic maladaptation and other disturbances (e.g., fire, insects, etc.). Three forest pathogens, *Phellinus noxius* (causes brown root rot), *Austropuccinia psidii* (causes myrtle rust), and *Armillaria solidipes* (causes Armillaria root disease) are used for bioclimatic prediction models (maps) of current and future distributions. These pathogens cause destructive diseases on diverse tree species that are integral to diversity of forest ecosystems, economies, and native culture in various global regions. Contemporary prediction models were produced using Maximum Entropy algorithms that coupled climatic data with 179, 403, and 382 georeferenced and DNA sequence-confirmed occurrence locations for *P. noxius*, *A. psidii*, and *A. solidipes*, respectively. Bioclimatic prediction models were also produced for one host (*Pseudotsuga menziesii*, Douglas-fir) of *A. solidipes* based on 12,000 georeferenced points. From the contemporary models, data were extrapolated through future climate scenarios to forecast areas at risk for invasion/emergence of these pathogens. The utility of bioclimatic models to predict current and/or future geographic distribution was successfully demonstrated for all three pathogens and one host of *A. solidipes*. Multiple genetic groups occur within *P. noxius* and *A. psidii*, and bioclimatic modeling showed that distinct genetic groups pose distinct threats, and understanding these distinct threats is critical for limiting damage from invasive pathogens. Predictions of current and future distributions of forest pathogens that consider genetic diversity and population structure will help to develop appropriate management/regulatory practices to minimize impacts on forest ecosystems.

Keywords: climate change; suitable climate space; species distribution modelling; tree maladaptation; disturbance

Myrtus communis in Europe threatened by the South African and pandemic strains of the myrtle rust pathogen *Austropuccinia psidii*

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Abstract

Austropuccinia psidii, the causal agent of myrtle rust, has emerged as a significant threat to Myrtaceae in planted and natural woody ecosystems. The first detection of *A. puccinia* on *Myrtus communis* was on a severely infected plant growing in a residential garden in South Africa. This raised concern that *M. communis*, the sole Myrtaceae species native to Europe and an important component of vegetation in Mediterranean regions could be threatened by the rust. In light of the potential threat to this unique species, seed was collected from 12 Italian provenances of *M. communis*, including mainland and island (Sardinia and Sicily) populations. We assessed the susceptibility of these provenances to both the South African and pandemic strains of *A. psidii*. Seedlings were artificially inoculated in South Africa with a single-uredinium isolate of the South African strain and all provenances showed high levels of susceptibility. In Colombia, where the pandemic strain of *A. psidii* is native, seedlings rapidly became infected by natural inoculum. It was however also possible to conduct artificial inoculations on plants for some provenances, with a single-uredinium isolate of the pathogen, and these were all susceptible to infection. Plants of each of the 12 provenances were planted and monitored in Florence, Italy, and these showed no signs of disease This study highlights significant threat that both the South African and pandemic strains of *A. puccinia*) pose to *M. communis* in Europe.

Keywords: biosecurity; emerging diseases; invasive forest pathogens; microbial invasions; sentinel plants

Posters

Early selection for rust resistance in *Eucalyptus* progénies

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Abstract

Myrtle rust caused by *Austropuccinia psidii* is one of the most important diseases affecting eucalypt (*Eucalyptus* spp.) plantations in Brazil. Currently, the disease management can be carried out with fungicides, but the planting resistant clones has been the most economical and effective measure for rust management in Brazil. Usually this selection is performed after choosing clones with high productive performance, considering industrial requirements. An alternative to avoid this expenditure of time and money in the selection of productive clones, but susceptible to rust, would be the early selection for resistance to rust before the deployment of clonal tests. Thus, the objective of this work was to develop a methodology for inoculation and evaluation of rust resistance in seminal seedlings in hybrid progenies. The production of seminal seedlings and the entire stage of inoculation and resistance assessment was carried out at Suzano in the nursery in Jacareí (SP). Three breeding populations were evaluated, with more than 20,000 plants being phenotyped. Plants were grown in a greenhouse with an average temperature of ca. 25°C (±5°C) and natural light. After 40-days age, the plants were inoculated with 2 × 104 urediniospores ml−1. The isolate of *A. psidii* was collected in the region of Jacareí and previously multiplied on young leaves of *Syzygium jambos*. The inoculum suspension, prepared with sterilized water with 0.05% Tween 20®, was homogenously sprayed on both adaxial and abaxial leaf surfaces using an electric compressor. To ensure efficiency of inoculation, highly susceptible plants of *S. jambos* were randomly distributed among the eucalypt plants. At 21 days after inoculation, the seedlings were classified as resistant or susceptible to rust based on the observation of symptoms on leaves and petioles. About 65% of the evaluated plants were classified as susceptible to rust, not needing, a priori, to be continued in the following stages of the Genetic Improvement Program. This phenotyping model makes it faster to exclude susceptible genetic materials, thus saving time and resources in the development of new clones.

Keywords: Phenotyping, control, *Austropuccinia psidii*, breeding, screening

Predicting the climatic risk of myrtle rust in a daily basis for Brazil. Loos, R.A.

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Abstract

Brazilian forest-based industries are supported by more than 7.5 million hectares of Eucalyptus growing under different climatic conditions with different degrees of favourability for myrtle rust (*Austropuccinia psidii*), affecting both new plantations and the regrowth of provenances, which are susceptible to this tree disease. Worse still, range shifts of infectious plant disease are expected under climate change. As plant diseases move, emergent abiotic-biotic interactions are predicted to modify their distributions, leading to unexpected changes in disease risk. Evidence of these complex range shifts due to climate change, however, remains largely speculative. Here, we developed a mobile APP to model the infection of myrtle rust in a daily risk assessment, based on climate forecast, advancing control strategies. The model used to estimate myrtle rust severity was applied to determine the spatial and temporal variability of this disease at Suzano´s cellulose company farms in Brazil. We customized the model using the night air temperature and night leaf wetness on the degree of disease infection and we normalized the infection severity in a risk index. Daily maps are presented in the APP forecasting the risk of rust up to two weeks ahead and an historical risk index database is fed each day with the respectively actual climate conditions. The daily scale allowed monitoring disease prevalence in plantations from Espírito Santo and Bahia States even when areas have been classified as low risk. Risk maps based on historical averages of disease occurrence steer the forest planning of resistant provenances in high risk areas, but a daily risk assessment becomes necessary to adopt complementary management measures to control the disease, especially for periods that become high risks in the present. The methodology used in this study can be implemented in other parts of the world.

Keywords: *Austropuccinia psidii*; *Eucalyptus* plantation; Rust

Session 12 Embracing the beauty of uncertainty and dealing with it in predictive models for forest pests and pathogens

Oral communications

i-Tree Pest Predictor: Challenges and triumphs using models to predict the next high-impact insect invaders in forests

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Abstract

In forest ecosystems around the world, introduced insects produce impacts that range from no or minor damage to regional host mortality and functional host extinction. Forecasting potential impact of insects that have not yet arrived in a novel range is a challenge, but is essential for identifying potential pests, improving biosecurity measures, and preventing the introduction and establishment of insects that are forecasted to have significant impacts on native host plants. In this study, we used available data from introduced insects currently established in North American forest ecosystems to evaluate drivers that could explain why some introduced insects are benign while others are catastrophic and develop models to predict the next high-impact insect invaders in forest ecosystems. To start, we considered four submodels: (i) insect traits, (ii) host traits, (iii) host evolutionary history, and (iv) insect evolutionary history. We found differences between factors that drive impact of insects that have a narrow native host breadth versus broad host breadth, feed on conifers versus hardwoods, and feed in different guilds (e.g., sapfeeders versus non-sapfeeders). As a result, we consolidated the significant submodels into five composite models that can be used to forecast which insects have a high probability of causing tree mortality in North American forests should they establish. These models have been built into the i-Tree Pest Predictor tool, which can help support insect risk assessment on 400+ hardwood and conifer trees native to North America, and aid decision-making by federal scientists and forest resource managers. The modeling approach used here may be adapted to develop predictive models for other forested regions across the globe.

Keywords: Evolutionary history; Information theoretic approach; Introduced insects; North America; Risk assessment

Uncertainty in forest pest models: The good, the bad, and the ugly

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Abstract

Forests are affected by various interacting natural disturbance agents that can alter their ability to provide valuable ecosystem services. There have been changes in the magnitude, frequency, and seasonality of disturbance agents, and this trend is expected to continue under changing climate conditions. To mitigate potential negative ecological and economic effects, research has sought to understand how disturbance agents interact with their physical environment and predict geographic locations at high risk. Forest risk models can be used to predict the spatial-temporal occurrence, abundance, or distribution of pest populations, and assess how changing climatic conditions may alter these dynamics. Historically, these analyses have ignored and/or been limited in their ability to address the inherent uncertainty present in risk assessments, mainly due to imperfect environmental and pest data. Thus, the goal of this work was to identify common sources of uncertainty and to illustrate its use with an example. The framework discussed here includes the assessment of forest pest risk using a hurdle model (i.e., risk = probability of occurrence x magnitude of occurrence), as well as its uncertainty. Prediction was accomplished using environmental and pest observations through a multi-step extreme gradient boosting algorithm. Interpolated prediction interval maps (i.e., uncertainty) were presented for each spatial-temporal pixel using quantile extreme gradient boosting. This technique used the uncertainty to identify the worst- and best-case scenarios for a given location. Threshold probabilities showing areas at major risk were calculated using Monte Carlo simulations. The results from this work show a path forward for practitioners to use predictive analytics to assess not only the risk, but also its likelihood. Such understanding of natural disturbances is critical for informing proper conservation policies and management decisions.

Keywords: Bark beetles; Disturbance; Forest health; Machine learning; Spatial Statistics

Incorporating habitat quality in reaction-diffusion models to improve predictions of invasive insect dispersal

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Abstract

Invasive insects are among the most severe threats to forest conservation and production. The design of efficient and effective strategies for prevention and control demands high-quality information and several predictive models and simulations of different scenarios, including different climatic conditions, land uses, or patterns of host distribution. During the design of containment strategies, it is essential to estimate the potential pattern of dispersal of the pest through the territory. Reaction-diffusion models are mathematical tools commonly used to model particles' dispersal in a medium. Some adaptations of these models have been used for modeling the dispersal of organisms in an area. Skellman and Fisher's models combine diffusion with population growth to predict the number of individuals at any point in time and space. Unfortunately, in their basic formulation, these models treat space as homogeneous and continuous, where dispersion is isotropic. This study evaluates the advantages of including heterogeneity to obtain more realistic predictions from these models. To achieve this aim, we include habitat quality (suitability) in the Fisher model and compare results with those obtained with the basic approach. We estimate habitat quality by fitting Species Distribution Models (SDM) using random forests and presence-only data and then including the suitability index as a covariable in the logistic section of the Fisher model. Our results show that, by combining reaction-diffusion and SDMs, we can obtain more realistic predictions of the dispersal of an invasive forest species and, therefore, improve the quality of the information needed for decision-makers during the design of control strategies. We demonstrate our application using data from recent insect invasions to Southern Chile.

Keywords: dispersal, reaction-diffusion models, species distribution models,

Understanding stand susceptibility to bark beetle attacks after a severe drought event

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Abstract

Several time-series analyses have demonstrated that after extreme summer drought bark beetle disturbances increased. However, studies predicting bark beetle susceptibility depending on local growing condition, topography, and landscape structure are still scarce because of technical constrains in obtaining spatially explicit data over large spatial extents. Using a unique dataset of georeferenced bark beetle infestation data, we tested whether the spatial variation of local growing conditions of forest stands, topography, and landscape structure modified the local occurrence of Ips typographus attacks after a severe hot drought in Central Europe in 2017 and 2018. We used residual autocorrelated logistic models and a multi model selection procedure to find significant parameters. We found that bark beetle attacks depended on the weather-induced aridity intensity, elevation, slope, and available water content. We showed that elevation interacted with growing conditions and topography: at low elevations, spruce forests growing on flat areas and wetter soils were more sensitive to the infestations, whereas forests located on steep slopes and on soils with low water availability were rarely attacked. Our findings are consistent with the growth-differentiation balance hypothesis predicting that trees growing under chronic dry conditions tend to be more resistant against biotic disturbances. Finally, our results suggest that the most productive stands at low elevations are those more exposed to bark beetle infestations in the initial phase of a drought-induced outbreak.

Keywords: *Ips typographus*; aridity; topography; water limitation

Predicting fusiform rust disease through spatial interpolation and machine learning

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Abstract

Fusiform rust is considered the most economically devastating forest disease for loblolly pine stands, which dominate southeastern US. A major part of alleviating disease pressure is to deploy genetically resistant families to sites with a high risk of disease incidence. Hazard maps largely determine this decision, which are solely based on the historical presence of the disease on a given area. However, these maps are imprecise and do not reflect the dynamic relationship between the environment, pathogen, and host. It is well-documented that these factors have a strong influence on disease, so we can use these climactic variables to infer future disease incidence. In this project, we use permanent plots distributed over the south east US which track the number of healthy trees over time. To create predictive maps we spatially interpolated maps of fusiform rust incidence in loblolly pine stands for separate age classes. A set of environmental and soil predictors were included in the model as selected by a Lasso regression (least absolute shrinkage and selection operator) and analyzed through a generalized additive model (GAM). The GAM allowed for relations between variables to be shown, providing a framework to investigate major trends as affected by each independent variable, modeling non-linear relations without the need to specify a parametric model. Spatial autocorrelation was accounted by a separate universal kriging model that was run over the residuals of the GAM model. As a result, we have generated four fusiform rust hazard maps that consider the most important climactic variables for different age classes. This work provides a more accurate and interactive resource for land managers as well as a framework for understanding and predicting disease based on environmental data.

Keywords: Breeding, Disease modeling, Fusiform rust, Machine learning, *Pinus taeda*

Integrating climatic suitability assessments of mountain pine beetle and its fungal symbionts

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Abstract

Climate-driven range changes and interactions among outbreak-prone bark beetle species and their fungal symbionts have the potential to shape the forests in the coming decades. Unfortunately, little attention has been given to biogeographical studies involving both the components (the bark beetle and the associated fungi) in this system for a proper risk assessment. Such an integrated analysis could help identify mutually conducive zones for the study bark beetle-fungal complex and highlight areas at a greater risk of outbreaks under climate change. The mountain pine beetle (MPB), Dendroctonus ponderosae, is an eruptive bark beetle species that continues to cause extensive pine mortality in North America. Unprecedented range expansions of MPB have occurred in recent times as previously untouched forests populated by novel host pines (e.g., Jack pine, *Pinus banksiana*) are being infested. This expansion, in part driven by warming climates, threatens the vast boreal forest of Canada. Three fungal species are commonly associated with MPB: *Grosmannia clavigera*, *Ophiostoma montium*, and *Leptographium longiclavatum*. These fungi, which provide fitness benefits to MPB and facilitate tree colonization, are sensitive to temperature extremes. Therefore, it is likely that warming temperatures due to climate change will differentially impact bark beetle-fungal co-occurrences. In this study, we used species distribution models to project the potential distributions of MPB and the associated fungi in Canada and the United States using biologically-relevant predictors. Georeferenced distribution data of MPB and the fungi were collected and the corresponding pseudo-absence points were generated using different methods for modelling. To minimize uncertainties in projections, we combined the outcomes from multiple models. Results suggest that MPB distributions in Canada and the United States are in agreement with the current climatic suitabilities. We will project the ensemble distribution models under two climate change scenarios (SSP2-4.5 and SSP5-8.5) and over multiple time periods. Potential distributions and range overlaps of MPB and the fungi under climate change will be discussed.

Keywords: Mountain pine beetle; *Grosmannia clavigera*; *Ophiostoma montium*; *Leptographium longiclavatum*; climate change

Posters

Modelling hotspots of forest pest introductions in Europe

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Abstract

Pests are a major driver of tree damage and loss in Europe. The increasing number of novel pests combined with the extensive time required for risk analysis leads to an interest in predicting where these species might be introduced using data of historic introductions. Densely populated areas are known to facilitate pest establishment through high propagule pressure via trade and availability of suitable hosts. For the first time, this study constructed a full statistical model to a dataset of first establishment sites of tree pests in European countries. We examined sampling bias by including citizen science data resembling the sampling process. We mainly predicted high probabilities of first establishment near cities, in highly populated areas, warmer temperatures and areas with higher densities of woody crops. Bias correction strongly reduced the importance of distance to cities and human population density. We thus caution that biases inherent to the collection methods for these data may overemphasize the importance of cities as starting points for pest invasions.

Keywords: BayeSDM, forest pests, invasion ecology, invasive species, Naive Bayes, pest introduction, urban hotspots

Modelling the population dynamics of CRISPR-Cas9 gene drive systems in Sirex noctílio

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Abstract

Sirex noctilio is an invasive pest of pine that has caused significant economic damage in South Africa and many other countries. Current management tools are not always efficient and consequently there is a need for more targeted control measures. An emerging tool for pest management is the use of gene drive systems. In this study we investigate the use of CRISPR-Cas9 gene drive systems in the management of *S. noctilio* in South Africa. As a first step, we developed a model for the population dynamics of *S. noctilio*, using historical data and incorporating the influence of two main biological control agents of the pest. We then modeled the influence of two different CRISPR-Cas9 models on the population dynamics of *S. noctilio*, namely a Toxin-Type CRISPR (TTC) model and Complementary Sex Determination CRISPR (CSDC) model. Each model is also used to simulate different introduction strategies to estimate the effectiveness of the gene drive system. Results suggest that both CRISPR-Cas9 gene drive systems would be effective at controlling the population growth of *S. noctilio*, but that the TTC model develops complete resistance after a few generations. The CSDC model develops no resistance by causing non-viability in individuals that contain a resistant allele. Overall, the models suggest that CRISPR-Cas9 gene drive systems would serve as a viable form of control, but that further research should be done to identify possible target genes and optimize introduction strategies.

Keywords: CRISPR-Cas9; Mathematical modelling; Pest management; *Sirex noctilio*

Session 13 A look to the future of the behavioural and chemical ecology of forest insects

Oral communications

Approaches in understanding forest insect outbreaks by combining behavioral and biochemical studies

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Abstract

Early this year, the IUFRO working party 7.03.16 organized a webinar series highlighting the research of graduate students from forest research institutions all over the world. This plenary talk will briefly introduce the multifaceted work presented in the seminars, mirroring the various aspects of forest insect behavior and chemical ecology. The combination of behavioral studies with tree and insect related chemical analyses delivers strong tools for the investigation of biotic disturbance events such as bark beetle mass outbreaks. Novel findings from field and laboratory studies have pushed forward our understanding of how environmental factors, host tree quality and infestation of Norway spruce forests by the European spruce bark beetle, *Ips typographus*, are interconnected. Embracing the multitrophic relationships between Norway spruce, bark beetles and associated micro-organisms, I will report on the latest outcomes of a rainfall exclusion experiment to examine the effects of drought on spruce defense capability and bark beetle attack success. Still, many open questions remain with regard to tree attractiveness and defense, bark beetle host choice and acceptance from landscape scale to the level of individual trees and bark tissue.

Keywords: forest insects, bark beetles, *Ips typographus*, laboratory and field bioassays, chemical analyses

Volatile-mediated between-plant communication in Scots pine and the effects of elevated ozone

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Abstract

Herbivore-induced plant volatiles (HIPVs) convey information between damaged plants and their neighbors. Ground-level ozone, an important phytotoxic pollutant, is known to negatively affect between-plant communications, but the extent of the effects has not been comprehensively investigated. Coniferous trees release substantial quantities of HIPVs upon bark-beetle attack, representing a strong indicator of herbivore presence. However, between-plant communications mediated by bark-feeding-induced volatiles have not been investigated. Furthermore, the mechanisms structuring between-plant communications are yet to be elucidated with respect to photosynthesis and VOC storage structures.

Here, we used a system comprising Scots pine (*Pinus sylvestris*) and the large pine weevil (*Hylobius abietis* L.) to test whether HIPVs can mediate between-plant communication in conifers and how ozone pollution affects this process.

We show that Scots pine seedlings exposed to HIPVs were more resistant to subsequent weevil feeding and received less damage. Receiver plants had both induced and primed volatile emissions and their resin ducts had an increased epithelial cell mean area and an increased number of cells located in the second epithelial cell layer. Moreover, we observed that HIPV exposure increased stomatal conductance and net photosynthesis rate of receiver plants. Under elevated ozone conditions, the plant responses were altered. However, the final defense outcome was not significantly affected.

These findings demonstrate that HIPVs modulate conifer metabolism through responses spanning photosynthesis and chemical defense. The responses are adjusted under ozone stress, but the defense benefits remain intact.

Keywords: Between-plant communication; ozone; photosynthesis; plant defense; Scots pine

Susceptibility of *Eucalyptus* trees to defoliation by the Eucalyptus snout beetle, *Gonipterus* sp. n. 2, is enhanced by high foliar contents of 1,8-cineole, oxalic acid, and sucrose and low contents of palmitic and shikimic acid

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Abstract

Gonipterus sp. n. 2 (Coleoptera, Curculionidae) is an invasive, commercially important weevil that causes large-scale defoliation of Eucalyptus trees. The weevil specifically feeds on young leaves and new shoots and thus substantially reduces tree growth rates. The weevil displays a very strong preference for certain Eucalyptus genotypes, however, this behaviour and the chemistry underlying it is poorly understood, thereby complicating the selection of resistant trees. To elucidate the feeding preference of *Gonipterus sp. n. 2*, we assessed the relative levels of susceptibility of 60 *Eucalyptus* genotypes from 26 species using a laboratory choice assay. This revealed large intraspecific variation in susceptibility to weevil feeding, which for certain species, exceeded the interspecific variation. A detailed metabolite profile analysis of 17 genotypes from three species revealed strong correlations of 10 metabolites to feeding damage. The behavioral effects of the identified compounds were assessed through an in vitro feeding preference assay using artificial diets as well as under field conditions, using 12 unique genotypes. This revealed three phagostimulants (1,8-cineole, oxalic acid, and sucrose) and two repellent compounds (shikimic acid and palmitic acid) for *Gonipterus sp. n. 2*. These chemical markers can be applied to tree breeding programs for the selection of resistant genotypes to reduce damage caused by *Gonipterus* weevils.

Keywords: artificial diet; feeding behaviour; feeding deterrent; phagostimulant; plant-insect interactions;

Mechanisms involved in the establishment success of non-native pine bark beetles

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Abstract

Bark beetles are a large and diverse group of insects commonly recognized as important forest pests and invaders. Understanding the mechanisms driving their establishment in new areas is important for predicting future invasions and improving management strategies. Our aim was to characterize population drivers of the establishment success of two recently arrived Eurasian pine bark beetle species in Patagonia (Argentina): *Hylurgus ligniperda* and *Orthotomicus laricis*. We performed field experiments to test how fitness of each species is determined by: (1) initial abundance - 1, 3, 5 or 10 couples- (Allee effects), (2) coexistence with other species (facilitation relationship) and (3) the use of ancient (*Pinus sylvestries*, *P. nigra*) vs novel (*P. contorta*, *P. ponderosa*, *P. radiata*) hosts. We found that *H. ligniperda* is able to establish a population starting from one couple only, and that the probability of leaving offspring was similar among all treatments (66-75% of 17 replicates). *O. laricis* was able to establish populations starting only from three or more couples, and left offspring in only 10% of the 18 incubated logs, suggesting it may be experiencing stronger Allee effects than *H. ligniperda*. We found that, despite the fact it is common to find both species together on the same stem, the fitness of each species did not improve when coexisting. On the contrary, *H. ligniperda* tends to leave more offspring when developing alone, while *O. laricis* showed similar performance with or in absence of *H. ligniperda*. We found that for *H. ligniperda* the probability of leaving offspring was similar when colonizing ancient vs. novel hosts, indicating that the previous history with pine trees is irrelevant in determining establishment. Our results show that *H. ligniperda* is more successful in establishing new populations than *O. laricis*, and could be explained by the difference in their life history traits and ecology. These findings contribute to a better understanding of the factors leading to successful bark beetle invasion events.

Keywords: Facilitation; *Hylurgus ligniperda*; host preference; *Orthotomicus laricis*; pine bark beetles

The role of pheromones and temporal mechanisms in the reproductive isolation of *Monochamus* spp. in Ontario

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Abstract

Among insects, reproductive isolation is believed to be an emergent property of the same mechanisms that facilitate mate location (e.g., the use of pheromones). Differences in pheromone blends contribute to species-specific mate recognition, preventing the deleterious costs of cross-attraction/hybridization. Surprisingly, monochamol has been demonstrated to be a sex pheromone/pheromone attractant to three sympatric *Monochamus* spp. in Ontario, namely *M. scutellatus*, *M. mutator* and *M. notatus*. The goal of this study was to investigate the role of long- and short-range pheromones and temporal mechanisms in the reproductive isolation of these three populations of *Monochamus* spp. in Ontario. The presence of long-range sex pheromone compounds from *M. scutellatus* and *M. mutator* was investigated in the laboratory by analyzing volatile extracts with GC-MS. The diel rhythms in long-range sex pheromone release from *M. scutellatus* was investigated in the laboratory by analyzing volatile extracts collected during photo- and scoto-phase periods with GC-MS. Phenological and diel patterns of flight activity were investigated with a field trapping experiment performed over three years. The role of short-range pheromones in mate recognition of *M. scutellatus* and *M. mutator* was investigated using mating and cross-attraction bioassays in the laboratory. Volatile collections of *M. scutellatus* confirmed the identity of the previously reported pheromone monochamol, which was released during both photo- and scoto-phase periods at similar concentrations. This study is the first to detect monochamol in the effluvia of male *M. mutator*. No differences were observed in male aeration extracts of these two species. There is considerable overlap in the distribution of the three species in the field during the months of the year and times of the day. Bioassays demonstrated that males only recognized conspecific females after antennal contact when cuticular profiles were intact or reconstituted on the elytra, or when conspecific female extracts were applied to heterospecific female cadavers. We conclude that cuticular compounds are necessary and sufficient for male mate recognition in these two species, and males can discriminate con- and hetero-specific females based on cuticular compounds.

Keywords: Behaviour, Sex Pheromones, Flight Activity Patterns, Ecology and Evolution, Forestry

Evolution of the pheromone of the small southern pine engraver through asymmetric tracking

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Abstract

Forest insects rely on chemical signals to mediate intraspecific interactions such as mate location and mate choice. The evolution of these signals has primarily been studied in the order Lepidoptera. However, given the impact of bark beetles on forest ecosystems, the evolution of pheromones of these insects should receive more attention. One way to study pheromone evolution is to compare the response of receiving individuals to the distribution of the signal produced by signaling individuals. Through trap captures, this study shows that changes in the enantiomeric ratio of ipsdienol, a pheromone component of *Ips avulsus*, affect trap captures of *I. avulsus* and reveals a broad profile of the receiver preference function. By identifying the enantiomeric ratio of ipsdienol produced by male *I. avulsus* through chiral GC-MS analyses, we found little variation in enantiomeric ratio among emitting individuals. The asymmetric tracking model hypothesizes that if the energy costs and predation risks associated with releasing pheromones are lower than the costs of searching for a signaler, responding individuals should respond indiscriminately to different emitted ratios. The model predicts that the evolution of the emitted signal would not be constrained by responding individuals. In this study the comparison of the receiver preference function and the distribution of signal phenotypes suggests that naturally occurring variation in ipsdienol enantiomeric ratio has no effect on the attraction of conspecifics. These results are consistent with the predictions of the asymmetric tracking model and indicate that the evolution of the pheromone of *I. avulsus* might be under external selection forces.

Keywords: Asymmetric tracking; Bark beetle; Enantiomeric ratio; Ipsdienol

Effect of trap design and canopy cover on pest beetle catch

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Abstract

Semiochemical-baited traps are important management tools for forest Coleoptera in North America. However, many factors such as trap position and/or design can influence their performance and the effects of these factors can vary among taxa. Here, we tested the effect of different intercept trap designs (black, white, and clear panel and multiple-funnel traps) placed in open and closed canopy habitats on the number of beetles captured. Almost 50 000 beetles were caught over seven weeks, including 6 *Monochamus* and 4 Curculionidae species. For most species of Curculionidae and *Monochamus*, traps placed in open canopy captured significantly more beetles. For most species, no effect of the trap design was observed. For three Curculionidae and two *Monochamus*, black panel traps captured significantly more individuals than at least one other trap type. In conclusion, this study provides additional evidence that horizontal gradients intersecting forest edges can influence beetle abundance and the performance of surveillance tools. In addition, we demonstrate that different colours of the same intercept trap design can affect numbers captured, suggesting that colour or contrast can improve trap efficiency for some forest insects.

Keywords: Beetle trapping, Semiochemical, trap colour, open canopy

Posters

Transcriptomics reveal chemosensory gene signatures for ecological niche and behavioural specialisation in *Sirex noctilio*

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Abstract

The evolutionary and ecological success of insects is partly ascribed to their sophisticated and uniquely adapted chemosensory systems. Insect chemosensation genes form some of the largest multigene families and are important in reverse genetics approaches to study the relationship between ecology, evolution and genomics of these organisms. The woodwasp, *Sirex noctilio*, poses a significant threat to pine plantations in various parts of the world. Genomics, transcriptomics, phylogenetics and differential expression analyses were used to investigate the chemosensory gene families of this economically important pest. Here, 129 chemosensation genes, including 45 ORs, 12 GRs, 48 IRs, 1 SNMPS, 14 OBPs and 9 CSPs, were identified and phylogenetically characterised in the genome of *S. noctilio*. Most of these genes exhibited basal phylogenetic clustering within the order Hymenoptera. RNA-sequencing of the olfactory and non-olfactory tissues of adult and pupal *S. noctilio* woodwasps allowed for expression profiling of the chemosensory genes identified. Tissue- and sex-specific expression patterns were observed in these data. Receptors potentially involved in recognition of ecologically relevant chemical stimuli, such as pheromones and host-tree volatiles, were identified. The phylogenetically basal position of this woodwasp within the Hymenoptera allows for a deeper understanding of the evolution of the multigene families associated with chemosensation in this ecologically and economically important order. This study broadens our understanding of the molecular processes involved in perireceptive events of *S. noctilio* chemosensation. Tissue- and sex-specific genes are promising targets for future deorphanisation studies which could aid the identification of novel attractants or repellents for biorational pest management.

Keywords: Chemoreceptors; Chemosensation; Differential expression; Hymenoptera; Woodwasp

Session 15 New and emerging vascular wilt diseases caused by the *Ophiostomatoid* fungi

Oral communications

Prevention of disease outbreaks by early detection: the case of *Ceratocystis platani* in Europe

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Abstract

In Europe, both Oriental plane and London plane trees are seriously threatened by the invasive fungal pathogen *Ceratocystis platani*, the causal agent of the canker stain disease (CSD) of plane trees, a lethal disease able to kill a mature tree in one-two years. The fungus is considered to be indigenous to North America and was unintentionally introduced into Europe during World War II, where it continues to spread. So far, the disease is reported in Italy, France, Switzerland, Greece, Albania and Turkey. The impact of CSD in Europe can be compared with notorious tree diseases such as Dutch elm disease, chestnut blight, and more recently ash dieback, which have all caused devastating losses to natural woody ecosystems and ornamental trees. Once the disease is introduced, if not promptly detected, it is hard to be eradicated, since it is highly infective and easily transported by occasional vectors, although humans, through the use of infected pruning tools and terracing machinery, are the main agents of spread.

To prevent further eastward or northward spread and to study the biology of the pathogen, several early detection tools, both biochemical and molecular based, have been developed and validated. Here we describe the different tools and their possible use to prevent the further spread of such a destructive disease.

Keywords: Real-time PCR, LAMP, volatile organic compounds, biomarker, portable diagnostic tools, pathways of introduction and spread

Ceratocystis wilt on *Acacia* and *Eucalyptus*: emerging evidence for host range expansions

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Abstract

Ceratocystis species are well-known pathogens, mostly of trees. In recent years, the incidence of vascular wilt and canker diseases caused by these fungi has increased substantially. This is to a point where *Ceratocystis* spp. are emerging as amongst the most important of all tree pathogens globally. For example, *C. manginecans* has caused the collapse of the extensive Acacia mangium plantations in Indonesia and Malaysia and is now also emerging as a pathogen of the replacement *Eucalyptus* species. In Indonesia, our population studies have shown that isolates from *A. mangium* and *Eucalyptus* are genetically related. This indicates a direct host range expansion of the pathogen from one important forestry genus to another. In South Africa, we recently reported the related *C. eucalypticola* causing a disease for the first time on a *Eucalyptus* hybrid variety. The origin of the fungus is unknown but it is genetically linked to isolates that have been present in the country for over a decade, including those on tree wounds not linked to disease. The emergence of this disease is apparently due to inadvertently planting a susceptible *Eucalyptus* variety in an environment conducive to infection. The wide geographic distribution of *C. eucalypticola* in South Africa, and its presence in the soil could act as a reservoir for infections of new and more susceptible host plants. The extent of host range and climatic parameters allowing for the spread of these *Ceratocystis* spp. have not been determined, but must be a high research priority in order to minimize the local and intercontinental distribution of these pathogens.

Keywords: Biological invasion, canker disease, Ophistomatoid fungi, population genetics,

Ceratocystis wilt affecting wood properties and tree growth of *Eucalyptus pellita* in Indonesia

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Abstract

Ceratocystis wilt is considered one of the most important diseases on eucalyptus. The most common symptoms are tree die-back, wilt, presence of epicormics shoots and xylem discoloration. Effective control can be obtained by screening eucalyptus clones for resistance. However, the high genetic and physiological variability of the pathogen may compromise the disease control. In Indonesia, the pathogen is wide spread in commercial eucalyptus plantations, especially in Sumatra Island. Thus, the present study aimed to determine the economic impact caused by this pathogen on tree growth and wood properties of susceptible clones of *Eucalyptus pellita* planted in the Riau Province, Sumatra. A total of nine compartment planted with a susceptible clone was monitored from 2018 to 2020 to assess the disease progress. In addition, wood and pulp properties of diseased trees were compared with healthy trees. When the disease monitoring started in 2018, the average of disease incidence in the nine compartments studied was 19.7%. Two years later, the disease progressed to 63.2% in average. The impact on wood volume varied according to the compartment studied, but there was a correlation (R2 = 65.0) between disease incidence and the amount of wood estimated, showing that the fungus is affecting the plantation performance. A decrease of 61% in wood volume was observed on plantations with a disease incidence of 74%. Also, all parameters used to assess the impact of Ceratocystis wilt in wood and pulp properties showed a negative effect in infected trees: density (-1,1%); total extractives (-181.1%); screened pulp yield (-10.9%); brightness (-7.1%); and specific wood consumption (+14.8%). Therefore, the information provided in this studied showed that Ceratocystis wilt is able to cause a severe impact on commercial plantations of E. pellita in Indonesia and that selection of resistant materials is crucial for the establishment of healthy forests.

Keywords: genetic resistance; wilt disease; wood properties

Posters

Mechanism of cycloheximide resistance in the tree-infesting and bark-beetle associated *Ophiostomatalean* fungi resolved

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Abstract

One of the more interesting characteristics of fungi in the Opiostomatales, which for example includes important tree pathogen species in the genera *Ophiostoma*, *Leptographium* and *Sporothrix* is their remarkable ability to tolerate high concentrations of the antibiotic cycloheximde in culture. Most of these fungi are found in the galleries of bark beetles that infest trees and where the environment is virtually devoid of other fungal groups. There is evidence that the ability to tolerate cycloheximide in the tree bark beetle niche is related to the presence of actinomycetes that also occur in this environment. In this regard, the relationship between bark-beetle associated Ophiotomatales, and cycloheximide tolerance represents an ancient evolutionary relationship. It is consequently interesting that the biochemical origin of this relationship has never been resolved. In this study the newly available genome data from species in the Ophiostomatales were utilised to determine the mechanism of resistance to Cycloheximide. Early research had shown that in yeasts such as *Kluyveromyces* similar resistance was the result of a transition mutation (P56Q) in one of the 60S ribosomal protein genes now known as eL42. We examined all the available genomes for the Ophiostomatales and discovered that a same transition mutation in the gene coding for ribosomal protein eL42, likely confers resistance to this antibiotic. This change across all genera in the Ophiostomatales studied suggests that the mutation arose early in the evolution of these fungi. These results also highlight the usefulness of using this antibiotic as a selective marker in the isolation of species in the Ophiostomatales as this is likely present across the order of a group of important tree-infecting fungi.

Keywords: Cycloheximide resistance, *Ophiostomatales*

Session 16 Pitch canker of pines: Emerging frontiers

Oral communications

Smell the disease – utilizing volatiles for the early, rapid and nondestructive detection of *Fusarium circinatum* in pine

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Abstract

Global forests are increasingly threatened by alien invasive species, and there is a great need to modernize the tools used for detection in order to safeguard our forests from such threats. *Fusarium circinatum*, causal agent of the lethal disease pine pitch canker, is on-the-horizon for many countries. This project investigated whether volatilomics can be used as a method for detecting the presence of *F. circinatum* in infected trees. Volatile organic compounds (VOCs). VOCs emitted during the in vitro fungal growth and in vivo plant infection were analyzed to distinguish unique chemical signatures of *F. circinatum*. Four *Fusarium* species (*F. circinatum*, *F. bulbicola*, *F. oxysporum* spp. *pini* and *F. graminearum*) were grown in vitro and their VOCs were sampled using solid phase microextraction fibers and analyzed using gas chromatography coupled with mass spectrometry (GC-MS). The four *Fusarium* species were readily distinguishable based on in vitro VOC profiles. To test if *F. circinatum* could be detected in trees, 1-year-old pine seedlings of three different *Pinus* species (*P. radiata*, *P. sylvestris*, *P. pinea*) were artificially inoculated with *F. circinatum* on the stem. Comparison of VOC profiles could differentiate infected *P. sylvestris* and *P. radiata* trees from mock-inoculated controls, and in *P. radiata*, this could be seen even before the onset of visual symptoms. *P. radiata* and *P. sylvestris* showed characteristic disease symptoms within 3 weeks following inoculation by *F. circinatum*, while *P. pinea* remained asymptomatic during the entire observation period (4 months) with a largely unchanging VOC profile. None of the VOCs produced by *F. circinatum* when grown on synthetic media were detected from the infected or control trees. The findings of this study suggest that VOC analysis has potential as a novel, affordable and quick identification tool for phytosanitary agencies for detecting important tree pathogens.

Keywords: *Fusarium circinatum*; *Pinus* spp.; VOCs; biosecurity; forest pathogens

Testing the Galician *Pinus pinaster* breeding population for susceptibility to Pitch canker disease

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Abstract

Maritime pine (*Pinus pinaster* Aiton) is a very important pine species in Spain due to both, its ecological and economic value. For this reason, there is a genetic breeding program in Northwestern Spain (Galicia). Genotypes of this breeding program are selected mainly by growth and stem form; lately, other important traits such as disease resistance are considered in the breeding program. Pitch canker disease, caused by the fungus *Fusarium circinatum*, is considered a quarantine pest in Europe. It can cause shoot dieback to pines when adults, but can also produce death on seedlings. In 2004 it was detected for the first time in Spain. Present work evaluates genetic variation in susceptibility to pitch canker disease among the half-sib families from the Galician *P. pinaster* breeding population. We performed artificial inoculations on these families to evaluate mortality and disease development, and their relationships to morphological traits assessed prior to inoculation. The results showed significant differences among families, indicating that susceptibility to *F. circinatum* is a heritable trait. Starting and ending of the disease symptoms occurred at 17 and 47 days after inoculation, respectively; the duration of the disease lasting for 30 days. Comparing to the P. radiata control seedlot used, the disease lasts almost the double for P. pinaster (30 days vs. 17 days), starting earlier and ending later. At the end of the assay, 95 DAI, the mean survival for the Galician breeding population was 29.5%, ranging from 0 to 81.3% depending on the family. In fact, there were sixteen families with survival levels equal or over 50% at the end of the assay. As a conclusion, it can be highlighted that a new genetic breeding line, "pitch canker resistance", could be developed within the Galician maritime pine breeding program, if it was considered necessary.

Keywords: Maritime pine, genetic variability, heritability, breeding program, *Fusarium circinatum*

Posters

Dual RNA-seq comparison of host responses to *Fusarium circinatum* challenge in tropical pines

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Abstract

One of the most important fungal pathogens to pine forestry worldwide is *Fusarium circinatum*. Variation in host resistance is present within and between species, so development of resistant varieties or hybrids has emerged as a promising control strategy.

The interaction of *F. circinatum* with two resistant (*P. oocarpa, P. tecunumanii*) and two susceptible (*P. greggii*, *P. patula*) *Pinus* spp. was investigated. Sequencing data originated from shoot tissue of ca. 6-month-old seedlings (mock & inoculated) at 3- and 7-days post inoculation (dpi). Induced host responses were investigated by identifying differentially expressed genes in inoculated relative to mock samples at each timepoint. Subsequently, a weighted gene co-expression network was constructed to compare changes in expression over time, and to investigate constitutive differences in expression between host species. In planta F. circinatum responses were investigated by comparing pathogen expression in inoculated samples between timepoints for each host, as well as between hosts at each timepoint.

Induced responses were similar between resistant hosts, indicating ethylene signalling at 3-dpi with further induction of jasmonate and salicylate (SA) signalling pathways at 7-dpi. In contrast responses in susceptible hosts were different. *P. greggii* showed suppression of host defences with attenuation of responses over time, while expression in *P. patula* showed delayed and compromised defence. Constitutively, susceptible hosts showed higher expression of SA metabolism and cold stress related genes, while resistant hosts had higher expression of genes involved in sulfur metabolism. Eight putative *F. circinatum* core virulence genes were identified from those expressed in all hosts at both timepoints. Likely gene functions include host attack as well as antimicrobial and host defence. Expression of these genes could aid in effective host colonisation and increased disease severity.

Keywords: Dual RNA-Seq, host-pathogen interaction, tropical pines, virulence factors

Using GWAS to identify genes associated with virulence in *F. circinatum*

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Abstract

Fusarium circinatum, commonly referred to as the pitch canker fungus is a globally important fungal pathogen causing serious disease globally in a wide range of *Pinus* species. Despite its economic importance, few studies have sought to determine the molecular basis of virulence and pathogenicity to its plant hosts. In this study, we aimed to identify genes and/or genomic regions associated with the pathogenicity and virulence of *F. circinatum* on *Pinus patula* by making use of a genome-wide association study (GWAS). For this purpose, we used a set of 80 *F. circinatum* isolates that are representative of the known diversity of the fungus in South Africa (i.e., based on mating-type, genotype, geographic origin, and *Pinus* host). The Ion TorrentTM sequencing technology was used to sequence the genomes of these isolates, while inoculations studies were used to determine their virulence on *P. patula* seedlings. To identify genes and/or genomic regions that are associated with virulence, a correlation between genotypic and phenotypic data was determined using GWAS. Various SNPs were significantly ($P < 0.05$) associated with this phenotype. Some of these SNPs were also located near genes that are known to be involved in fungal virulence/pathogenicity. These included genes encoding a putative pathogenicity factor and thioredoxin peroxidase which were located downstream of a gene encoding CYB2-lactate dehydrogenase cytochrome b2 that harbored a significant SNP. The association of these sets of genes/genomic regions identified in this study would therefore contribute to the existing knowledge of virulence/pathogenicity of *F. circinatum*. Future research will seek to functionally characterize these genes to ultimately improve our understanding of the molecular processes underlying pathogenicity in this important fungus.

Keywords: Pitch Canker, pathogenicity genes

Terpene profiling in the defence response of moderately resistant *Pinus pinaster* to infection with *Fusarium circinatum*

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Abstract

Fusarium circinatum is a fungal pathogen causing Pine Pitch Canker (PPC). In Europe, it is an invasive pathogen established in northern Spain and Portugal where the disease affects forest stands of *Pinus radiata* and *P. pinaster*. Of these, *P. radiata* is an exotic species used extensively in the forestry sector, and it is considered one of the most susceptible Pine species to PPC disease; *P. pinaster* is a native forest species to the Mediterranean basin with economic and environmental importance which has shown a moderate disease resistance. Besides, *P. pinaster* is the Iberian species from which resin is yielded. Defence mechanisms employed by conifers is the production of terpenoid oleoresin, which acts as a physical and chemical barrier against insect and pathogen attacks. The aim is to investigate the regulatory mechanism of resin synthesis to identify involved genes. As a first step, we study differences in terpene profile between *P. pinaster* and *P. radiata* resin that can explain differences in PPC disease susceptibility. For this, a controlled experiment in a P2 greenhouse was carried out inoculating *F. circinatum* on seedlings of both species. Terpene profile (170 metabolites) was analyzed by GC-MS after 11 and 18 dpi. Principal Component Analysis on metabolite abundance indicates a major effect of species (PC1 component explaining 46.8% of variance). Using PLS-DA for each species, VIP score was used to identify the most relevant metabolites in the groups of inoculated vs mock-inoculated plants. A group of 10 metabolites (majorly resin acids) was less abundant in mock-inoculated plants of *P. pinaster* at 11 dpi, while the other 15 (within monoterpene and diterpene groups) were less abundant at 18 dpi. In *P. radiata* 16 most important metabolites (DT and ST) were less abundant in inoculated than in mock-inoculated plants at 11 dpi, and vice versa at 18 dpi.

Keywords: PPC disease; Disease resistance; Oleoresin

Session 17 Precision pest management in plantation forestry

Oral communications

Precision Pest Management in plantation forests

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Abstract

New and emerging technologies allow for greater precision in forest pest management, from high throughput sequence, chemical and digital sensor data collection, to the integration of data across fields. These technologies allow for the development of much more precise tools for pest management than has ever before been possible; an approach that we define as Precision Pest Management (PPM). These tools also allow for more rapid development of IPM tools, as well as early recognition and response to changes in pest population densities and distributions. We argue that there is an urgent need to develop practical PPM approaches, not only to improve current control measures, but especially to deal with the scale and complexity of pest management in the future.

Keywords: Precision Pest Management, Invasive Pests

Disease and insect pest challenges to plantation forestry in Colombia

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Abstract

Approximately 600.000 ha of plantations have been established in Colombia. These include various native tree species as well as non-native *Pinus* and *Eucalyptus*. Over time, plantations have been increasingly threatened by insect pests and pathogens, including those native to the country and others that have been, or will most likely be introduced from elsewhere in the world. Emerging diseases caused by the shoot and needle pathogens *Diplodia sapinea*, *Dothistroma septosporum*, *Lecanosticta pharomachri*, *Calonectria* spp. and canker caused by *Fusarium circinatum* represent threats to *Pinus* species. In *Eucalyptus* plantations, damage due to canker and wilt pathogens *Chrysoporthe cubensis* and *Ceratocystis neglecta* as well as rust caused by *Austropuccinia psidii* has increased substantially in recent years. In addition, the appearance of insect pests such as *Pineus boerneri* on *Pinus* spp. and *Glycaspis brimblecombei*, *Monalonion velezangeli* and *Gonipterus platensis* on *Eucalyptus* are having a negative impact. These diseases and pests have become a matter of national concern because they threaten the economic viability and long-term sustainability of the forestry industry as well as environmental and social stability. Consequently, private forestry companies and universities have re-structured the forestry sector to include strategies that will help prevent new outbreaks and to implement control measures for those that do occur. This is mainly focused on breeding and selection of tolerant planting stock, and Integrated Pest Management. Prospects for future management include robust surveillance measures and the implementation of policies to reduce the introduction of pathogens and pests that threaten plantation forestry in Colombia.

Keywords: emerging diseases, surveillance, planting stock, pest

Sentinel trials in commercial plantations, opportunities and challenges

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Abstract

Early detection of new pest and pathogen introductions is important to pest and disease management. Eradication of new introductions depends on early detection but typically fails due to a lack of effective surveillance and monitoring systems. Various approaches to the use of sentinel plantings have received attention as possible early detection system for pest and disease threats. In commercial forestry there is interest in the use of such plantings to detect novel pests and diseases and investigate the susceptibility of different tree genotypes to insects and pathogens. A series of pine and eucalypt sentinel plantings were established in two plantation growing regions in South Africa. One eucalypt trial included different varieties with the goal of detecting novel pests and diseases. A second eucalypt trial was established with a mix of pure species to determine their susceptibility to known diseases present in South Africa that are not yet known in other eucalypt growing regions. The pine trials included a mix of species and hybrids with the goal of detecting novel pests and diseases. These plantings have resulted in valuable information, but their monitoring, data collection and processing, as well as maintenance have been challenging. Due to the fast growth of trees, accurate surveillance of canopy pests and pathogens are increasingly challenging and time consuming as trees age. The recognition of unknown/novel pest and pathogen signs and symptoms is only possible by highly skilled and experienced staff. Processing of samples collected requires the skills of experienced taxonomists and access to molecular diagnostics and skills associated with phylogenetic analyses and interpretation of data. Experience to date, shows that although valuable and informative, the more traditional ways of implementing and managing sentinel plantings in plantation forestry is not feasible for many companies/countries, and alternative strategies need to be implemented.

Keywords: Early detection; Monitoring; Pest surveillance

Variation in Performance of Intercept Trap Designs: Prospects for Improved Performance

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Abstract

Intercept traps are used extensively to survey forest Coleoptera. A recent meta-analysis reported a significant amount of heterogeneity in the effect of trap type on flying forest Coleoptera. Much of this heterogeneity can be attributed to species-specific differences in responses to predictor variables. We tested the ability of morphological traits to explain why the effectiveness of flight-intercept trap design varies among beetle species by including nine morphological traits as moderators in meta-analysis models. Traits were selected based on hypotheses relating to beetle movement, manoeuvrability, and sensory perception, while closely correlated traits were omitted. We compared the performance of morphological traits as moderators, versus guild, taxonomic family, and null models. Morphological traits improved model fit, reduced within-study variance, and explained more variation for the effect of trap type on beetle capture rates, compared with null, guild, and taxonomic family models. Trap type effects could be due to differences in i) trap active space; ii) the proportion of beetles that initiate upwind flight and contact the trap; iii) the proportion of beetles that contact the trap surface and are captured; and iv) retention of captured individuals. Therefore, we observed the approach of large woodborers (*Monochamus* spp.) to pheromone-baited traps in the field. Most beetles oriented upwind to traps and we saw no differences in the number of beetles approaching to within 5 m downwind of panel and multiple-funnel traps or in the proportion of beetles orienting from 5 to 1 m downwind of traps. A significantly higher proportion of those beetles that oriented to 1 m downwind were captured by panel traps than by multiple-funnel traps. These studies suggest that morphological traits can be a valuable tool for understanding species-specific differences in trap type effects and that these differences are due to mechanisms active in the near field of traps.

Keywords: Survey and Detection; Monitoring; Integrated Pest Management; Cerambycidae

Strip canker disease caused by *Fusarium* spp. – a resurrected threat to *Populus* x *euramericana* plantations

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Abstract

Populus x *euramericana* is the most widely grown and economically important woody crop cultivated in short rotation coppices in Europe. Since 2019, tree and branch cankers have been observed in plantations of *P.* x *euramericana* in the lowlands of Serbia, South Eastern Europe. An initial disease symptom is characterized by longitudinal strips in the bark that resemble "cat scratches". Soon after canker edges are usually raised and appear swollen with a sunken center. Cankers often enlarge and girdle the stem causing tree death. Samples of cankered tissues were collected, surface sterilized, and plated on AMEA. Fusarium-like fungal cultures were consistently recovered from the samples and the aim of this study was to identify these fungi and test their pathogenicity. The isolates were first screened using ITS rRNA and representatives of each species complexes were further identified using a combination of TEF 1-alpha and RPB2 genes. The isolates were identified as *Neocosmospora solani*, *Fusarium sambucinum* and *Fusarium oxysporum* species complexes. Rooted cuttings of the two *P.* x *euramericana* clones, i.e. I-214 and *Pannonia* were wound inoculated under controlled conditions to test pathogenicity of the fungi. Koch's postulates were fulfilled, confirming pathogenicity of the three species complexes on both clones. This is the first report of N. solani, *F. sambucinum* and *F. oxysporum* species complexes as pathogens of *P.* x *euramericana* in Serbia, and the first report of *F. oxysporum* and *F. sambucinum* species complexes as pathogens of *P.* x *euramericana* in Europe, and the world. Strip canker disease is a serious threat to poplar plantations and results of this study represent a starting point in developing and implementing guidelines for the disease management.

Keywords: *Populus* x *euramericana*, *Fusarium*, poplar tree diseases, canker diseases

Trachymela sloanei (Coleoptera: Chrysomelidae), a new pest affecting *Eucalyptus* in Portugal

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Abstract

The tortoise beetle *Trachymela sloanei* (Coleoptera: Chrysomelidae) is an Australian insect that has become invasive in New Zealand, USA (California), China, Spain, and Portugal. Both adults and larvae of *T. sloanei* feed on the leaves of several *Eucalyptus* species, causing defoliation and reduction in wood productivity. Despite its widespread distribution, few studies targeting this insect have been performed, possibly because its introduction in important areas for eucalypt wood production is recent. Therefore, little is known on its biology, ecology, and control. In 2019, T. sloanei was first recorded in Portugal, a country where eucalypts are economically important. Since its detection, we have been monitoring its spread annually throughout the country. *Trachymela sloanei* has dispersed rapidly, and in only two years it occupied the southern half of the country. Nevertheless, the first serious attacks to commercial plantations were recorded only at the end of 2021. We have studied its basic biology and have performed preference tests of adults and larvae towards fifteen *Eucalyptus* species. Here, these results are presented and discussed. Also, we discuss the management strategy that is being developed against this pest.

Keywords: *Eucalyptus globulus*; Tree susceptibility; Host preference; Defoliation; Pest management

Posters

Black crust on rubber tree: development of sexual and asexual spores of the causal agent under different temperatures

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Abstract

The black crust, caused by *Rosenscheldiella heveae*, has become a concern in the rubber tree culture, as it is rapidly advancing in rubber tree cultivation areas in Brazil, causing an early fall of rubber tree leaves, causing it to decrease. its latex production. This work aimed to evaluate the development of R. heveae, in its sexuais and asexual form (*Cladosporum* sp.) in rubber tree leaflets, under the influence of different temperatures. The experiments performed with sexual and asexual spores were set up separately. In both experiments, rubber tree leaflets were inoculated with a suspension of 107 spores.ml-1, being kept in a humid chamber (about 90% relative humidity) at 10, 15, 20, 25, 30, 35 and 40± 1ºC in the dark, with a leaf wetness period of 24 hours. Circular samples, 5 mm in diameter, were obtained from the inoculated areas at predetermined time intervals (4, 6, 12, 24, 48 and 72 hours) and fixed in a Karnovsky solution and then evaluated under a microscope. scan, with five repetitions for each treatment. The evaluation of the fungus development on the leaf surface was qualitative, observing and comparing the development of the pathogen in the different treatments. In the images obtained in a scanning electron microscope, it was observed that the asexual spores, Cladosporum sp., have a rapid development, because 4 hours after the fungus inoculation it is possible to verify the germination and penetration of the conidia at temperatures from 10 to 20 ºC. It was verified in the asexual phase, at temperatures from 10 to 25 ºC, the formation of phialides, from six hours after inoculation, indicating that the fungus is already in the reproductive period. In the sexual phase, in the scanning microscopy images, from four hours after inoculation, it was possible to verify the formation of small protuberances, which must be the beginning of stroma formation, at temperatures between 10 and 20 ºC. These black dots, at these temperatures, evolve over the periods evaluated, to protruding circular black spots, similar to the symptoms of black crust, with apparent formation of spores on them. These initial observations improve the understanding of the fungus cycle, since there is little information about this disease in rubber trees.

Keywords: *Hevea brasiliensis*; environment; *Rosenscheldiella heveae; Cladosporum*

Endosymbionts prospection in *Leptocybe invasa* (Hymenoptera: Eulophidae) in Brazil

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Abstract

The eucalyptus gall wasp *Leptocybe invasa* (Hymenoptera: Eulophidae), one of the main pests in Brazil, causes damage to nurseries and field plantations of Eucalyptus spp. Endosymbiont bacteria present in insects can be related to different types of interactions, such as food processing, reproduction and even the defense of insects against infections by pathogens, and can influence their biology, behavior and interaction with other organisms and the environment. The aim of this study was to prospect for endosymbionts present in a population of larvae and two populations of adults of *L. invasa* in Brazil. Larvae populations and a population of adults of *L. invasa* were obtained from rearing in the greenhouse belonging to the Forest Pest Biological Control Laboratory, from FCA/UNESP – Botucatu Campus – São Paulo State, and a population of adults of *L. invasa* were obtained from *Eucalyptus* plantations in the field located in the city of Casa Branca, São Paulo State. The genera of endosymbionts analyzed were: *Rickettisia*, *Wolbachia*, *Hamiltonella*, *Arsenophonus*, *Serratia*, *Sodalis*, *Spiroplasma*, *Regiella*, *Carsonella*, and *Cardinium*. For this, molecular analyzes were performed, the DNA of the populations was extracted by Chelex at 10% and PCR amplification was performed. In all populations, the presence of *Rickettisia* and *Serratia* was detected. This study shows us the possible diversity of endosymbiont bacteria present in different populations of *L. invasa* in Brazil. The effects of interactions between endosymbionts and *L. invasa* need to be investigated.

Keywords: Bacteria; *Eucalyptus* spp.; Gall wasp

Session 18 Current status of viruses and phytoplasma of forest and urban trees

Oral communications

Towards the forest virome: next-generation-sequencing expands our knowledge on virosphere in temperate forest ecosystems

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Abstract

Thanks to the development of Next-Generation-Sequencing (NGS) technologies, a vast amount of genetic information on the virosphere of temperate forests has been gained the last seven years. To estimate the qualitative/quantitative impact of NGS in forest virology, we have summarized viruses affecting major tree/shrub species and their fungal associates, including fungal plant pathogens, mutualists and saprotrophs. Reviewed data about viral presence in holobionts, allowed us to address the role of the virome in the holobionts. Genetic variation is a crucial aspect in hologenome, significantly reinforced by horizontal gene transfer among all interacting actors. Through virus-virus interplays synergistic or antagonistic relations may evolve, which may drastically affect the health of the holobiont. Novel insights of these interplays may allow practical applications for forest plant protection based on endophytes and mycovirus biocontrol agents. The current analysis is conceived in light of the prospect that novel viruses may initiate an emergent infectious disease and that measures for avoidance of future outbreaks in forests should be considered.

Keywords: Next-Generation-Sequencing, virome, holobiont, forest pathogens

Emaraviruses in forest tree species – new viruses are associated with long-known diseases

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Abstract

Emaraviruses (Order Bunyavirales; Family Fimoviridae) are an emerging group of worldwide-distributed, plant pathogenic viruses with an economic impact. Members of the genus have been discovered in numerous host plants, fruit and field crops as well as ornamental and wild plant species. These viruses are naturally transmitted by gall mites and in some cases by mechanical means. Emaraviruses contain a segmented, single-stranded, negative-sense RNA genome comprising four core components and a highly variable number of additional segments encoding non-structural proteins. Considering the core genome, emaraviruses are grouped into several main clades.

By High-throughput sequencing (HTS), we recently identified novel emaraviruses affecting important deciduous tree species at forest, landscape and urban stands in Northern and Central Europe. These viruses can now be assigned to long-known diseases observed in oak, sycamore, ash and aspen. We applied RT-PCR with genus- and species-specific primer pairs to consistently detect the different viral RNAs in collected leaf material. This revealed a correlation between virus detection and observed symptoms on diseased trees including chlorotic ringspots, mottle, mosaic and shoestring. Full-length PCR strategy with the generic primer pairs contributes to the completion and identification of emaraviral genome segments which help to shed some light into the genome organization of this variable virus ensemble. In-depth analysis and homology researches indicate links between the protein composition and the phylogenetic placement of emaraviruses into certain clades. Insights into the group of emaraviruses with the focus on the genomic composition of these multipartite RNA viruses are presented.

Since long-lasting viral infections significantly contribute to the health status of plants, which is especially true for permanently stressed urban trees, further characterization of emaraviruses is needed as a prerequisite for an effective disease management.

Keywords: Fimoviridae, deciduous trees, symptoms, genome organization, phylogenetic relations

Emaraviruses in forest trees – relationships between viruses, host species and putative vectors

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Abstract

Emaraviruses are a large group of plant viruses which have been recently identified in a growing number of woody host species worldwide. Apart from fruit trees and ornamental species where they are responsible for quality and yield losses in production, emaraviruses cause diseases in several important forest tree species. Leaf symptoms such as mosaic, mottle, chlorotic ringspots, line pattern and leaf deformations for instance in *Acer pseudoplatanus*, *Amelanchier* sp., *Fraxinus* spp., *Quercus robur*, *Populus tremula* and *Sorbus* spp. are characteristic for the different diseases. Virus-affected trees also often display decline. Most of the diseases in the different forest tree species have been known for a long time. But only by the recent availability of novel identification and diagnostic tools, the respective diseases could be linked with newly identified virus species, i.e maple mottle-associated virus (MaMaV, Emaravirus aceris) in sycamore maple, common oak-ringspot-associated virus (CORaV, E. quercus) in penduculate oak, aspen mosaic-associated virus (AsMaV, E. populi) in Eurasian aspen as well as the yet unassigned novel ash shoestring-associated virus (ASaV) which affects common and manna ash in Europe.

Currently, we are investigating the relationships between the identified novel viruses, their host range, geographical distribution and modes of transmission, which are key features to estimate the impact of the respective viruses on their woody hosts. Special focus is given on the suspected natural mode of transmission of emaraviruses by different eriophyoid gall mites. Results are presented about the morphological and molecular identification of gall mite species associated with diseased tree species, which may be involved in the effective dissemination of these viruses in the population of their woody hosts.

Keywords: eriophyid gall mites; forest tree species; plant virus; transmission

Viruses in street trees – a study on the occurrence in the Hamburg metropolitan region

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Abstract

In Hamburg, nearly 225,000 street trees and around 600,000 park trees contribute to the perception of the city as a green metropolis on the waterfront. These city trees are not only exposed to more difficult living conditions due to compacted soils, sealed surfaces or mechanical injury to above- and below-ground plant parts, pathogens and pests; increasing summer heat and dry periods as well as storms also pose new challenges for urban trees and urban planners. While monitoring of general canopy vigor of urban street trees is common, studies focusing on plant viruses in trees in urban areas are rare.

In the focus of this preliminary study is a comparison of the occurrence and incidence of plant viruses on native and non-native urban tree species. The survey covered the tree genera Acer sp., *Amelanchier* sp., *Betula* sp., *Fraxinus* sp., *Quercus* sp., *Sorbus* sp., and *Ulmus* sp. Both traditionally used species and those discussed as so-called climate trees (e.g. *F. ornus*, *Q. frainetto*, *Q. palustris*, and *A. arborea*) were considered. The latter may be better able to cope with the extreme conditions of inner-city sites exacerbated by climate change.

From 2018 to 2021, extensive visual surveys were conducted at more than 40 sites in Hamburg, leaf samples were collected from trees, and laboratory analyses were performed by bioassay, enzyme-linked immunosorbent assay (ELISA), and/or reverse transcriptase-polymerase chain reaction (RT-PCR).

Plant viruses could be detected in about 6% of the street trees. Thus, viruses occur not only in native tree species, but also in introduced species of the same genus. The viruses detected belong to the genus Emaravirus, Idaeovirus, Badnavirus and Carlavirus. However, cherry leaf roll virus, apple mosaic virus and arabis mosaic virus could not be detected. Against the background of the virus infections shown, we are currently developing recommendations for sustainable management of urban trees in the Hamburg metropolitan region. This includes the investigation and assessment of the potential of beneficial bio agents to strengthen street trees against plant diseases.

Keywords: climate trees, city trees, Emaravirus, distribution, incidence

Viruses in urban trees- studies on distribution of nutrients in leaves of flowering ash (*Fraxinus ornus*)

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Abstract

In contrast to the native common ash (*Fraxinus excelsior*), the flowering ash (*F. ornus*) is considered resistant or tolerant to ash dieback caused by the fungal pathogen *Hymenoscyphus fraxineus*. The flowering ash, originated in Southern Europe, belongs to the so-called "climate tree species". These are non-native robust trees which are considered to be more tolerant to hot and dry conditions expected due to climate changes in Germany.

The suitability of climate change trees, which have already been planted in many German cities, must be evaluated in regard to their susceptibility to diseases in the urban environment. For this purpose, we are conducting studies within the framework of various research projects with a particular focus on virus diseases.

A three-year survey (2018-2020) on the occurrence of virus-suspected symptoms on urban trees in the Hamburg metropolitan region revealed that 12-20% of flowering ash trees (F. ornus) showed ash shoestring-associated virus (ASaV)-related symptoms. ASaV-infected ash trees show chloroses as chlorotic line pattern, in some cases additional leaf deformations up to shoestrings were found.

The virus symptoms suggest disturbances in the physiological processes of the trees. Therefore, ASaV-infected flowering ash trees were chosen as a model host-pathogen system in this study to investigate the overall nutrient composition (ionomics) and their respective distribution of leaves by different X-ray fluorescence spectroscopy-based methods. The knowledge of the disturbances of the plant's nutrient balance caused directly or indirectly by a pathogen could be used for indirect pathogen detection by mobile measuring devices; further, it could also provide the basis for developing an appropriate plant nutrition strategy to increase tree vitality to counteract the negative influences of virus infection in urban green.

The discrepancy of the nutrient distribution in the leaves of ASaV-infected flowering ash trees compared to the control are presented and discussed.

Keywords: Ash shoestring associated virus (ASaV), climate tree species, *Fraxinus ornus*, Ionomics

Abiotic factors influencing pollen and allergen production in birch trees

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Abstract

Allergy is the most common chronic disease in Europe and poses a major risk to human health. This problem may even become worse: Climate change related effects have already been observed in the timing and duration of the pollen season, airborne pollen concentration and pollen production, pollen allergenicity, and plant and pollen distribution. Pollen production and allergenicity might be influenced not only by temperature but also by other abiotic and biotic factors. In this stuy, we focus on Betula (birch) pollen and gather, distribute and integrate analyses of important environmental factors influencing pollen production and allergenicity in order to study the importance of single factors and the correlation to viral infection by CLRV (Cherry leaf roll virus) in the trees. We investigate abiotic factors, e.g., air temperature, relative humidity, air pollutants (O3; NOx), along different temperature gradients (longitudinal/latitudinal, altitudinal gradients). Data from birches growing under different climate conditions, geographic origins in Europe and genetic background are opposed to data from birch pollen allergic volunteers and infection by CLRV. Cloned individuals in International Phenological Gardens or in a seed plantation are incooperated to exclude genetic variations in natural environments. The dataset is complemented by analyses of plant traits such as tree height and stem circumference, leaf area index, specific leaf area and chlororphyll content of leaves. The results of this study support the hypothesis of an interaction between climate, viral infection and pollen production and allergenicity.

Keywords: birch; clones; CLRV; pollen characteristics.

Detection of the influence of abiotic and biotic stressors on common ash using multisenorial and multitemporal data

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Abstract

The focus of our research is to detect and delineate the health status of ash trees associated to viral and fungal diseases with multisensory and multitemporal data at an early stage of infection. In previous studies, we have already documented the degree of damage caused by ash dieback at various study sites in Germany. However, the influence of viral infections on the health status of ash trees has not been examined yet. Therefore, we conduct a continuous and high spatial resolution monitoring of ash trees. The analysis of multisensory pictures (via permanently installed camera systems, UAV flights) allows a comprehensive assessment of vitality from single leaf sections up to whole stands. Color-based and thermal indices are correlated to field measurements (chlorophyll, chlorophyll-fluorescence, leaf morphology, phenology and soil analytics) and used to develop predictive models and to detect dependences of terrestrial and remote sensing data. Algorithms are applied to identify and delineate impacts as well as to document the magnitude of damage. The survey of camera-based features during different phases of infections with fungi or viruses allows concluding on the cause of damage at early stages (with weak or low differentiated symptoms) in the retrospective and can be used as an early warning system in monitoring. Finally, comprehensive knowledge will be gained for single stress factors and the interactions among them, which can be used for formulating quality criteria for seed harvesting and recommendations for action for forestry practice.

Keywords: Fixed cameras; Multispectral; Thermal; UAV

Molecular monitoring of *Hymenoscyphus fraxineus*

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Abstract

Our research in the field of ash dieback focuses on the development of biological control systems to regulate the causal agent of ash dieback, *Hymenoscyphus fraxineus*, its host interaction and the diagnostics of the causative agent. Investigations of the ash microbiome enabled the identification of the first antagonists of *H. fraxineus*. Differences between the communities of symptomatic and asymptomatic ash trees became apparent. The pathogen quantity on and in *Fraxinus excelsior* as well as the co-occurrence with other pathogens such as the so far insufficiently characterised viruses associated to ash remains unclear. Molecular monitoring of the fungal pathogen in ash leaves, leaf litter and rhizosphere will provide information on pathogen dynamics in the stand and on individual trees. Quantitative and qualitative detection of fungal DNA and RNA markers by real-time PCR allows characterisation of the pathogen pressure in the respective samples. Furthermore, the detection of transcripts enables differentiation between non-active old and acutely progressing infections, the growth of the pathogen in the leaves of the last summer and the accumulation in the soil. The aim of our project is therefore to obtain a differentiated picture of pathogen pressure and to analyse this information together with data on aerobiological spore concentration, viral infection, genetic background, environmental factors and disease development.

Keywords: ash dieback, qPCR, rhizosphere, leaf litter

Posters

Discovery of a novel mycovirus in *Ceratocystis fimbriata*

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Abstract

Ceratocystis fimbriata is a plant pathogenic fungus that causes black rot in sweet potatoes (*Ipomoea batatas*) and is closely related to a number of well known tree pathogens such as *C. manginecans* and *C. eucalypticola*. Black rot is typically controlled by means of chemical fungicides, but these are known to harm both the environment and human health. Mycoviruses (fungal viruses) are being investigated as possible alternatives to fungicides, as some have the ability to induce hypovirulence (reduced virulence) in their hosts. In this study, transcriptomic data derived from *C. fimbriata* was used to detect and characterize novel mycoviruses. After de-novo contig assembly, Putative mycoviral contigs were identified through Pfam searchers for RNA-dependent RNA polymerase and viral helicase domains, which are hallmarks of RNA viruses and endornaviruses, respectively. BLAST screening revealed the presence of a putatively novel member of the Endornaviridae family, within the Alphaendornavirus genus. The provisional name *Ceratocystis fimbriata* endornavirus 1 (CeFEV1) has been proposed. The putative length of the genome is 10 723 nucleotides with the single ORF encoding for a polyprotein gene that shares 24.87% amino acid homology with Helianthus annuus alphaendornavirus. Phylogenetic analysis confirmed the putative classification of CeFEV1 as an alphaendornavirus. This study represents the first report of a mycovirus in *C. fimbriata* and may contribute to the future development of a biocontrol strategy for this fungus and relatives.

Keywords: mycovirus, Endornavirus

Session 19 Worldwide trends in tree mortality: The roles of climate change, insects and pathogens and their interactions

Oral communications

Regional impacts of forest insect and disease invasions on tree mortality in the US

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Abstract

Worldwide, forests are increasingly affected by nonnative insects and diseases, some of which cause substantial tree mortality. Forests in the United States have been invaded by a particularly large number (>450) of tree-feeding pest species. While information exists about the ecological impacts of certain pests, region-wide assessments of the composite ecosystem impacts of all species are limited. Here we analyze 92,978 forest plots distributed across the conterminous United States to estimate biomass loss associated with elevated mortality rates caused by the 15 most damaging nonnative forest pests. We find that these species combined caused an additional (i.e., above background levels) tree mortality rate of 5.53 TgC per year. Compensation, in the form of increased growth and recruitment of nonhost species, was not detectable when measured across entire invaded ranges but does occur several decades following pest invasions. In addition, 41.1% of the total live forest biomass in the conterminous United States is at risk of future loss from these 15 pests. These results indicate that forest pest invasions, driven primarily by globalization, represent a huge risk to US forests and have significant impacts on carbon dynamics.

Keywords: tree mortality, biological invasion, forest inventory, impact

The role of insects and pathogens in tree mortality in Europe

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Abstract

Insects can be an important factor in tree mortality, either by themselves or in connection with other factors which weaken tree defences. In this presentation I will provide a short review of the role of insects in instances of tree mortality in Europe. While some native insects are well known for their potential to cause tree mortality, such as the spruce bark beetle Ips typographus, most native tree-feeding insects do not kill trees. In the case of Ips typographus, severe outbreaks are typically preceded by predisposing events such as windthrow or drought. However, during an outbreak, even healthy trees can be killed because their defences are overcome by mass attacks. By contrast, several non-native insects can kill trees irrespective of the occurrence of other predisposing factors. While this issue does not yet reach the magnitude of tree-killing pathogens in Europe (such as the invasive pathogen causing ash-dieback) or invasive tree-killing insects in North America, the incidence of such invasions has been increasing as a result of globalisation and increasing intercontinental trade. Most insect invasions have occurred without an apparent role of climate change, contrary to popular belief. Examples of cases of tree-killing insects will be presented along with a typology of causes and mechanisms. The relative importance of insects and other causes of tree mortality will be discussed.

Keywords: insects, insect-pathogen interactions, pathogen vectors, tree mortality

Mass mortality of pine (*Pinus sylvestris*) forests in Belarus and Ukraine

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Abstract

Many large outbreaks of bark beetles and other saproxylophagous insects were observed in the forests of Ukraine and Belarus in the recent years. Mass mortality of pine (*Pinus sylvestris*) trees led to severe impacts on forest ecosystems across large areas in a magnitude which were not observed elsewhere in the region before.

Various methods (from on site evaluation to remote sensing tools) were used to assess the impacts on the affected areas. Monitoring systems were set up to define the population density and swarming behaviour of the bark beetles.

We present how pine mortality progressed in the two countries over the last ten years. We analyse the main causes, the time course of the mortality, the major insect species involved, and the different forest management and protection procedures used. We highlight the role of pine bark beetles (*Ips acuminatus*, *I. sexdentatus*, *Tomicus pininperda* and *T. minor*) in the pine dieback and the possible solutions.

The study also provides a comparison of the methods used in the two countries in assessment, monitoring, and reforestation.

Keywords: bark beetle damage, control and management, pine mortality, *Pinus sylvestris*

Defense strategies among long-lived pines and vulnerability to Bark beetles

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Abstract

Several high-elevation five-needle pines that grow in the western United States (US) are among the longest-lived conifers worldwide. Three species, *Pinus longaeva*, *P. balfouriana*, and *P. aristata*, are close relatives in the Balfourianae or bristlecone subsection. Each species has a relatively disjunct distribution that does not overlap with the other Balfourianae species. *Pinus flexilis*, however, commonly occurs in the same stands with each of these Balfourianae species, but it also has a much larger distribution that extends across the western US and Canada. The four species have varying susceptibility to a key herbivore, the mountain pine beetle (MPB, *Dendroctonus ponderosae*). *Pinus longaeva* and *P. balfouriana* are attacked by MPB less frequently than the other two species, and in a laboratory study very few MPB adults (N=54) emerged from *P. longaeva*, compared to *P. flexilis* (N=4389). These results suggested that the long-lived *P. longaeva* may have reduced threat from climate change-induced increases in bark beetle activity at high elevations. We investigated constitutive and induced defenses in these species and analyzed potential tradeoffs in defense strategies. The influence of climate on defense strategies among sites sampled within each species was also investigated. Finally, climate change factors contributing to recent and unexpected mortality in *P. longaeva* will be discussed.

Keywords: bark beetle, bristlecone pine, climate change, mountain pine beetle, ips

The scent of easy prey - The importance of olfactory signals in bark beetle host selection

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Abstract

Past decades have been marked by rising tree mortality triggered by more frequent extreme weather events like drought, heat or storms. Such events can also lead to an increased susceptibility of trees towards biotic agents like bark beetles. Despite a rich body of research, reaching back more than 250 years, major knowledge gaps concerning ecological interactions like host tree selection still remain. In early spring, overwintering pioneer beetles start swarming to search for new potential breeding sites and being exhausted from hibernation and food deprivation, they can often find suitable hosts in stressed and weak spruce trees that offer less defences against attackers. Because seasonal population growth depends on the success of the pioneer beetles, such a targeted host selection is of high ecological relevance. In our study we assumed that bark beetles (*Ips typographus*) use olfactory and visual signals emitted by spruce trees (*Picea abies*) for identifying and localizing suitable hosts. We measured volatile organic compounds emitted from stems in trees that were weakened and damaged by drought or windthrow and compared volatile emission profiles with those from vital trees. As measurements are currently ongoing, results will become available shortly before the meeting in September. We will then provide a first overview of our results, hopefully indicating a specific signal of declining vitality that can be used in subsequent R&D project as criteria for seed tree selection and/or in tree breeding programs to produce beetle-unattractive tree planting material.

Keywords: Bark beetle, host selection, Norway spruce, host attractiveness, volatiles

Semi-mechanistic modelling of tree mortality induced by an invasive pathogen at the landscape level

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Abstract

Forest diseases are often perceived as minor agents in forest dieback and mortality. However, many examples show that forest invasive pathogens can cause high ecological and economic damage. While a few studies have addressed the impact of forest diseases in terms of growth loss, a mechanistic understanding of disease damage and pathogen-induced tree mortality at the landscape scale is lacking.

We used the ash dieback disease, caused by the invasive pathogen *Hymenoscyphus fraxineus*, as a model to understand the impact of an invasive pathogen in tree mortality and its temporal dimension. We obtained data from 2008 to 2018 on stand variables (such as host basal area, hydric index, tree diameters) from the French Forest National Inventory, climatic data from Météo France and annual data on the progression of the ash dieback disease in France from the Forest Health Department. We fitted a Bayesian hierarchical model with ash mortality as a response variable as a function of time of disease presence, tree diameter, and branch mortality, which in turn was modelled as a function of time of disease presence, and variables that are known to impact disease severity: ash basal area and stand hydric index.

The hierarchical model allowed a better understanding of the mortality process by attributing each variable to either the disease-induced branch mortality process (higher host basal area and higher stand humidity leading to higher branch mortality) or the mortality process (larger trees being more resilient). The model also output the temporal dynamic of the invasive disease. At the stand level, significant branch mortality started to occur 2 years after disease arrival, and ash mortality started to increase once the disease had been present in the stand for 4 years. We showed that total ash basal area was in an expansion phase in France before disease arrival, and that the emergence of ash dieback stopped this increasing trend.

Keywords: Pathogen invasions; tree mortality; *Fraxinus excelsior*; *Hymenoscyphus fraxineus*; Bayesina hierarchical model

The impact of elevated CO_2 on mature oak and seedling defence against powdery mildew

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Abstract

Levels of Carbon Dioxide (CO_2) are at the highest point in 2 million years due to human activity and it is predicted to increase in the next few decades. Forest trees play a major role in mitigating rising CO_2 levels therefore, efforts focus on woodland conservation and planting. In the UK, strategies include oak species, however they are highly affected by the biotrophic fungus Erysiphe alphitoides, the causal agent of oak powdery mildew (PM). The high susceptibility of seedlings to PM is considered a limiting factor in oak woodland regeneration. Using a unique Free Air CO_2 Enrichment (FACE) facility, we have identified that elevated CO_2 (eCO_2) enhances seedling susceptibility further. Mature trees however are able to tolerate infections by PM and eCO_2 does not increase susceptibility. Using untargeted metabolomics, we have assessed the impact of eCO_2 in seedling and mature oaks throughout a growth season (where PM develops). Results show that eCO_2 does not impact the metabolome of mature trees massively, but differences emerge as the season and the disease progress. In contrast, huge impact is observed in the metabolome of seedlings exposed to eCO_2 and this is further pronounced in infected samples. Pathway enrichment has found that key defence pathways are downregulated by eCO_2, therefore explaining the susceptibility phenotype. The next step is to understand the differences between the perception and signalling of eCO_2 by mature trees and seedlings to develop solutions to protect this keystone species and support woodlands.

Keywords: Imaging; *Quercus robur*; Powdery mildew; metabolomics

Diversity and enzymatic activity of the rhizosphere microbiome of European beech trees with contrasting health status

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Abstract

In recent years, some populations of European beech (*Fagus sylvatica*) have been suffering a progressive decline due to biotic and abiotic factors. It is widely known that plants are able to attract some beneficial microorganisms to their rhizospheres through specific plant-microbe interactions. However, the role of the soil microbiome in forest declines is far from being completely understood. In this work, we investigated rhizosphere and bulk-soil microbial communities and their enzymatic activities in declined and healthy beech trees from central Spain. The composition and diversity of fungal and bacterial assemblages in the rhizosphere and bulk soil were analysed by metabarcoding tools. In addition, the enzymatic activity of seven hydrolytic enzymes related to C, N and P mobilization was quantified from these soil samples using a fluorometric microplate assay. A clear rhizosphere effect in enzymatic activities was found in both declined and vigorous trees, with the rhizosphere showing higher activities than the bulk soil. This effect was stronger in declined than in healthy trees, especially in enzymes related to N mobilization. This might indicate the selection of certain nitrifying microorganisms by declining trees. The taxonomic composition of microbial communities is still under analysis, but preliminary results suggest a rhizosphere effect, with the bulk soil showing higher fungal diversity than the rhizosphere, but without differences between the two health statuses. This study could help to expand our current understanding of the role of soil microbes in the health of European beech trees.

Keywords: Enzyme activity; Metabarcoding; Microbial community; Rhizosphere

Posters

Survey of pathogenic microorganisms on woody Fabaceae plants in Lithuania

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Abstract

The plants of the Fabaceae family are widespread throughout the world and belong to the third largest flowering plant family. Few representatives of this family are included in the lists of invasive plants in Lithuania. These plants create a threat to the diversity of native plant species and ecosystems, as well as the microorganisms directly associated with them where various pathogens can cause diseases to invasive Fabaceae plants. So far, there are not enough studies yet to evaluate what kind of influence can be made to local flora by microorganisms that spread with invasive plant species in the territory of Lithuania.

The aim of the study was to identify and characterize the microscopic fungi found in plants of Fabaceae family growing in natural ecosystems.

Plant material was collected in 2017-2021 in 28 different areas of Lithuania. A total of 159 plants belonging to the three genera (*Cytisus*, *Robinia* and *Caragana*) were collected. A total of 664 microscopic fungi were isolated from 133 plants.

Analysis of several genes such as actin (ACT), calmodulin (CAL), translation elongation factor 1-α (TEF1) and beta-tubulin (TUB) (White et al., 1990; Carbone, Kohn 1999; Glass, Donaldson 1995) and fragments of the internal transcribed spacer region of nuclear ribosomes (ITS) were used to identify fungal microorganisms by species.

Studies have shown that the most commonly found genera of phytopathogenic fungi were *Diaporthe*, *Kalmusia* and *Fusarium*, where several species were found in each genus. Whereas, representatives of other fungi such as *Alternaria*, *Chondrostereum*, *Epicoccum*, *Eutypa*, *Mortierella*, *Paraphaeosphaeria*, *Pezicula*, *Stagonospora*, *Thyronectria* were less frequently detected. Work on the threat assessment of pathogenic fungi to native plant species is continuing.

Keywords: *Diaporthe*, Genetic analysis, Fabaceae, Invasive plants, Plant pathogenic fungi

Pilot studies to evaluate bark temperature as an indicator of tree health

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Abstract

It is widely known that the temperature of the assimilatory apparatus is a good indicator of plant health. However, obtaining quality data for higher plants, such as trees, isn't easy without using expensive and time-consuming methods. Therefore, we present this work as an overview of a pilot study to evaluate bark temperature as an indicator of tree health.

We have chosen the Norway spruce (*Picea abies* [L.] Karst.) as the tree that is currently one of the most endangered tree species in Central Europe because of periods of extreme drought often followed by bark beetle outbreaks. We collected data from two different research plots using a handheld thermal camera. One plot was in a forest environment where bark-beetle infested trees were present, and the other one was represented by trees in a controlled experiment with simulated drought stress. So both experiments included healthy trees as well as trees in weakened health condition.

Our work has two purposes: (a) We compared the bark temperatures of trees and evaluated the potential of a handheld thermal camera as a tool for rapid health assessment; (b) We used data to develop a predictive model (based on Random Forest analysis) to discriminate between the health status (more focused on drought stress) of the trees.

The results show that the temperature of trees with weakened health is statistically higher than that of healthy trees and therefore is a good indicator of three health. Moreover, we found that the results of the predictive model are promising and can be used to predict health status (drought stress) based on bark temperature with high probability.

We believe that this methodology could be integrated into practice, but additional research is still needed.

Keywords: Temperature, thermal camera, health status, Random Forest

Novel fungi in *Pewenomyces* (Coryneliaceae) associated with cankers and die-back of *Araucaria araucana* in Chile

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Abstract

Pewén or the monkey puzzle tree (*Araucaria araucana*) is an ancient conifer endemic to the Chilean and Argentinian mountain ranges. These long-lived trees adapted to extreme conditions including long periods of snow cover, are slow-growing, and have both a sacred and economic importance to indigenous communities in both countries. Between 2015 and 2016, an unprecedented dieback disease was discovered on A. Araucana trees of all ages and throughout most of their natural distribution. Surveys conducted in natural protected and private areas in the Chilean mountain ranges showed that among the most important symptoms were girdling cankers on branches and stems resulting in copious resin exudation. The cause of the cankers and an significant contributor to the death of the trees was recently shown to be a novel genus and species in the *Coryneliaceae* and described as *Pewenomyces kutranfy*. Three additional and novel *Pewenomyces* species have also been found in the samples bearing branch cankers. Their identity was confirmed with DNA sequence data for seven gene regions and morphological comparisons with herbarium specimens from species previously known from this host, including *P. kutranfy* and species in the closely related genus *Caliciopsis*. Culture growth studies showed that these fungi are well adapted to colder temperatures typical of the environment where *A. araucana* occurs, a feature also found in *P. kutranfy*. It is unclear whether these novel fungi play a role in the occurrence or development of the canker disease. However, the existence of a great diversity of this group of fungi associated with *A. araucana*, and adapted to the conditions where these trees occur, reinforces the hypothesis that this is a native disease system that may have been altered due to changes in climatic conditions.

Keywords: Climate change, monkey puzzle, native disease, *Pewenomyces kutranfy*

Session 20 Can pathogen behaviour within a native range inform management decisions following pathogen

Oral communications

Update on the status of *Dothistroma septosporum* in Western Canada

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Abstract

Dothistroma septosporum, one of the causal agents of Dothistroma needle blight, has a broad host range within the family Pinaceae and a large geographic distribution. In British Columbia, disease expression on lodgepole pine (*Pinus contorta* var. *latifolia*) has increased and is hypothesized to be linked with changes in local weather driven by changes in climate. In Alberta, an outbreak of disease within an important lodgepole pine clone bank at the Alberta Tree Improvement and Seed Centre necessitated management intervention and it has been confirmed that the pathogen can cause disease on jack pine (*Pinus banksiana*) as well as limber pine (*Pinus flexilis*). Genetic diversity is present within the population of pathogen, and damage has traditionally not been severe, suggesting that the pathogen has existed in balance with its host. This is in contrast to the situation in countries where the pathogen is known to have been introduced, resulting in severe disease within pine plantations composed of exotic species.

Keywords: Dothistroma needle blight, *Dothistroma septosproum*

Evidence of new introductions and the sexual stage of *Dothistroma septosporum* in Colombia

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Abstract

Dothistroma needle blight (DNB) is one of the most important diseases of *Pinus* spp., especially in Southern Hemisphere plantations. In Colombia, Dothistroma septosporum has emerged as a serious constraint to the sustainable propagation of tropical *Pinus* spp. At the time of the first major outbreak of this disease in 2008, two geographically distinct and clonal populations, each with a different mating type, were characterised. Since then, new outbreaks of the disease have occurred in different farms. The aim of this study was to characterize isolates from these new outbreaks and to consider changes that have occurred in the D. septosporum population subsequent to its first detection in Colombia. A total of 12 forestry farms were surveyed that included new areas reported with DNB. Microsatellite and mating type markers were used to determine the structure and diversity of the isolates collected from infected trees. A total of 238 isolates were collected from five tropical Pinus spp. and three hybrids, in which 193 unique multilocus haplotypes (MLH) were identified. Results showed that over a ten year period, the population has changed substantially where D. septosporum now exhibits a high level of genetic diversity and new alleles, indicative of new introductions of the pathogen. In addition, there was evidence for sexual recombination in the population which was supported by the discovery of *D. septosporum* sexual structures on infected needles. This is the first report of the sexual state of the pathogen in Colombia. Structure analysis suggested a high level of gene flow mainly driven by sexual reproduction that could potentially result in novel haplotypes representing a substantial risk for other pine species in the future. Overall, these results demonstrate the importance of continual DNB monitoring at a population level and the risk that this pathogen represents to forestry globally.

Keywords: ascomycete, haploid, sexual reproduction

Understanding the enemy: why is *Austropuccinia psidii* (myrtle rust) so invasive?

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Abstract

Austropuccinia psidii, the casual agent of myrtle rust, is a globally invasive rust fungi that has devastated Myrtaceae wherever it has established. The rust originates from South America where more than eight strains of the pathogen have been identified; the most globally invasive strain is known as the “pandemic biotype”. Unlike most rust pathogens that have limited host ranges, A. psidii is known to infect in excess of 480 species from 69 genera of Myrtaceae worldwide. The different strains have different host ranges, although the pandemic strain has the widest known range.

A. psidii was first found in Aotearoa-New Zealand in 2017 and artificial screening against different strains has shown varying symptomology and frequency for resistance within species tested. Under natural conditions, the pathogen initially tended to mainly affect the most susceptible plant species (*Lophomyrtus* spp.) but as the pathogen has spread more disease has been reported on other species. Recent research has investigated the host-pathogen system, with fungal and plant transcripts analysed to gain insight into the infection process. With the largest fungal genome sequenced to date, which is likely to allow A. psidii to infect a large of plant species, this pathogen provides a unique case study to understand the characteristics that make it invasive and could be useful for protecting forests from other exotic pathogens that establish outside of their native range.

Keywords: *Austropuccinia psidii*; myrtle rust; invasive; Myrtaceae; susceptibility screening

Mechanisms of pine disease susceptibility under experimental climate change

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Abstract

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 concurrent 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, confirming what we expected a priori. 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 that likely contribute to enhanced tree susceptibility to pathogenic attack.

Keywords: Austrian pine, Climate change, *Diplodia*, enhanced susceptibility, metabolism

Session 21 Research progress on ash dieback and emerald ash borer

Oral communications

Cherry trees to the rescue: the metabolomic fingerprint of enhanced resistance to ash dieback by neighbouring cherries

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Abstract

The outbreak of pathogenic ash dieback (AD) disease in the European continent from 1992, caused by the fungus *Hymenoscyphus fraxineus*, has resulted in the death of millions of European ash trees, a species with huge economic, ecological and social relevance due to its versatility, fast growth and tolerance to different environments. *H. fraxineus* infects all species of ash and no full genetic resistance to the disease has been identified. However, partial resistance has been documented. Here, we report on how mixed species forest plantations affect the expression of resistance. We assessed disease incidence in over 300 ash trees growing in a mixed plantation consisting of 7,000 trees of 23 different species. The results have unravelled different levels of disease resistance to AD in the plantation. Also, we have identified a potential role of neighbouring tree species in disease resistance. We found that disease expression was at its highest when ash trees were neighbouring with Tilia cortata (lime) and at its lowest when ash trees were cohabiting with *Prunus avium* (cherry). Ash leaf samples from trees with neighbouring cherry or lime were subjected to untargeted LC-MS/MS metabolome analysis. Spectra were filtered using XCMS R-package. According to our results, 17304 and 14657 putative masses were detected in ESI-pos and ESI-neg, respectively. Subsequently, a Kruskal-Wallis analysis showed 2499 significant global differences ($p<0.05$). Co-expression analysis and clustering analyses were applied to determine the specific metabolites associated with resistance/susceptibility. A total of 106 putative identifications for 54 different masses were obtained. Further research is needed to identify the metabolites and elucidate the mechanisms by which these metabolites alter disease resistance to AD disease. Nevertheless, these results provide guidance to policy makers and woodland owners to increase the number of thriving forests in the years to come.

Keywords: Ash dieback, metabolomic approach, neighbouring effect, priming of defence

Divergent phloem chemistry in green v. white ash across tree sizes: potential impacts on Emerald ash borer performance

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Abstract

The Emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae; EAB), threatens to extirpate ash (*Fraxinus* spp.) in its introduced range in North America and Europe. Rapid growth of EAB populations and high rates of ash mortality have been attributed to the beetle's novel association with highly susceptible, "naive" host trees with no prior selection for resistance or tolerance to EAB attack. Top-down control of beetle populations via introduced biological control agents (particularly *Tetrastichus planipennisi* and *Spathius galinae*) together with growing evidence of reduced susceptibility in small trees provides hope that ash may be preserved as a component of the forest. Little is known about the potential for ontogenetic (age-related) resistance mechanisms in ash. We collected and analyzed chemical constituents (using HPLC) of over 300 phloem samples from green and white ash ranging from 3-15 cm diameter at breast height. We evaluated the impacts of defensive induction across tree size classes by applying the plant hormone methyl jasmonate v. as well as by experimentally infesting trees with EAB (including appropriate controls). Preliminary analyses suggesting that chemical signatures in the phloem differ significantly by ash species, tree size, and induction status are currently being confirmed. EAB growth and survival was primarily affected by species (larvae developed faster and survived better on white ash) while few performance differences were evident as a function of tree size. Parasitism rates were also higher on white ash, but only on smaller trees. These results represent an important and unique characterization of defensive chemistry in green and white ash across size classes with apparent impacts on EAB and parasitoid performance. With ongoing refinement, this research has the potential to improve understanding of key drivers of beetle and parasitoid population dynamics within this important size cohort of ash.

Keywords: *Agrilus planipennis*; *Fraxinus* spp.; HPLC; Insect invasion; phloem chemistry

Do soil related factors and sustainable soil amendments influence ash dieback of *Fraxinus excelsior*?

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Abstract

The ash dieback fungus (*Hymenoscyphus fraxineus*) is decimating European ash (*Fraxinus excelsior*) populations across the continent, with few options currently available to reduce disease severity. Soil related factors play a key role in plant health and improving soil health may reduce tree susceptibility to pests and diseases. Altering soils with sustainable soil amendments such as biochar and composts has been reported to lessen pathogen effects on crop plants, but few studies to date have considered their influence on tree diseases. A field trial and three pot trials were established in 2020 at two locations in England to assess the effects of biochar, compost, comfrey tea and willow woodchip applied at different volumes on ash dieback in young ash saplings. 320 ash saplings were planted in 20 plots in a randomised complete block design for the field trial, and 140 saplings were grown with 13 treatments and a control, replicated ten times in each of three pot trials, totalling 420 saplings. Ash saplings in the field and pot trials were scored for ash dieback disease symptoms in late summer and early winter 2021. Soil samples were taken for the field trial in December 2021, and in spring 2022 for the pot trial. Initial results suggest the effects of the treatments on ash dieback are not apparent in very young trees, particularly those protected by tree guards (saplings <45 cm that had not grown above the top of the tree guard (45cm) had significantly lower disease scores). A more influential factor appears to be the retention of the rachis following leaf senescence, with saplings that had retained ≤1 rachises in December 2021 having significantly higher disease scores than saplings that had lost all their rachises. The results of these trials should help reveal the role soil related factors play in the progress of disease in the above ground parts of trees.

Keywords: Ash dieback; biochar; *Hymenoscyphus fraxineus*; soil amendments; soil health

Expansion of Ash Dieback towards the scattered *Fraxinus excelsior* range of the Italian peninsula

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Abstract

Hymenoscyphus fraxineus, causal agent of ash dieback, is posing a threat to *Fraxinus excelsior* survival in Europe since the 1990s. In southern Europe, optimal climatic conditions for H. fraxineus become scattered as the host density, thus reducing disease spread rates. Our team has recently described the ash dieback diffusion in central Italy, where native *F. excelsior* is present as small fragmented populations.

This work aims to report the outcomes of the disease monitoring undertaken during the last two years on a national scale in Italy. It describes the current expansion of ash dieback in Italy and it shows an updated and detailed mapping of the natural distribution range of the host species across the Apennine Mountains in central and southern Italy. Our investigation also presents considerations about the consequences of further local disease spread with regards to the loss of *F. excelsior* genetic resource.

A bibliographic search of *F. excelsior* floristic reports and a direct interviewing to people were conducted for the creation of a detailed range map. *F. excelsior* leaves samples were collected for genetic mapping in twenty areas in northern and central Italy during 2020 and 2021. All the symptomatic *F. excelsior* plant specimens were analyzed with a culturomics and a quantitative PCR approach. The combined use of both techniques confirmed the presence of H. fraxineus in all the sites of central Italy where host plants were symptomatic. These new records represent the southern limit of the current known distribution of this pathogen in Italy, and together with Montenegro, in Europe.

Our results suggest that further spread of ash dieback in southern Italy is a realistic scenario. This poses a threat not only to the southern European provenances of *F. excelsior*, but to the species as a whole, should ash dieback lead to the loss of genetic material adapted to warm climates, which could become an increasingly valuable resource in the face of climate change.

Keywords: *Hymenoscyphus fraxineus*, *Fraxinus excelsior*, Central-Southern Italy, Disease spread

Greenhouse infection assays with the ash dieback pathogen *Hymenoscyphus fraxineus* on common ash (*Fraxinus excelsior*)

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Abstract

As it is nowadays known, *Hymenoscyphus fraxineus*, the causal agent of the ash dieback was introduced in the 90ies in Poland. Since then it colonized almost the whole geographic range of common ash *Fraxinus excelsior* in Europe. While most ash genotypes show disease symptoms at different degrees, some few ash trees are still looking healthy in between diseased ones. There is a gradient in disease severity from fully healthy individuals up to dead ones. Around 5-10 percent of the common ash trees in forests show no or only few symptoms of the disease in Switzerland. To ensure that these ash trees are really tolerant against ash dieback and did not avoid it due to environmental reasons, we tested some ash trees under greenhouse conditions. We performed stem and rachis infections by the ash dieback pathogen on grafted replicates of phenotypically selected ash trees from the field (*F. excelsior*). A total 20 trees from 10 forest sites in Switzerland were tested whereas 10 trees seemed to be tolerant and 10 trees (control) seemed to be susceptible to ash dieback after field assessments. At the end, 5 ash individuals out of the 10 tolerant ones were proved to be highly tolerant to ash dieback in the greenhouse experiments. Surprisingly some few susceptible trees were less vulnerable than expected. These trees may suffer from other abiotic or biotic stressors additionally to ash dieback in the field. These results are very encouraging because 1) 50% of the seemingly tolerant ash trees in the field show a really high tolerance against ash dieback in greenhouse assays and 2) the highly defoliated ash trees in the field may not all suffer from ash dieback. Different conservation and breeding programs will build up on these first experiments and the role of some interplaying environmental factors in the field will be further studied.

Keywords: ash dieback (*Hymenoscyphus fraxineus*); common ash (*Fraxinus excelsior*); stem and rachis infections; tolerance; susceptibility

European ash genotypes with increased resistance against the ash dieback disease may also be more resistant against the emerald ash borer

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Abstract

The invasive emerald ash borer *Agrilus planipennis* (EAB) has killed millions of ash trees in North America over the past 20 years. In 2003, the beetle was also introduced into Russia and has since been slowly but steadily spreading towards Central Europe. The advance of the EAB poses an huge potential threat to European ash *Fraxinus excelsior*, which is already severely weakened by the widespread ash dieback (ADB) disease. In an ongoing research project, we are investigating different European ash genotypes to test wether (1) these differ in their resistance to EAB, and (2) if tree genotypes with an increased EAB-resistance also possess an increased resistance against ADB (cross-resistance). Mature trees of two ash genotypes were selected at each of 10 sites throughout Switzerland. At all sites, the ADB-disease was fully established. At each site we selected one genotype that showed severe symptoms of ADB (ADB-susceptible genotypes), and one genotype that showed no signs of ADB infections despite of growing adjacent to ADB-infested trees (genotypes with an increased ADB-resistance). Shoots were grafted from all 20 genotypes on ash rootstocks. Four grafted shoots per genotype were infested with EAB larvae and different larval performance parameters were assessed on them. EAB larval mortality, developmental stage, and final weight varied significantly among ash genotypes 18 days after EAB-infestation. Later larval developmental stages and higher final weights were observed on ADB-susceptible ash genotypes than on ADB-resistant genotypes. Our results show that *F. excelsior* exhibits significant intraspecific variation in EAB-resistance. Moreover, the factors leading to an increased EAB-resistance also appear to increase resistance to ADB. Identifying and selectively promoting ADB-resistant ash genotypes will therefore not only help with ADB-disease management but could also slow down the spread of EAB in Europe.

Keywords: Cross-resistance, invasive species management, intraspecific variation, emerald ash borer, *Fraxinus excelsior*

Vertical spread of *Hymenoscyphus fraxineus* propagules

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Abstract

Hymenoscyphus fraxineus is an invasive ascomycete fungus causing the Ash dieback. The natural spread of the fungus is intermediated by its airborne ascospores. The ascospores are released from matured apothecia formed on infected leave litter of the previous growing season. The ascospores mainly spread during summer season, have limited viability and ability to be spread for long distances. The only possibility to get spread in long distances can be the vertical spread and consequent transport by air masses. Previous studies recorded spores three meters above the source of the inoculum and other studies detected spores of H. fraxineus in aerobiological samples of the Pollen Information Service network, which are sampled at height of 10 – 20 m. Focus of our study was to prove whether there are detectable amounts of *H. fraxineus* propagules in higher heights which can be transmitted for long distances. Our study was conducted in a municipal locality (Boršov nad Vltavou) with tens of infected ash trees (*Fraxinus excelsior*) in South Bohemia (SW Czechia). The infected trees surround an agricultural silo where five rotating arm spore traps (rotorods) were mounted for ten consequent 48h samplings during the peak of the sporulating season (17th July to 6th August 2020). The spore traps were mounted 48, 37, 25, 14 and 0,3 meters above ground. Samples were quantified by qPCR. Results clearly proved the ability of the spores to reach the height of 48 meters. Whether the ascospores would be viable after such intensive transport by wind remains unclear. The *H. fraxineus* DNA was detected from all five spore traps during all ten samplings. Generally, the amount of detected spores showed decreasing trend with the height and varied a lot. The difference between the highest recorded concentration (lowest spore trap) and lowest concentration (highest spore trap) was more than four orders. This variability was determined by weather conditions.

Keywords: Air sampling; Ascospores; Ash dieback; Rotorods; Spore traps

Specificity and sensitivity of LAMP assays for early detection of two *Agrilus* pests: Emerald ash borer (*A. plannipennis*) and bronze birch borer (*A. anxius*)

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Abstract

Among forest pests, Buprestids are an emerging threat to broadleaf forests across the world. Species like emerald ash borer (*Agrilus planipennis*) and bronze birch borer (*A. anxius*) pose serious threats to ash (*Fraxinus* spp.) and birch (*Betula* spp.) stands, respectively, where they are currently established. If these beetles were introduced to Europe, native populations of their host tree species would suffer dramatic losses. Due to their cryptic lifestyle, feeding on vascular tissue of their host plants, Buprestids and other woodborers can be difficult to observe or detect. Early detection tools are vital to swiftly implement eradication measures, and prevent the establishment of introduced species. Detection methods using PCR or qPCR assays to target specific taxa can take hours to prep and run the results. However, loop-mediated isothermal amplification (LAMP) eDNA assays are highly specific and sensitive and can provide results within 30 min after extraction. In this study, we designed a novel LAMP assay for *A. anxius* and investigated the specificity and sensitivity of both this assay and a previously developed LAMP assay for A. planipennis for its use as an early detection tool in European forests. We found that both assays were specific to the targeted species when tested against 12 other European *Agrilus*, five other Buprestids, two Scolytinae, and five Cerambycid woodborers. The sensitivity of the two assays varied with the A. planipennis assay amplifying at a concentration as low as 0.02 pg/µl, whereas the *A. anxius* assay amplified at 3.2 pg/µl. These results demonstrate that both assays make for a highly specific and sensitive tool that can be used to detect and monitor for the spread of A. anxius and A. planipennis, if or when, respectively, they are introduced to European forests.

Keywords: early detection, emerald ash borer, bronze birch borer, environmental DNA

Novel RNA viruses from the native range of *Hymenoscyphus fraxineus* to control ash dieback in Europe

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Abstract

A major threat to forest ecosystem is emerging diseases. Besides climate change and intensive global trade, forest pathogens are one of the contributing factors to these diseases and fungi comprise a major group of these pathogens. Traditional control strategies (resistant breeding and use of fungicides) are not as effective to overcome this problem. In this context the use of mycoviruses as biocontrol agents offer an effective and self-sustainable alternative. Since the successful control of the devastating forest pathogen chestnut blight fungus (*Cryphonectria parasitica*) by a hypovirus several studies were undertaken to search for such biocontrol agents to control forest diseases caused by pathogenic fungi. Since the last two decades *Hymenoscyphus fraxineus*, the causal agent of lethal ash dieback, has spread across Europe. This pathogen was likely introduced from Asia, where it occurs as a saprophyte. In the present study, total RNA of 116 H. fraxineus isolates from Japan were sequenced by using the Illumina RNA-seq platform. Novel RNA viruses belonging to different families were detected including a unique mitovirus (family Narnaviridae) with a circular genome, two dsRNA viruses belonging from the family Patitiviridae and genus Botybirnavirus and, a ss (+) RNA virus from the family Endornaviridae and a ss (-)RNA virus from the proposed family “Mybuviridae”. The infection frequency among the Japanese isolates was 11.5 %. The mitovirid was transmitted to virus-free isolates by using the paired culture technique and effects of virus on the host fungus were investigated. Furthermore, we assessed virulence of virus-free and virus-infected isolates, infected with mitovirus, on European ash (*Fraxinus excelsior*) trees by conducting inoculation experiment in the greenhouse.

Keywords: Invasive pathogens, mycovirus, circular mitovirus, biological control

Hymenoscyphus fraxineus and *H. albidus* genomes differ in secondary metabolites and transposable elements

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Abstract

In this study we analyzed the genomes of the ash dieback fungus *Hymenoscyphus fraxineus* and *H. albidus*, the native but, now essentially displaced, non-pathogenic sister species, and compared them with other members of Helotiales. The focus of the analyses was to identify signals in the genome that may explain the rapid establishment of *H. fraxineus* and displacement of *H. albidus*. Results: The genomes of *H. fraxineus* and *H. albidus* showed a high level of synteny and identity. The assembly of *H. fraxineus* is 13 Mb longer than that of *H. albidus*', most of this difference can be attributed to higher dispersed repeat content (i.e. transposable elements [TEs]) in H. fraxineus. In general, TE families in *H. fraxineus* showed more signals of repeat-induced point mutations (RIP) than in H. albidus, especially in Long-terminal repeat (LTR)/Copia and LTR/Gypsy elements. Comparing gene family expansions and 1:1 orthologs, relatively few genes show signs of positive selection between species. However, several of those did appeared to be associated with secondary metabolite genes families, including gene families containing two of the genes in the *H. fraxineus*-specific, hymenosetin biosynthetic gene cluster (BGC). Conclusion: The genomes of *H. fraxineus* and *H. albidus* show a high degree of synteny, and are rich in both TEs and BGCs, but the genomic signatures also indicated that H. albidus may be less well equipped to adapt and maintain its ecological niche in a rapidly changing environment.

Keywords: Ash dieback, invasive species, Secondary metabolites, Transposable elements

Life stage specific developmental rates of emerald ash borer, *Agrilus planipennis*, in naïve ash, *Fraxinus*, host trees

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Abstract

The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an invasive wood-boring beetle from Northeast Asia killing ash (*Fraxinus*) trees across eastern North America and European Russia. To better inform management decisions and reduce tree death, we tracked the stage-specific development of emerald ash borer across biologically-relevant temperatures (7-30 °C) in naïve green and European ash. These growth rates will inform models to mitigate range expansion and predict long-term impacts. For eggs and larvae, 7 and 10 °C appear to be below developmental thresholds with zero eggs hatching and no larvae progressing to second instars. Egg development duration was shorter as temperatures increased from 15 to 35 °C (62 versus 8 days). Larval development within *F. pennsylvanica* was fastest at 30 °C with more than 50% of larvae completing development from eclosion to prepupae at 30 °C in 45 days. At 30 °C, larvae took residence in mini-bolts at a higher rate in *F. pennsylvanica* (3.31 ± 0.19 larvae/mini-bolt) compared to F. excelsior (1.13 ± 0.13). Larval development to the first prepupa took 50% longer in *F. excelsior* than *F. pennsylvanica*. These growth rates will be used to determine where a life cycle can completed and to enhance biological control release timing.

Keywords: Invasive Species; Emerald Ash Borer; Wood Borer; Development; Buprestidae

Modeling seasonal variability in *Agrilus planipennis* (Coleoptera: Buprestidae) larval instar distribution for improving biological control

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Abstract

Emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an invasive pest of ash trees in North America and western Russia. In efforts to manage emerald ash borer in North America, four hymenopteran parasitoids from its native range in China and the Russian Far East were approved and released. Three of these species are gregarious multivoltine larval parasitoids that attack only the later feeding emerald ash borer larval instars (i.e., 3rd and 4th instar). Emerald ash borer is a semivoltine species in its most northern distributions, and populations transition to univoltine at more southern latitudes. The seasonal availability of emerald ash borer larval stages that are suitable for parasitism varies spatiotemporally and is critical for parasitoid establishment and persistence. In North America, emerald ash borer parasitoids are being released across climates that differ significantly from those of their endemic distributions. The objective of this study was to develop a phenology model for predicting the seasonal larval stage distribution across the current and potential geographic range of emerald ash borer. We reared all emerald ash borer life stages at a range of temperatures in the laboratory and measured their development rates and their variability. These data were fit to development rate and cumulative probability functions and incorporated into a computer program that tracked development of each cohort from EAB adult emergence through the mature larval stage using hourly temperature data from specific geographic locations. Model simulations illustrate the spatiotemporal variation in EAB voltinism and availability emerald ash borer larval instars that are suitable for parasitism depending on geographic location. Our model will be useful for determining parasitoid release times, predicting the potential distribution of introduced parasitoids, and modeling parasitoid-host population growth rates across emerald ash borer’s current and potential distribution.

Keywords: classical biological control, invasive species, phenology model, introduced parasitoids

Establishment, impact and dispersal of parasitoids for control of emerald ash borer in Canada

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Abstract

Parasitoids have been released in Canada for the biological control of emerald ash borer, Agrilus planipennis, (EAB) since 2013. Release sites were established in Ontario, Quebec, New Brunswick and Nova Scotia and used to determine if parasitoids could successfully establish in Canada and exert regulation on EAB populations. As of 2019 two species, *Tetrastichus planipennisi* and *Oobius agrili*, were shown to be established in 81% (13/16) and 29% (4/14) of release sites, respectively. A third species, *Spathius galinae* has also been released in Canada but establishment has not been demonstrated. Using the network of release sites in Ontario, we did not observe an increase in parasitism rates by *T. planipennisi* in sites 1-4 years post-release but did observe a slight decrease in parasitism rates by native EAB parasitoids. In 2019 and 2021 we evaluated dispersal of *T. planipennisi* from a subset of release sites using a network of yellow pan traps deployed during the summer months. From these sites we observed dispersal rates of 2.3-3.2 km per year, consistent with previous laboratory and field observations from the United States. The maximum observed dispersal distance was 48 km from a four-year old release site in Northern Ontario. These results suggest that *T. planipennisi* has successful established in Canada and has been able to disperse to exploit EAB populations beyond those at the initial release sites.

Keywords: Biological control, dispersal, Emerald ash borer, invasive species

Posters

FraxForFuture—The German research network on Ash Dieback

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Abstract

Ash dieback is the most important threat to Common Ash (*Fraxinus excelsior*) in Europe. The non-indigenous fungal pathogen *Hymenoscyphus fraxineus* is causing severe damage to trees, stands, ecosystems, and forestry. We are running a transdisciplinary research network to secure ash and its economic usability in forestry, funded by two German Ministries (Environment, Nature Conservation, Nuclear Safety and Consumer Protection; Food and Agriculture) within the Waldklimafonds under the Fachagentur für Nachwachsende Rohstoffe e.V. It comprises of five sub-networks: FraxConnect fulfills a dual function: (i) It ensures the communication and coordination of the entire FraxForFuture research network; (ii) conducts economic analyses and is responsible for its transfer to practitioners. FraxGen focuses on the genetic aspects within the research project. Genetic variability of both ash trees and fungal pathogens will be explored. One focus of this collaborative effort is the selection of plus trees. These are considered sustainable because they currently show no or hardly any disease symptoms and can be seed trees of tolerant subsequent generations. FraxMon is dedicated to the monitoring of ash stands, i.e. the ongoing monitoring of disease development. Thus, this sub-network lays the foundations for management concepts and area-related considerations for dealing with ash as a commercial tree species. FraxPath investigates the relationships between ash as a host tree species and the pathogen *H. fraxineus*. The various symptoms of ash shoot dieback (e.g. collar rots, shoot infections, leaf necrosis) are being investigated and innovative approaches to pathogen management are being sought. FraxSilva pursues different research approaches in natural environments (in situ) and outside of them (ex situ). The main objective is to test and develop silvicultural treatment options for practical use (forest owners and managers).

Keywords: *Fraxinus excelsior*; transdisciplinary research network; monitoring; fungal pest

Evaluating tolerance of Ash genotypes to ash dieback disease in the gene bank collection in Ireland

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Abstract

Ash (*Fraxineus excelsior*) is ecologically, economically and culturally one of the most important native broadleaf tree species in Ireland. However, its survival is increasingly threatened by ash dieback disease, which has spread nationally and results in severe damage and mortality. Ash dieback disease is caused by an invasive fungal pathogen; *Hymenoscyphus fraxineus*, which originated in Asia and was first noted in Ireland in 2012. In Teagasc, a breeding programme to select and screen ash dieback tolerant genotypes began in 2015 and we have now established two gene banks consisting of more than 200 genotypes collected from Ireland and across Europe, which were pre-screened to show varying level of tolerance in their place of origin. Current research is focused on screening these genotypes under natural disease infestation conditions. Preliminary data illustrates that a varying level of tolerance to the disease exists within the collection. Interestingly, ~15% of the genotypes in the ash gene banks are showing higher levels of tolerance to the disease compared to the control susceptible genotype Kilwa. The findings from this project will advance our knowledge on the extent and phenomenon of disease tolerance in different ash genotypes, and positively guide the ash tree improvement programme in Ireland.

Keywords: Chalara, dieback of ash, tree improvement, disease tolerance, plant-microbe interaction

Session 22 Use of RNA strategies for the control of forest pests and diseases

Oral communications

Viral diversity in Mediterranean forest ecosystems

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Abstract

Climate crisis, human pressure and globalization alter the conditions of forestry habitats and facilitate the entry of new pathogens that don't share a co-evolution history with the forest. Due to urban massification, the human population is particularly exposed to the appearance of new infectious diseases, such as the current coronavirus pandemic. For this reason, the study of viruses, and specifically RNA viruses in the forest ecosystem, is essential to understand how viral flow across different hosts might occur, and to prevent possible outbreaks of human diseases in the future. In this work the viral diversity found in trees, insects and fungi from Spanish Mediterranean forests is described. To this extent, three habitats in Castile and Leon characterised by a key Mediterranean tree species (*Quercus ilex, Castanea sativa* and *Pinus radiata*) were sampled and RNAseq was performed on above-ground tree tissues, insects and fungi cultured from the same materials. 161 viral sequences were detected by searching for matches to conserved motifs of the RNA-dependent RNA polymerase (RdRP) using Palmscan. Tentative taxonomy was assigned to well-supported contigs by taking a consensus of the top 10 hits above 90% identity threshold against PALMdb. Up to 15 viral families were identified, with Botourmiaviridae (25%) and Partitiviridae (6%) being the most abundant. Viruses belonging to families with cross-kingdom capabilities such as Hypoviridae (1), Mitoviridae (7) and Narnaviridae (5) were also found. Distribution of viruses across ecosystem was: *Q.ilex* (57%), *P.radiata* (27%) and *C.sativa* (16%). Interestingly, 40% of RdRP sequences had no matches in currently available viral databases, thus constituting a starting point to search for novel viruses that might be participating in unknown infectious pathways within forests and potentially posing a threat to the human being.

Keywords: Next generation sequencing, palmprint, Serratus

The Chestnut blight fungus and its *Hypovirus 1* (CHV-1) in England

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Abstract

Cryphonectria parasitica was first detected in 2011 in a nursery in England (UK) and in the wider environment on semi mature trees in 2016. Surveys were carried out from 2017 to 2020 and the disease was detected at different sites in Berkshire, Buckinghamshire, Cornwall, Derbyshire, Devon, Dorset, London, West Sussex, and the island of Jersey. A total of 759 samples were collected from cankers, and 350 samples tested positive for *C. parasitica* by both quantitative real-time PCR and by isolation. All isolates were tested for mating type, vegetative compatibility group (VCG) and the presence of *Cryphonectria hypovirus 1* (CHV-1). Both mating types were detected although usually at different sites and only occasionally on the same site. However, perithecia were never detected in any of the samples collected. A total of 12 VCGs were confirmed amongst the isolates. The CHV-1 virus was detected in a total of ten isolates. The concentration of the CHV-1 was low in all isolates and in all cases the CHV-1 belonged to the subtype I. Investigations were carried to find a CHV-1 infected C. parasitica isolate that potentially can be used as an effective biocontrol agent. Inoculation trials with candidate *C. parasitica* isolates highly infected with CHV-1, achieved by transmissions, were tested under controlled conditions. Additionally, different studies looking at colony morphology, mutability, satellite RNA, and the stability of the mycovirus depending on the fungal mycelium age, temperature and growing medium type were performed. The results will be discussed together with the efficiency of a newly developed real-time PCR method for the detection of the CHV-1 virus.

Keywords: chestnut blight; diagnostics; disease control; hypovirus; stability

A past mycovirus infection caused a residual effect in the Pine pitch canker pathogen *Fusarium circinatum*

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Abstract

Studies on the long term effects of viral diseases have predominantly focused on human infections. The effects of mycoviruses on their fungal hosts are not well studied and the residual effect on a fungus after the degradation of a mycovirus is even less well understood. Fungi infected with mycoviruses are known to be difficult to cure but their spontaneous loss occurs commonly, meaning that the effects of their loss is not easily studied in a methodological way. There are diverse explanations for the disappearance of mycoviruses in their hosts including repeated transferring of cultures, other forms of stress and posttranscriptional silencing processes. In this study, we analysed the residual effect on the transcriptome of the pathogenic fungus *Fusarium circinatum* after the loss of the mitovirus FcMV1. Isolates infected with this mitovirus were found to no longer harbour the virus after 4 years in storage. The transcriptome of these isolates was compared with isolates that did not have the virus. Only 14 genes were determined to be differentially expressed and all were related to cell cycle regulation and amino acid metabolism. The results showed a slight acceleration in the metabolism of the host that had lost the mycovirus by the up-regulation of genes involved in essential functions for the fungal development. To the best of our knowledge, this is the first time the residual effect of a virus post-infection has been studied in a fungus. The residual effect provoked by the loss of a mycovirus in the host transcriptome reflects the difficulty in obtaining unaltered isolates for further studies. Future research focused on the generation of isogenic lines of F. circinatum with and without mycoviruses by transfection methods is required in order to better understand the feasibility of virocontrol of this forest pathogen.

Keywords: Tree diseases, *Pinus radiata*, transcriptomic, forest pathology, biocontrol

Preventing mycelial spread of *Heterobasidion* using dsRNA virus treatment combined with fungal biocontrol

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Abstract

Heterobasidion spp. is one of the most important fungal pathogens of boreal forests. It spreads by aerial spores to freshly cut stump surfaces and the established mycelium is able to infect living trees, mostly conifers, via root connections. New infections can be controlled by chemical or biological stump treatments, but this does not prevent vegetative spread that can only be restricted by changing the tree species to a resistant one. The bisegmented dsRNA virus HetPV13-an1 (a member of genus *Alphapartitivirus* in Partitiviridae family) considerably restricts the growth of Heterobasidion strains and alters the expression of nearly 700 genes of its host, Heterobasidion annosum, making it a promising candidate for preventing Heterobasidion damage. We tested if the spread of an already established Heterobasidion infection could be inhibited with a combined biocontrol approach: treating stumps around H. annosum disease centres by the biocontrol fungus *Phlebiopsis gigantea*, and application of the debilitating mycovirus HetPV13-an1 to stumps already infested with Heterobasidion decay. This field investigation showed that the enlargement of disease centres was reduced by *P. gigantea*, and that this biocontrol effect was enhanced by the virus treatment. However, in laboratory conditions, tolerance against the detrimental effects of HetPV13-an1 is common among strains of *H. annosum*, and mixed infections by other viruses may reduce or enhance its transmissibility and phenotypic effects on the host. Therefore, efforts to commercialize the virocontrol approach still need further investigation.

Keywords: Conifer, mycovirus, root rot, stump treatment

RNAi-based forest protection products against the European spruce bark beetle, *Ips typographus* (Coleoptera: Curculionidae, Scolytinae): current status and prospects

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Abstract

Norway spruce (*Picea abies* L. Karst) is one of the primary forest production species in Europe and the Czech Republic. However, there has been a drastic reduction in *P. abies* population due to climate change and outbreaks of European spruce bark beetle, *Ips typographus* (*I.typ*). Existing conventional methods for managing *I.typ* failed to stop the recurrent outbreaks, and hence, there is a need for aggressive alternatives for efficient bark beetle management. Research on the RNA interference (RNAi) tool has gained significant interest in controlling coleopteran forest insect pests due to its sequence-specific gene-silencing mechanism and efficiency. However, there is no progress in using RNAi against European tree-killing bark beetles (Coleoptera: Curculionidae, Scolytinae). With the genome sequence availability, we postulate that RNAi can serve as an efficient and aggressive method to control *I.typ*. To determine the functionality and bio-pesticide potency of RNAi against *I.typ*, target gene silencing was performed by force-feeding dsRNA. Further RNA-seq studies revealed RNAi-core machinery gene expression dynamics in *I.typ* and its downstream consequences. Our findings exposed the potential application of RNAi technology against *I.typ* and other coleopteran wood-boring forest pest management.

Keywords: *Ips typographus*, RNA interference, dsRNA, Coleopteran forest pest management, RNA-seq.

Spray Induced Gene Silencing as new and sustainable protection strategy for *Quercus suber*

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Abstract

Charcoal and canker diseases on cork oaks have been raising in the last years. The increase of these two diseases is enhancing the rapid decline of cork oak (*Quercus Suber*) population. The lack of a specific treatment for these two fungal diseases and the progressive ban of chemical fungicides in the EU, calls for new and innovative alternatives. Recent biotechnological techniques using double-strand RNA (dsRNA) to inhibit the expression of homologous essential genes, have been reported. One of these techniques is named Spray Induced Gene Silencing (SIGS). The SIGS involves the inhibition of plant pathogens through a direct spray of dsRNA targeting specific pathogen genes. In this work, preliminary results suggest that when targeting essential genes through SIGS, the normal development of cork oak pathogens is affected, due to a growth rate reduction and development of morphological anomalies. Given the high specificity of this approach, the use of target-specific RNAi strategies offers a great potential as a new and sustainable plant protection strategy.

Keywords: Fungal diseases; *Quercus suber*; RNAi; SIGS; Sustainable;

The use of clay particles prolongs RNA interference-mediated plant protection against fungal diseases

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Abstract

The spray-induced gene silencing (SIGS) technique has been found to be effective for controlling fungal plant diseases. SIGS is a method for exogenously applying double-stranded RNAs (dsRNAs) targeting essential or virulence-related pathogen genes to plants or postharvest products to cause RNA interference (RNAi) and inhibit fungal growth and disease. By delivering biologically active dsRNA as layered double hydroxide (LDH) or clay particles, a formulation referred to as BioClayTM, it can prolong RNA durability on plants, thereby protecting them from pathogens. In this study, we demonstrated that dsRNA delivered as BioClay can extend protection against Botrytis cinerea, a major necrotrophic plant pathogen, on tomato leaves and chickpea mature plants. Comparing to naked dsRNA, BioClay increased the protection window on tomato leaves from one week to three weeks. In the same way, tomato fruit was protected when BioClay was applied ten days before infection, double that of naked dsRNA. In addition, chickpea plants treated with BioClay were protected for up to 4 weeks, covering the critical period of pod formation, whereas those treated with naked dsRNA were protected for only a few days. As a result of this research, our ideas about SIGS as an alternative to traditional fungicides for use in forestry are significantly improved, with BioClay having the potential to provide extended protection against a wide range of fungi but also to pests.

Keywords: RNA interference; SIGS (Spray-induced gene silencing); BioClay; fungal pathogens; plant protection

Posters

Plant disease management by RNA Strategies: mycoviruses and RNA interference

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Abstract

A new approach based on the use of RNA-based strategies is being explored for the management of plant diseases as an alternative to the use of chemical applications, which not only leave harmful residues in the environment, but also result in a proliferation of resistance in pathogens. Mycovirus (mostly with RNA genome) can be used as one of these strategies since several of them have been known to cause hypovirulence in fungal pathogens. This acknowledgement has been exploited to manage some diseases caused by pathogenic fungi. However, research in this area is crucial due to the limited discovery of hypovirulent mycoviruses in pathogenic fungi, as well as the lack of protocols that allow effective and systemic infection. Another alternative strategy in the treatment of plant diseases is based in RNA interference (RNAi), the eukaryotic cellular system that recognizes RNA sequences and specifically degrades them or prevents their translation. The occurrence of cross-talk between host and pathogen and the ability of most pathogens to capture RNA from the environment have allowed scientists to control plant diseases using two different approaches: host-induced gene silencing (HIGS) and spray-induced gene silencing (SIGS), respectively. HIGS strategy requires the construction of transgenic plants, which limits its applicability in Europe and in forest species, which are difficult to transform, so our work has focused on the study of the possibilities and handicaps of SIGS. In addition, using SIGS, we have applied exogenous dsRNA to silence essential genes of the pathogen *Phytophthora cinnamomi*. These first quick analyses confirmed its silencing effect in *Arabidopsis thaliana*, and now we intend to test it in *Quercus* plants.

Keywords: Plant pathogen control, hypovirulence, host-induced gene silencing (HIGS), spray-induced gene silencing (SIGS), *Phytophthora cinnamomi*

Session 23 Forest pathologist and entomologists unite! Results of innovative collaborations that improve our understanding and management of forest pest invasions

Oral communications

Why entomologists and pathologists should and can join forces to control invasive forest pests and pathogens

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Abstract

Forest pathologists and entomologists have long worked in parallel on forest health issues. These complementary but not very collaborative studies were due to different conceptual frameworks and methodological approaches.

However, several factors make closer cooperation between the two disciplines and their actors necessary. Global change is accelerating the decline of many forests, bringing biotic risks to the forefront of concerns. A growing number of examples show that biological invasions and diebacks in forests are caused by positive interactions between insects and fungi or microbes, while antagonisms can occur at the host tree level. Finally, more and more research projects show that the same methods of detection, identification, eradication or control could be applied to both forest insect pests and pathogens.

However, we still have to think together about how to promote this convergence and make it operational to better protect forests against biological invasions. We will see that this could require a better understanding of the mechanisms of biotic interactions, the sharing of innovative investigation methods, the development of common concepts in risk analysis and management, and the multidisciplinary training of young researchers.

Keywords: forest protection; insects; interdisciplinarity; invasion; pathogens

Comparison of intercept trap fluids and aerial spore collectors to survey fungal spores

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Abstract

Surveillance for early detection of non-native, invasive pathogens requires simple, sturdy, and easy to use collecting devices. In this study, we compared the fungal species detected in wet collection cups of Lindgren traps versus those detected on slides with oiled cheesecloth as aerial spore collectors. DNA was extracted and amplified from both using the primers ITS1F - gITS7G, and Illumina sequencing was used for metabarcoding of fungi present in samples. In 90 samples there were 1277 fungal operational taxonomic units (OTUs). For fungal OTUs only detected by one collection method, insect traps had three times the number of fungal OTUs compared to slides, and this pattern persisted when analyses were restricted to pathogens and forest pathogens. Annually, thousands of insect traps are deployed in North America and the associated trap fluids have added value in forest disease research and monitoring.

Keywords: pest management, survey and detection, sampling, forest biosecurity

Insect-fungal joint invasions and biological control: Lessons from the global *Sirex noctilio* invasion

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Abstract

Sirex noctilio and its symbiotic fungus, *Amylostereum areolatum*, are important invasive pests of pine trees in various parts of the world. Studies on the genetic diversity and population structure of the woodwasp and fungus over the past two decades have revealed that the pathways of introduction and establishment are not always aligned between these organisms, despite their obligate, mutualistic association. We have found that invasive populations of the fungus are largely clonal from apparently limited introductions, while the wasp populations appear to reflect multiple introductions from different regions. In contrast, populations of the biocontrol nematode *Deladenus siricidicola* range from nearly clonal populations in some countries to others that contain unexpected diversity stemming from historical releases, multiple introductions and admixture between disparate lineages. These patterns of diversity not only reflect the signatures of variable quarantine systems, but also the unintended and unconsidered complexities of developing biological control programs. These studies demonstrate the importance of considering the potential complexity of multiple invasion patterns when developing biological control programs.

Keywords: Invasive forest pest, symbiosis, biocontrol

Towards a global sentinel plants research strategy to prevent new invasive forest pest and pathogen introductions – The experience of HOMED

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Abstract

The early detection of pests and diseases using sentinel plants has been exploited in some experimental work, validating the tool for the identification of (unknown) plant pests and pathogens prior to introduction into new countries through international plant trade. Sentinel plantings can have different objectives: in-patria plantings estimate infestation rates of associations of native insects and native tree species that may be exported with that particular commodity, whereas ex-patria plantings are relevant to assessing new pest-host associations prior to the introduction of the pests, such as possible host shifts of non-native pests to a native host in the importing country that would otherwise be impossible to predict. In the frame of the EU project HOMED we have implemented this concept, widening the use of this tool simultaneously to many different countries and continents. Sentinel plantations were established in Italy, France, Switzerland, China and South Africa, according to a common experimental design.

Tree species of European origin (in-patria) and of Chinese and South African origin (ex-patria) were planted in Europe, while in China using mostly Chinese species (in-patria) and in South Africa using European species (ex-patria) were planted. Over three years, insects and fungi were collected on a frequent basis from all trees and identified. Moreover, surveys of pests and pathogens in sentinel arboreta in South Africa, Europe and production plantations in New Zealand were also conducted. The results presented demonstrate that the monitoring techniques developed and used in HOMED are extremely effective: regularly monitoring of a diversity of tree species over a period of three years has yielded a multitude of pest-host associations. Our work presents the associations and discuss them in the context of risks associated with the global movement of live plants.

Keywords: Sentinel planting, Exotic insects and pathogens, invasive species, Early warning

Refining guidelines for managing overland spread of oak wilt by Nitidulidae (Coleoptera) beetles in Canada

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Abstract

Nitidulid beetles transmit oak wilt, an invasive fungal disease present along Canada's border. The fungus is spread overland when beetles contaminated with the fungus visit fresh oak wounds. Prevention of overland spread is the most cost-effective form of control, e.g., by using regulatory controls to reduce the risk of oak wounding during the active flight season of vectors. Between 2018 and 2021, a series of studies were carried out in New Brunswick, Manitoba, and Ontario to determine: which Nitidulidae species were potential vectors, their seasonal flight periods, and the most effective traps for their detection. We collected 12 potential vector species from oak wounds and developed degree day models (base 5ºC) using 3-years of weekly trap catch. Cumulative degree days at 1, 5, and 95% of flight were determined using historical temperature data. Risk of oak wilt infections by known vectors, *Carpophilus sayi* and *Colopterus truncatus*, is greatest between April and August. If *Epuraea avara* proves to be an efficient vector, the risk period extends into September. Trap choice will influence the success of detection programs. Trap types (wind-vane, funnel, modified funnel, Multi-trap) differed significantly in mean catch of certain species and relative ranking varied among nitidulid species. Currently, the role of timing and environmental factors in nitidulid beetle attraction to oak wounds is being investigated in Michigan and Canada.

Keywords: *Bretziella fagacearum*, *Quercus*, sap beetles, degree days, traps

Session 24 Impacts of global change on forest defoliating insects

Oral communications

Spread of *Panolis flammea*, *Lymantria monacha* and *L. dispar* in Far North of Europe: old maps and new trapping results

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Abstract

Climate change affects distributions of forest insects in North Europe. There is relatively good observation record of moths done by Finnish Lepidopterologists. In addition, several good quality distribution maps have been published in Finland. Currently this information is available as open species data on the internet provided by Natural History Museum of Finland. However, the data may have bias towards more densely populated areas and biotopes hosting rare moth species, whereas changes in presence of common species living in species poor forest biotopes may be overlooked.

We studied the current occurrence and abundance of two potential forest pests, *Panolis flammea* and *Lymantria monacha* in Finland in 2018-2021 using pheromone traps and compared the trapping results to available open species data and old distribution maps. In addition, a survey of *Lymantria dispar* was done in Southern Finland in summer 2020.

The results of pheromone trappings of 2018-2021 showed that *P. flammea* is very abundant throughout most of the Finland up to 67° 30’ N. *L. monacha* was very abundant in southern parts of Finland up to 62° 30’ N. Comparison of new results from pheromone trappings and available old species observation data shows that continuous distribution of these two defoliating moths has moved some 200 km (*L. monacha*) to 300 km (*P. flammea*) northwards since 1990. No single specimen of L. dispar was found in survey of 2020.

The trap catches varied from few up to 202 individuals of *P. flammea* and up to 1000 individuals of L. monacha per flight season. High frequency of *P. flammea* records in pheromone traps in Northern Finland suggests that Open Species Data largely underestimates presence of the species in the north. In pheromone trap data, the northern limit of *L. monacha* is approximately 100 km more north than in Open Species Data.

Keywords: Climate Change; Distribution; Finland; Moths

Larval tent construction and social behaviour of pine processionary moths (*Thaumetopoea pityocampa*)

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Abstract

Social organisms require organisation to be efficient and functional as a colony. Throughout the whole larval life, *Thaumetopoea pityocampa* live gregariously in their communal tent with siblings and conspecifics from other colonies. In this study, we explored the social behaviour of *T. pityocampa* caterpillars associated with tent construction and foraging. The location of the tent built on the host tree and silk application on the tent were both predominant in the south where there is maximal insolation. The caterpillars can determine spatial orientation/direction from early larval instar through a pair of specialised stemmata that detect skylight polarisation patterns, which they used for tent construction and maintenance. Tent maintenance occurred when environmental temperatures were > 10 °C at sunset whereas, foraging generally occurred when it was < 10 °C in complete darkness. By understanding how *T. pityocampa* caterpillars constructed the tent and foraged, it can give us an insight into the potential impacts of changing global temperatures on *T. pityocampa* colony behaviour.

Keywords: Animal behaviour, cooperation, processionary caterpillar, sex differences, social organization

Pine processionary moths in Turkey, their distribution and management

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Abstract

Pine processionary moths in Turkey are distributed along the coastal regions of the country where winters are milder in comparison to more continental inner regions. The occurrence of the species in southern Anatolia has been known since the Hellenistic periods. There are also records from the northern Anatolia from the early 19th century. Until recently, the species that spreads in the country was identified as *Thaumetopoea pityocampa*; whereas molecular studies showed that the main pine processionary moth species in Turkey is *T. wilkinsoni* which has been also known to occur in the Middle East, Cyrpus, and Crete. In previous studies, Ipekdal et al. (2015, 2021) discovered the exact limits if the two species in Turkey; thus showed the easternmost limit of *T. pityocampa* distribution along with the northernmost limit of *T. wilkinsoni* distribution. These studies also revealed a case of hybridization between the two sibling species in northwestern Turkey. Species distribution modeling studies conducted in Turkey by Ipekdal et al. (2017) predicted a northeastward range expansion of *T. wilkinsoni* which could end in an other contact zone with *T. pityocampa* that was recently introduced accidentally to a Russian locality on the eastern Black Sea coast.

As spraying insecticides in Turkish forests is forbidden since early 2000s, management of pine processionary moth populations, which make massive outbreaks once in a four to five years period, is persued through mechanical, biochemical, and biological strategies. Mechanical management strategies include egg mass and larval nest removal, whereas biochemical strategies rely on using pheromone traps. Biological strategies can be classified under two main topics: (1) conservative biological control which includes supporting the populations of egg and larval parasitoids by keeping the mechanically collected egg masses and larval nests in cages or artificial islands in forests; (2) inundative biological control which includes releasing a lab-reared larval predator, Calosoma sycophanta, during outbreak years. On the other hand, none of the control measures mentioned above had a proven success in significantly decreasing outbreak populations.

Keywords: *Thaumetopoea wilkinsoni*, *T. pityocampa*, hybridization, biological control

Pine processionary moth: a good model species to explore the interrelation between range shifts and phenological changes

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Abstract

Climate change is impacting species in various ways, notably their distribution and the timing of life stages. The pine processionary moth (PPM), *Thaumetopoea pityocampa*, is an important pine defoliator, triggering sanitary problems for humans and animals. The PPM is currently expanding northwards and toward higher elevation in Europe, and the causal relationship between this expansion and climate warming during the cold season has been clearly demonstrated. This range expansion appears to be spatially heterogeneous, notably in France where the northern edge of PPM distribution was assessed every 5 years from 2005-2006 to 2020-2021. Furthermore, a recent study revealed that the southern edge has been retracting from South Tunisia due to higher temperatures recorded in summer-autumn. A habitat shift was found in Bulgaria, accompanied with a southern retraction as well. As a result, the PPM distribution may be shifting rather than expanding. Since distribution and phenology are closely linked, we explored the effects of climate change on the PPM phenology as well. We monitored PPM phenology in France and Bulgaria (at the western and eastern sides of the PPM northern front, respectively) to explore atypical phenologies. Furthermore, a phenology model was developed to describe the development of eggs and the 5 larval instars, until pupation procession time. This model revealed that climate warming could effectively shorten the period of larval development and eventually lead to atypical early processions (before the end of December instead in Spring, typically). This phenological shift in relation with climate warming, combined with different stage-specific thermal constraints, could alter survival rate positively or negatively. Thanks to the knowledge gained about its distribution and phenology shifts, and the corresponding long time-series available, the PPM appears as a very good model to explore the inter-relation between species distribution and species phenology, and better understand the resulting effects of climate change.

Keywords: *Thaumetopoea pityocampa*; range expansion; range retraction; phenology shift; climate change

Synergistic disturbances in a warming environment: the western spruce budworm and Douglas-fir beetle

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Abstract

Biotic disturbance in North American forests is caused primarily by eruptive herbivorous insects. Most commonly, these disturbances are the result of outbreaks by species of lepidopteran defoliators and bole-infesting bark beetles. Our understanding of the factors affecting biotic disturbance has largely been derived from the isolated study of individual species; however, forests are spatially and temporally complex systems in which disturbances are unlikely to act independently. For example, during defoliator outbreaks, impacts to the photosynthetic capacity and overall vigour of trees are generally expected to increase their susceptibility to subsequent infestation by bark beetles. However, not all defoliation events are followed by bark beetle attacks and not all bark beetle outbreaks are preceded by defoliation, therefore the conditions under which these two disturbance types interact remain unclear.

We conducted a study to quantify the potential for two biotic disturbance agents to interact over large forested landscapes in western Canada. Using long-term forest health survey data together with a vegetation resource inventory dataset for the province of British Columbia, we assessed the Douglas-fir – western spruce budworm – Douglas-fir beetle interaction to determine the potential for a defoliator outbreak to predispose conifer stands to attack by an aggressive bark beetle. Evidence suggests that repeated defoliation events have differential impacts on conifer resin defense systems and phloem quantity, whereby defenses decline in the short term in response to defoliation (acute effects) before reductions in phloem thickness manifest (chronic effects). Therefore, using general additive models we tested the prediction that acute defoliation will be most closely associated with subsequent Douglas-fir beetle infestation. In support of our prediction, the probability of Douglas-fir beetle being present increased as western spruce budworm defoliation duration and cumulative severity increased, reaching a maximum at approximately 11 years duration and declining thereafter. We conclude that the pattern of bark beetle disturbances as a function of defoliator outbreaks may be predictable based on detailed information regarding forest conditions and defoliator impacts.

Keywords: *Choristoneura freemani*; *Dendroctonus pseudotsugae*; Disturbance synergy; Outbreaks

Using paleoecology to understand the dynamics of SBW outbreaks and their impact on the landscape

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Abstract

An important challenge we face with climate change is the future dynamics of SBW outbreaks, their impact on the landscape and consequently on forest management. A mean to better understand the dynamics of future outbreaks is to look at the dynamics of outbreaks throughout the Holocene past changing climates. Dendrochronology has been very useful to reconstruct chronologies of SBW outbreaks across its range using living trees and trees from old buildings. However, since the host species don't live so old and that building built with wood are relatively recent in North America, other techniques must be developed to compare chronologies for several centuries and even millennia. To get back further in time, we have developed a paleoecological technique using a new proxy indicating the importance of insect populations: lepidoptera wing scales. These resistant structures are well preserved in lake sediments, produced in phenomenal quantities during outbreaks, and can be analyzed in conjunction with charcoal and pollens. Sediment collectors indicate that the amount of scales deposited annually is directly proportional to the defoliation level surrounding the lakes. Five chronologies of SBW outbreaks covering the whole Holocene has been reconstructed for the first time and compared to fire chronology in different climate environments. All the chronologies indicate an inverse relationship between the frequency of fire occurrence and the frequency of outbreaks occurrence.

Keywords: Srpuce budworm, Paleoecology, Holocene chronologies

Session 25 Role of plant symbiotic organisms on plant resistance to insects and pathogens

Oral communications

Forest *Phytophthora*: ecology, diversity and management

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Abstract

Damage to trees in forests and urban environments caused by *Phytophthora* species are an increasing threat to the vitality and productivity of these ecosystems in many parts of the world, including Sweden. *Phytophthora*, which seriously damage the fine roots of trees and several other woody plants, is efficiently introduced to new environments through imported plant material (mainly non-native plants through the nursery trade). A study was conducted in collaboration with stakeholders in Southern Sweden investigating potential soil microbiome interactions with Phytophthoras. The idea is to develop new biocontrol methods to manage *Phytophthora*-damage in different forest settings. To uncover ecological interactions, the microflora present in soil samples were quantified using a new approach combining high-throughput qPCR Biomark Fluidigm chip technology optimised for oomycete analysis and Next Generation Sequencing. The fungal communities in the soils included a wide range of pathogens and ectomycorrhizal taxa. Amongst the most abundant oomycetes, *Phytophthora plurivora*, *Phytophthora cactorum* and *Phytophthora gonapodyides* were identified. A specific taxon in the genus *Trichoderma* was strongly correlated with scarcity of pathogenic Phytophthora spp. The ongoing meta-analysis of the soil microbiome represents an excellent tool supporting the development of biocontrol strategies for the management of diseases caused by *Phytophthora* spp.

Keywords: Soil microbiome; Forest Phytophthoras; Meta-analysis; Biocontrol development strategies

Fungal symbionts increase growth rate, alter defensive chemistry, and reduce disease severity in five-needle pines

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Abstract

Hundreds of asymptomatic fungal taxa live inside healthy white pine tissues. Recent studies suggest that these fungi can influence the frequency and severity of infections by fungal pathogens such as *Cronartium ribicola*, which causes white pine blister rust. In a full-factorial experiment, we inoculated western white pine (*Pinus monticola*) from six seed families with foliar fungal endophytes (FFE) or ectomycorrhizal fungi (EMF, genus: *Suillus*), as well as a combined fungal treatment (FFE+EMF), and a control treatment (no inoculation). The six seed families consisted of half-siblings and full-sib progeny, with both high and low levels of known disease resistance. Four months post-inoculations, we infected a subset of seedlings from all four treatments with the rust to determine the individual and shared effects of FFE and EMF on resistance to white pine blister rust. We measured tree defensive chemistry (terpenes) for all treatments immediately before, and four months after infection, as disease symptoms began to appear. In trees never exposed to the pathogen, seedling growth rate was higher in all treatment groups compared to controls, indicating a benefit to hosting fungal symbionts. Fungal treatments also increased foliar diterpene concentrations both before and after pathogen infection. This coincided with an initial reduction in bark infections on both mainstems and branches of seedlings. After two years, treatment effects have started to decline, however, the initial reduction in disease severity seen early on may have meaningful impacts in natural settings where pathogen pressure is much lower.

Keywords: *Cronartium ribicola*; ectomycorrhizal fungi; fungal endophytes; *Pinus monticola*

Endophytes in forest protection – a nature-based solution or a new eco-catastrophe?

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Abstract

The potential of endophytic fungi as protective agents in forest trees has been intensively explored during the past decades. Accumulating evidence suggests that several mechanisms exist, potentiating the capacity of endophytes to antagonize pathogens, but we still lack technical solutions to harness this potential into practical forestry. However, while these technical issues related to the use of endophytes in forest protection may be fixed within a reasonable time, the questions of ecological constraints are likely to be harder to resolve. In this presentation, the ecological implications of the use of endophytes in forest protection are discussed, with special attention to the potential risks and their mitigation.

Keywords: Endophytes, forest protection, risks

Plant growth promoting rhizobacteria associated to date palms for controlling pathogens

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Abstract

Date palm (*Phoenix dactylifera*) is one of the most important crops in the south of the Mediterranean (e.g. Tunisia and Algeria) due to fruit production. This plant species is currently affected by different biotic and abiotic factors that are threatening date palms sustainability, including the spread of fungal diseases (e.g. *Phytophthora palmivora*, *Fusarium oxysporum*) and extreme environmental conditions (drought, temperature). In this work, soils of healthy and diseased date palms were compared in terms of bacterial diversity and composition. Bacterial isolates with plant growth promoting characteristics (production of organic acids and siderophores, as well as phosphate solubilization capacity) were selected and molecularly identified by using 16S barcode region. The same soils were used for pursuing a metabarcoding strategy using the bacterial barcode (16S). The results from this work revealed differences in bacterial diversity of healthy and diseased date palm soils. A collection of bacterial isolates was created and will be further used for searching methods that allow date palms to cope with pathogens. The use of native microbial communities to control disease progression and ameliorate stresses, coupled with growth promotion features, could be a promising eco-friendly and sustainable strategy for date palm production.

Keywords: Bacterial diversity; Date palm; PGPR traits

Elucidating the role of foliar fungal endophytes against an insect defoliator in white spruce (*Picea glauca*)

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Abstract

As a wide-spread boreal tree species, the white spruce is under a constant threat by outbreaks of defoliators including spruce budworm (*Choristoneura fumiferana*). Historically, application of microbial insecticides and silvicultural tactics have been used to improve white spruce stand resistance and tolerance against spruce budworms. More recently, variations in chemical defenses and fungal endophytes have received a particular attention in forestry due to their potential roles in herbivory resistance. Currently, we do not know the role of fungal endophytes in white spruce resistance to the spruce budworm, neither how the endopythic communities vary among different populations of white spruce. Here, we characterized the variations in white spruce foliar terpenes and fungal endophytes from a two progeny trial sites in western Canada. The monoterpene, sesquiterpene, and fungal endophyte profiles were varied between the two sites. Furthermore, we tested the impact of host terpenes and fungal endophytes on performance of the budworm larvae on a dose-dependent manner. Briefly, we prepared an artificial diet amended with fungal endophytes and exposed the budworm larvae to fungal volatiles. These results indicate that fungal endophytes have both positive and negative impacts on the spruce budworm performance depending on the species of the fungal endopythes and concentrations. Overall, the outcome of this study can enhance the current knowledge on the microbial and chemical defenses in tree resistance by identifying fungal endophytic communities and their roles in the plant resistance. Furthermore, the results from this study might be a useful component of integrated pest management against the spruce budworm.

Keywords: Fungal endophytes; Insect-plant interactions; secondary compounds, spruce budworm, white spruce

Posters

Variations in the intraradical AMF communities associated with *Cryptomeria japonica* along with altitude and slope gradients

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Abstract

Arbuscular mycorrhizal fungi (AMF) have a broad host range and are ubiquitous in all terrestrial ecosystems, but it was unclear whether the same AMF species colonize the same host plant species at different altitude and slope positions. *Cryptomeria japonica*, an AMF host tree of the Cupressaceae, is one of the most planted trees in East Asia, including Japan, where the natural landscape features mountains and valleys. We collected the roots of *C. japonica* trees in the summer of 2021 to investigate the changes in its intraradical AMF community along with altitude and slope gradients. Using the Illumina next-generation amplicon sequencing targeting the SSU rDNA, we found that the AMF communities in *C. japonica* roots were similar among the altitude and slope positions, regardless of the difference in soil AMF community among sampling positions. The bigger size of the trees in the downslope compared to the middle and upper slope positions may be solely related to water availability rather than effects of the related AMF community, soil pH, or soil EC, which did not vary significantly among positions. These structural and compositional conservations in the intraradical AMF community of *C. japonica* suggested that the host roots were selectively colonized by the same AMF, which have an affinity to the host species, and they can fully get the mycorrhiza-related protection and benefits regardless of the altitude or slope positions.

Keywords: Intraradical, Extraradical, AMF community, Seasonal variations

Enhanced resistance against root pathogens by rhizobacteria in *Pinus densiflora* prior to ectomycorrhiza formation

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Abstract

Plant roots are exposed to various biotic and abiotic stresses, especially pathogen invasion and infection, which causes mortality in the early germination stage. Woody plants, including Fagaceae and Pinaceae, acquire resistance to them by associating with ectomycorrhizal (ECM) fungi. However, there is a temporal gap between germination and ECM symbiosis because ECM fungi can colonize only fine roots branched from lateral roots. Therefore, a defense mechanism to pathogens different from ECM symbiosis should function in the host root prior to ectomycorrhiza formation. In this study, we performed dual- and triple-culture assays in vitro with a *Pinus densiflora* seedling, two root rot pathogenic fungi (*Fusarium oxysporum* and *Trichoderma* sp. strain PS-2), and three bacterial isolates (*Bacillus cereus*, *Paraburkholderia sediminicola*, and *Streptomyces* sp. strain STS-13) to evaluate the influence of rhizobacteria on host tree resistance to the pathogens at the early germination stage. In the dual culture antagonism tests, *P. sediminicola* and *Streptomyces* sp. strain STS-13 significantly suppressed the mycelial growth of the two pathogens, while *B. cereus* showed no inhibitory effect. *Paraburkholderia sediminicola* showed the highest efficacy when it was inoculated into *P. densiflora*, mitigating root browning and elongation inhibition and dampening the pathogen's penetration into the primary root. Whereas *Streptomyces* sp. strain STS-13 largely prevented fungal infection but strongly inhibited host root elongation. Moreover, microscopic observation indicated that root extracellular traps (RETs), consisting of cells shedding from the root cap and exudates such as root mucilage released from them, contributed to the colonization of these bacteria into the rhizosphere. Our results suggested that rhizodeposition with rhizobacteria functions as the first line of defense response against root pathogens in the early germination stage of a pine seedling.

Keywords: Japanese red pine; root rot fungi; root extracellular traps (RETs); rhizobacteria; *Paraburkholderia*

The fungal microbiome associated with *Phytophthor*a-lesions is shaped by the pathogen presence and time of sampling

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Abstract

Forest diseases are an increasing threat to forest ecosystems, causing a huge economic and environmental impact on forests worldwide. One of the most serious concerns in European forests and nurseries is the upsurge of damages caused by invasive, fungal-like, oomycetes *Phytophthora* species. Recent research has revealed the importance of microorganisms for the plant holobiont, i.e. the functional entity composed of the plant and its associated microbiome; endophytic (internal) and epiphytic (external) microbes. In our study, we have tested whether the lesion produced by *Phytophthora* infection could be related to the quality or quantity of endophytes. European beech seedlings (*Fagus sylvatica*, 2yr-old seedlings) were inoculated with 4 isolates of *P. plurivora* and 4 isolates of *P. cambivora* to induce lesion formation (control plants had agar without inoculum) in the greenhouse. Half of the plants were harvested at week 3 and the other half at week 8 post-inoculation. Plants were brought to the lab where the necrosis length was measured and total DNA was extracted and sent for the sequence with the Illumina MiSeq platform to characterize the fungal community. Our results showed that necrosis length was dependent on the isolate used and the time where the plants were sampled (week 3 or week 8). The community composition of the fungal microbiome was also dependent on the presence of the pathogen since control plants differed from inoculated ones. The time of sampling was also a significant factor, indicating that the lesion length was correlated with the fungal community composition. The results confirmed our initial hypothesis that the presence of the pathogen changes the fungal community composition around the lesions and over the time of infection.

Keywords: Fungal microbiome, endophytes, forest pathogens, biocontrol, lesion

Session 26 Bark and ambrosia beetles

Oral communications

Mass trapping of bark beetles (Coleoptera: Curculionidae, Scolytinae) in the pine forest of Samothraki (Greece) – a preliminary insight

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Abstract

Bark beetles (Scolytinae, Curculionidae, Coleoptera) comprise some of the most notorious pest of forests worldwide. The frequency and intensity of their population outbreaks have been considerably increased in the last decades, resulting in extended damages to forest and natural reservoirs. Due to their biology and intensity of population outbreaks, the only plausible and efficient management approach is to log and remove dead and infested trees. By doing so, both the available food sources and insects' population level are drastically reduced.

Recently, pine trees (*Pinus brutia*) infested by different bark beetle genera (e.g., *Tomicus* sp., *Orthotomicus* sp., *Pityophthorus* sp.) have been detected in the pine forest of Samothraki (Greece). The unique traits of this forest (natural isolation from other pine-covered areas, relatively small area) allow the establishment and assessment of mass trapping to control bark beetles. In total, 77 Theysohn® traps are installed and equipped with kairomone lures (KaiPin ®), that will be replaced every 40 days to retain their attracting capacity. After only two sampling periods (June 2021 / August 2021), more than 15.000 bark beetle individuals belonging to six different Scolytinae genera have been collected. The ultimate goal of this 2-year project, is to drastically suppress the population of bark beetles. Simultaneously, we will assess the potential of mass-trapping as a complementary and sustainable management tool, that in concert with sanitation loggings, will effectively protect similar forests from bark beetles.

Keywords: Scolytinae; Samothraki island; *Pinus brutia*; Greece; *Orthotomicus* sp.

Ips typographus damages nationwide patterns and causes in Finland

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Abstract

The European spruce bark beetle, *Ips typographus* (Coleoptera: Curculionidae), is the most important forest insect pest in Central and Northern Europe as it causes destructive damage in Norway spruce (*Picea abies*). As a result of ongoing climate change, its damages have increased and shifted northwards, affecting wood production and other ecosystem services. Therefore, we need locally adapted forest management practices, which consider increasing risk of damages and enhance preparedness.

To find out patterns and causes for *I. typographus* damages in Finland, we compared forest and stand characteristics, and environmental factors in non-attacked *P. abies* stands with those in stands harvested due to *I. typographus* damage. Further, we analysed the distance between the nearby (0-5 km) recent clear-cuts (age 0-4 years) and the stands harvested due to wind or I. typographus damage. For this, we used forest-declaration-use, showing bark beetle damage maps and forest-stock data freely accessible from the Finnish Forest Centre (Metsäkeskus).

During 2012–2020, the total number of declarations of harvesting operations due to *I. typographus* damage were 4691, with an epidemic phase (2013–2016), aggregated in south-eastern Finland and showing a northward distribution shift. We found evidence that bark beetles don't select *P. abies* stands at random, in terms of "forest variables" (soil type, fertility class, development class or mean diameter of tree stand, and distance to recent clear-cuts). Also, the "beetle pressure" variable "distance to earlier attacks" in some cases increased the frequency of *I. typographus* damages, unlike distance to earlier wind damages — probably due to the quick remotion of wind damaged trees, prompted by law.

A substantial part of the *P. abies* stands may be vulnerable to *I. typographus* attacks. Our findings may provide necessary support for adapting forest management practices, which consider increasing risk of outbreaks and enhance preparedness.

Keywords: beetle pressure variables; climate warming; forest variables; *Picea abies*

Do pheromone traps help to reduce new attacks of *Ips typographus* at the local scale after a sanitary cut?

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Abstract

The spruce bark beetle, *Ips typographus*, is causing severe economic losses during epidemic phases triggered by droughts and/or windstorms. Sanitation felling and salvage logging are usually the most recommended strategies to limit the damages. However, any additional control method to limit the economic impact of an outbreak would be welcome. In this respect, the efficiency of pheromone trapping is still controversial or poorly documented.

In a two-year study (2020-2021), at the peak of a severe outbreak in Belgium, we quantified the wood volume and presence/absence of new attacks at 126 sites attacked during the previous year and within 100 m from the initial attack. We compared three treatments, randomly allocated to experimental sites: 1) three crosstraps baited with pheromones, 2) one tree-trap baited with pheromones and treated with an insecticide and 3) control sites with no trapping device.

We found a strong decrease of the attacks relative to the previous year in all treatments, including the controls (more than 50% of the control sites had no new attacks). In both years, new attacks were more frequent (presence/absence) in sites with crosstraps than in sites with a tree-trap and in control sites. In 2020, the attacked volumes were slightly higher in sites with crosstraps than in control sites and no significant difference was found with tree-trap sites. In 2021, there were no significant differences between the volumes attacked in the control sites, crosstraps sites and tree-trap sites.

Overall, we found no evidence in favor of the efficacy of pheromone trapping to reduce Ips typographus damages at the local scale when combined with sanitation felling and during a severe outbreak. The use of baited crosstraps could even be hazardous, as it seemed to increase the occurrence of new attacks. The exact impact of sanitation felling remains to be confirmed, but it likely contributed to the important decrease in attacks relative to the previous year.

Keywords: Pest management, Pheromone trap, *Ips typographus*

Chemoecology of verbenone: Effects on the colonization behaviour of two sympatric bark beetle species on Norway spruce

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Abstract

The European spruce bark beetle (*Ips typographus*) and to a lower extent the six-toothed spruce bark beetle (*Pityogenes chalcographus*) are the most important pests on Norway spruce (*Picea abies*) in Europe. In the last four years of extraordinary drought and heat (2018-2021), sanitation and salvage logging claimed more than 7.5 M cubic meters in the state of Baden-Württemberg (SW-Germany) alone. During endemic phases, disturbance events like windthrows can be a starting point for mass outbreaks. The volatile compound verbenone is a putative component of the anti-aggregation pheromone of *I. typographus*. It inhibits the attraction of both beetle species to their own aggregation-pheromones. Here, we present results of a set of experiments aiming at protecting windfelled spruce trees from bark beetle infestation using SPLAT®Verb, a novel technology to apply verbenone for long time spans in the field. SPLAT®Verb has been repeatedly shown to protect pine trees from the mountain pine beetle (*Dendroctonus ponderosae*) in the US. We monitored spruce bark beetle infestation in control and verbenone treated spruce trees in weekly intervals at study sites in the Black Forest (SW Germany) and analysed the gallery systems at the end of the experiment. We then studied the effect of stem-wise (2 years) or area-wise (1 year) verbenone application on the infestation probability and density of both beetle species and their competition for breeding habitat. Analysis of air samples allowed a spatio-temporal description of the verbenone distribution around the dispensers in the field. This approach was supplemented by a study on the verbenone emission of beetle infested spruce stems under controlled conditions. Despite heavy infestation pressure in some years and rather low effect sizes, the treatment with SPLAT®Verb reduced the infestation probability and density of both bark beetle species and may thus provide an additional tool for the future management of spruce bark beetle in Europe.

Keywords: bark beetle management, semiochemicals, spruce bark beetle, verbenone, windthrow

Bark beetle outbreaks and meta-population dynamics of woodpeckers in Switzerland

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Abstract

Resource pulses can affect animal populations in various ways. One such pulse is the increased availability of spruce bark beetle (*Ips typographus*), the most common forest pest in Central and Northern Europe, following disturbances such as storms and dry summers, and the subsequent increase of other wood-boring insects. Woodpeckers are considered important predators of bark beetle, but their relationship has not been studied thoroughly. Therefore, we investigated the bark beetle-woodpeckers system using a multi-decade long dataset to understand whether increases in population size of woodpeckers can be related to bark beetle outbreaks, and whether the relationship is density-dependent. We also investigated the role of standing deadwood and salvage logging as moderators of woodpecker responses to bark beetle outbreaks. We infer on the importance of deadwood for woodpecker per se, and as a pulsing resource linked to bark beetle outbreaks. We expect that bark beetle outbreaks have positive direct effects on the abundance of woodpecker species over short time periods. Our model can be used to estimate the role of bark beetle outbreaks in influencing bird population trends and whether birds contribute to stabilizing or even controlling bark beetle infestations over time.

Keywords: forest pest; resource pulse; prey-predator

Assessing efficacy of vegetation management to reduce tree mortality caused by bark beetles in the western United States

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Abstract

Forest trees in the western United States have experienced widespread mortality due to bark beetle outbreaks over the last two decades. While vegetation management has mitigated bark beetle-caused tree mortality in small stands, the efficacy of these treatments at larger scales is poorly understood. This study aims to determine if thinning treatments reduce bark beetle-caused mortality at larger spatial scales, especially at the scale of forests and geographic regions. We focused on two of the most prevalent and productive conifer species in western forests: ponderosa pine (*Pinus ponderosa*) and douglas-fir (*Pseudotsuga menziesii*) which are predominantly attacked by mountain pine beetle (*Dendroctonus ponderosae*) and douglas fir beetle (*Dendroctonus pseudotsugae*), respectively. The study was conducted within national forests in eight states (AZ, CO, ID, MT, NM, SD, UT, WY) in the western U.S. Within each forest, four sites were selected in areas that had been exposed to bark beetle pressure, were free of fire damage, and had been treated within the last 15 years. At each treated and corresponding untreated site, tree mortality and growth data were measured in seven sample plots. A total of 96 pairs of sites within 24 forests were sampled during 2021-2022. We found that in drier areas of the southwestern U.S. there were minimal differences between treated and untreated sites, while in the remainder of sampled western states treatments reduced focal tree mortality. Our results demonstrate that for the majority of the western U.S. forest thinning treatments reduce bark beetle-caused tree mortality at larger spatial scales, although additional environmental factors in the southwest U.S. may reduce the efficacy of such treatments.

Keywords: Vegetation management; bark beetles; tree mortality; *Pinus ponderosae*; *Pseudotsuga menziesii*

The effect of the new forest gap creation on Norway spruce phloem and airborn monoterpene composition and host acceptance by *Ips typographus*

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Abstract

The formation of the new opened Norway spruce forest edge changes the climatic conditions. Trees are suddenly exposed to higher sunlight radiation and higher temperatures, resulting in higher emissions of primary bark beetle attractants. Therefore, new forest edges are thought to have a higher predisposition for the infestation by bark beetle *Ips typhographus* (L.), the most important pest in European Norway spruce forests. Our work aimed to understand better the mechanisms behind these phenomena that are still not fully known. Our objective was to find the differences in (i) bark monoterpene volatiles, (ii) internal phloem monoterpene composition and (iii) drilling activity of bark beetles between spruce trees at the new created forest edge (FE) and trees in the forest interior (FI). Our research took place one month after creating the new forest edge in June 2021 in a 90-year-old healthy Norway spruce (*Picea abies* (L.) Karst.) stand in the Western Carpathians, Central Slovakia, Forest district Očová. The set-up included two groups of trees (FE and FI), each consisting of six spruce trees and together 24 inoculation boxes were installed, two boxes per tree. After 24-hour inoculation of 10 bark beetles (five males and five females) in each inoculation box, we found a statistically significant difference (t-test, $p < 0.037$) in the mean values of drilled bark beetle individuals per one tree between FE and FI: 2.58 ± 0.7 and 0.83 ± 0.44 (±SE), respectively. Total drilling activity was 17.5 %. Solid-phase micro-extraction (SPME) samples of spruce bark volatiles obtained from the internal space of inoculation boxes and hexane extracts of phloem samples extracted 1 meter above the inoculation boxes were analysed for their monoterpene content using gas chromatography and mass spectrometry (GC–MS). SPME analysis showed that the trees at FE released significantly higher amount of monoterpenes alpha-pinene, beta-pinene, beta-phellandrene compared to the trees in FI. Also we found increased values of delta-3-carene, limonene and myrcene at FE. Similarly, spruce phloem internal composition analysis showed higher concentrations (from 1.2 to 8.0 fold) of most represented monoterpenes alpha-pinene, beta-pinene, beta-phellandrene, delta-3-carene, limonene, myrcene and sabinene at FE compared to FI. We can conclude that after creating a new forest edge, the trees at FE change in monoterpene amounts in the phloem tissues, more monoterpene volatiles are released and are more attractive for bark beetles during their host acceptance. Our work supports the hypothesis that the newly created forest edges could have an enhanced predisposition to bark beetle attacks in compared to closed forest stands.

Keywords: Host acceptance; *Ips typhographus* (L.); Monoterpenes; New forest edge stand; *Picea abies* (L.) H. Karst.

Susceptibility of forest stands to the spruce bark beetle

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Abstract

The spruce bark beetle (*Ips typographus*) is one of the most severe pest insects, and the current outbreak in Europe is the largest of its kind. Both the frequency and magnitude of spruce bark beetle outbreaks are expected to increase in the future, mainly as a result of a warmer climate. However, not all forest stands are equally susceptible to spruce bark beetle attack. Moreover, forest management actions may alter forest stand susceptibility. To support decision making in forestry, we have developed a spruce bark beetle susceptibility index. The index is based on stand characteristics known or hypothesised to affect bark beetle susceptibility, and can be implemented in forest decision support systems. The index can be used to compare the susceptibility of a stand to bark beetle infestation, relative stands with other properties (such as tree species composition or soil moisture) or under different management or climate scenarios. We demonstrate the use of the index by implementing it in Heureka and running different management scenarios, assessing how that affects the index as well as important forestry variables such as harvest volume. We simulate three scenarios: current practice, longer rotation time, and mixed forest for an area of 2451 ha consisting of 751 stands. We aim to continue developing the index, first by using real data (collected during the ongoing outbreak in Sweden) to validate the it. We will analyse how different stand properties relate to occurrence and rate of infection, and analyse if that corresponds with stands that score a high susceptibility index in simulations.

Keywords: Insect pests; *Ips typographus*; *Picea abies*; Stand susceptibility

Platypus cylindrus F. (Coleoptera: Curculionidae) attack dynamics in cork oak stands (*Quercus suber* L.), on Ponte de Sor region

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Abstract

The cork-oak forests cover 737.000ha, mainly in southern regions of Portugal. Cork oak stands are a complex ecosystem that after the 1980's started showing a decaying process. Climate changes and biotic factors are the main causes, with special emphasis on the ambrosia beetle Platypus cylindrus. This pest was associated to dead or weakened trees, however in the last decades began to attack apparently healthy trees.

To mitigate *P. cylindrus* impact on cork oak forests, it is important to better understand the dynamics and patterns of the attacks in space and time. For this purpose, two plots with 2 ha each, were delimited in three cork oak stands (6 plots overall) at Ponte de Sor, a strong cork producing region. The selection was made according to the sanitary conditions and the incidence of *P. cylindrus* attacks. A total of 1222 trees were geo-referenced and dendrometric, exploitation and phytosanitary variables were assessed twice a year, from 2018 to 2021.

The plots revealed an heterogeneous structure: Tree Breast Height Perimeter from 11 cm to 318 cm, exploitation (debarking in 2018, 2019 and 2020) and phytosanitary conditions (most trees showing defoliation level between 11-60%). Concerning *P. cylindrus* attacks, it was present in 2.9% of trees before 2018, and in the following years revealed a significant cumulative increase: 4.4% in 2018, 8% in 2019, 12,3% in 2020 and 20.5% in 2021. The survey also revealed that spatial distribution of this pest attacks is not clustered, based on previous attacks, but associated with other variables such as higher defoliation degree, bigger tree dimension, recent debarking activity and the existence of large wounds.

This data provides novel insights and confirms the trend suggested on previous studies. Moreover, it provides fundamental knowledge to develop a more adequate management strategy that could directly improve cork oak stands sanitary conditions.

Keywords: Ambrosia beetle; Economic impact; Oak decline; Population ecology

Posters

Hazard rating of Japanese larch forests for larch bark beetle (*Ips subelongatus*) using self-organizing map (SOM) and random forest approaches

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Abstract

The objective of this study was to evaluate the hazard ratings of Japanese larch forests to Ips subelongatus by considering environmental factors. Factors related to thinning, geographical factors and forest stand condition were collected at 16 study sites in Japanese larch stands where thinning was implemented. Using machine learning approaches, self-organizing map (SOM), one of the unsupervised classification methods, and random forest model, which predicted the probability of the tree mortality caused by *I. subelongatus*, hazard rating of Japanese larch forests after thinning was analyzed. Our results showed that stand age, thinning history, slope orientation, and soil moisture are the major factors influencing on abundance of dead trees killed by the beetles after thinning. There were more dead trees killed by in older forests than in younger forests, suggesting that tree vigor can influence outbreak of the beetles. The slope orientation was related to occurrence of dead trees, which was more on southeast-facing slope than at sites facing other directions. Also, the dead trees were more occurred in the forests with dry soil during the first 3 years after thinning. These results can provide useful information for improved the Japanese larch forests management against *I. subelongatus* after thinning.

Keywords: hazard rating, *I. subelongatus*, self-organizing map, random forest

Selecting modelling techniques for landscape scale analysis of bark beetle outbreaks in conifer forests

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Abstract

It is always a concern which variables and modelling techniques to choose for analyzing bark beetle outbreaks on a larger scale (i.e. several thousand hectares of forests). Some variables are relatively easily available, such as digital elevation model (DEM) and variables derived from DEM (aspect, slope). Other variables can be obtained from local and regional stakeholders such as meteorological stations and forest databases. We combined different combinations of explanatory variables to analyze publicly available spatial data on bark beetle-caused infestations in conifer forests. One of the challenges associated with landscape scale bark beetle outbreaks are the changing conditions during the outbreak, when some variables remain stable (such as elevation) whereas others are hard to model on a larger scale (temperatures, stand characteristics). Several techniques are available for modelling. The basic two concepts are statistical models and machine learning methods. We used surface range envelop (SRE), classification tree (CT), random forests (RF), multiple adaptive regression splines (MARS) and flexible discriminant analysis (FDA). Rreceiver operating characteristic (ROC) were used to assess model performances. We received contrasting results between different modelling techniques and the different years of the analyzed time span (from 2007 to 2014). Higher precisions were received at the beginning of an outbreak (ROC > 0.74) and at the end of the study period (ROC > 0.68) in comparison with lower values of ROC (about 0.5) in the middle of the epidemics.

Keywords: Bark beetles, Modelling, National park, Outbreak, Spruce

Investigating pheromone biosynthesis among life stages of Eurasian spruce bark beetle – *Ips typographus*

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Abstract

The Eurasian spruce bark beetle *Ips typographus* is known for its devasting attack over host tree *Picea abies*, a common conifer in Europe. The beetle's various life stages and respective pheromones production plays vital role in successful attack over the host. The beetle uses various pheromone component (2-methyl-3-buten-2-ol and cis-verbenol) for mass aggregation to overcome the tree defence mechanism. Though the role of pheromones in the aggregation behavior of the beetle is known for few decades now, gene level understanding behind the biosynthesis of these compounds are unknown yet. Our recent findings have paved way for understanding the de novo aggregation pheromone biosynthesis and its possible candidate gene families from the gut tissue from various life stages of *I. typographus* (Ramakrishnan et al 2022). Interestingly, our approach using metabolomic and transcriptomic methods of analyzing the beetle gut tissue for the specific pheromone biosynthesis has revealed the vital life stages and possible gene candidates. Our extensive analysis using quantitative real time PCR have revealed isoprenyl di phosphate synthase (IPDS) as a potential candidate, which have significantly expressed in fed stage where 2-methyl-3-buten-2-ol, a vital aggregation pheromone compound also produced in large amount (238 ng/gut) of I. typographus. This approach would lead us to elucidate the molecular basis of pheromone biosynthesis and the derived knowledge from this study would lead to an eco-friendly pest management for this aggressive pest.

Keywords: *Ips typographus*, bark beetle, pheromone biosynthesis, gene relative expression

Behavioural interaction of spruce oxygenated monoterpenes with *Ips typographus* (Coleoptera: Curculionidae) in field test

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Abstract

Causing a considerable infestation in Europe, Ips typographus has cost only the Czech Republic around 23 million m³ of trees damaged per year between 2015 to 2021. The bark beetle outbreak is attributed to anthropogenic factors and climate changes. According to the hypothesis, the I. typographus presumably possess for selecting of suitable host tree (Norway spruce, Picea abies) a semiochemical communication mediated by host tree VOCs and in the later phase by aggregation pheromones produced by successfully boring pioneer males. With the use of EAG techniques were selected oxygenated monoterpenes and volatile phenolics in the complex VOCs mixture emitted by Norway spruce. The 1,8-cineole, α-terpineol, camphor, carvone, estragole, trans-4-thujanol, terpinen-4-ol, pinocamphone and isopinocamphone are in the spruce bouquet in the low concentration in comparison with the main terpenes but elucidate strong response on *I. typographus* antenna. Their content grows in cut trees, after herbivore/pathogen attacks or in acute drought conditions. In this study, we tested the relevance of these compounds to dispersing *I. typographus* using a long-distance field trapping experiment. The nine compounds in three doses (with the nominal release rate of 0.1; 1; 10 mg/day) were tested in combination with *I. typographus* pheromone in pheromone traps. The number of caught beetle was compared with catches to pheromone only as a reference. Among the compounds tested, the 1,8-cineol in high dose revealed according to previous experiments a significant role as an anti-attractant, with a low catch number of 0.71% (N= 852 SD 60.1) and differing among all the compounds tested, except estragole in low dose with 1.8% (N=2158 SD 142.4). These two compounds attracted significantly less than the control pheromone with 6% (N=7208 SD 308.9). The significance of other tested oxygenated monoterpenes depended on the tested dose. Understanding the scenarios of attraction and anti-attraction of oxygenated monoterpenes and volatile phenolics brings the ecology finding and is a promising cue for developing alternative eco-friendly methodologies in bark beetle management using Semiochemicals.

Keywords: Anti-attractant; Bark beetle; Insect communication; Monoterpenes

Determination of biogenic organic volatiles emitted by bark beetles infested Norway spruce trees

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Abstract

The Eurasian Spruce Bark Beetle (*Ips typographus*, Coleoptera, Scolytinae) is a devastating pest of spruces in the Eurasian forest. Various reasons, such as long-term drought, reduce defence mechanisms in stressed trees and allow to develop of bark beetle outbreaks. Functional prevention of the bark beetle calamities on spruce is the early detection of freshly infested trees based on personal observation by foresters. Then trees must be removed from the forest before the next bark beetle generation will emerge. Here, we present the development of an alternative early attack detection method, based on changes in VOC emission by the freshly infested trees. We optimized tree emission detection in the surrounding model logs in the laboratory by using either the headspace technique to sorb VOC to cartridges or SPME fiber and analyzing them by GC-FID. From the quantity of chosen characteristic compounds (α-pinene, 2-methyl-3-buten-2-ol) was constructed, the 3D VOC cloud. Optimization of technique on the model system will allow the measurement of a freshly infested tree in a real forest, and when compared with non-infested trees, it will have the perspective of being used on the large scale detection along with remote sensing methods.

Keywords: Early attack detection, bark beetle management, VOC, Eurasian spruce bark beetle, *Ips typographus*

Fungal volatiles as semiochemicals for monitoring and management of bark and ambrosia beetle pests

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Abstract

Native and invasive bark and ambrosia beetles present a threat to global forest health and productivity. Semiochemicals are often used to monitor and manage these pests, but these compounds can be ineffective at monitoring low-level populations or in reducing population densities. We tested the hypothesis that fungal volatiles play a role in host colonization and can modify attraction of these beetles to monitoring lures. Over three years (2017-2020) a suite of fungal and other volatiles were tested in trapping experiments in Washington and Indiana states, with the walnut twig beetle (*Pityophthorus juglandis* Blackman) and exotic ambrosia beetles [*Xylosandrus crassiusculus* (Motchulsky), *Xylosandrus germanus* (Blandford), and *Xyleborinus saxesenii* (Ratzeburg)] as the focal species at each location, respectively. Two fungal alcohols, both emitted by a symbiotic fungus, consistently enhanced attraction of walnut twig beetles to their pheromone lure. Conversely, one fungal and two other volatiles reduced attraction to the same pheromone lure. The response of ambrosia beetles differed by species, but all behaviorally active compounds reduced attraction to their current monitoring lure (ethanol). Attraction of *X. crassiusculus* to ethanol was reduced by four fungal alcohols, while attraction of the congeneric *X. germanus* was reduced by three fungal alcohols and one non-host volatile. The attraction of *X. saxesenii* to ethanol was also reduced by two fungal alcohols, although this response was not consistent through all experiments. These findings demonstrate that fungal, as well as host and non-host, volatiles may play a role in improving the efficacy of semiochemical-based monitoring and management tactics for bark and ambrosia beetle pests.

Keywords: Fungal volatiles; Semiochemicals; Monitoring; *Pityophthorus juglandis*; Ambrosia beetles