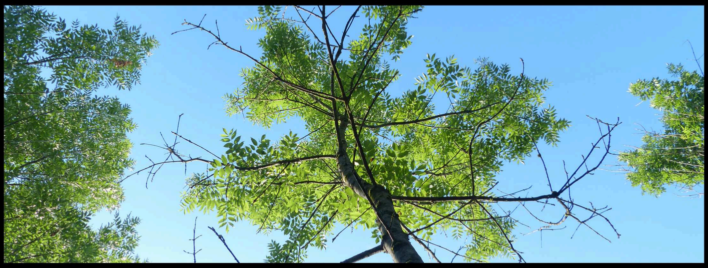
INRA
SCIENCE & IMPACT

correspondant
observateur
département de la santé des forêts

UNIVERSITE D'ORLEANS

5th International Workshop on the
Genetics of Tree-Parasite Interactions
Orléans, France, 23-28 August 2015

Book of Abstracts



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5th International Workshop on the Genetics of Tree-Parasite Interactions

Orléans, France, 23-28 August 2015

Organizers:

Véronique JORGE & Arnaud DOWKIW – INRA

Stéphanie BANKHEAD – Université d'Orléans

François-Xavier SAINTONGE – Ministère de l'Agriculture, Département de la Santé des Forêts

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EDITO

COLLOQUE INTERNATIONAL SUR LA GENETIQUE ARBRES-PARASITES

Du 23 au 28 août 2015 à Orléans

Comment ne pas être fier d'accueillir, en cette terre ligérienne, nourrie par le 1er fleuve sauvage de France et entourée par la plus vaste forêt domaniale de France, le prochain **Colloque International sur la Génétique Arbres-Parasites.**

Du 23 au 28 aout prochains, cette 5ème édition d'une série de rencontres entre éminents spécialistes et chercheurs du monde entier, qui œuvrent pour la préservation de notre éco système, se réunira à Orléans. Leurs réflexions et leurs échanges porteront sur la question cruciale de la protection du patrimoine forestier mondial. Dérèglement climatique, mondialisation qui rendent les arbres plus sensibles aux maladies : les réponses sont difficiles à apporter tant les espèces sont nombreuses et leurs comportements différents selon les climats. Quelle stratégie adopter entre la sélection avec le déploiement d'arbres résistants et la diversité en laissant opérer la nature ? Questions auxquelles la communauté scientifique s'attache actuellement à trouver des réponses durables.

Orléans ne pouvait que répondre présente à cette invitation de par son précoce et solide engagement dans le domaine du développement durable. Dotée d'un Agenda 21 dès 2006, elle est l'une des premières villes françaises à avoir adopté, en 2009, un Plan biodiversité pour préserver, restaurer et valoriser la biodiversité sur tout son territoire et particulièrement en milieu urbain : réalisation d'une trame verte et bleue, sensibilisation du grand public à travers différentes publications, notamment par la charte de l'arbre urbain, prise en compte de l'impact des pratiques sur la biodiversité. En outre, notre ville a été choisie pour accueillir, en 2016, les 6èmes assises Nationales de la Biodiversité.

Grâce à ce terreau fertile, Orléans a vu de nombreux laboratoires de recherche, dont certains instituts de renom tels que le BRGM, le CNRS… et plus particulièrement l'INRA qui conduit des recherches très poussées sur le peuplier, le frêne et la chenille processionnaire du pin. L'INRA qui, en partenariat avec l'Université d'Orléans et le Département de la Santé des Forêts du Ministère de l'Agriculture organise ce remarquable colloque et je tiens ici à les remercier.

Bienvenue à Orléans, Ville d'Art et d'Histoire, Cité Johannique rayonnante qui offre à ses habitants une douceur de vivre remarquable auprès de son fleuve royal, inscrit au patrimoine de l'Unesco. Elle vous dévoilera toute l'élégance d'une ville-jardin au cœur de France.

Bienvenu !

Olivier CARRE
Maire d'Orléans
Député du Loiret

EDITORIAL

INTERNATIONAL WORKSHOP ON THE GENETICS OF TREE-PARASITE INTERACTIONS 23-28 August 2015

Who would not be proud to welcome, to this region of the Loire marked by the longest, untamed river and by the largest forest estate in France, the next **International Workshop on the Genetics of Tree-Parasite Interactions?**

This 23 to 28 August, the 5th meeting of eminent specialists and researchers, all striving to protect our ecosystem, will take place in Orléans. Their reflections and exchanges will focus on the crucial issue of protecting the world's forest heritage. Climate change and globalisation make trees more vulnerable to disease: solutions are difficult to find in the face of so many species and their varied reactions in different climates. How to choose between selective manipulation of resistant species and diversity by giving nature its way? These are questions to which the scientific community is determined to find sustainable answers.

Orléans could only accept such an invitation in light of its early and strong commitment to sustainable development. Endowed with an Agenda 21 as early as 2006, it is one of the first, major French towns to have adopted, in 2009, a Biodiversity Plan to protect, restore and promote biodiversity throughout its territory and particularly in urban environments: implementing the French green and blue framework (*introduced by the French Environment Summit of 2007*), raising public awareness via various publications, notably the urban tree charter, and taking account of the impact common practices have on biodiversity. Furthermore, our town has been chosen to host the 6th National Biodiversity Convention.

Thanks to this fertile ground, Orléans has known many research centres, including renowned institutes such as the BRGM *(Bureau de Recherches Géologiques et Minières)*, the CNRS *(Centre National de la Recherche Scientifique),* – and of course the INRA, which is leading advanced research on the poplar, ash and the pine processionary caterpillar. The very INRA who, in partnership with Orléans University and the Forest Health Department, has organised this remarkable event and I sincerely thank them.

Welcome to Orléans, a town of Arts and History, Joan of Arc's radiant city which offers its residents a remarkably sweet life on the banks of its royal river, now a Unesco world heritage site. You will enjoy all the natural elegance of a town-garden in the heart of France.

WELCOME!

Olivier Carré
Mayor of Orléans
Loiret Member of Parliament

	Monday 24		Tuesday 25		Wednesday 26	Thursday 27		Friday 28	
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SESSION 1: EMERGING or NON-NATIVE pests and pathogens

Saving Our Forests – Developing Durable Resistance to Non-Native Pathogens

Richard A. SNIEZKO

U.S. Forest Service, Dorena Genetic Resource Center, 34963 Shoreview Dr., Cottage Grove, Oregon 97424, USA

World-wide, native forest ecosystems, as well as managed forests, are besieged by non-native, invasive forest pathogens or insects. Infestation by these biotic agents threatens the viability of some tree species, disrupts ecosystems services, and impacts the sustainable production from managed forests for societal needs. Although many of these invasions are human-mediated and extremely destructive, there are actions that society can take to help restore some balance. Once a potentially destructive biotic agent is introduced into a suitable environment, a tree species' first line of defense is genetic resistance. However, in many cases, the frequency or level of this genetic resistance is too low to maintain healthy levels of tree populations in the forests affected. Basic research into resistance and the plant–microbe interactions is important, but applied research and the operational development of resistant populations of a species should proceed concurrently or even without waiting for all the answers from basic research. In at least some species there are forms of both complete and partial resistance. The complete resistance may prevent infection or damage, at least beyond an initial stage and is often due to a single, major dominant gene. It is often referred to as major gene resistance (MGR). In contrast, progeny of parent trees with partial resistance usually show a much more quantitative spread in resistant phenotypes, such as fewer infections, lower severity of infection, latency of infection, and tolerance, than progeny of MGR parents. Tree species are long-lived organisms, and resistance must be durable to have utility. Although MGR is impressive and can be used immediately, it is often ephemeral, being rendered ineffective by the evolution of a virulent race of the pathogen. Partial resistance is often more durable but is more difficult to evaluate, and breeding is often needed to enhance the level. To have a chance of mitigating the damage these biotic agents cause world-wide, the scientific and management communities need to become more efficient in moving applied resistance programs forward. Examples from two of the most advanced applied programs for genetic resistance development will be used to illustrate some of the components of an operational program and where they may be in developing durable resistance.

Keywords

Durable resistance, major gene resistance, partial resistance

Genetic variability for susceptibility to the invasive ascomycete *Hymenoscyphus fraxineus* in a common Ash (*Fraxinus excelsior*) provenance and progeny trial indicates significant potential for breeding

Arnaud DOWKIW[1], MUNOZ F[1], MARÇAIS B[2], BONNE F[3], ROUSSELET V[3], DUFOUR J[1]

[1] Institut National de la Recherche Agronomique, UR AGPF 0588, 2163 avenue de la Pomme de Pin, ARDON CS4001, France.
[2] INRA, Université de Lorraine, UMR 1136 Interactions Arbres – Microorganismes (IAM), F–54280 Champenoux, France
[3] Institut National de la Recherche Agronomique, UE UEFL 1261, 54280 CHAMPENOUX, France

Severe dieback of European common ash (*Fraxinus excelsior*) was first reported in Poland and Lithuania in the early 1990s. It was fourteen years after the first reports that an ascomycete, now referred to as *Hymenoscyphus fraxineus* (Queloz et al. 2011), was finally identified as the primary causal agent (Kowalski 2006). Several findings pledge for the species being of Asian origin and acting as an invasive pathogen in Europe (Husson et al. 2011, McKinney et al. 2012, Bengtsson et al. 2012, Zhao et al. 2013). Detailed quantifications are scarce, but consequences of the disease can be severe. In Lithuania, 10 years after the first report, over 30,000 ha of common ash stands were reported to be affected and mortality was estimated to be 60% state wide while in some parts of the country only about 2% of the trees remained visually healthy (Juodvakis and Vasiliauskas 2002). First detection of the disease in France occurred in 2008, in the North-Eastern part of the country. Since then, the disease has been progressing southwestwards at a pace of approximately 50 km per year. The fungus now occupies half of the country. Eventhough millions of Fraxinus excelsior trees died from the disease, recent results from clonal or progeny trials raised hopes that resistant/tolerant trees could be selected. Significant heritability estimates and provenance and/or family effects seem to indicate that breeding programs could be implemented against the disease (Pliura et al. 2011, Kjaer et al. 2012). The present study is only third to analyze the genetic variability of common ash for resistance/tolerance to *H. fraxineus* using open-pollinated progenies. It is based on a 20-year old field trial with 23 half-sib families from 3 French provenances. The trial is located very close to the place where the disease was first detected in France. Because the stand has been monitored every year since 2010 for disease incidence, spatio-temporal components of disease spread could be taken into account to avoid confounding disease escape and resistance. Contrary to previous studies, a Bayesian approach was used instead of the classical frequentist analysis. Bayesian methods accommodate spatio-temporal structures in a more straightforward way. They are also more suitable for modelling data which deviates from normality as commonly observed during early stages of disease spread. This study is also the first to report on genetic parameters for collar lesions and on the genetic correlation with crown defoliation. Collar lesions due to *H. fraxineus* have been described in France only recently (Husson et al. 2012) and this symptom has certainly been overlooked in countries with a longer infection history.

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Keywords

Fraxinus excelsior, European Ash dieback, resistance, tolerance, breeding, progeny trial, genetic variability.

Hymenoscyphus fraxineus on introduced ash species in Estonia: research in perspective of ongoing COST Action FP1103 FRAXBACK

Tiia DRENKHAN, DRENKHAN R, ADAMSON K

Estonian University of Life Sciences, Institute of Forestry and Rural Engineering, Kreutzwaldi, 5, 51014, Tartu, Estonia

Hymenoscyphus fraxineus is an invasive ascomycetous pathogen that causes dieback of common ash (*Fraxinus excelsior*) in Europe. The fungus originates presumably from East Asia, where it is associated with Mandshurian ash (*F. mandshurica* Rupr.) and Korean ash (*F. rhynchophylla* Hance). In Japan, China and South-Korea, *H. fraxineus* has not caused any losses to these ash species (Zhao et al. 2012, Zheng and Zhuang 2013, Han et al. 2014). The pathogen may have been introduced to Europe with imported seedlings or seeds of the Asian host trees (Zhao et al. 2012). *H. fraxineus* was infected *F. nigra*, *F. pennsylvanica*, *F. americana* and *F. mandshurica* in environment of Estonia (Drenkhan and Hanso 2010). We found that in Estonia the ash species *Fraxinus sogdiana*, growing naturally in the “bottleneck” of that belt, in Central Asia, is susceptible to *H. Fraxineus* (Drenkhan et al 2015). It is the first record of *H. fraxineus* on a Central Asian ash species and was found also in Estonia. The research is supported by Norwegian-Estonian EEA cooperation project (EMP162).

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Keywords

H. fraxineus, ash

Susceptibility of other *Fraxinus* species to *Hymenoscyphus fraxineus* - the origin of the pathogen and its putative future spread

Lene Rostgaard NIELSEN, MCKINNEY LV, KJÆR ED

Geosciences and Natural Resource Management, University of Copenhagen, Rolighedsvej 23, 1958 Frederiksberg C, Denmark

Unintended introduction of organisms to new areas can develop into novel infectious diseases often with substantial consequences to the native flora and fauna. In Europe, the widely distributed native forest tree species (*Fraxinus excelsior*) is currently under threat by an aggressive invasive pathogen, *Hymenoscyphus fraxineus*, throughout most of its distribution area. The pathogen is believed to have been introduced from Asia, and is reported to have a harmless relationship to the native *Fraxinus mandshurica* in Japan. Presently it is unknown to what extent North American species of *Fraxinus* will respond to the pathogen if introduced to North America.We compare the susceptibility of different *Fraxinus* species from Asia, Europe and North America based on a living collection from a Danish arboretum. Health assessment of mature trees in the arboretum revealed different levels of susceptibility among the species. Species from Asia, where *H. fraxineus* is believed to be native, were in general less affected by the disease while both European and North American species showed significantly higher levels of disease symptoms. Same patterns were seen after controlled inoculation with mycelial wood plugs on grafts of the species. Grafts of the different species were also exposed to ascospores of H. fraxineus in a closed chamber, and the level of *H. fraxineus* DNA content quantified with real-time PCR. Only two of the six assessed Asian species showed high levels of *H. fraxineus* DNA in their leaves suggesting that not all Asian ask species serve as host for *H. fraxineus*. However, most of the European and North American species had high levels of *H. fraxineus* DNA in the leaves and/or showed symptoms that resembled *H. fraxineus* caused symptoms. Apothecia development on leaves from the species was detected. Several North American species developed apothecia thus confirming that they can act as host for the pathogen. Or results support an Asian origin of the pathogen and that introduction to North America would have severe consequences for North American *Fraxinus*.

Keywords

Ash dieback, Coevolution, invasive, emerging infectious disease

Update on Breeding and Identification of Emerald Ash Borer-Resistant North American Ash

Jennifer L. KOCH[1], CAREY DW[1], KNIGHT KS[1], POLAND TM[2], RONALD WG[3], HERMS DA[4], MASON ME[4]

[1] USDA Forest Service, Northern Research Station, Delaware, OH 43015 USA
[2] USDA Forest Service, Northern Research Station, Lansing, MI 48910 USA
[3] Jeffries Nurseries Ltd, Portage La Prairie, MB, R1N 3B7, Canada
[4] Department of Entomology, The Ohio State University, Ohio Agricutural Research and Development Center, Wooster, OH 44691 USA

The emerald ash borer (EAB, *Agrilus planipennis*) has killed tens of millions of North American ash (*Fraxinus* spp.) trees since it was introduced into Michigan, USA, in the 1990s. EAB has now been confirmed in 25 states in the U.S. and two Canadian provinces (as of March 2015). Initial attempts to eradicate EAB were unsuccessful and current management strategies are focused on slowing ash mortality (SLAM) and reducing the EAB population through biocontrol. Three EAB parasitoids identified in China are currently being reared and released in the U.S. However, high mortality rates of North American ash trees planted in China have been reported despite the presence of parasitoids and EAB attacks on Asian ash trees are typically limited to stressed trees, indicating that successful control of EAB will also require at least some level of host resistance to achieve the population steady state necessary for long term survival of North American ash.

Our program is currently utilizing both hybrid and traditional breeding approaches to breed for resistant black (*F. nigra*), green (*F. pennsylvanica*) and white (*F. americana*) ash, the most prevalent of the ash species in North America. Hybridization efforts have focused on breeding black ash with the EAB-resistant Asian species, *F. mandshurica* (Manchurian ash) because F1 hybrids, ‘Northern Treasure’ and ‘Northern Gem’ are commercially available. Additional F1 hybrids have been generated as well as putative F2 hybrids (open-pollinated F1) and F1 backcrosses to both parent species. EAB egg bioassays have shown that the F1 hybrids have an intermediate level of EAB-resistance and are currently being used to assess resistance of the putative F2 hybrids. To identify additional EAB-resistant species for use as hybrid parents, we accessioned 15 Asian ash species from a wide geographical and ecological range and established them in research plantings.

Annual surveys of monitoring plots located in heavily EAB-infested forested areas in southeast MI and northwest OH, USA, identified a small number of North American ash trees that maintain a healthy crown for at least two years after 98 % of the ash in the stand have died. These surviving trees were propagated and assessed using bioassay experiments. Adult feeding preference studies identified genotypes that were significantly less preferred than susceptible controls and egg bioassays identified genotypes with significant differences in the number of larvae killed by host defenses, overall larval development and/or larval weight. Replicated clone tests for evaluation of field performance are currently being established.

Keywords

Fraxinus; *Agrilus planipennis*, bioassay

Genomic analysis of bacteria associated with Acute Oak Decline

James DOONAN[1], DENMAN S[2], PACHEBAT JA[3], GOLYSHIN PN[1], MCDONALD JE[1]

[1] School of Biological Sciences, Bangor University, Deiniol Road, Bangor, LL57 2UW, United Kingdom
[2] Centre for Forestry and Climate Change, Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, United Kingdom
[3] Institute of Biological Environmental Rural Sciences, Aberystwyth University, Penglais, Aberystwyth, Ceredigion, SY23 3DA, United Kingdom

Both species of native oak trees in the UK (*Quercus robur* and *Quercus petraea*) are affected by a Decline syndrome termed Acute Oak Decline (AOD). Black weeping patches on the stems of mature trees are the primary symptom of AOD and indicate the presence of necrotic lesions in the underlying tissue. Two newly described bacterial species, *Gibbsiella quercinecans* and *Brenneria goodwinii* are consistently isolated from necrotic lesions. It has been suggested that these bacteria have a central role in tissue necrosis, possibly via genome encoded pathogenicity islands, plasmids or phage encoded enzymes. The aim of this investigation is to identify the presence of genes associated with bacterial pathogenicity. Contemporary sequencing analysis allows high-throughput in silico analysis of novel pathogens providing large volumes of data and the starting point for the generation of hypotheses. This study used the Illumina MiSeq second generation and Pacific Bioscience third generation sequencing platforms, for the de novo assembly of *Gibbsiella quercinecans* and *Brenneria goodwinii*. Resultant assemblies were annotated using the RAST web server, PROKKA, a prokaryote specific annotation tool and the CAZy database. This genomic analysis has revealed a number of potential virulence factors. These include Plant Cell Wall Degrading Enzymes (PCWDE), numerous iron-acquisition siderophores such as enterobactin, virulence mediating quorum sensing systems and assorted secretion systems. Similar studies of bacterial associated plant pathogens have indicated that the synchronous relationship of these genes in for example *Pectobacterium carotovorum* leads to maceration of host cell tissue. The annotated genes encompass a suite of virulence factors providing *G. quercinecans* and *B. goodwinii* with the tools to compromise oak tree health. To ensure the accuracy of the genome annotation, and analyse differential gene expression at various time points of infection, complete mRNA expression analysis (transcriptomics) and laboratory based testing of putative pathogenic genes and operons is currently underway. A combination of second and third generation sequencing analysis has provided substantial evidence implicating *Gibbsiella quercinecans* and *Brenneria goodwinii* as putative pathogenic contributors to the Acute Oak Decline syndrome.

Keywords

Acute Oak Decline, *Brenneria goodwinii*, *Gibbsiella quercinecans*, genomics, virulence factors

Interaction between two invasive chestnut pathogens: does the chestnut blight fungus *Cryphonectria parasitica* benefit from the presence of the gall wasp *Dryocosmus kuriphilus*?

Joana MEYER[1], GALLIEN L[2], RIGLING D[1], PROSPERO S[1]

[1] Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Phytopathology, Zürcherstrasse 111, CH-8903 Birmensdorf, Switzerland
[2] Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Dynamic Macroecology, Zürcherstrasse 111, CH-8903 Birmensdorf, Switzerland

Impact of invasive fungal pathogens and pests on trees are often studied individually, thereby omitting possible interactions. In this study, we investigated the ecological interaction between the chestnut blight fungus *Cryphonectria parasitica* and the Asiatic gall wasp *Dryocosmus kuriphilus*.Both are invasive organisms in Europe where they affect European chestnut (*Castanea sativa*). The gall wasp drastically reduces the production of leaves and fruits thereby weakening the trees, whereas *C. parasitica* causes bark cankers on stems and branches. Field observations in southern Switzerland where both organisms co-occur suggest that the moribund tissue of abandoned *D. kuriphilus* galls can be colonized by *C. parasitica*. From the abandoned galls the fungus may spread into the adjacent twig, leading to the formation of new cankers. Here, we tested the hypothesis that old *D. kuriphilus* galls are frequently colonized by virulent (i.e. hypovirus-free) local strains of *C. parasitica*. For this, we randomly sampled a total of 1973 abandoned galls from 200 chestnut trees located in eight stands and characterized the fungal community on these galls. In total, *C. parasitica* was isolated from 7.2% of the galls and in a stand from 0.4-19.2% of the galls were colonized. Linear regression analysis showed that the incidence of *C. parasitica* on the galls significantly correlated with the time period since the appearance of *D. kuriphilus* in a stand. All but one *C. parasitica* cultures were virulent, even in stands where hypovirulence is well established. Consequently, abandoned galls may really act as a source of virulent inoculum of *C. parasitica* which may increase the damages caused by chestnut blight. The same vegetative compatibility types occurred in the cankers on living trees and on the galls, indicating that the latter are colonized by local strains of *C. parasitica*. From the abandoned galls several other fungal species were isolated, most of them being known saprotrophic or endophytic fungi. The predominant species was the chestnut endophyte *Gnomoniopsis castanea* whose abundance influenced negatively that of *C. parasitica*.

Keywords

invasive organisms, interactions, chestnut blight, gall-inducing insect, fungal community, Gnomoniopsis castanea

The causes of the reemergence of larch canker disease in Central France: can we decrease health risk by planting resistant provenances?

Cécile ROBIN[1], FEAU N[1], WAGNER S[1], FABREGUETTES O[1], PIOU D[1,2]

[1] INRA, UMR 1202 BIOGECO, F-33612 Cestas Cedex France
[2] French Forest Health Department (DSF), F-33612 Cestas Cedex, France

To reduce the vulnerability of forest plantations, forest managers may use plantation sources known for their resistance or tolerance to a given parasite. In Europe, one example of such risk management strategy is provided by the plantations of larch (*Larix decidua*), very susceptible outside of its natural range to the fungus *Lachnellula willkommii*, the causal agent of larch canker. It appeared, after many provenance trials, that alpine provenances are more susceptible than provenances of Central Europe. For nearly 20 years, the European larch has been planted extensively in France in the Massif Central. In accordance with the recommendations, managers required provenances from Central Europe, especially provenances from Sudetenland, located between the Czech Republic and Poland. Despite this, a high incidence of canker symptoms was recently observed in many planted stands. Because the *Lachnellula* genus includes many cryptic species which can develop on larch, identity of the causative agent of cankers observed in this outbreak had to be confirmed. This was done by developing genetic markers which allowed *Lachnellula* species discrimination by using DNA directly extracted from apothecia. An epidemiological survey carried out on 55 larch plantations revealed that disease prevalence was partly explained by site (humidity) and host factors (age), but not by the genetic origin of trees indicated by the provenance certificates. We then used microsatellite markers recently developed for *L. decidua* to check the origin of the trees planted in infected stands (Wagner et al. 2012). Seventeen plantations consisted partly or entirely of larch alpine origin whereas only eight plantations consisted partly or entirely of larch Central European origin. These latter plantations were lightly impacted by larch canker disease, confirming the best resistance of this origin.The discrepancy between origin certificates and genetic results can be explained by the within-range translocations and associated admixture which have been recently evidenced in *L. decidua* (Wagner et al. submitted). This case study illustrates the need for better traceability of forest reproductive material to manage a health risk by choosing resistant sources.

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Keywords

Lachnellula willkommii, population genetics, resistance

Interactions between two invasive *Phytophthora* species and the defoliator *Lymantria dispar* mediated by oak trees

Slobodan MILANOVIĆ[1,2], NAVAZO G[3], SOLLA A[3]

[1] Faculty of Forestry, Belgrade University, Kneza Višeslava 1, 11000 Belgrade, Serbia (slobodan.milanovic@sfb.bg.ac.rs);
[2] Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemedelska 3, 61300 Brno, Czech Republic (slobodan.milanovic@mendelu.cz)
[3] Ingeniería Forestal y del Medio Natural, Universidad de Extremadura, Avenida Virgen del Puerto 2, 10600 Plasencia, Spain

Oak decline is a complex phenomenon that requires the combination of several factors to occur. Although declining trees are often damaged by several biotic stressors, the interaction among these organisms has not been investigated. The talk provides evidence about interactions between two pathogenic *Phytophthora* species and the gypsy moth defoliator (*Lymantria dispar*) mediated by oak trees. In a first experiment, the suitability of Q. rubra leaves to L. dispar was assessed in trees infected by *P. plurivora* and trees free of infection. In choice tests, the consumed area of leaves from trees infected by *P. plurivora* was four times larger than that from non-infected trees. Larval performance was best if *L. dispar* was fed by leaves from *Phytophthora*-infected trees. These differences in preference and performance were explained by increased values of N, soluble protein, and water content observed in the leaves of infected trees (Milanović et al. 2015). In a second experiment, *Q. ilex* plants were untreated (C), defoliated by *L. dispar* (L), or infected by *P. cinnamomi* (P). Twenty days later plants were treated again, so that nine scenarios (CC, LC, CL, LL, PC, CP, PP, LP, PL) were tested. Final plant mortalities were 8, 3, 3, 21, 22, 27, 44, 93 and 31%, respectively, thus root infections preceded by defoliation cause a significant synergistic effect on plant mortality. In a third experiment acorns from declining *Q. ilex* trees naturally infected with P. cinnamomi and from non-declining trees free of infection were germinated. At the age of 6 months, seedlings were individually defoliated by larvae of *L. dispar*. Defoliation was more intense in seedlings obtained from healthy trees than in seedlings obtained from declining-infected trees ($P<0.01$). The first two experiments provide evidence that tree-mediated cross-effects may provide feedback to oak decline processes. Results from the third experiment indicate that the offspring of *Phytophthora*-infected trees are less suitable for *L. dispar* than the offspring of healthy trees, suggesting that a kind of transgenerational induction of resistance may occur.

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Keywords

Gypsy moth, non-native invasive pathogen, oak decline, trophic interactions

Heritable genetic variation in response to *Dothistroma* needle blight in native Scottish Scots pine (*Pinus sylvestris* L.)

Annika PERRY[1], WACHOWIAK W[1], BROWN A[2], COTTRELL J[3], ENNOS R[4], CAVERS S[1]

[1] Centre for Ecology and Hydrology, Bush Estate, Penicuik, Midlothian, EH26 0QB, UK
[2] Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK
[3] Forest Research, Northern Research Station, Roslin, Midlothian, EH25 9SY, UK
[4] Institute of Evolutionary Biology, University of Edinburgh, Ashworth Building, King's Buildings, Edinburgh, EH9 3JF, UK

The threat from pests and pathogens to native and commercially planted forest trees is unprecedented and expected to increase under climate change. How trees and forests respond to threats depends in large part on their adaptive capacity: their ability to adapt to changes in their environment. This in turn is determined largely by naturally present resistance mechanisms and, crucially, the extent to which variation in resistance is under genetic control. The most significant threat to Scots pine is currently *Dothistroma* needle blight (DNB), a foliar pathogen causing growth retardation and mortality in severe cases. A progeny-provenance trial of four-year-old *P. sylvestris* trees comprising six Scottish provenances each with 3-5 families in 7 blocks was artificially inoculated using a single isolate of *Dothistroma septosporum*. DNB severity, defined as the percentage of total needles that were not green and healthy, was measured regularly during the 61 days following artificial inoculation when plants were retained in conditions ideal for infection development (warm with high humidity and leaf wetness). Results showed that there is huge variation in the severity of symptoms both within and among provenances and families. Provenance means of DNB severity correlate negatively with key water-related environmental variables at the site of origin: trees from sites with wet climates (high suitability for *D. septosporum* and development of DNB) are less susceptible to DNB than those from drier climates. This relationship suggest that *D. septosporum* may be endemic to Caledonian pinewoods in Scotland: Scots pine provenances have had time to evolve in response to pathogen pressure, particularly in areas where environmental conditions have meant that pathogen pressure is high. Furthermore, there are significant differences in DNB severity among families within provenances indicating that variation in resistance is heritable, with high estimates of narrow sense heritability (0.38 to 0.75) and evolvability (genetic coefficient of variation; 23.47), providing additional support that *D. septosporum* is endemic to Britain and has co-evolved with native Scots pine. These data tentatively suggest that native Scots pine provenances contain the necessary genetic diversity to allow an increase in their resistance to *D. septosporum* to occur by adaption through natural selection in response to elevated prevalence of this pathogen.

Keywords

Scots pine, *Pinus sylvestris*, *Dothistroma septosporum*, artificial inoculation, adaptive capacity, heritable, resistance

Asymptomatic potted plants for planting carry invasive soil-borne pathogens

MIGLIORINI D[1,2], **Luisa GHELARDINI[1],** LUCHI N[1], SANTINI A[1]

1 Institute of Sustainable Plant Protection, C.N.R., Via Madonna del Piano, 10, 50019 Sesto fiorentino, ITALY.
2 DiSPAA, Piazzale delle Cascine, 28, 50144 Firenze, ITALY

The most common pathway for the movement of plant pests across boundaries is the trade of live plants, especially pot ornamentals. Invasive soil-borne pathogens carried in pot plants have more chances to survive transportation, and may more easily become established once at destination. The EU has an open phytosanitary system, under which a commodity that is not specifically regulated can be imported. Inspections are concentrated on a small number of economically important plant pests, and limited to visual check of the crown. Inspections fail to detect other pests; especially if these are internal to the tissues or in the soil; if plants are asymptomatic or incipient symptoms are limited to the roots. We have chosen *Phytophthora*, a soil-borne pathogen known worldwide to be particularly harmful, but poorly regulated in Europe, to illustrate the risk inherent to nursery pathway. Aim of this paper was to assess the level of infestation by *Phytophthora* in nursery ornamentals largely traded from and within Europe as plants for planting. Since phytophthoras are not easily isolated, we developed a real-time PCR assay based on a genus-specific TaqMan MGB probe in order to detect small quantities of DNA of the pathogen from plant tissues and soil before symptoms occurrence. We also identified *Phytophthora* species by isolation and ITS sequencing. *Phytophthora* was detected in 87% of the pots tested through qPCR and in 70% of the asymptomatic pot plants. Pot plants in soil can carry several *Phytophthora* species without showing any external symptom.The results of this study claim for a more careful European legislation on the trade of woody plant in pot. Since eradication of soil-borne organisms is difficult or impossible, prevention of introduction is the only advisable measure against spread of these pathogens

Keywords

early detection, international trade, invasive microorganisms, molecular diagnostics, nursery pathway, *Phytophthora*, qPCR, soil-borne disease, soil pathway

Sub-lethal effects of genetic resistance and pathogen infection on host performance

Anna W. SCHOETTLE[1], VOGAN PJ[1,2]

[1] USDA Forest Service, Rocky Mountain Research Station, 240 W. Prospect Rd, Fort Collins, CO USA; aschoettle@fs.fed.us
[2] Mountain Studies Institute, Silverton, CO USA

The strong directional selection pressure on native host populations imposed by introduced pests and pathogens, or the management to mitigate them, may have lasting genetic and performance effects on host species that need to be considered when planning restoration strategies in a changing climate. It is well known that disease resistance to native pathogens can carry a strong fitness cost to the host in the absence of the pathogen. However, we hypothesize that in the absence of the pathogen, genetic resistance to a non-native pathogen, which the host species has not co-evolved, may be neutral or carry a benefit to the host via a function that offers adaptive benefit for environmental factor(s), biotic or abiotic, under which it did evolve. In the presence of the pathogen, native or non-native, there is often a disease resistance – carbon relations trade-off. Working with the *Pinus-Cronartium ribicola* pathosystem, we tested for (1) constitutive difference in stress tolerance and growth traits between susceptible and resistant seedling families as well as for (2) carbon costs of resistance expression following pathogen infection. While resistance to *C. ribicola* is the most important adaptive trait determining the sustainability of North American white pine populations into the future, the loss of other traits correlated with disease susceptibility and the altered physiological performance of individuals expressing disease resistance may shift both the fundamental and realized niche of the pine host species. This research highlights how plant adaptations to biotic and abiotic stresses are intertwined and understanding these relationships can inform successful management of natural populations challenged by non-native pests and pathogens.

Keywords

Cronartium ribicola, *Pinus flexilis*, *Pinus aristata*, cost of resistance, white pine blister rust, trade-offs

Genetic and phenotypic changes in *Cryphonectria parasitica* populations along a colonisation gradient

Cécile ROBIN, SAINT-JEAN G, GUILLEDON F, FABREGUETTES O, DUTECH C

INRA, UMR 1202 BIOGECO, F-33612 Cestas France

Intercontinental transports of plants often result in the accidental introduction and spread of pathogens into new areas. These spillover phenomena, which often cause devastating epidemics, present the opportunity to study evolution during rapidly changing range limits.*Cryphonectria parasitica*, the causal agent of chestnut blight, originating from Asia, is now established into different continents. *C. parasitica* has been officially reported for the first time in 1958 in France, and has rapidly spread in all the southern part of the country. Multiple introductions resulted in changes in the genetic variation and structure of populations in the introduced areas (Dutech et al. 2012). During this expansion in new ranges, *C. parasitica* was subjected to different selection pressures but evolutionary changes have not been yet investigated. We took advantage of *C. parasitica* recent emergence in northern France to study the changes in population structure and in phenotypic traits along this colonization gradient. Our work hypothesis was that northern populations were derived from Southern ones. In such a case, evolutionary changes occurring during this range expansion could be studied by analyzing phenotypic changes between populations with the same putative origins but having experienced or not a range shift. Fifty *C. parasitica* populations (427 isolates) were sampled in Northern France and analyzed with 10 microsatellite markers. In parallel, in vitro growth rate was measured for a subsample. Our results indicate that *C. parasitica* invasion mostly occurred through a clonal expansion since nearly 50% of the isolates belong to dominant clonal lineages established in Southern France (Dutech et al. 2010), and recent Northern populations are related to more ancient Southern ones. Northern isolates had higher in vitro rate growth at 12 and 15°C and smaller rate growth at 28 and 32°C than southern isolates belonging to the same clonal lineages. These results suggest the evolutionary potential of *C. parasitica* lineages along a temperature gradient and that clonal evolution is not a limit for rapid adaptation for invasive fungal species.

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Keywords

chestnut blight, genetic population, evolutionary potential

Is *Pinus pinea* L a host species for pinewood nematode?

Isabel CARRASQUINHO[1], INÁCIO L[1], PEREIRA JP[2], OLIVEIRA V[2], PEREIRA H[2], MOTA M[3]

[1] Instituto Nacional de Investigação Agrária e Veterinária, I.P., Av República, Quinta do Marquês, 2780-152 Oeiras, Portugal
[2] Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda 1349-017 Lisboa, Portugal
[3] NemaLab/ICAAM, Herdade da Mitra, Valverde, Universidade de Évora, 7002-554 Évora Portugal

The pinewood nematode (PWN) is the causal agent of pine wilt disease (PWD) and is ravaging pine forests all over the world. Maritime pine (*Pinus pinaster* Ait.) is the main host in Portugal. Current knowledge provides insufficient information on the susceptibility of the Mediterranean stone pine (*Pinus pinea* L.) to PWN (EFSA, 2012). According to Evans et al (1996), this species is considered to be moderately susceptible whereas maritime pine is susceptible. However, *P. pinea* has not been affected in the mixed stands where the disease was first identified in Portugal. The purpose of this study was to compare the susceptibility to PWN of three years old seedlings from two *Pinus pinea* L. provenances and one *Pinus pinaster* Ait provenance. The trial was established in a greenhouse, using 50 plants from each of the provenances. All the plants were measured for their total height and diameter at the base and inoculated with the PWN. Plants were monitored for symptoms during five weeks, and weekly, ca. 30 plants were randomly selected for extraction and counting of living nematodes, using the modified Baermann funnel method. A preliminary study was also carried out to determine the potential use of near infrared (NIR) spectroscopy to distinguishing between these two pine species and their respective families. Further studies will be needed to assess if NIR is suitable for discriminate the pine susceptibility to PWN and determinate which are the chemical compounds involved.

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Keywords
Pinewood nematode, Pine wilt disease, *Pinus pinaster* Ait., *Pinus pinea* L., Near infrared (NIR) spectroscopy

Effects of different Factors on *Neonectria neomacrospora* infection on cut *Abies nordmanniana* branches

Jing XU[1], NIELSEN UB[1], TALGØ V[2], THOMSEN IM[1], HANSEN OK[1]

[1] IGN, Department of Geosciences and Natural Resource Management, University of Copenhagen, Rolighedsvej 23, 1958 Frederiksberg, Denmark
[2] Bioforsk - Norwegian Institute for Agricultural and Environmental Research, Plant Health and Plant Protection Division, Høgskoleveien 7, 1430 Ås, Norway

Nordmann fir (*Abies nordmanniana*) is a widely used Christmas tree species in northern Europe. Around 65 million commercial Christmas trees are used every year in Europe, half of them Nordmann firs. Since 2009 the bark parasite *Neonectria neomacrospora* has caused a canker epidemic on true firs (genus *Abies*) in Norway and Denmark, including Nordmann fir Christmas tree stands (Talgø et al. 2013). Typical symptoms are dead branches, canker wounds with dead tissue below the bark, heavy resin flow, cream spore pustules (*Cylindrocarpon cylindroides* anamorph) and red fruiting bodies (perithecia) (Talgø et al. 2011). Such novel invasive pathogens are becoming an increasing global problem. Susceptibility of N. neomacrospora has been studied in a set of clones included in the Danish breeding program, and the first evaluations document strong clonal differences in severity of attack. It is therefore hypothesized that it is possible to breed more non-susceptible trees within the species, and integrate this feature into the multi-trait selection of an already tested and best-performing group of plus-trees from the breeding program. Currently, we are aiming at exploring the most efficient methods for screening of non-susceptible trees within the species Nordmann fir. Effects of inoculum age, branch age and size (length and diameter), branch position in the tree (upper, lower part, sunny or shade), inoculation method (bud infection or infection through needle scars of removed needles) and duration and clone resistance on the development of canker wounds caused by *N. neomacrospora* were evaluated. Inoculation tests with *N. neomacrospora* isolates were carried out on cut branches from upper, lower, sunny and shade area in trees of 13 Nordmann fir genotypes. A wound was created by removing a needle from the shoot, and then covered with a potato dextrose agar (PDA) plug (0.5 mm in diameter) from the *N. neomacrospora* culture, according to V. Talgø (unpubl.). Inoculation duration ranged from three to six weeks after inoculation at room temperature. To evaluate infection of N. neomacrospora on the inoculated branch, disease severity was rated based on a 0 to 2 scale, where 0= no visible symptoms; 1= discoloration at inoculation points and 2= canker wounds occurred around inoculation points. Current results show no significant differences in susceptibility to *N. neomacrospora* among different ages, sizes and positions of branches. Longer inoculation duration caused more severe canker wounds. Significant differences in susceptibility to *N. neomacrospora* were found among the 13 tested Nordmann fir genotypes.

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Keywords

Abies nordmanniana, *Neonectria neomacrospora*, canker wound, inoculation method

Genetic variation of *Fusarium circinatum* resistance in maritime pine and its relationship with water stress tolerance

ELVIRA-RECUENCO M[1], GASPAR MJ[2], **Eugenia ITURRITXA[3],** MAJADA J[4], ALIA R[5], RAPOSO R[1]

[1] Departamento de Selvicultura y Gestión de Sistemas Forestales, INIA-CIFOR, Ctra. Coruña km 7.5, 28040 Madrid, Spain
[2] Departamento de Genetica e Biotecnologia, Universidade de Tras os Montes e Alto Duro, Vila Real, Portugal
[3] NEIKER, Granja Modelo-Arkaute, Vitoria-Gasteiz, Spain
[4] CETEMAS, Finca Experimental La Mata, Principado de Asturias, Spain
[5] Departamento de Ecología y Genética Forestales, INIA-CIFOR, Ctra. Coruña km 7.5, 28040 Madrid, Spain

Fusarium circinatum, which causes pitch canker disease in *Pinus* species, is an exotic pathogen of recent introduction in Spain threatening Maritime pine (*P. pinaster*) stands. To predict the impact this disease will have on maritime pine, we examine the host resistance and the genetic architecture of this trait. Resistance phenotyping was done in a provenance/progeny trial, on three-year-old seedlings artificially inoculated with the pathogen and maintained under controlled environmental conditions. A total number of 700 ramets were assessed, distributed in ten provenances, with a total of 47 families, and 2 to 5 halfsibs per family. High genetic variation was found at the three hierarchical levels studied: provenances, family and individual, being important both additive and non-additive effects. Narrow-sense and broad-sense heritability estimates were relatively high, with respective values of 0.43-0.58 and 0.51-0.8, depending on the resistance traits measured: lesion length (LL), lesion length growth per day (LLRATE), time to first symptoms of wilting above inoculation point (T_W) and proportion of plants without wilting at the end of experiment (SV). These values suggest high capacity of evolutionary response of this pine species to *F. circinatum* pathogen. We concluded that there is considerable genetic variation within populations to allow pine adaptation to pitch canker disease, through natural or artificial selection. We also explored the correlation between pitch canker resistance and water stress tolerance at three hierarchical levels (population, family and individual) from the same provenance/progeny trial. The traits studied for water stress tolerance were as described in Gaspar et al. 2013: days to reach 50% mortality (T50) and survival at 100th day (S100) in a polyethylene glycol-osmotic induced stress experiment; survival at mesic site, age 1 after planting (M_SV) and survival at xeric site, age 1 after planting (X_SV) in a field testing. The traits studied for pitch canker resistance were as described above. At population level, significant correlations values were found for the water stress tolerance traits T50, S100 and X_SV with all the traits described for pitch canker resistance, with negative correlation values for LL and LLRATE, and positive for T_W and SV. At family level significant positive correlation was found between S100 and S and none significant correlation was found at individual level. These results suggest an interaction between drought tolerance and disease resistance.

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Keywords

pitch canker disease, genetic variation, genetic architecture, disease resistance, adaptive potential, drought tolerance

The Pacific madrone (*Arbutus menziesii*) common garden study: sentinel trials for monitoring genetic resistance to pathogens, insects, and potential impacts of a changing climate

Marianne ELLIOTT[1], CHASTAGNER G[1], KANASKIE A[2], SNIEZKO R[3], SAVIN D[3], HAMLIN J[4], HARRINGTON C[5], RUSSELL J[6]

[1] Washington State University, Research and Extension Center, 2606 West Pioneer, Puyallup, WA, 98731, USA
[2] Oregon Department of Forestry, 2600 State Street, Salem, OR, 97310, USA
[3] USDA Forest Service - Dorena Genetic Resource Center, 34963 Shoreview Road, Cottage Grove, OR, 97424, USA
[4] USDA Forest Service - Umpqua National Forest, 2900 NW Stewart Parkway, Roseburg, OR, 97471, USA
[5] USDA Forest Service – Pacific Northwest Research Station, Olympia, WA, 98512, USA
[6] British Columbia Ministry of Forests and Range, Cowichan Lake Research Station, Mesachie Lake, BC, V0R 2N0, Canada.

Pacific madrone is a wide ranging evergreen hardwood species in western North America, covering a large latitudinal gradient from California to British Columbia. Very little is known about the genetics of this species, such as its variation in resistance to pathogens, and adaptation potential in the face of changing climate. The WSU Pacific madrone seed collection contains seed from 320 families in 7 ecoregions. Using this seed collection, common garden plantings consisting of 119 families were planted at seven locations in California (1), Oregon (2), Washington (2), and British Columbia (2). Common gardens in the US (California, Oregon, and Washington) were established in 2011 and the two sites in British Columbia were planted in 2013. These sites are located in 4 of the 7 ecoregions where seed was collected. Assessments were made of growth, disease incidence and severity, cold damage, and phenology for each site on a yearly basis.This project uses the range-wide collection of Pacific madrone to examine genetic variability. Some specific objectives are the following: 1) Screen for resistance to multiple pathogens such as *Phytophthora ramorum*, *P. cinnamomi*, and endemic canker and foliar pathogens. 2) Examine variation in growth and adaptive traits. 3) Identify seed sources or populations that may contain individuals that are best adapted to climate change and for urban and restoration plantings. 4) Identify seed zones for this species. 5) Compare data collected on seedlings in the nursery with plants grown in the field, to determine whether some traits can be predicted from nursery measurements. Some preliminary results will be summarized. These trials serve as invaluable sentinel plantings to detect impacts of insects, pathogens and climate change and the potential genetic resistance within the sites and any interaction over environments.

Keywords

North America, forest ecosystem

Tubakia jejuensis on *Quercus serrata*, a new species from Korea

Hye Young YUN and KIM YH

Department of Agricultural Biotechnology, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul, South Korea

The survey project of Tubakia leaf blight on oak trees in Korea has been conducted since May, 2013. We found a severe leaf blight disease occurring on *Quercus serrata* in Jeju city and Seogwipo city, Jeju province, where no serious oak leaf diseases have been found until the time of the survey. The fungal isolates collected from the blighted leaves of *Q. serrata* were examined for the species identification based on its morphological characteristics and analysis of gene sequences in ITS (internal transcribe spacer) regions of rDNA. Pycnothyria, conidiomata typical to the genus *Tubakia*, were formed on the blighted areas of mostly upper or sometimes both leaf surfaces with scutella of 40-129μm with irregular margins. Conidiospore from the pycnothyria were hyaline, subglobose and ellipsoid, 12-18 × 6-14μm, which is smaller than *T. seoraksanensis* that was recently reported in Korea. This indicates the present pathogen should be different from the previous one. Also the analysis of ITS rDNA sequences of the present fungal isolates deposited in GenBank (HY1385, HY1420, HY2790 and HY2797) showed that they belong to a monophyletic lineage independent of other *Tubakia* species. It is suggested that the Korean *Tubakia* is a new species morphologically and genetically different from other *Tubakia* species that cause leaf spots on oak trees. Given that the new species was collected from Jeju province, it was named as *Tubakia jejuensis* sp. nov. This disease occurred from seedlings to mature trees of *O. serrata* over 20m high.

Keywords

Tubakia leaf blight, oak trees, Jeju province

SESSION 2: Resistance/ tolerance and virulence / aggressiveness MECHANISMS and their genetic determinants

Poplar-Root-Knot nematode interaction: a model for perennial woody species

Fabien BALDACCI-CRESP[1], TWYFFELS L[2], VERMEERSCH M[2], PEREZ-MORGA D[2], DE ALMEIDA ENGLER J[3], EL JAZIRI M[1], BAUCHER M[1]

[1] Université Libre de Bruxelles, Laboratoire de Biotechnologie Végétale, Rue des Professeurs Jeener et Brachet, 12 6041 Gosselies, Belgique.
[2] Université Libre de Bruxelles, Center for Microscopy and Molecular Imaging-CMMI, Rue des Professeurs Jeener et Brachet, 12 6041 Gosselies, Belgique.
[3] Institut Sophia Agrobiotech, INRA PACA, 400 Route des Chappes 06903 Sophia Antipolis, France

Plant Root-Knot nematode (RKN) interaction studies are performed on several host plant models representing a broad spectrum of angiosperms and gymnosperms. However, even if RKN interact with trees, no perennial woody model was developed so far. This work aims to demonstrate that poplar (*Populus tremula x P. alba*) is an appropriate model plant to study tree-RKN interactions. We show that poplar grown in vitro is susceptible to *Meloidogyne incognita* as this nematode is able to penetrate, to develop and to reproduce in poplar roots. In our growth conditions, *M. incognita* completed its life cycle by producing viable eggs in about 6 weeks. In order to characterize the interaction at the molecular level, quantitative RT-PCR analysis was developed to study changes in poplar gene expression. First, several housekeeping genes allowing comparisons of gall versus root and of galls at different developmental stages were identified. Second, several marker genes reported for other plant-RKN interaction models were evaluated. Finally, a down-regulation of two genes involved in the lignin biosynthetic pathway, HCT and F5H, was evidenced in galls versus non-infected roots. Together, this study shows that poplar can be considered as a model for tree-RKN interaction.

Keywords

Poplar, Root-Knot Nematode

Overlaying phenotypic, genotypic and metabolomics: a novel approach applied to the *Populus – Sphaerulina* pathosystem

Ahmed NAJAR[1], Isabel N[3], LeBoldus JM[2], Strelkov S[4], Thomas BR[1], Erbilgin N[1]

[1] Department of Renewable Resources, University of Alberta, Canada
[2] Department of Plant Pathology, North Dakota State University, USA
[3] Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, Canada
[4] Department of Agriculture, Food and Nutritional Sciences, University of Alberta, Canada

Cankers remain some of the least understood forest diseases causing considerable damage particularly in hybrid poplar plantations. In Canada the causal agent of *Septoria* canker and leaf spot, *Sphaerulina musiva* Peck., is endemic to the eastern provinces (Ontario and Quebec), home to *Populus deltoides* (Bartr.) Marsh and *P. balsamifera* L. In the Northwestern range (Saskatchewan, Alberta and British Columbia), home to mainly *P. balsamifera* and *P. trichocarpa* Torr. & Gray, the pathogen is found sporadically in shelterbelts, nurseries and plantations, depending on the poplar varieties. Isolates of *S. musiva* identified in Alberta have been shown to be unique relative to isolates found in eastern Canada.While *P. deltoides* is resistant to the pathogen in North America, *P. trichocarpa* and *P. balsamifera*, two closely related species, are highly susceptible. We expect this susceptibility to increase in sympatric zones where *P. trichocarpa* and *P. balsamifera* hybridize. Through the use of species-specific SNP, we selected genotypes ranging from pure *P. balsamifera* to *P. trichocarpa* and their hybrids that show different levels of admixture (25:75, 50:50, 75:25). We then infected these genotypes of pure and admixed individuals with selected isolates of *S. musiva*. After 8 weeks, the following phenotypic measurements were then taken: necrotic area, secondary defensive compounds (phenolic glycosides) and primary chemistry (fatty acids). The combined use of phenotypic and genotypic data and the inclusion of metabolite analysis in the measures of phenotypic responses to infection will help the development of valuable tools to inform breeders and managers in selection programs, and shed more light on the poorly understood pathosystem.

Keywords

Sphaerulina musiva, *Populus* sp., chemical phenotype, genomics, metabolomics

Phenol related gene activities and metabolic pools vary intra-specifically in Aspen

Vicki Huizu GUO-DECKER[1], BANDAU F[1], COLE CT[2], ALBRECTSEN BR[1]

[1] Department of Plant Physiology, Umeå Plant Science Centre, Umeå University, Umeå SE 90187, Sweden
[2] Division of Science and Mathematics, University of Minnesota, Morris, MN 56267, USA
[3] Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, DK 1871 Frederiksberg C, Denmark

Phenolics such as condensed tannins (CTs) and salicinoids are the main defense compounds derived from the phenylpropanoid pathway (PPP) in aspen and they vary intra-specifically as an effect of soil nutrients and determined by genotype specificity. In this work, we investigated those phenol-related gene activities, the metabolic pools of five PPP related phenolic classes (CTs, salicinoids, lignins, intermediate flavonoids and anthocyanins), and their interactions from eight aspen genotypes categorized as high- and low- intrinsic foliar tannin groups under simulated ambient, deposition, and boreal forest fertilized N levels. Our results showed that the expression of PPP genes was higher in high tannin group and the expression patterns differed across three nitrogen environments. The levels of phenolic classes increased along leaf age, but for tannin precursors. Different classes responded to nitrogen addition accordingly, for instance lignin precursors, flavonoids and tannin precursors are very plastic, in contrast to the stable salicinoid level. Our low tannin plants appeared a more diverse expression no matter at transcription level or metabolic level. However, it is obscure how genetic differences driving plant chemical defense responds to N input from the transcriptional level to metabolic level related to leaf age. Our results suggested the theoretical defense models could explain a portion of our aspen's intra-specific variation regard to the expression of genes and phenolics and the pattern we observed, however the diverse expression could be caused by the discrepancy in the responses of their distinct biosynthetic/accumulation origins and/or their metabolic costs during evolution.

Keywords

Aspen; genotype; condensed tannins; phenylpropanoid pathway; phenolics; genotypic variation; nitrogen addition; lignin; *Populus*; salicinoids

Back to the wild: identification and genetic mapping of resistance factors to *Melampsora larici-populina* in *Populus nigra*

Véronique JORGE[1], DOWKIW A[1], EL-MALKI R[1], ALBERT E[1,4], PEGARD M[1], SEGURA V[1], GUERIN V[1], RIDEL C [1], POURSAT P[3], ALMEIDA J [3], FAIVRE-RAMPANT P[2], BASTIEN C[1]

[1] INRA, UR0588, Amélioration Génétique et Physiologie Forestières, F-45075 Orléans, France.
[2] INRA, US1279, EPGV Etude du Polymorphisme des Génomes Végétaux, F-91057 Evry, France.
[3] INRA, UE0995, Génétique et Biomasse Forestières Orléans, F-45075 Orléans, France.
[4] Present address: UR1052 GAFL Génétique et Amélioration des Fruits et Légumes, F-84143 Montfavet, France

In Europe, resistance to *Melampsora larici-populina* (*Mlp*) leaf rust is a major breeding objective for cultivated poplars. Up to now, qualitative resistances inherited from American species deployed in commercial hybrids were all overcame by the fungus. Breeder strategy has now switch to the identification of quantitative resistances in natural populations of black poplar (*Populus nigra*) coevolving with the populations of *Mlp*. The objective of the present study is to unravel the genetic determinism of partial resistance in *P. nigra* using a range of *Mlp* strains from cultivated areas and from natural populations and to locate and validate resistance factors in different genetic backgrounds. Artificial inoculation tests were carried out to measure three epidemiological components (latent period, uredinia number and size) using four *Mlp* strains in 7 *P. nigra* individuals from natural population and their F1 progenies organized in a factorial mating design. These F1 progenies were genotyped at high density in order to perform QTL detection. A quantitative variation of the resistance, a high genetic variability and significant genotype by strain interaction were observed in the parental genotypes and their F1 progenies. Major QTLs, with component and strain- specific effects, explained up to 86 % of the phenotypic variability observed for the epidemiological components. A fourth resistance component "pseudo-quantitative" expressing a hypersensitive reaction was identified. This reaction was observed for the first time in *P. nigra*. The QTL involved in the genetic control of the different components were located in genomic regions rich in NBS-LRR resistance genes, contributing to blur the line between qualitative and quantitative resistance.

Keywords

Populus nigra L., *Melampsora larici-populina* Kleb., leaf rust resistance, partial resistance, strain-specificity, QTL mapping

Mapping QTL of aggressiveness traits in the poplar rust fungus

PERNACI M, DE MITA S, FABRE B, DUPLESSIS S, HALKETT F, **Pascal FREY**

INRA, Université de Lorraine, UMR 1136 Interactions Arbres – Microorganismes (IAM), F–54280 Champenoux, France

Poplar is an important product in Europe, both for the wood industry and for its increasing contribution to energy production systems. The main disease limiting the growth of poplar is poplar rust, caused by the basidiomycete *Melampsora larici-populina*. The selection of poplar cultivars with qualitative resistance has led to repeated failures since 1980, due to the breakdown of all the resistance types through the rise of new virulence factors. The search for more durable resistance has led breeders to focus on quantitative resistance. Meanwhile, pathologists have focused on the potential for rapid evolution of several fungal quantitative traits related to aggressiveness and dispersal potential (Lannou 2012). We used a quantitative genetics approach to determine whether such traits are heritable and directly impact the fitness of the pathogen, which can lead to rapid changes in pathogen populations in response to selection exerted by the host. Mapping the traits of interest requires the construction of a high-resolution genetic map of the fungus and the measurement of segregating traits in progeny. We adopted a strategy of genetic mapping by the re-sequencing, based on approximately 145,000 SNPs, of a S1 progeny derived from selfing of the 98AG31 strain (reference genome, 462 scaffolds). A preliminary analysis on 47 offspring allowed us to bring together 81 scaffolds in 23 linkage groups (Pernaci et al. 2014). A further analysis of 85 offspring allowed the completion of a high-density genetic map, comprising 18 chromosomes, with a total genetic length of 4,138 cM. A single virulence trait against *Populus deltoides* ‘L270-3’ (virX) segregates in the progeny, and was mapped on chromosome 17. Aggressiveness traits (latency period, infection efficiency, sporulation capacity, lesion size) and morphological traits (spore volume, length/width ratio) exhibit a clear segregation in this progeny. So far, three QTL for lesion size were mapped on chromosomes 7, 9 and 14.

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Keywords

Genetic mapping, QTL, quantitative genetics, life history traits, aggressiveness, fitness

What makes *Hymenoscyphus fraxineus* aggressive?

Jan STENLID[1], ELFSTRAND M[1], BRANDSTRÖM DURLING M[1], CLEARY M[2]

[1] Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Box 7026, 750 07 Uppsala, Sweden.
[2] Southern Swedish Forest Research Centre, Swedish University of Agricultural Sciences, Box 49, 230 53 Alnarp, Sweden.

Recently, a new, invasive fungal pathogen (*Hymenoscyphus fraxineus*) affecting common ash (*Fraxinus excelsior*) has emerged as a serious forest health problem in Europe. The fungus is believed to be native in eastern Asia where it is acting as an endophyte in *F. mandschurica*. Population genetic work indicate that H.fraxineus is outcrossing but has experienced recent population bottlenecks. We have sequenced the genomes of *H. fraxineus* and its European congeneric *H. albidus* and also analysed gene expression following inoculation into ash trees. In our analysis we have identified gene families expanded in these species compared to other members of Helotiales. *H. fraxineus* and *H. albidus* differ in their repertoir and gene expression of carbohydrate active enzymes. Moreover, *H. fraxineus* has an active gene cluster likely to be involved in secondary metabolism that is non-functional in *H. albidus*. Differences in gene expression beween the species when inoculated into ash will be discussed in light of the contrasting interaction outcomes with the host tree.

Keywords

Ash Dieback, genome, virulence.

Battle amid the ashes: Phloem defense expression of resistant and susceptible ash species and associated physiological responses of emerald ash borer larvae

David N. SHOWALTER[1], RIGSBY CM[2], CIPOLLINI DF[2], HERMS DA[3], WIJERATNE S[4], WIJERATNE A[4], BONELLO P[1]

[1] Department of Plant Pathology, The Ohio State University, 2021 Coffey Road, Columbus, OH 43210, USA
[2] Department of Biological Sciences, Wright State University, 3640 Colonel Glenn Highway, Dayton, OH45435, USA
[3] Department of Entomology, Ohio Agricultural Research and Development Center, The Ohio State University, 1680 Madison Avenue, Wooster, OH 44691, USA
[4] Molecular and Cellular Imaging Center, Ohio Agricultural Research and Development Center, The Ohio State University, 1680 Madison Avenue, Wooster, OH 44691, USA

The alien, invasive wood boring beetle known as emerald ash borer (EAB, *Agrilus planipennis* Fairmaire) is devastating native ash (*Fraxinus* spp.) populations in North American natural and urban forest settings, with annual damage estimates approaching $3.5 billion. The Asian beetle has also become established near Moscow, Russia and further threatens European ash resources already facing ash dieback. A critical part of landscape-scale, long-term EAB management will be deployment of host resistance traits, which are present in coevolved Asian ash species, but have not yet been characterized. We hypothesize that Asian species are resistant because they are able to recognize and respond to an attack more rapidly and/or deploy specialized, inducible defenses that are not available to non-coevolved species. To test these hypotheses, we have conducted a field experiment in which we are profiling gene transcription, hormone signaling, and phenolic metabolism in phloem and cambial tissue from resistant Asian Manchurian ash (*F. mandshurica*) and susceptible North American white ash (*F. americana*), both before and immediately after EAB larval attack.Additionally, we have attempted to identify major categories of ash defenses by examining the developmental and physiological responses of EAB larvae feeding on the two contrasting ash species. We have found that larvae feeding on resistant Manchurian ash exhibited slower development and higher activities of various quinone-protective and anti-oxidant enzymes compared to larvae feeding on susceptible white ash. Additionally, Manchurian ash-fed larvae had lower β-glucosidase activity than white ash-fed larvae. These results suggest that pro-oxidant compounds and phenolic glycosides (e.g. verbascoside) are important components of an effective ash defense response against EAB. Ongoing ash phloem transcription profiling is identifying orthologous gene transcripts that are differentially expressed between the two ash species, either constitutively or following EAB attack. Discovery of ash genes of interest, with support from larval enzymatic studies, and future ash hormone signaling and defense metabolite network analyses, will allow us to identify targets for ash resistance breeding programs. More broadly, this research will significantly improve understanding of the genetic basis of angiosperm tree defenses against wood boring insects.

Keywords

wood-boring insects, transcriptomics, host plant resistance, oxidative stress

Constitutive phenolic biomarkers identify *Quercus agrifolia* (coast live oak) resistant to the invasive pathogen *Phytophthora ramorum*

CONRAD A[1], MCPHERSON B[2], WOOD D[2], **Pieluigi BONELLO[1]**

[1] The Ohio State University, Department of Plant Pathology, Columbus, OH 43210, USA
[2] University California, Department of Environmental Science, Policy, & Management, Berkeley, CA, 94720, USA

Sudden oak death is a devastating disease in native populations of red oak (*Quercus* spp.) and tanoak [*Notholithocarpus densiflorus* (Hook. & Arn.) Manos et al.] in coastal California and southwestern Oregon. The disease is caused by the invasive alien pathogen *Phytophthora ramorum* Werres et al., which can cause stem girdling, lethal cankers on oak and tanoak, as well as foliar lesions on tanoak. Long-term monitoring plots (15 years) show that 52% of tanoak and 40% of coast live oak (CLO—*Quercus agrifolia* Née) trees have died following infection with P. ramorum. Additionally, variability in CLO susceptibility has been linked to variation in the concentration of certain phenolic compounds, and four putative phenolic biomarkers have been identified in asymptomatic phloem tissue collected from artificially infected CLO. To determine whether constitutive concentrations of phenolic compounds can be used as biomarkers of resistance, phloem samples were collected from asymptomatic (i.e. apparently healthy) CLO in naïve areas. Trees were then artificially inoculated with *P. ramorum* and classified into groups based on disease symptom expression. Phloem samples collected from trees, classified as resistant or susceptible 10 months following inoculation with *P. ramorum*, were analyzed using high performance liquid chromatography coupled with photo diode array detection. Sparse linear discriminant analysis and backwards stepwise logistic regression analysis were used to identify the most important phenolics for discriminating between resistant and susceptible CLO. Constitutive concentrations of four phenolics, including the flavonoid myricitrin and three other incompletely characterized flavonoids, discriminated between resistant and susceptible CLO. These four phenolic biomarkers were also used to estimate the probability of survival of trees, classified as resistant or susceptible 44 months following *P. ramorum* infection, using Weibull and Cox Proportional Hazards analyses. We show that these approaches could be used to identify naturally resistant CLO, which could then be used to breed resistant germplasm or as part of conservation and preservation strategies. The approaches utilized in this study may be useful in other forest pathosystems where both non-native and native invasive organisms are of concern.

Keywords

Phenolics, chemical profiling, constitutive resistance, sudden oak death, *Phytophthora ramorum*

A preliminary view of *Leptocybe invasa*-induced gall development and host transcriptional responses in a susceptible *Eucalyptus grandis* hybrid clone

Caryn OATES[1], MYBURG A[1], SLIPPERS B[1] NAIDOO S[1], DENBY K[2]

[1] Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), Genomics Research Institute (GRI), University of Pretoria, Private bag x20, Pretoria, 0028, South Africa
[2] School of Life Sciences and Warwick Systems Biology Centre, University of Warwick, Coventry, CV4 7AL, United Kingdom

Eucalyptus species comprise some of the world's most widely grown and economically valuable plantation trees. Currently, one of the biggest threats to the industry is the Australian gall-inducing wasp, *Leptocybe invasa* Fisher & La Salle (*Hymenoptera*: *Eulophidae*). The larvae are endophytic herbivores that induce gall development in immature midribs, petioles and stems (Mendel et al. 2004). An infestation can cause a range of symptoms including stunted growth, die-back and in some cases cause the failure of industrially important clones. Plants have evolved a sophisticated defence system that protects them against biotic stress; however, knowledge regarding *Eucalyptus* immunity in this host-pest interaction is lacking. The aim of this study was to investigate the development of the *L. invasa*-induced gall and to discern the transcriptional landscape following oviposition in a susceptible clone. Histochemical staining of cross-sections of mature galls shows a prosoplasmatic arrangement. Nutritive tissue surrounds the larval chamber followed by redifferentiated vascular tissue. The outer layer of the gall is heavily lignified to provide protection or structure (Stone and Schönrogge 2003). The transcriptional responses showed up-regulation of defence mechanisms such as secondary metabolite biosynthetic pathways as well as modifications to the primary metabolism. An integration of microscopic and transcriptomic responses over time will provide a more in-depth understanding of the Eucalyptus immunological network and allow the identification of exploitable biotechnological targets and improve the knowledge base on immune functioning of long-lived woody plants and trees in general.

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Keywords

Eucalyptus, Leptocybe, gall development, transcriptome

Transcriptional profiling of *Eucalyptus grandis* and *Chrysoporthe austroafricana* elucidates host defence mechanisms and putative pathogenicity strategies

Ronishree MANGWANDA[1,2], VAN DER MERWE A[1,2], MYBURG A[1,2], NAIDOO S[1,2]

[1] Department of Genetics, University of Pretoria, Pretoria, 0001, South Africa.
[2] Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0001, South Africa.

Eucalypts are extensively propagated for their desirable wood properties, but this species is also being investigated as a potential source for biofuel production. These properties make eucalypts a highly valuable commodity for the international forestry industry. Throughout their lifetime, these woody species are exposed to various pathogens that can cause severe losses to the industry. Among these pathogens is *Chrysoporthe austroafricana* which causes the development of stem cankers on eucalypts. This fungal pathogen has undergone a host shift from native *Syzygium* spp. (waterberry) to non-native *Eucalyptus* spp. in Africa. The established pathosystem of *E. grandis* and *C. austroafricana* can be used as a model system to elucidate the defence strategies of the host as well as to decipher pathogenicity mechanisms of the pathogen. This can be achieved by using the available genome sequences of *E. grandis* and *C. austroafricana*. To investigate the defence responses of *E. grandis*, a susceptible clone (EgrS) and a moderately resistant clone (EgrR) were inoculated with *C. austroafricana* and stem material, harvested 3 days post inoculation (dpi), was sent for transcriptome profiling. In vitro growth of fungi on differential media can mimic a stress response and reveal genes involved in pathogenicity. Thus, transcriptome profiling was performed on *C. austroafricana* grown in vitro on nutrient limited media and nutrient rich media. A Cuffdiff analysis was performed between the two media compositions as well as between the nutrient rich media and the in planta samples to identify potential pathogenicity mechanisms towards *Eucalyptus*. Data analysis of *E. grandis* challenged with *C. austroafricana* revealed 1539 and 1495 differentially expressed genes in EgrR and EgrS respectively when compared to the controls. Further investigation of these genes suggested a role for gibberellin signaling in facilitating susceptibility. Additionally, expression profiling of selected candidate genes at 7dpi indicated a possible delayed defence response in EgrS. Pathogenicity mechanisms elucidated by transcriptome profiling of *C. austroafricana* included cell wall degrading enzymes, fungal effectors and candidates that attribute to virulence. Candidates of interest that emerged include an isocitrate lyase, non-ribosomal peptides as well as genes such as ent-kaurene oxidase and salicylate hydroxylase that may manipulate gibberellin and salicylic acid signaling respectively. The ability of a host to fine-tune its defence responses is crucial in determining the outcome of a pathogen incursion and the responses identified in this study provide a glimpse into the complexity of these responses activated in *Eucalyptus*.

Keywords

Transcriptome profiling, host defence, gibberellin, pathogenicity

Conifer defence against fungi: Terpene analysis and transcript profiling of the response of *Pinus sylvestris* to infection by *Heterobasidion annosum* under field condition

KERIÖ S[1], JABER E[1], RAFFAELLO T[1], KOVALCHUK A[1], LORENZ W[2], HOLOPAINEN J[3], DEAN J[4], **Fred O. ASIEGBU[1]**

[1] Department of Forest Sciences, University of Helsinki, P.O. Box 27, FIN-00014 Helsinki, Finland
[2] Warnell School of Forestry and Natural Resources, The University of Georgia, Athens, GA 30602, USA
[3] Department of Environmental Science, University of Eastern Finland, P.O. Box 1627, FIN-70211 Kuopio, Finland
[4] Department of Biochemistry, Molecular Biology, Entomology & Plant Pathology, Mississippi State University Mississippi State, MS 397672, USA

In conifer forests of Northern Europe, the pathogenic fungus *Heterobasidion annosum* attacks the roots of Scots pine and causes mortality (1). Infected trees grow slower and produce less timber with reduced quality. Presently, no conifer tree has been identified with absolute resistance against this pathogen. A number of chemicals and defence genes have been postulated as major contributory factor to conifer defences (2). We studied stem and root responses of mature naturally regenerated Scots pine trees to the infection. Based on stem phloem lesion size, we selected one highly susceptible and one least susceptible trees that were used for detailed terpene and transcript profiling analysis in order to ascertain the mechanistic basis for the differential responses. Terpenes were analysed with the aid of GC-MS while micro-array was used to analyse Pine transcriptome (36.4K cDNA elements). The highly susceptible tree accumulated ten times more terpenes compared to the least susceptible tree. The absolute concentrations of 3-carene was higher in the least susceptible tree. The concentration of delta-3-carene was negatively correlated with lesion length. The microarray analysis indicated that transcripts involved in terpene pre-cursor synthesis through the methyl-erythritol-phosphate (MEP) pathway were up-regulated. Alpha-pinene synthases were up-regulated in the high susceptible tree but not in the least susceptible tree. The potential relevance of delta-3-carene and alpha-pinene synthases as markers for screening Scots Pine trees for *H. annosum* resistance will be discussed.

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Keywords

Fungi, terpenes, gene expression, Scots pine;

General patterns of early within plant allocation of chemical defences and defensive strategies in Paleartic and Neartic pines (Subgenus *Pinus*)

Luis SAMPEDRO[1], MOREIRA X[1], CARRILLO-GAVILAN A[2], VILA M[2], ZAS R[1]

[1] Misión Biológica de Galicia (CSIC), Pontevedra, Galicia, Spain
[2] Estación Biológica de Doñana (CSIC), Sevilla, Spain

Plant hormones involved in the signaling of wounding herbivore, and biotrophic pathogens damage, respectively. We use phylogenetic comparative methods to control for the lack of statistical independence among species. We found a marked general model in the quantitative allocation of constitutive defenses along the plant, specifically for the stem oleoresin (increased concentration upward), and for total phenolics and condensed tannins in the needles (increased concentrated downwards). Specifically, constitutive allocation to pine chemical defences seems to be well preserved across the phylogeny (Carillo-Gavilan et al., 2015). In contrast, the inducibility of chemical defensive traits was evolutionarily labile and may depend more on the particular climate, biotic interactions and resource availability in each species' range. We did not find evidences of trade-offs between inducibility in response to the jasmonic acid and salicylic acid pathways. Even more, we found positive significant relationships between the inducibility by the two routes for needle phenolics and stem resin. That is, species more inducible by jasmonate application were also more responsive to salicilate. Defensive strategy was associated with growth rate such that slow-growing species invested more in constitutive defence while fast-growing species invested more in inducible defence (Moreira et al., 2014). Relative position of pine species along the constitutive-induced trade-off axis was in turn associated with geography; moving poleward and to higher elevations, growth rate and inducible defences decreased, while constitutive defence increased.

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Keywords

Induced defences, defensive allocation, *Pinus*

Priming of plant immunity: the molecular mechanisms of defense priming in Norway spruce

Paal KROKENE[1], VIVIAN-SMITH A[1], FOSSDAL CG[1], CROSS H[1], ZHAO T[2]

[1] Norwegian Institute of Bioeconomy Research, P.O.Box 115, N-1431 Ås, Norway
[2] Ecological Chemistry Group, Department of Chemistry, Royal Institute of Technology, SE-100 44 Stockholm, Sweden

The tree-killing bark beetle *Ips typographus* and its associated pathogenic bluestain fungi represent one of the most devastating forest health problems in Europe, causing great economic and ecological losses in Norway spruce forests. The host tree's natural defenses are the most important factor maintaining the beetle-fungus complex at low, endemic levels. Recent work has shown that spraying of spruce trees with the plant hormone methyl jasmonate (MeJA) can enable trees to respond more quickly and strongly when challenged several weeks after treatment, although initial changes in defense responses is not always observed. This phenomenon, known as defense priming, does not directly increase tree resistance to pests, but rather enables a more rapid or effective induced response when an attack occurs. In recent field experiments we observed no obvious anatomical responses to methyl jasmonate treatment in Norway spruce, no induction of terpene levels, and very little upregulation of various transcript markers of resistance. Still, trees with no obvious anatomical or chemical induction were also resistant to bark beetle attack and fungal infection, suggesting that tree resistance had been primed by methyl jasmonate. In this presentation we will discuss the chemical and transcriptomal aspects behind defense priming in Norway spruce.

Keywords

Plant-insect-pathogen interactions, induced defenses, defense priming, conifer defense, transcriptomics, terpenoids

Aleppo pine provenances vary in susceptibility and secondary chemical response to the infection of *Gremmeniella abietina*

Carmen ROMERALO[1], WITZELL J[2,3], DIEZ JJ[1]

[1] Sustainable Forest Management Research Institute, University of Valladolid-INIA, Avda. Madrid 44, Building E, 34004, Palencia, Spain
[2] Southern Swedish Forest Research Centre, Swedish University of Agricultural Sciences, Box 49, 23053, Alnarp, Sweden.
[3] University of Eastern Finland, Faculty of Science and Forestry, School of Forest Sciences, P.O. Box 111, 80101 Joensuu, Finland.

Aleppo pine is a thermopile species that due to its heat and drought tolerance has become a popular tree species in restorations even beyond its natural habitat. To guarantee the success of restorations the most appropriate plant material, including the provenance region, with good tolerance to different threats should be chosen. In 1999 the pathogenic fungus *Gremmeniella abietina* was isolated for first time in Aleppo pine plantations in Spain living outside its natural habitat, but it is not clear if the provenances differ in their resistance to *G. abietina* infections The main objective of this study was to analyze the variation in susceptibility to the infection of *G. abietina* among Spanish Aleppo pine seedlings from five provenance regions. Artificial inoculations were performed in the seedlings from different regions with eight *G. abietina* isolates in two times of inoculations to validate the results. After several months, the symptoms of the disease started to appear and at the end of the experiment all the seedlings were cut and brought to the lab. The susceptibility of the provenances was evaluated by recording the severity of visual symptoms and stem internal necrosis produced by the pathogen. In addition, we quantified the concentration of two flavanone compounds as putative indicators for resistance of the plants. The provenances exhibited significantly different levels of necrosis. The relation between the geographic origin of the provenances and their resistance was not straightforward, but the highest degree of necrosis was found in one of the southern provenances and the lowest in a northern provenance. The content of a naringenin flavanone was significantly different among provenances, suggesting a possible indicator value for the resistance of the provenances. Our results show that the provenance dependent variation in susceptibility of Aleppo pine to *G. abietina* should be considered among the factors that may determine Aleppo pine’s success in regenerations and restoration.

Keywords

Provenance trials, severity, forest health, phytopathogens, flavonoids

Mechanisms of drought-induced susceptibility of Austrian pine to *Diplodia sapinea*

SHERWOOD P[1], VILLARI C[1], CAPRETTI[2], **Pierluigi BONELLO[1]**

[1] Department of Plant Pathology at The Ohio State University, Columbus OH 43210, USA
[2] Dipartimento di Scienze Produzioni Agroalimentari e dell biente, Università degli Studi di Firenze, 50144 Firenze, Italy

Drought is perhaps the most important abiotic stressor for plants due to its directly deleterious effects, but also because it can predispose plants to infection and exacerbate disease. The biochemical basis for this predisposition is largely unknown, but likely results from changes in host physiology that create favorable conditions for pathogens. In this study we investigated two very common plant responses to drought: the production of reactive oxygen species (ROS) and the accumulation of proline. Both of these responses are also involved in plant defense against pathogens, and thus, likely play a central role in concurrent host responses to abiotic and biotic stress. For example, H_2O_2 (a key ROS) is part of the early signaling processes in plant defense against pathogens, while a hyperactive proline synthesis cycle is a known activator of programmed cell death (PCD), which would facilitate the necrotrophic lifestyle of *D. sapinea*. Using the *Pinus nigra-Diplodia sapinea* pathosystem, we measured the effects of drought on disease severity, the amounts of H_2O_2 and free amino acids, and the activity of key proline cycle genes in healthy and inoculated shoots. In both droughted and watered trees, infection reduced the levels of H_2O_2 and increased the levels of proline (the only amino acid to increase upon inoculation). Using in vitro assays, we demonstrated that exogenous H_2O_2 is toxic to *D. sapinea*, but the fungus responded by increasing mycelial catalase and peroxidase activity that can scavenge H_2O_2, thus protecting the fungus from direct oxidative damage and potentially inhibiting host defense responses. Proline was found to be a preferred in vitro N source, compared to inorganic sources and other amino acids, and protected hyphae from exogenous H_2O_2 by acting as an oxidant scavenger. Therefore, proline accumulation during drought may further contribute to inhibiting the oxidative burst during host defense responses to pathogen infection. The proline synthesis genes pyyroline-5-carboxylate synthetase and reductase were both upregulated in diseased shoots, while a proline catabolism gene, proline dehydrogenase, had lower expression compared to watered controls. This suggests that fungal infection increases proline levels in the shoots via greater proline synthesis coupled with lower catabolism, which could promote PCD. A new model integrating interactions of proline and H2O2 metabolism with drought and nectrotrophic fungal infection of plants is proposed.

Keywords

Abiotic stress, necrotroph, tip blight, Pinus nigra, aggressiveness, reactive oxygen species, hydrogen peroxide, antioxidant, proline

Quantifying genetic parameters for tolerance / resistance to pinewood nematode in *Pinus pinaster* Ait. half-sib progenies

Isabel CARRASQUINHO[1], GONÇALVES E[2], LISBOA AF[1,2], AGUIAR A[1], LIMA A[2]

[1] Instituto Nacional de Investigação Agrária e Veterinária, I.P., Av República, Quinta do Marquês, 2780-152 Oeiras, Portugal
[2] Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda 1349-017 Lisboa, Portugal

Pine wilt disease (PWD), caused by the pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is a major concern to maritime pine forest in Portugal due to its economical, environmental and social impacts. It is also regarded as a major threat to European forests as this pathogen is listed as a quarantine pest. A mass selection program was initiated in 2009 in the south of Portugal. About 500 trees were selected as candidate to plus trees at Comporta, an area with one of the highest incidence of the disease. Two years old half-sib progenies, obtained from 96 of those selected trees, were inoculated in greenhouse to evaluate their resistance/tolerance to PWN according to a randomized complete block design. The experiment was initiated in September 2014 and lasted for 22 weeks. For each plant, total height and diameter at the base were measured. The plants were monitored four times for external symptoms. Four levels were considered as a percentage of coverage of dead needles in relation to the total amount of needles and associated with plant vitality (1- 0 to 25%; 2 – 26 to 50% 3 – 51 to 75%; 4 – 76 to 100% of the plant symptomatic). Additionally, a dicotomical criteria (0 – healthy and 1 – dead plant) for plant disease symptom was also considered.The methodology for statistical analysis followed the mixed models theory. Genetic parameters for tolerance/resistance to pinewood nematode, for two years old *Pinus pinaster* Ait. half-sib progenies, were evaluated.

Keywords

Pinewood diseases; *Pinus pinaster* Ait.; Genetic parameters; Mixed Models

Variation in defoliation by *Lymantria dispar* within- and between-populations of *Quercus ilex* in Spain

SOLLA A[1], **Slobodan MILANOVIĆ[2,3],** BUENO A[1], CÁCERES Y[1], CORCOBADO T[1,4], PÉREZ A[1], GALLARDO A[5], MORCUENDE D[5], QUESADA A[6], MORENO G[1], PULIDO F[1]

[1] Ingeniería Forestal y del Medio Natural, Universidad de Extremadura, Avenida Virgen del Puerto 2, 10600 Plasencia, Spain
[2] Faculty of Forestry, Belgrade University, Kneza Višeslava 1, 11000 Belgrade, Serbia
[3] Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemedelska 3, 61300 Brno, Czech Republic
[4] Federal Research Centre for Forests, Natural Hazards and Landscape (BFW), Seckendorff-Gudent-Weg 8, 1130 Vienna, Austria
[5] Instituto Universitario IProCar, Universidad de Extremadura, Avenida de la Universidad s/n, 10003 Cáceres, Spain
[6] Facultad de Veterinaria, Avenida de la Universidad s/n, 10003 Cáceres, Spain

Herbivory is not distributed uniformly across available hosts between and within tree populations. Comparison of the responses to defoliation in conspecific populations with different origin represents a common experimental strategy for studying defoliation resistance. It is hypothesized that variability of herbivory may be higher within- rather than between- tree populations, as normally occurs with genetic diversity in forest tree species. Within- and between-population variation in defoliation by *Lymantria dispar* was studied in six populations of *Quercus ilex* in Spain. Seedlings grown from field-collected acorns from six holm oak populations were subject to defoliation by gypsy moth larvae. We used a randomized complete block design including 8 trees per population and 40 seedlings per tree. On May 20 2014, 20 out of the 40 seedlings per tree were individually defoliated during six days by placing third-instar larvae of *L. dispar*. To prevent larvae from escaping, tubular cages (one per plant) were used. The most defoliated tree population was 'Almería' (3.6±1.0 cm2 of defoliated area per plant), followed by 'Mallorca' and 'Cádiz' (3.4±0.6 and 2.8±0.4 cm2, respectively). The previous two populations were significantly more defoliated than 'Monfragüe' (2.1±0.4 cm2; $P<0.002$). Nineteen per cent of the total variability of defoliation was accounted for between-population variation, and four per cent of the total variability of defoliation was accounted for within-population variation. The high variability of defoliation between populations is in accordance to the high genetic diversity among *Q. ilex* populations reported before (Michaud et al. 1995). Most parameters of larval performance related positively to defoliation, but some of these relationships were significantly conditioned by the origin and the fitness of seedlings. Results suggest differences in the suitability of holm oak populations to *L. dispar* which could be interpreted as different strategies of defoliation resistance. Acknowledgments. Funded by Ministerio de Agricultura, Alimentación y Medio Ambiente of Spain (project 956, 'Determinants of biotic resistance in a model tree species: a new tool for adaptive management in national parks') and Ministry of Education, Science and Technological Development of the Republic of Serbia (project 43007, 'Studying climate change and its influence on the environment: impacts, adaptation and mitigation').

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Keywords

Gypsy moth, holm oak, defoliation resistance

A study of phenylpropanoid gene expression and associated metabolic pools in various aspen genotypes

Vicki H. GUO-DECKER[1], BANDAU F[1], COLE C[2], ALBRECTSEN BR[1,3]

[1] Department of Plant Physiology, Umeå University, Umeå SE 90187, Sweden
[2] Division of Science and Mathematics, University of Minnesota, Morris, MN 56267, USA
[3] Department of Plant and Environmental Sciences, University of Copenhagen, DK 1871 Frederiksberg C, Denmark

Condensed tannins and salicinoids are the main defense compounds in aspen. These phenolic compounds are derived from the phenylpropanoid pathway like lignins, intermediate flavonoids and anthocyanins. The biosynthesis and accumulation of tannins is in a genetic variant fashion and influenced by environmental factors such as nutrient addition. However, it is obscure how genetic differences driving plant chemical defense responds to N input from the transcriptional level to metabolic level related to leaf age. We selected eight representative aspen genotypes producing high- and low- intrinsic foliar condensed tannins and investigated 1) the gene activities of genes involved in phenylpropanoid pathway; 2) the expression of phenolic metabolites, in response to soil nutrients. We found intra-specific variation regard to the expression of genes and phenolics, and theoretical defense models could explain a portion of the pattern we observed.

Keywords

Aspen; genotype; condensed tannins; phenylpropanoid pathway; phenolics; genotypic variation; nitrogen addition; lignin; *Populus*; salicinoids

Implications of rust resistance selection on other traits of interest in *Eucalyptus* breeding

Daniele AA ARRIEL[1], GUIMARAES LMS[1], MAFIA RG[2], RESENDE MDV[3], ALFENAS A[1]

[1] Universidade Federal de Viçosa, Laboratório de Patologia Florestal- BIOAGRO, s/n, Avenida Peter Henry Rolfs, Viçosa, 36570-900, Brazil
[2] Fibria S.A. Centro de Tecnologia, Aracruz, 29197-900, Brazil
[3] Empresa Brasileira de Pesquisa Agropecuária, Centro Nacional de Pesquisa de Florestas. Estrada da Ribeira, Km 111, Brazil

Myrtle rust, caused by the fungus *Puccinia psidii*, is currently one of the main diseases of eucalypts. In planted forests the disease can lead to losses of about 27% of the volume of wood, depending on environmental conditions and geographic location. Due to these factors, the selection of resistant genotypes is a constant practice in Eucalyptus breeding programs. In general, this selection is performed after selection for others traits of productive interest such as diameter at 1.30 m (DBH), height and volume. However, at the end of these procedures, it may happen that superior genotypes for production-related traits, that took time and money to be identified, are excluded due to susceptibility to the disease. The objectives of this study were to evaluate the influence of rust resistance on the performance of progenies and to verify whether the selection for rust resistance in hybrid-progeny tests (i.e. before the selection of potential individuals for clonal tests) leads to loss of genetic gain in other variables of interest in *Eucalyptus* breeding. For this, 70 progenies of *Eucalyptus* spp, with different numbers of individuals, were evaluated for rust resistance and planted in the field. At 28 months after planting, the variables height and DBH were measured and the volume of all plants was estimated. For these three traits, the best progenies were ranked using three different averages: taking into account all the trees, only the resistant trees and only the susceptible trees. For the selection of individuals, we simulated two situations. First, the top 50 individuals considering all the plants of the experiment were selected. In the second, the top 50 individuals considering only the resistant plants were selected. In both situations we estimated heritability, genetic variance and genetic gain by the methodology of mixed models. Regardless of the rust resistance phenotype, there was no change in the classification of the best progenies for the three variables. In individual selection, the gains were higher when considering all plants of the population compared to only the resistant plants. In addition, approximately 90% of the population of superior plants were susceptible to rust. The values for heritability and genetic variance were similar in both scenarios. These results indicate that the performance of progeny was not affected by the presence of susceptible plants and that the selection for rust resistance before selecting for other productivity-related traits may lead to the loss of superior individuals.
Acknowledgements: CNPq, Fibria.

Keywords

Puccnia psidii, resistance, eucalypts, breeding

Differentially expressed genes and metabolites of *Abies nordmanniana* involved in the response to silver fir woolly adelgids

Katharina Birgit BUDDE[1], NIELSEN UB[1], HESKES AM[2], HAMBERGER BR[2], HANSEN OK[1]

[1] IGN, Department of Geosciences and Natural Ressource Management, University of Copenhagen, Rolighedsvej 23, 1958 Frederiksberg, Denmark
[2] PlEn, Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg, Denmark

Nordmann firs (*Abies nordmanniana*) are an economically important tree species grown for the Christmas tree industry in Europe. Christmas tree plantations often suffer damage from silver fir woolly adelgids (*Dreyfusia nordmannianae*). *Abies nordmanniana* and *D. nordmannianae* naturally co-occur in mountainous regions along the east coast of the Black Sea. The main host of *D. nordmannianae* is *Picea orientalis*, which also occurs in this region but is absent from Europe. As a result the adelgids are restricted to asexual reproduction on *A. nordmanniana*. Visible damage caused by these sap-sucking insects, such as needle curling and needle shedding, can be an economic disaster for Christmas tree growers. Therefore, insecticides are employed on a regular basis during the lifetime of a plantation. In order to reduce the use of insecticides, the identification and specific cultivation of more tolerant provenances or specific genotypes is needed. An earlier study found that different genotypes show distinct levels of susceptibility and that tolerance to aphid feeding is a heritable trait in Nordmann fir (Nielsen et al. 2002); however, detailed knowledge about the genetic architecture of this trait is so far lacking.In the present study we aimed to identify plant metabolites and candidate genes involved in the tolerance to adelgid feeding in *A. nordmanniana*. Trees naturally infested with adelgids and showing different degrees of tolerance were sampled from a clonal seed orchard in Denmark. GC-MS and LC-MS metabolite profiling of needles was performed as well as transcriptome sequencing to identify genes differentially expressed between the susceptible and tolerant clones. The levels of expression of these candidate genes were confirmed in a total of 115 trees with real time PCR. We present candidates for differentially expressed genes and metabolites which may be involved in the tolerance to aphid feeding in Nordmann fir.

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Keywords

Abies nordmanniana, *Dreyfusia nordmannianae*

Fourier-transform infrared (FT-IR) spectroscopy discriminates *Chamaecyparis lawsoniana* (Port-Orford-cedar) individuals that are resistant and susceptible to invasive pathogen *Phytophthora lateralis*

VILLARI C[1], **Richard A SNIEZKO[2],** CONRAD AO[1], SAVIN DP[1], RODRIGUEZ-SAONA LE[3], BONELLO P[1]

[1] Department of Plant Pathology, The Ohio State University, Columbus, OH, USA
[2] U.S. Forest Service, Dorena Genetic Resource Center, Cottage Grove, Oregon 97424, USA
[3] Department of Food Science and Technology, The Ohio State University, Columbus, OH, USA

Phytophthora lateralis (Tucker & Milbrath) is a soil-borne non-native, invasive pathogen that causes root rot of Port-Orford-cedar (*Chamaecyparis lawsoniana* (A. Murr.) Parl.) (POC). POC is native to Oregon and California but is also used in horticulture and urban forestry worldwide. The pathogen was first reported in the Pacific Northwest in 1920 and remained confined to western North America for decades, raising concerns about the viability of POC forest ecosystems and greatly diminishing POC use in horticultural plantings. But after its detection in Europe towards the end of the 1990s, concerns grew over POC's viability in horticultural settings there as well. *P. lateralis* is currently included in the EPPO A2 list of invasive species. The finding that POC expresses at least two types of genetic resistance (qualitative, including families with high survival; and quantitative, characterized by high phenotypic variability in survival time within and between families) has prompted the USDA Forest Service and the USDI Bureau of Land Management to start a large selection program for resistant genotypes to be used for reforestation and restoration. However, the identification of resistant trees based on classical greenhouse screening and testing techniques alone can take several years. The goal of this study was to determine if Fourier-transform infrared (FT-IR) spectroscopy, a chemical fingerprinting technique, can be used for the identification of resistant POC prior to infection. The methanol extracts of roots and twigs of rooted cuttings of parent trees used to produce 63 self-pollinated POC families, previously screened for resistance, were analyzed. Twigs were included in the analyses because they are far more accessible than roots, and would allow for less invasive sampling. Soft independent modeling of class analogy (SIMCA) was used to discriminate between resistant and susceptible trees, while partial least squares regression (PLSR) was used to predict mortality rates in the progenies. Analyses are currently in progress, but preliminary results suggest that FT-IR is able to correctly classify the two resistant types and susceptible POC individuals using both root and twig extracts. The implementation of this technique has the potential to facilitate the selection and breeding of resistant POC trees, for instance by allowing quick pre-selection of resistant genotypes before greenhouse screening.

Keywords

Fourier-transform infrared (FT-IR) spectroscopy, disease resistance, *Phytophthora lateralis*, chemical fingerprinting

Identification of European ash resistant to the invasive pathogen *Hymenoscyphus fraxineus* using Fourier-transform infrared (FT-IR) spectroscopy

Michelle CLEARY[1], STENER LG[2], STENLID J[3], ENDERLE R[4], METZLER B[4], KIRISITS T[5], DOWKIW A[6], PLIURA A[7], KJÆR ED[8], VILLARI C[9], BONELLO P[9]

[1] Swedish University of Agricultural Sciences, Southern Swedish Forest Research Centre, Sundsvägen 3, Alnarp, Sweden
[2] Skogforsk, Ekebo 2250, Svalöv, Sweden
[3] Swedish University of Agricultural Sciences, Dept. Forest Mycology and Plant Pathology, Uppsala BioCenter, Almas Alle 5, Uppsala Sweden
[4] Department Forest Protection, Wonnhaldestrasse 4, Freiburg Germany
[5] Institute of Forest Entomology, Forest Pathology and Forest Protection, Peter-Jordan-Strase 82, Vienna, Austria
[6] INRA Val de Loire, UR AGPF, 2163 avenue de la pomme de pin Ardon – CS40001, Orleans, France
[7] Institute of Forestry, Lithuanian Research Centre for Agriculture and Forestry, Liepu str. 1, Girionys, Kaunas, Lithuania
[8] University of Copenhagen, Rolighedsvej 23, Copenhagen, Denmark
[9] Ohio State University, Dept. of Plant Pathology, 201 Kottman Hall, 2021 Coffey Road, Columbus, OH, USA

Over the last two decades common ash (*Fraxinus excelsior*) has been threatened by an alien invasive pathogen *Hymenoscyphus fraxineus* (syn. *H. pesudoalbidus*; *Chalara fraxinea*) introduced to Europe from East Asia. As with most introduced pests and pathogens where the host plant lacks a history of co-evolution, the damage is devastating. The very existence of ash is now under threat as large populations of ash are disappearing from forest ecosystems and urban landscapes. Genetic resistance can be an important tool for disease management and to conserve the species from further demise. Even with high infection and mortality rates, variation in ash susceptibility to the pathogen has been observed within natural populations in field studies [1-3]. These studies show that disease expression is under strong genetic control (a heritable trait) which suggests considerable gain through selection and long-term breeding. While the mechanisms of ash resistance to *H. fraxineus* are unknown, some preliminary work supports the notion that plant specialized secondary metabolites are important for ash defense against *H. fraxineus* [4].The objective of this work will be to determine if using a state-of-the-art chemical fingerprinting technique known as Fourier-transform infrared (FT-IR) spectroscopy can identify ash resistant to *H. fraxineus*. FT-IR spectroscopy has been tested successfully in other forest-tree pathosystems to, for example, identify putative phenolic biomarkers in coast live oak against the deadly invasive pathogen *Phytophthora ramorum* [5], and discriminate between elm clones of differing levels of susceptibility to the Dutch Elm disease pathogen *Ophiostoma novo-ulmi* [6]. In 2015, we collected material from a select number of known susceptible and resistant ash genotypes from genetic trials in six countries (Sweden, Germany, Austria, France, Lithuania, and Denmark). Therefore, the objectives of this project will be to use FT-IR spectroscopy to 1) discriminate between resistant and susceptible genotypes and 2) predict the concentration of putative phenolic biomarkers of resistance. The implications of these results could significantly advance our current screening protocol and aid in the development of a more resistant ash population for future planting in forests, cities, and landscapes.

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Keywords
ash dieback, phenolic biomarkers, *Fraxinus excelsior*, *Hymenoscyphus fraxineus*, FT-IR spectroscopy

Defensive strategies in *Pinus pinaster*: interprovenance variation in constitutive and induced allocation to chemical defences

LÓPEZ-GOLDAR X, SAMPEDRO L, **Rafael ZAS**

Misión Biológica de Galicia (MBG-CSIC). Apdo. 28, 36080 Pontevedra, Galicia, Spain

Maritime pine can respond to antagonistic interactions with herbivores and pathogens by producing a modified phenotype with increased resistance that usually involves quantitative and qualitative changes in chemical defences. Maritime pine harbour a large intraspecific variation in life history traits, however little is still known about across population genetic variation in constitutive and induced allocation to chemical defences. Here we studied, in ten populations covering the north-south range of distribution, the constitutive allocation to chemical defences and the inducibility of those defensive traits in response to real herbivory and to simulated damage signaling. We performed a greenhouse experiment with young pines belonging to the Pinus pinaster CLONAPIN® clonal collection (Serida, Asturias, Spain), consisting of 25 genotypes from each of 10 provenances covering the whole range of the species distribution, with 4 clonal copies per genotype. Clones were subjected to either real herbivory by the large pine weevil (*Hylobius abietis*) or treated with methyl jasmonate (MeJa), a phytohormone analogue involved in damage signaling and defensive responses. We quantified pine defences (non-volatile resin and total polyphenolics) in stem and needles and the damage caused by the pine weevil in subsequent herbivory bioassays. There were significant differences across populations for both non-volatile resin and total phenolics. Both MeJa and real herbivory significantly increased the concentration of non-volatile resin in stem and needles. MeJa application decreased total phenolics in stem and needles, and no effect was detected for herbivory in stem phenolics. We found that debarked area by the weevil decreased after MeJa application, and that there were significant differences between populations in resistance against the pine weevil. Results suggests a latitudinal genetic variation in resistance to the herbivore northward across sites of origin of the populations. We detected significant genetic variation between and within populations only for constitutive defences but not for inducibility. The percentage of total variation explained by differences between populations or between families depended on the considered defensive trait. This study contributes to understanding the geographic structure and genetic differentiation of chemical defences between and within populations along the evolutionary history of this model pine species. Further work should address possible geographical or climatic patterns in the divergences observed in the defensive capabilities and in plasticity across populations.

Keywords

Intraspecific genetic variation; induced resistance; pine weevil; methyl jasmonate;

Effect of gall rust disease to *Falcataria moluccana* genetic resources in Indonesia

Sri RAHAYU

Department of Silviculture, Faculty of Forestry, Universitas Gadjah Mada, Bulaksumur, Yogyakarta 55281, Indonesia

Pathogens are posed to be a major threat to genetic variation of forest trees species. In the case of exotic pathogens, the balanced between host and pathogen does not exist, thus its more likely cause epidemic that decimate population and erode their genetic diversity. *Falcataria moluccana* (batai, sengon) occurs naturally in Indonesia (Moluccas and Irian Jaya islands), Papua New Guinea, New Britain and the Solomon islands. The trees have been planted and established more than fifty years in the community forest and more than twenty years in the plantation forest in Java Island, Indonesia. In 2004, outbreaks of gall rust disease on *F. moluccana* caused by Uromycladium falcatarium fungus have been occurred in East Java and persist in the entire of Java Island up to now. The disease causing severe damage to all growth stages of the plant from seedlings in the nursery to mature trees in the field. Due to almost all the genetic resources of batai trees have been devastated by the gall rust fungus, thus, it is needed to assess their genetic diversity and to ascertain their relationship to gall rust disease severity. Based on RAPDs technique, it was found that their genetic diversity was small, with 1.04 to 1.1 effective alleles, 34-55 polymorphic loci, 0.12-0.19 of Shannon Diversity Index and 0.2-0.3 of Nei's Diversity index. In addition the genetic distance among the seed sources assessed was narrow (0.04 to 0.15). All seedlings from Brumas seed sources (R02, R05, R2001 and 2S/75) were closely related to those from East Timor, East Flores, Moluccas and Java (Kediri, Jasinga, Ampel), but were distant from Wamena, Irian Jaya. Fortunately, seedlings from Wamena also showed more tolerant to gall rust disease than other seed sources as well as trees in the fields. Based on hystopathological assessment, it was found that epidermis cells and also primary floem of Wamena provenans, were thicker than others. Hence, mechanical defense such as tylosis were formed intensively. Thus, in situ and ex situ gene conservation from native population of Irian Jaya, particularly Wamena is required in order to prevent the loss of low frequency alleles that may be genes that confer protection against gall rust fungus.

Keywords

Uromycladium falcatarium, *Falcataria moluccana*, genetic resources

SESSION 3: Host-parasite CO-EVOLUTION and DURABLE breeding

Ecology and evolution of co-infection

Anna-Liisa LAINE

Metapopulation Research Group, Department of Biosciences, University of Helsinki, FI-00014
Helsinki, Finland

Co-infections by multiple pathogen strains are common in the wild. Theory predicts co-infections to have major consequences for both within- and between-host disease dynamics, but data are currently scarce. Here, we study co-infection dynamics of powdery mildew *Podosphaera plantaginis* in its host populations of *Plantago lanceolata* in the Åland Islands, SW Finland. We find coinfection to be common yet spatially structured in the wild. A common garden experiment showed that disease prevalence was higher in co-infected treatments both at the host genotype and population levels than in singly-infected treatments. Our experimental findings are confirmed in natural pathogen populations—more devastating epidemics were measured in populations with higher levels of co-infection. Moreover, populations supporting high levels of co-infection had higher pathogen survival between seasons, and genetic diversity is increased suggesting increased opportunities for outcrossing in the pathogen. Jointly, our results confirm the predictions made by theoretical and experimental studies for the potential of co-infection to alter disease dynamics across a large host–pathogen metapopulation.

Are patterns of *Populus nigra* geographical differentiation for resistance to *Melampsora larici-populina* dependent of genetic variation of the pathogen populations?

Catherine BASTIEN[1], DOWKIW A[1], FAIVRE-RAMPANT P[2], ALBERT E[1,5], VILLAR M[1], POURSAT P[3], ALMEIDA J[3], RIDEL C[1], GUERIN V[1], VIGUIER B[1], STEENACKERS M[4], JORGE V[1]

[1] INRA, UR0588 Amélioration Génétique et Physiologie Forestières, Orléans, France
[2] INRA, US1279, EPGV Etude du Polymorphisme des Génomes Végétaux, F-91057 Evry, France.
[3] INRA, UE0995 Génétique et Biomasse Forestières, Orléans, France
[4] INBO-VLAGEW, Research Institute for Nature and Forest, Geraardsbergen, Belgium
[5] Present address: UR1052 GAFL Génétique et Amélioration des Fruits et Légumes, F-84143 Montfavet, France

Breeding poplars for more durable resistance to *Melampsora larici-populina* (*Mlp*) leaf rust is a real challenge, especially in an European context where *Mlp* populations exhibit high genetic diversity in black poplar-larch sympatric areas and where selective pressures from the domesticated interspecific host (*P. xeuramericana* and *P.xinteramericana*) are both rapid and strong.Several approaches combining field evaluation under natural infection and artificial inoculation tests with control of the pathogen diversity were more recently directed at the study of genetic variation for partial resistance (QR), in the co-evolved parental species *P. nigra* L. Quantitative genetic studies of the genetic control of partial resistance were developed on one association population composed of more than 800 clones from European natural populations and genotyped with more than 8,000 SNPs.The existence of a large reservoir of variation for QR in different natural populations of P.nigra was demonstrated for several epidemiological parameters, with some evidence of geographical patterns. Significant variation for strain-specificity was observed at clonal level within a subset of high field-resistant natural poplar genotypes.The characterization of selection pressures exerted by host resistance on pathogen population provides useful insights into the dynamics of host-parasite co-evolution processes and is crucial for the design of both breeding and deployment strategies. Preliminary experiments suggested local adaptation of Mlp populations to partial resistances present in *P.nigra* populations. All these results will be discussed in the framework of poplar breeding strategies and poplar cultivar deployment.

Keywords

Poplar breeding, *Populus nigra* L., *Melampsora larici-populina* Kleb., leaf rust resistance, partial resistance, strain-specificity

A real-time evolution experiment with oak and powdery mildew

BARRES B[1,2], BODENES C[2], BURBAN C[2], DUTECH C[2], FIEVET V[2], GARNIER-GERE P[2], LEPOITTEVIN C[2], SAINT-JEAN G[2], **Marie-Laure DESPREZ-LOUSTAU[2]**

[1] ANSES, Laboratoire de Lyon, Unité RPP, 31 avenue Tony Garnier, 69007 Lyon, France
[2] INRA-Université de Bordeaux, UMR1202 BIOGECO, 69 route d'Arcachon, 33610 Cestas, France

Since Haldane's seminal communication (1949), struggle against disease has been widely acknowledged as a potent evolutionary agent in populations. Theoretical predictions have been made on the evolution of immune defense traits under natural selection (Seppala 2015). However, empirical studies investigating such questions are still few, especially for plants. Here, we designed a field experiment with several sub-populations of oaks (*Quercus robur*), including a set of progenies, submitted to contrasted levels of exposure to powdery mildew (*Erysiphe alphitoides*) in order to assess the effect of disease on phenotypic and genetic trajectories. More than one thousand seedlings were monitored during five years for traits related to parasite encounter (phenology), infection, and host response to disease impact (compensatory growth), i.e. avoidance, resistance and tolerance, as well as for growth and survival, considered as fitness proxies. All seedlings were also genotyped at 819 SNP (in genes putatively related to biotic and abiotic stress response) and 9 micro-satellite loci. A large phenotypic variation was found for all traits and significant differences between progenies were evidenced for the various components of tree resistance (sensu lato) and for growth and survival. Several genetic markers (SNP) were found to be significantly associated with avoidance, resistance or tolerance in genetic association studies. The contribution of the different traits to tree fitness was investigated. Finally, the phenotypic and genetic trajectories of the sub-populations submitted to low and high pathogen pressure were compared.

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Seppala O (2015) Natural selection on quantitative immune defense traits: a comparison between theory and data. J . EVOL. BIOL . 28: 1–9

Keywords

Erysiphe alphitoides, resistance, tolerance, avoidance, natural selection, ecoimmunology

Quantitative trait evolution within a qualitative resistance breakdown

Katherine HAYDEN[1,2], FABRE B[2], PÉTROWSKI J[2], HALKETT F[2], FREY P[2]

[1] Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh EH3 5LR, UK
[2] INRA, Université de Lorraine, UMR 1136 Interactions Arbres – Microorganismes (IAM), F–54280 Champenoux, France

Evolution of the fungal poplar rust pathogen *Melampsora larici-populina* (*Mlp*) has been shown to have been dramatically influenced by the deployment of qualitative resistance genes in commercial poplar plantations. After one notable breakdown event (that of the qualitative R7/virulent7 complex), virulent7 rust genotypes swept across France between 1994-1998, and resulted in the complete replacement of a lineage of *Mlp* (Xhaard et al. 2011, Persoons et al. in prep). We demonstrate that the virulence landscape continues to be dominated by the sweep described by Xhaard et al (2011), with regions heavily planted with poplar resistance type R7 dominated by the corresponding virulent7 type, along with those types that “hitch-hiked” across the landscape during the original sweep. These genotypes persist, despite a reduction in planting of R7, and a near-absence of resistance types corresponding to the hitchhikers, but with some evidence of the reduction in virulent7 after the initial sweep. Next, we present results of experimental tests of trade-offs in pathogen aggressiveness and reproduction, in relation to differences in host quantitative resistance (resistance measured by degree). Comparing traits across isolates collected before and after the landscape-wide sweep of the virulent7 rust lineage, we demonstrate rapid evolution of fungal aggressiveness traits in response to the quantitative resistance traits of the most widely deployed R7 cultivar.

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Keywords

Poplar rust, virulence evolution.

Environmental maternal effects on the early phenotype and resistance of *Eucalyptus grandis* and the structuring of fungal endophytic communities

Maria VIVAS, KEMLER M, SLIPPERS B

Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Cnr Lynnwood and University Road, Hatfield 0083, Pretoria, South Africa

The environmental experience of plants can influence the phenotype and stress tolerance of its offspring. This impact on the progeny is not related with modifications to the DNA sequence, but through processes of seed provisioning and epigenetic modifications. These maternal effects can modulate the development and interaction of the progeny with other organisms, which have significant effects on trees and their associated ecosystems. This experiment aims to study early performance and resistance to the wasp, *Leptocybe invasa* of *Eucalyptus grandis* seedlings from different maternal environments and genetic backgrounds. We also consider the potential impact on the fungal endophytes communities of the seedlings.Leaves and seeds were collected from two *E. grandis* plantations with the same genetic material (replicated clones), but different site qualities. A total of 18 leaf samples were collected from maternal trees (2 maternal environments x 3 genotypes x 3 ramets). Furthermore, a total of 1620 seeds were sown (2 maternal environments x 3 genotypes x 3 ramets x 90 seeds). Seed size and weight, as well as seedling development were measured. In addition, the response of the seedlings to natural infection with *L. invasa* was calculated as the rate between the number of galled leaves and the number of total leaves per plant. Finally, the composition of endophyte communities in the maternal leaves were assessed using Ilumina Miseq amplicon sequencing of the ITS rDNA.Height was different in seedlings grown from the contrasting maternal environments ($P<0.01$) and genotypes ($P<0.01$), but the interaction between all three was not significant ($P=0.277$). *L. invasa* infection was 2% different between seedlings grown from the contrasting maternal environments ($P=0.671$), and ranging from 37-57% of infection between maternal genotypes ($P<0.01$). The interaction for infection between maternal environments and genotypes was not significant ($P=0.205$). Moreover, preliminary sequence data from endophytes from one of the tree genotypes showed substantial differences in the composition of the endophytic communities between the two maternal environments. Our findings suggest that maternal effects may influence the early phenotype of *E. grandis* seedlings. Although not significant, there is a trend that shows seedlings from one of the maternal environments to be more susceptible *L. invasa* than seedlings from the other. Additionally, the fungal communities of maternal plants correlated strongly with the geographic locality. Ongoing work will characterize the response of the seedlings from different environment to pathogens and total fungal endophyte communities. A better understanding of the influence of the maternal environment on offspring and their interaction with other organisms might contribute to predict the response of trees to stress conditions and to enhance the efficiency of tree breeding programs.

Keywords

maternal environmental effects, phenotypic plasticity, epigenetic, microbiome, endophytes

Genotype by Inoculum Interactions and Deployment of Fusiform Rust Resistant Loblolly Pine Families in the Southeastern United States

Steve MCKEAND, SPITZER J, WHETTEN R, ISIK F

NC State University Cooperative Tree Improvement Program, Campus Box 8002 Raleigh, NC 27695-8002, USA

Fusiform rust (caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme*) is the most economically important disease of loblolly pine (*Pinus taeda* L.) in the southern United States. Deployment of resistant families is the only reasonable control practice available to the landowners who plant approximately 300,000 hectares of loblolly pine each year in high rust-hazard regions. Estimates of family resistance to different strains of the rust are critical for deployment decisions, because 95% of loblolly pine plantations are now established with individual families (85% open-pollinated families, 8% full-sib families, 2% clones). If families show significant interactions with different pathogen inocula, deployment of some families to different regions may need to be adjusted.

Comparisons of rust disease incidence measurements from field trials and controlled inoculation studies provide ample evidence of virulence variation in the rust inocula. There are also many examples of host family by inoculum interactions, but the predictive power of these observations is low; we cannot yet easily determine which planting sites might harbor rust pathotypes capable of overcoming the genetic resistance present in a specific pine family.

To estimate the risk that this lack of predictability entails, we compared rust breeding values of 56 loblolly pine families estimated from two independent sets of trials. We regressed the rust incidence breeding values of the families estimated from broadly-based field tests (14 sites with all 56 families tested across a broad geographic range) on breeding values of the same families estimated from narrowly-based tests (multiple test series with subsets of the families each with 4 tests). The model F test was highly significant (Pr<0.001). Breeding values based on local testing explained 75% of the variation in breeding values based on wide-geographic testing (R2=0.75), indicating that locally derived rust breeding values are relatively reliable predictors of the families' performance across a broad range of sites. Linear mixed models fit to the two data sets also showed that variance components due to genotype by environment interactions were negligible. The rankings of families were highly consistent across test sites within broadly- and narrowly-based testing schemes as shown by type-B genetic correlations of 0.95 and 91, respectively. We conclude that in the absence of a cost-effective assay for pathogen virulence factors, field-testing provides a reasonably accurate prediction of the operational value of pine families for deployment in regions with a high hazard for fusiform rust incidence.

Keywords

Pinus taeda, *Cronartium quercuum f.sp. fusiforme*, genotype x environment interaction, virulence variation

Changes in pathogenecity of *Uromycladium falcatarium* affected by pyroclastic cloud from merapi volcano, following its invasiveness on *Falcataria moluccana* in Indonesia

Sri RAHAYU

Department of Silviculture, Faculty of Forestry, Universitas Gadjah Mada,Jl. Agro No.1
Bulaksumur, Yogyakarta 55281, Indonesia

Falcataria moluccana (Sengon) is mostly planted monoculturally in community forests at valley of Merapi volcano Sleman, Indonesia. The presence of gall rust disease caused by the rust fungus, Uromycladium falcatarium, is able to reduce sengon productivity. The disease causing severe damage to all growth stages of the plant from seedlings in the nursery to mature trees in the field. Group of rust fungi have strong mechanism for adaptation and change their pathogenecity under critical condition, including very high or low temperature, relative humidity. The objectives of the research were to determine the morphological characteristics, survival and pathogenecity of U. falcatarium in sengon affected by pyroclastics cloud after Merapi eruption in November 2010.Inoculums of *U. falcatarium* were taken from sengon trees in the Southern hill of Merapi volcano which showed gall symptoms on 3 different affected area by pyroclastic cloud, i.e. danger (3-7 km), alert (>7-11 km) and warning (>11-15 km) from the top of Merapi. Three areas or locations from each distance then were randomly choose, namely fire location (directly affected), border locations (indirectly affected) and green location (completely uninfluenced) by pyroclastic cloud. Based on artificial inoculation test, pathogenecity of each inoculum were observed, while germination, penetration and infection ability of each inoculums were accessed using free hand section of disposable samples in order to get microscopic samples, and acquired using scope photo software. The color, size and shape of teliospore as well as texture and color of gall were observed using qualitative and quantitative assessment. Survival and aggressiveness of *U. falcatarium* in the fire area were lower than in the border or green location in all area including danger, alert and warning area. However, in the border location itself, the spores of *U. falcatarium* has more pathogenic and agresive, represent by higher percentage of germination, penetration, and infection also their ability to form the infective gall symptom than in the fire and green locations. This result was also supported by the characteristic of teliospores that were bigger, brighter, had better integrity shape (round) in the border location. In addition, *U. falcatarium* from the border location also had high ability to induce gall become bigger than in the fire and green locations. One of obvious case due to local environmental changing caused by Merapi eruption was the increasing of invasiveness of the gall rust disease caused by *U. falcatarium* on legume, particularly on *F. moluccana*.

Keywords

Uromycladium falcatarium, pyroclastic cloud, changes pathogenicity

Advanced generation clonal testing for multiple pest resistance and growth in *Thuja plicata*

John H. RUSSELL[1], YANCHUK AD[2]

[1] BC Ministry of Forests, Cowichan Lake Research Station, 7060 Forestry Rd., Mesachie Lake, British Columbia, Canada V0R 2N0
[2] BC Ministry of Forests, Forest Genetics Section, Tree Improvement Branch, 727 Fisgard St., Victoria, British Columbia, Canada V8W 9C1

An advanced generation breeding strategy for *Thuja plicata* is currently being implemented with the goal of developing a population with durable, cross resistance to multiple pests, and above average growth (Russell and Yanchuk 2012). As a part of this strategy, clones selected for reduced deer palatability through enhanced foliar monoterpene concentrations from F1 families, were established in five field and nurserybed sites in southern British Columbia (BC). Included in the trial were multiple F1 seedling controls that were resistance to either deer browse or cedar leaf blight (CLB; *Didymascella thujina*). In addition, there were multiple wildstand seedlots both susceptible and resistant to CLB, and seed orchard lots selected for enhanced growth and CLB resistance. Two of the sites were established in ecosystems with high deer browsing and foliar pathogen infection while the other two field sites and the one nurserybed were free from these pests, and conducive to optimal growth. Early results from one of the ”pest” sites indicated that, overall, clones were less palatable to deer compared to F1 seedlings, despite both genetic groups having been selected for high foliar monoterpenes. In addition, the clones were also less susceptible to CLB than both wildstand and seed orchard seedlings. Increased clonal resistance could possibly be related to a more mature ontogenetic stage. Clones were selected for resistant to both deer browse and cedar leaf blight while maintaining above average growth rates using clonal breeding values and independent culling. Selections from this study and future clonal trials will be used for breeding a F2 population with the goal of selecting for durable, cross resistance to pests, and above average growth. Clones resistant to heartwood rots, an important economic trait in Thuja plicata, cannot be evaluated for 20+ years. Inclusion of these clones will ideally be accomplished through genomic selection which is currently being developed. As climates change and pests, known and unknown, become more abundant, we anticipate that this breeding strategy will better accommodate shifts in environmental stresses.

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Keywords

Thuja plicata, clonal trial, cross resistance, breeding

Exploring native accessions, introductions and indigenous clones of *Hevea* spp. for disease resistance genes.

Chaendaekattu NARAYANAN, MYDIN KK

Crop Improvement Group, Rubber Research Institute of India, Ministry of Commerce and Industry, Govt. of India, Kottayam 686009, Kerala, India

Hevea brasiliensis (the Para rubber tree; family, Euphorbiaceae; 2n=36), native to the Amazon forests of Brazil, is the sole global source of natural rubber. The tree is monoecious, entomophilic, and predominantly out-crossing and freely hybridizing with other *Hevea* spp. Natural rubber latex has thousands of industrial uses and has a very high socio-economic value. Crop improvement in *Hevea* resulted in spectacular genetic enhancement of rubber yield increasing the yield per hectare from 300 kg/ha using seedlings to more than 3500 kg/ha through superior clones. International exchange and introduction of native germplasm accelerated genetic improvement of the species through hybridization and selection.Abnormal leaf fall (ALF) and shoot rot caused by *Phytophthora* spp., pink disease by *Corticium salmonicolor*, leaf infection by *Corynespora*, and powdery-mildew by *Oidium* sp. are the major fungal diseases capable of significantly reducing yield of rubber leading to severe economic losses upto 40% [1]. In severe cases, pink and shoot rot lead to complete drying of young plants considerable reducing stand density. South American leaf blight (SALB), caused by *Microcyclus ulei* (=*Dothidella ulei*) is a devastating fungal disease which almost wiped out rubber plantations in Brazil epidemic outbreaks; SALB is a looming threat to the global rubber cultivation. Every year, thousands of tons of fungicides are being used for prophylactic spraying in plantations not only for prevention of major disease outbreaks but also ensure good crop. However, long-term applications of fungicides have several environmental and socio-economic issues besides the huge costs. Rising trend in yield-loss due to diminishing resistance levels among popular clones, increased cost of chemical fungicides, coupled with acute scarcity in labour, mandate breeding for disease resistance as a high-priority area of research in *Hevea*. Many *Hevea* clones show variable levels of resistance to fungal pathogens; none of the clones are completely resistant/tolerant [2, 3, 4, 5, 6, 7]. Screening germplasm accessions revealed operation of high levels of resistance to fungal pathogens [7, 8]. Evaluation of hybrids (generated from Wickham clones and Amazonian accessions) in a mature field trial showed improvement of resistance to abnormal leaf fall among hybrids. Evaluation of hybrids showed maximum high leaf retention ranging from 59 % in family RRII 105 x RO 142, to minimum leaf retention in HP 168 of family RRIM 600 x RO 87. Wild accessions could successfully transfer their resistance traits to high-yielding clones without any compromise on yield. The study showed that the accession from Rondonia, RO 142, is possibly a potential source for genes for tolerance to ALF disease in Hevea, and hence, a good candidate for future disease resistance breeding.The high-yielding hybrids with appreciable levels of disease resistance have subsequently been advanced to final stages of their selection through participatory plant breeding approach before release for large-scale planting. Few introduced clones have also shown considerable levels of resistance to major fungal diseases. Few clones including PB 217, PB 260, and RRIM 600 from Malaysia displayed comparatively more tolerance to leaf infection by *Corynespora*. Similarly, clone Fx 516 from Brazil has been found to be highly tolerant to ALF by *Phytophthora* and moderately tolerant to powdery mildew by *Oidium* sp. Hence, clone Fx 516 were used for hybridization with high-yielding clones like RRII 414, RRII 430 and RRII 105, to recover high-yielding recombinants with enhanced levels of resistance. In addition, open pollinated progenies were also collected and for raising nursery evaluation trials in order to rapidly recover progenies with high-yield and tolerance to ALF disease. Among indigenous clones, RRII 5 and RRII 33, the clones developed by RRII, have been found to be more tolerant to diseases including powdery mildew caused by Oidium sp. In additional to above, many germplasm accessions have also been found to be tolerant to major fungal diseases [7]. Hence, these clones and germplasm accessions can also be used in breeding for disease resistance in *Hevea*. Since SALB is a global threat to rubber cultivation, it is important to develop strategies to prevent pandemic outbreak, for which a global network resistance breeding programme needs to be developed and implemented on a priority basis through international collaboration.

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Keywords

Resistance breeding, fungal pathogens, Hevea, germplasm, introduced clones, South American Leaf Blight, global network disuse resistance programme

Genetic characterization in scots pine provenances regarding resistance / tolerance against mistletoe and *Diplodia* tip blight

Franziska PETERS[1], VORNAM B[2], DOUNAVI A[1], SCHUMACHER J[1]

[1] Forstliche Versuchs- und Forschungsanstalt Baden-Württemberg, Wonnhaldestr. 4, 79100 Freiburg, Germany
[2] Abteilung Forstgenetik und Forstpflanzenzüchtung, Büsgen-Institut, Georg-August-Universität Göttingen, Büsgenweg 2, 37077 Göttingen, Germany

In recent years, the forest area affected by European pine mistletoe (*Viscum album* ssp. austriacum) has increased steadily in southwestern Germany, reaching threatening proportions for the standing crop in some cases, whereas the importance of this hemiparasite is relatively slight in the northern and eastern pine forests. To date, the cause of this increase cannot be scientifically ascertained, but there is evidence indicating that the effects of climate change (dry summers, warmer temperatures) are factors promoting infection with mistletoe. *Diplodia* tip blight of pine, caused by the thermophilic microfungus *Sphaeropsis sapinea*, has developed into an increasing problem impairing growth and health of native scots pine in Germany. For this pathogen, the beneficial effects of climatic change have been scientifically ascertained. It has also been shown that different provenances of scots pine occurring in Germany differ significantly in susceptibility to *S. sapinea* (Schumacher, 2012). Although a valid question, since both pathogens share the same host on similar environmental habitats, it has so far not been studied if primary infection with either mistletoe or *S. sapinea* will make scots pines more vulnerable towards the other pathogen.In this project we are analyzing the difference in susceptibility to *S. sapinea* of different pine provenances on a molecular level. We are also examining if susceptibility to mistletoe, which is not an important parasite on pine in large areas of Germany, can also be ascribed to a difference in provenance. Information on specific host-parasite interaction with regard to tolerance and resistance characteristics of different German pine provenances gained during this project is to assist us in stemming a further spread and an increase in severity of these forest disturbances in future by a selection of tolerant provenances.

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Keywords

Diplodia tip blight, mistletoe, pine provenance, genetic characterization, tolerance

Analysis of molecular traits enhancing tolerance of oak roots for feeding of forest cockchafer grubs

Julia TEPLY[1], DOUNAVI A[1], DELB H[1], CREYAUFMÜLLER CF[2], KREUZWIESER J[2]

[1] Forstliche Versuchs- und Forschungsanstalt Baden-WürttembergWonnhaldestraße 4D-79100 FreiburgGermany
[2] Institut für Forstbotanik und BaumphysiologieUniversität FreiburgGeorges-Köhler-Allee 53/54D-79110 FreiburgGermany

Forest cockchafer larvae (*Melolontha hippocastani*) are among the most destructive insect pests in many European forest ecosystems by causing severe damages to host roots. White oak species are among the favoured food sources, so that in areas of high grub population density the survival of oak progeny is endangered. Therefore, our work aims to elucidate the below-ground interactions of forest cockchafer grubs and pedunculate oak (*Quercus robur*) by identifying specific molecular traits that promote tolerance against grub feeding on roots.Soil-dwelling cockchafer grubs are found to be attracted by certain volatile organic compounds (VOCs) emitted by host roots. However, it is still unknown in which way different oak provenances differ in VOC composition and quantities and how this influences grub behaviour and fitness. Therefore, we are characterizing several German provenances at a chemical and at a genetic level. To this end, we will first challenge 4-year-old seedlings of pedunculate oak with cockchafer grubs and measure the changes of marker gene expression, of metabolite composition, and of VOC emission into the rhizosphere. Second, we also plan to assess the olfactory preference of grubs towards root volatiles of different provenances in a choice test. Finally, in order to define the genetic background of the oak seedlings, we use polymorphic microsatellites for detailed population genetic analyses. Hence, this work should allow us to make recommendations for the selection of provenances for areas of high cockchafer grub density and for marker-assisted breeding.

Keywords

Quercus robur, *Melolontha hippocastani*, VOC and metabolite profiling, gene expression, provenance

SESSION 4: POPULATION GENETICS of parasites and their vectors

Population genetics and genomics of insect pests: lessons from the pine processionary moth

Carole KERDELHUÉ

INRA, UMR CBGP (Centre de Biologie pour la Gestion des Populations), 755 avenue du Campus Agropolis, CS30016, F-34988 Montferrier-sur-Lez Cedex, France

In the last decades, population genetics has proved to be a powerful tool to study the neutral evolution of living organisms at different spatial and temporal scales. Classical molecular markers, mostly mitochondrial sequences and microsatellite loci, were successfully used in a range of species to characterize the natural structure of populations, to identify the environmental factors favouring or impeding gene flow, and to understand the driving forces of genetic differentiation, possibly leading to speciation. In the context of current global changes, population genetics was also used to disentangle the colonization routes of introduced species, and to decipher the demographic processes during invasions and expansions. By using appropriate markers and sampling designs, these approaches allow to identify the main past events (in phylogeny or phylogeography for instance) and/or the contemporary patterns. The recent advent of Next Generation Sequencing (NGS) technologies has revolutionized the field of population genetics, and now allows the development of genome-wide approaches even in non-model organisms. These major improvements provide most powerful tools to analyse the evolution of both neutral and adaptive genetic diversity.

I will illustrate the presentation through the case study of a major insect pest, the pine processionary moth *Thaumetopoea pityocampa* (*Lepidoptera*; *Notodontidae*). It is well known as a forest pest, and it is also a public health concern because the urticating larvae can cause severe allergic reactions. This insect belongs to a Mediterranean species complex which shows genetic spatial structures at different scales. Phylogeographical and population genetics studies have been conducted at different spatial scales, and allowed to identify strong geographical structures and mosaic contact zones between clades. More, the species is currently expanding its range northwards and to high altitude due to climate warming. Molecular markers have allowed to dissect various expansion processes and to understand the dispersal patterns. Finally, a very peculiar case of allochronic differentiation in Portugal will be presented. I will end by showing how recently developed NGS markers will soon be used to improve our knowledge of both the macro- and the micro-evolution of the studied species.

The importance to combine landscape-level replicates and multiple scales in landscape genetics analysis: implication for inference of gene flow in insects vectors of forest pathogens

Julien HARAN[1], PAJARES JA[2], SOUSA E[3], ROSSI JP[4], ROQUES A[1], ROBINET C[1], ROUX G[1]

[1] INRA, UR633 Zoologie Forestière - 2163 Avenue de la Pomme de Pin 45160 Ardon, France. Email: julien.haran@gmail.com
[2] University of Valladolid, Escuela Técnica Superior de Ingenierias Agrarias, Avd Madrid s/n, 34071 Palencia, Spain.
[3] INIAV, Avenida da Republica, Quinta do Marquês 2780-159 Oeiras, Portugal.
[4] INRA, CBGP, Campus International de Baillarguet, Montferrier-sur-Lez, France

The pine wood nematode (PWN), *Bursaphelenchus xylophilus* (Steiner & Burher) Nickle (*Nematoda*, *Aphelenchoididae*) is the causal agent of the pine wilt disease (PWD), a virulent syndrome killing susceptible pine trees within few months. From its native area in North America, it has been introduced in several Asian and European countries causing considerable damage to native pine forests. The PWN was detected for the first time in Europe in Portugal in 1999. From its introduction site, it has rapidly expanded its range to a large part of the country and entered into Spain. In Europe, the native longhorn beetle *Monochamus galloprovincialis* (Olivier) is the only known vector for this nematode. This beetle performs its larval development in the wood of declining pine trees and spread the PWN when it emerges from infected wood by feeding or ovipositing on surrounding trees. Thus, natural dispersal of the PWN is highly depending on beetle dispersal. Given the rapid range expansion of this pest and the threat to forests, identifying potential barriers to dispersal of *M. galloprovincialis* is a critical parameter to define suitable pest management strategies.Based on the genotyping of 1043 individuals from Iberian Peninsula, we conducted a landscape genetic analysis to uncover the landscape features affecting dispersal of *M. galloprovincialis*. We have combined multiple scale and landscape-level replications in order to explore landscape effect in its whole extent. This method was used to account for the complexity of landscape changes in the study area and the uncertainties regarding the scale of gene flow in this species. We also used this method to avoid potential confounding effect of historically clustered lineages.Our results support that various landscape features and climatic parameters (winter temperature, elevation and pine density) explain genetic structure of *M. galloprovincialis*. We discuss to which extent these features could restrict the dispersal of this species and its associate nematode. We show that this species is not homogenously structured according to the landscape over the area of study. The scales and the replications considered for analysis deeply influenced the inference of landscape effect on dispersal. This result stresses the importance to assess landscape effect in its whole in order to avoid misleading interpretation, especially for historically clustered species exhibiting good flying abilities.

Keywords

Invasions, barriers, dispersal, *Monochamus galloprovincialis*, *Coleoptera*, *Cerambycidae*, Pine Wood Nematode.

Population genetics of partial asexuality

Katja REICHEL, ROUGER R, MASSON JP, STOECKEL S

INRA, Institute for Genetics Environment and Plant Protection (IGEPP), Domaine de La Motte, 35650 Le Rheu, France

The ability to reproduce both sexually and asexually is very widespread and may well represent the ancestral state of all modern eukaryotes. In tree-parasite interactions, understanding the population genetic consequences of this complex mode of reproduction can be of vital importance for either side: While most plant parasites reproduce vegetatively during the growing season and only resort to sex when establishing their overwintering stage, some trees also engage in clonal reproduction. Using a mathematical model, we endeavored to understand the mechanisms by which partial asexuality affects the evolution of genetic diversity compared to the well-known sexual case. Our results show that time plays a crucial role: partial asexuality slows down the approach to the Hardy-Weinberg equilibrium, and thus random fluctuations due to genetic drift or demographic events remain visible for a much longer time. For markers with a finite number of alleles (SNPs, SSRs), we found no evidence for a mutation-based “Meselson effect”, as any increase in heterozygosity was due to genetic drift only. Finally, we also compared different “partially asexual” life cycles – and found that the effects of clonality in and “on” trees are not quite the same.

Keywords

Clonality, cyclic parthenogenesis, FIS, Meselson effect, Markov chain model

Microsatellite analysis of clonality in the emergent woolly poplar aphid *Phloeomyzus passerinii* in several French poplar stands

Stéphanie BANKHEAD-DRONNET, POINTEAU S, NAVASSE Y, DE FERAUDY D

Université d'Orléans, Laboratoire de Biologie des Ligneux et des Grandes Cultures EA 1207,
Rue de Chartres, BP 6759, 45067 Orléans cedex 2, France.
stephanie.bankhead@univ-orleans.fr

The emergence of endemic forest insects may result in severe outbreaks as well as changes in their distribution range, which could be favoured by global warming, range-expansion of natural host tree species, introduction of new host trees, or human-mediated dispersion. Life-history and ecological traits can also play a role in the capacity of insects to quickly adapt and spread in the new environment. In aphids, clonal and/or sexual reproduction modes are also known to have important consequences on these processes.The woolly poplar aphid *Phloeomyzus passerinii* (*Hemiptera*, *Aphididae*) is a tree-dwelling aphid that develops on the trunks and at the base of branches of above six-year-old and about 90 cm circumference trees. It reproduces mainly by apterous virginoparous females, but it can also produce sexual alates in autumn. Severe outbreaks mostly damage *Populus x canadensis* Moench (*P. nigra* x *P. deltoides*) hybrids, leading to tree growth reductions and massive mortality with significant economic losses. It has become the most significant pest for twenty years in poplar stands of southern Europe, Northern Africa and the Near-East. Here we aim at better understanding the reproductive modes and population genetic structure of *P. passerinii* in poplar stands from several French regions. We report on exhaustive sampling strategy involving accurate geopositionning and microsatellite genotyping of aphid colonies (using 12 loci identified in Pointeau et al. 2012, completed by 3 additional loci). We analyze genetic diversity by discriminating the number of multilocus genotypes (MLGs) and measuring several diversity indexes to address clonality (Arnaud-Haond et al. 2007).

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Keywords

Tree-dwelling aphid, *Populus*, clonality, emergent species, population genetics.

Genome evolution of clonal lineages after a recent introduction of an invasive fungal pathogen (*Cryphonectria parasitica*) in Europe.

Cyril DUTECH[1,2], FIEVET V[1,2], GOUZY J[3], ROBIN C[1,2]

[1] INRA, UMR1202 BIOGECO, 69 route d'Arcachon, F-33610 Cestas, France
[2] Univ. Bordeaux, BIOGECO, UMR 1202, Bât. B2 Allée Geoffroy St-Hilaire, F-33615 Pessac, France
[3] UMR441 LIPM, INRA, 31326, Castanet-Tolosan, France

The chestnut blight fungus (*Cryphonectria parasitica*), an Ascomycete infecting bark and cambial tissues of its host is a prominent example of an invasive plant fungal pathogen that has had a dramatic impact in its introduced range, North America and Europe (Anagnostakis 1987). Previous genetic studies have clearly identified two major introductions in Europe, one directly from Asia, the native area of the species, and the other from North America where the species was previously introduced (Dutech et al. 2012). In each case, a limited number of clonal lineages have successfully spread from southern to northern part of Europe suggesting questioning the importance of recombination events as a major driver for adaptation during the invasion (Dutech et al. 2010). In order to identify the main genetic changes which have occurred during the introduction step and the expansion step of these clonage lineages, we analyzed the genomes of 18 strains sampled from seven clonal lineages widely spread in France using the Ion Torrent sequencing technology. We identified some variations indicating that few recombination events occurred along the genome within each clonal lineage. This genomic study allowed us to identify the origin of major genetic changes in clonal lineages, and to explore their putative effects on the adaptation of the fungal pathogen for its new environment.

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Keywords

Exotic fungal pathogen, NGS, asexual reproduction, Chestnut blight canker.

Thousand cankers disease research progress in the United States and Italy

Denita HADZIABDIC[1], VITO L[1], WINDHAM M[1], LAMBDIN P[1], GRANT J[1], WIGGINS G[1], WADL P[1], OWNLEY B[1], STATON M[1], MOULTON J[1], MERTEN P[2], KLINGEMAN W[3], PSCHEIDTJW[4], CRANSHAW W[5], TISSERAT N[5], FACCOLI M[6], TRIGIANO RN[1]

[1] University of Tennessee, Department of Entomology and Plant Pathology, 370 Plant Biotechnology Building, Knoxville, TN 37996, USA
[2] USDA Forest Service, Forest Health Protection, 200 W.T. Weaver Boulevard, Asheville, NC 28804, USA
[3] University of Tennessee, Department of Plant Sciences, 2431 Joe Johnson Dr., 252 Ellington Plant Sciences Building, Knoxville, TN 37996, USA
[4] Oregon State University, Department of Botany and Plant Pathology, 1089 Cordley Hall, Corvallis, OR 97331, USA
[5] Colorado State University, Bioagricultural Sciences and Pest Management, 307 University Ave., Fort Collins, CO 80523, USA
[6] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padua, Viale dell'Università, 16, 35020 Legnaro, PD, Italy.

The plant pathogenic fungus, *Geosmithia morbida*, vectored by the walnut twig beetle (WTB), *Pityophthorus juglandis*, has been associated with a disease complex of walnuts, *Juglans* spp., known as thousand cankers disease (TCD). Infected trees initially display wilting and yellowing foliage, branch dieback and eventually tree mortality within 3–4 years after symptoms develop. As the WTB and the pathogen move within the phloem and spread throughout the tree, multiple dark brown- to- black cankers form, coalesce, and girdle twigs and branches, hence the name “thousand cankers” to describe the disease. TCD was originally described from the western U.S. and now has expanded to the native, eastern range of black walnut in the U.S. TCD has recently been discovered in northwestern Italy on both black and native *J. regia*, English walnut. Current research indicates that we have two overlapping WTB generations per season in the U.S. and Italy. Due to pathogen movement and possible global distribution, there is a critical need to understand genetic diversity and population structure of both the fungal pathogen, *G. morbida*, and its vector, WTB. Although species of the genus *Geosmithia* is distributed worldwide, *G. morbida* and most recently *G. pallida*, are the first members of the genus to be described as plant pathogens. Furthermore, the latest findings of co-infection of *G. morbida* with *Fusarium solani* species 25 indicated an uncommon synergistic two-fungus pathosystem that could weaken the host. We used microsatellite loci to investigate population structure and spatial distribution of *G. morbida* in the U.S. and Italy. Our results indicated high genetic diversity among *G. morbida* subpopulations with evidence of gene flow and a significant correlation between geographic and genetic distance. Analysis of molecular variance (AMOVA) indicated that most of the genetic variation was attributed to individual variation rather than divergence across sampling localities. Bayesian clustering analyses identified four distinct genetic fungal clusters in native and non-native regions of black walnut with no evidence of sexual reproduction or genetic recombination. Our results support the hypothesis that *G. morbida* was disseminated to different regions of the U.S. multiple times from multiple sources. Understanding the genetic composition, host-pathogen-vector interactions and demography of this complex can provide insight into future predictions of TCD occurrences.

Keywords

Geosmithia morbida, walnut twig beetle*, Pityophthorus juglandis*, *Juglans nigra*, *Juglans regia*, black walnut, English walnut, thousand cankers disease, population structure, microsatellite loci

Genetic structure and diversity of *Armillaria ostoyae*, agent of an emerging fungal disease in the recent south-western French maritime pine forest

Frédéric LABBE, ROBIN C, DUTECH C

INRA, UMR1202 BIOGECO, F-33610 Cestas, France
Univ. Bordeaux, BIOGECO, UMR 1202, F-33600 Pessac, France

The homogenisation of ecosystems and the recent intensification of agricultural and forestry methods are known to be important causes of emerging fungal diseases (Anderson et al. 2004). This process is less often described in forest likely because their degree of artificialization is generally low. The Landes de Gascogne forest in south-western France is an interesting example of barely monospecific maritime pine (*Pinus pinaster*) planted forest for 150 years and for which emergences of several pests are assumed for less than fifty years (Thiveaud 1992; Dupuy 1994; Vallauri et al. 2012). Out of these emergences *Armillaria ostoyae* causing significant decline in maritime pine was reported shortly after the impressive landscape transformation of the region (Guyot 1928; Lévy and Lung-Escarmant 1998; Aumonier 2007). In addition to these reports, recent epidemiological studies suggested that this disease has emerged from the ancestral coastal forests of the region, which appear to be the source of the pathogen populations. Under the hypothesis of expansion of *A. ostoyae* populations from the western coastal area to the new planted forests, we tested whether a signal of genetic expansion was detected by using 221 isolates sampled on 240 000 ha, 14 microsatellite and 27 SNP loci. We especially assessed the interest of spatial genetic analyses to identify putative directional migration from the ancestral to new founded populations, and approximate Bayesian computation analyses to infer the importance and the timing of demographic expansion of the fungal pathogen species.

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Keywords

Fungi, Parasite, Population genetics, Population dynamics

A population genetics approach yields an enhanced understanding of the *Dothistroma* needle blight invasion in Colombia

Irene BARNES[1], RODAS CA[2], GRANADOS GM[3], WINGFIELD MJ[1]

[1] Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria (UP), Pretoria 0002, South Africa
[2] Forestry Protection Programme, Smurfit Kappa Colombia. Calle 15 # 18-109, Yumbo, Valle, Colombia
[3] Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), Pretoria 0002, University of Pretoria (UP), South Africa

The red band needle blight pathogen *Dothistroma septosporum* has had a profound effect on plantation forestry in the southern hemisphere for 50 years (Barnes et al 2014). The tree species most seriously affected has been *Pinus radiata*, one of the first *Pinus* spp. to be deployed as a non-native to establish plantation industries in the southern hemisphere. As new and promising *Pinus* spp. are being tested for their suitability for planation forestry, new and unexpected disease problems are emerging. An example is the recent and serious outbreak of Dothistroma needle blight (DNB) caused by *Dothistroma septosporum* in Colombia on non-native *Pinus tecunumanii* plantations (Rodas et al 2015). DNB was first detected in the Central Zone in 2008 on 2.5 year-old trees. Within 2 years the disease had spread throughout all three forestry zones in the North, Central and South. Data collated over a three-year period showed the disease incidence and severity was highest in the North and Central zones with the South zone being less affected and recovering over time. No consistent relationship could be found between the age of tree, elevation or precipitation with disease severity. Population genetic analyses, however, conducted on 160 isolates collected from 13 farms across all zones indicated that two, very distinct clonal populations of the pathogen exist; one in the North and Central zone and the other in the South zone. The increased severity in the North and Central zone could be due to the presence of this single unique haplotype. Disease susceptibility trials indicated that while *P. keysia* and *P. tecunumanii* low elevation was highly susceptibleto the haplotype in the North and Central zone, *P. tecunumanii* high elevation and *P. maximinoi* were tolerant. These trees could be used, in future, as alternative species to plant for disease resistance against *D. septosporum* in Colombia and elsewhere.

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Keywords

Clonal invasion, *Mycosphaerella pini*, population genetics.

Population genetic analyses reveal the origin and reproductive mode of the quarantine pathogen *Lecanosticta acicola* in Europe

Josef JANOUŠEK[1], WINGFIELD MJ[2], MARMOLEJO-MONSIVAIS JG[3], JANKOVSKÝ L[1], STAUFFER C[4], KONEČNÝ A[5], BARNES I[2]

[1] Department of Forest Protection and Wildlife Management, Faculty of Forestry and Wood Technology, Mendel University in Brno, Brno 613 00, Czech Republic
[2] Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa
[3] Facultad de Ciencias Forestales, UANL, Carretera Nacional Km. 145, Apartado Postal 41, C.P. 67700, Mexico
[4] Forest Pathology and Forest Protection, Department of Forest and Soil Sciences, University of Natural Resources and Life Sciences, Vienna (BOKU), Vienna, Austria
[5] Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno 625 00, Czech Republic

Lecanosticta acicola (sexual state: *Mycosphaerella dearnessii*) is a heterothallic ascomycete that causes brown spot needle blight on pines. Severe infections can lead to retardation of growth and death of pines. *L. acicola* has a global distribution and infects both native and non-native *Pinus* spp., particularly in North America and Europe. The objective of this study was to consider the origin of European populations of *L. acicola* and to determine the mode of reproduction of the pathogen in Europe. To confirm the identity of the isolates as *L. acicola*, part of the Translation Elongation Factor 1-α gene was sequenced. For the population genetic analyses, eleven microsatellite loci were screened and the mating type idiomorphs were determined for 201 *L. acicola* isolates from 29 locations (19 countries). Microsatellite data analyses distinguished two distinct lineages in North America: northern and southern lineage. Approximate Bayesian Computation (ABC) analyses supported independent introduction of these lineages into Europe. Interestingly, these two European populations reflect the same geographic distribution as that of the North-American populations, which is probably related to climatic and/or host adaption. Microsatellite and mating type analyses revealed that the European populations undergo sexual reproduction. The dissemination of *L. acicola* by windborne ascospores could, therefore, explain its long distance dispersal and current epidemics in Central Europe. *L. acicola* is clearly an invasive alien in Europe that reproduces both sexually and asexually.

Keywords

Approximate Bayesian Computation, fungus, reproductive mode, population genetics

Population structure of the ash dieback pathogen *Hymenoscyphus fraxineus* in the UK.

Elizabeth S ORTON[1], BRASIER C[2], BANSAL A[1], BILHAM L[1], WEBBER J[2], BROWN JKM[1]

[1] John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK
[2] Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK
(elizabeth.orton@jic.ac.uk)

Common European ash trees, *Fraxinus excelsior*, have been subject to heavy dieback and mortality in their native range caused by the pathogenic ascomycete fungus, *Hymenoscyphus fraxineus*. *H. fraxineus* was first observed in the UK in 2012 in the east of England, although it is likely to have been present for many years before this. It is assumed that the pathogen has infected trees in the UK via two routes; through windborne sexual spores from continental Europe and by planting infected saplings imported from European nursery stock. In this study to determine the population structure of *H. fraxineus* in the UK, three sites were identified as 'established' where it was estimated that the pathogen population has been established over an extended period by natural spread from Europe. Three sites were identified as 'planted' which were locally isolated from other ash woodlands and were away from the predicted zone for wind dispersal and had been planted with ash saplings from nursery stocks in the previous 10-20 years. It was hypothesised that the genetic differentiation of the isolates collected from 'planted' sites would show a less diverse population structure than that of the 'established' sites based on the assumption that fewer individual isolates would have started the local epidemic. Fungal isolates were collected from lesions on stem samples collected in 2014 and one isolate per individual lesion was used for analysis. Vegetative compatibility among the isolates was tested to determine the population structure. A total of 30 SNP markers based on KASP™ genotyping chemistry were developed using the sequence information of 43 *H. fraxineus* isolates from across the Europe including the UK to study genetic differentiation among local populations and between planted and established sites.

Keywords

Fraxinus excelsior; *Hymenoscyphus fraxineus*; vegetative compatibility; SNP marker; genetic differentiation

Genetic diversity of populations of *Ceratocystis fimbriata* from *Eucalyptus* spp. in Brazil

Leonardo SS OLIVEIRA[1], VALDETARO DCOF[1], PIMENTA LVA[1], GUIMARÃES LMS[1], TERRA J[1], ZAUZA EAV[2], MAFIA RG[3], NEVES DA[4], ALFENAS AC[1]

[1] Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, MG 36570-000, Brazil
[2] Suzano Papel e Celulose, Centro de Pesquisa, Itapetininga, 18207-780, Brazil
[3] Fibria S.A., Centro de Tecnologia, Aracruz, 29197-900, Brazil
[4] Veracel Celulose S. A., Centro de Pesquisa, Eunápolis, 45820-970, Brazil

Ceratocystis wilt caused by *Ceratocystis fimbriata* is currently one of the most important diseases of *Eucalyptus* in Brazil. Planting resistant genotypes, previously selected by artificial inoculation under controlled conditions, is the main strategy for disease control. Although the selection of resistant eucalyptus clones is effective, recent studies have revealed a high genetic and pathogenic variability among isolates of *C. fimbriata*. Thus, the present study aimed to determine the genetic diversity of populations of *C. fimbriata* from *Eucalyptus* spp. in Brazil. Fungal cultures, used in this study, were obtained from recently dead or wilted plants showing typical symptoms of the disease, in the states of Bahia, Mato Grosso do Sul, and São Paulo by using carrot slices as bait. Thirty-six isolates from Bahia, 32 from Mato Grosso do Sul, and 29 from São Paulo were used for microsatellite analysis. Among 13 markers analyzed, nine were polymorphic and four were monomorphic, which allele size ranged from 131 to 397. We identified 25 genotypes from Bahia, 23 from Mato Grosso do Sul, and 21 from São Paulo, with Nei's gene diversity (H) of 0.2144, 0.2177 and 0.1736, respectively. The UPGMA trees showed that populations from São Paulo and Mato Grosso do Sul are closely related to each other and both grouped separately from the Bahia population. When compared to natural populations previously studied, the relatively high values of H observed in populations of C. fimbriata used in the present study, especially those from Bahia and Mato Grosso do Sul, indicate that the fungus is native to these areas. Because of the high genetic variation observed in populations of *C. fimbriata* on *Eucalyptus*, it is expected that high variability in aggressiveness may also occurs and have a direct impact in selection and breeding for resistance in eucalypt.

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Keywords

population genetics, microsatellite, *ceratocystis* wilt

Residents or Foreigners- origin of the *Dothistroma septosporum* outbreak in Scotland

Marta Joanna PIOTROWSKA[1], RIDDELL C[2], BADEN RS[3], TUBBY CV[3], HOEBE P[1], ENNOS R[2]

[1] Scotland's Rural College, Crop and Soil Systems Research Group, West Mains Road, Edinburgh, EH9 3JG, UK
[2] The University of Edinburgh, Institute of Evolutionary Biology, Charlotte Auerbach Road, Edinburgh, EH9 3FL, UK
[3] Forest Research, Alice Holt Lodge, Farnham, GU10 4LH, UK

The fungal pathogen *Dothistroma septosporum* is a causal agent of *Dothistroma* Needle Blight (DNB) on pines and other conifers worldwide. It has been causing damage in the UK since the mid to late 1990s and it has already seriously affected exotic Corsican pine (*Pinus nigra* ssp. laricio) and lodgepole pine (*P. contorta*) plantations and is now affecting Scots pine (P*. sylvestris*) plantations previously thought to be resistant. In 2010 DNB infection was found in Scottish nurseries supplying Scots pine for restocking, and more recently *D. septosporum* has been recorded widely on natural regeneration throughout the iconic Caledonian pinewoods. The aims of this project are to determine the origin of the *D. septosporum* population now present on native pine within Scotland (whether indigenous or introduced), and investigate possible causes of the disease outbreaks in forest nurseries. Populations of *D. septosporum* have been collected from 7 forest nursery outbreaks, from regeneration on 5 native populations of Scots pine and from stands of lodgepole pine adjacent to these native pine populations. Microsatellite marker and mating type variation will be assessed and compared among these populations to test three hypotheses for the origin of *D. septosporum* in native pinewoods; transfer from exotic plantations, transfer from infected planting stock, or an indigenous origin. To investigate possible causes of disease outbreaks in forest nurseries we will explore the hypothesis that this has been facilitated by evolution of resistance to site specific fungicides routinely applied to control disease in forest nurseries. Resistance of isolates from nursery outbreaks and elsewhere will be compared using an in vitro assay. The research will clarify the origin of *D. septosporum* on native pine, and the genetic diversity and structure of populations now present within Scotland.

Keywords

Dothistroma septosporum, Scots pine, population genetics, fungicides

SESSION 5: New GENOMIC tools and resources

Forest Health Research and Education Center: Leveraging forest tree genomics and genetics resources to mark and identify genes for resistance to important forest tree pathogens and pests

Albert ABBOTT[1], ZHEBENTYAYEVA T[2], JEFFERS S[2], JAMES J[3], SISCO P[4], PERKINS M[5], HEBARD F[4], GEORGI L[4], STATON M[5], LANDHAM R[1], DAVITT J[5], BODENES C[6], SANTOS C[7], COSTA R[7], NELSON CD[8]

1 University of Kentucky, USA
2 Clemson University, USA
3 Chestnut Return Farm, USA
4 American Chestnut Foundation, USA
5 University of Tennessee, USA
6 Institut national de la recherche agronomique-Pierroton, France
7 Instituto Nacional de Investigação Agrária e Veterinária, Portugal
8 USDA-Forest Service, USA

The recently established Forest Health Research and Education Center at the University of Kentucky (Lexington, Kentucky, USA) is focused on utilizing genetics and comparative genomics resources of key forest and fruit tree species to identify, mark and characterize genes for resistance to major tree pathogens such as *Phytophthora cinnamomi* (*Pc*) the oomycete pathogen causing *Phytophthora* root rot (Prr) of chestnut. In addition to being a serious tree pathogen, *Pc* is one of the most important plant pathogens worldwide. This pathogen infects ~1000 plant species including agricultural crop plants, landscape plants, and forest trees, causing significant losses both in the wild and under cultivation. At the Fourth International Workshop on the Genetics of Host-Parasite Interactions in Forestry, diseases incited by *Phytophthora* spp. were identified as the #1 threat to forests world-wide (Alvin Yanchuk, personal communication). Currently, no resistance has been identified in most of the important forest and plantation tree species. We have identified resistance to *P. cinnamomi* in hybrid progenies of American chestnut (susceptible) and Asian chestnut species (resistant) that appears to be conferred by one or a few resistance locus/loci. In this report, we present our QTL mapping data and comparative genomics analyses to mark and characterize the genetics of resistance to this important forest tree pathogen in chestnut and discuss the potential strategies we are investigating to rapidly mobilize this resistance into susceptible chestnuts.

Keywords

Forest Health Center, disease resistance, *Phytophthora cinnamomi*, genetics, genomics, QTL mapping, comparative genomics

Microbiome analysis of UK oak: towards an understanding of Acute Oak Decline in the UK

James E. MCDONALD[1], RANSOM-JONES E[1], DOONAN J[1], PACHEBAT JI[2], GOLYSHIN PN[1], DENMAN S[3]

[1] School of Biological Sciences, Bangor University, Deiniol Road, Bangor, Gwynedd, LL57 2UW, UK.
[2] Institute of Biological Environmental Rural Sciences, Aberystwyth University, Penglais, Aberystwyth, Ceredigion, SY23 3DA, UK.
[3] Forest Research, Centre for Ecosystems Society and Biosecurity, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK.

Acute Oak Decline (AOD) is a complex Decline-disease affecting both native oak species (*Quercus robur* and *Q. petraea*) in the UK. The presence of visible stem bleeds from cracks in the outer bark plates that overlie areas of necrotic tissue in the inner bark are the primary symptoms of AOD, and the predominance of bacteria in necrotic tissue implicates them as one of several putative contributors to the Decline. Two novel bacterial species, *Gibbsiella quercinecans* and *Brenneria goodwinii*, are consistently isolated from these necrotic lesions, revealing a suite of potential pathogenicity factors, including secretion systems and plant cell wall degrading enzymes. However, microbiome studies are also necessary to gain a greater understanding of the structure and function of microbial communities associated with healthy and AOD-affected trees. To investigate the role of the total oak microbiome in AOD,we developed methodologies for the extraction and enrichment of microbial nucleic acids from oak tissue and applied a combination of Illumina 16S rRNA gene amplicon sequencing and shotgun metagenomics to healthy oaks and AOD-affected trees at different stages of the syndrome (early, mid and late stage). In addition, we sequenced the total metatranscriptome (rRNA and mRNA) of the microbiome associated with necrotic lesions on AOD affected trees. These data provide important insights into the taxonomic and function composition of the oak microbiome, and ultimately, their role in AOD.

Keywords

Microbiome; Oak; bacteria; pathogen; metatranscriptome; metagenome

Identifying genomic resources against pests and pathogens in tree genera: a case study in *Fraxinus*

Laura J KELLY[1], LEE S[2], KOCH J[3], JEPSON P[4], CARLSON JE[5], ROSSITER SJ[1], CROWTHER W[1], BUGGS RJA[1]

[1] School of Biological and Chemical Sciences, Queen Mary University of London, Mile End Road, London E1 4NS, UK
[2] Forest Research, Northern Research Station, Roslin, Midlothian, EH25 9SY, UK
[3] U.S.D.A. Forest Service, Northern Research Station, Delaware, OH 43015, USA
[4] School of Geography and the Environment, University of Oxford, Oxford, OX1 3QY, UK
[5] Department of Ecosystem Science and Management, Pennsylvania State University, University Park, PA 16802, USA

Fraxinus (*Oleaceae*) is a genus of c. 48 species, which have a northern hemisphere distribution and include taxa of ecological and economic importance. *Fraxinus* species face severe threats from both an invasive insect pest and a fungal pathogen. Within North America, the emerald ash borer (EAB) beetle, *Agrilus planipennis*, has killed tens of millions of trees since its accidental introduction in the 1990s. EAB also occurs in mainland Europe, and is extending its range westwards from Russia at a rate of approximately 25 miles/40 kilometres per year. Added to this, *Hymenoscyphus fraxineus*, which causes ash dieback disease (ADB), is currently devastating populations of F. excelsior throughout Europe. In this project, we aim to develop a new approach for identifying genes conferring resistance to tree pests and pathogens based on detecting evidence of convergent molecular evolution in species with reduced susceptibility to ADB and EAB. Preliminary evidence indicates that species with reduced susceptibility to both threats occur in separate clades within *Fraxinus*, suggesting convergent evolution in these phenotypic traits and raising the possibility of convergent sequence evolution in the genes underlying these phenotypes.In order to screen for genome-wide evidence of convergent molecular evolution associated with reduced susceptibility to ADB and EAB, we are conducting whole genome sequencing (WGS) and assembly for a large number of *Fraxinus* species (c. 35), which are spread across the genus and include representatives of each of the major taxonomic groups. In parallel to this, data on the susceptibility of each *Fraxinus* species to ADB and EAB will be generated from experiments in the UK and the USA, respectively. To date, WGS has been performed for 14 individuals from 13 species, using a combination of the Illumina HiSeq and NextSeq platforms to generate approximately 35x genome coverage. The recent availability of the *F. excelsior* genome sequence (ashgenome.org) has provided a high quality reference that is being used to facilitate analysis of our genus-wide WGS data. In this talk, we will give an overview of our progress in the project to date, including the analysis of our newly generated genomic data and plans for sequencing of additional *Fraxinus* species, including those with large and complex polyploid genomes.

Keywords

Fraxinus, whole genome sequencing, ash dieback, emerald ash borer

Generation of a genetic map for *Armillaria ostoyae* to be used for locating a natural mutation that severely affects mycelial growth and is possibly associated with non-virulence

Renate HEINZELMANN, RIGLING D

WSL Swiss Federal Research Institute, Zuercherstrasse 111, CH-8903 Birmensdorf, Switzerland

The basidiomycete fungus *Armillaria ostoyae* is widespread in forest ecosystems of the northern hemisphere. As saprotrophic white rot fungus it is an efficient decomposers of dead wood. But at the same time it acts as aggressive pathogen and causes root and butt rot of healthy trees, reducing their timer value, productivity and stability. To date, *Armillaria* root disease can hardly be controlled. Previous inoculation tests revealed a large variation in virulence among *A. ostoyae* strains, ranging from non-virulent to highly virulent (Prospero et al. 2004). When basidiospores of an non-virulent *A. ostoyae* strain where plated, approximately half of the haploid progeny showed a completely abnormal phenotype. The mycelium of these cultures grew extremely dense and hardly expanded. This phenotype was maintained on different natural and artificial substrates and the normal phenotype could be restored upon mating of an abnormal strain with a normal haploid strain. Altogether these observations suggest that the abnormal strains are likely carrying a mutation in a single gene, which severely affects mycelial growth. Any such gene might be interesting, not only in *Armillaria*, but also in other fungi, because its dysfunction leads to drastically reduced growth and therefore also to reduced virulence. Double digest restriction-site associated DNA sequencing (ddRADseq) was used to sequence about 200 haploid progenies of the non-virulent *A. ostoyae* strain mentioned above. The obtained sequence data will be used to establish a set of 2000 to 3000 single nucleotide polymorphisms (SNPs) distributed over the entire genome. In a next step a linkage map for *A. ostoyae* will be constructed and then used to find the approximate genome location of the altered gene causing abnormal mycelial grow.

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Keywords

Armillaria ostoyae, ddRADseq, genetic map, non-virulence, abnormal mycelial growth

Elucidating the quantitative nature of *Mycosphaerella* spp. virulence using high throughput automated image analysis

Ethan STEWART

Institute of Integrative Biology (IBZ) Plant Pathology, ETH Zürich, Universitätsstrasse 2
LFW8092 Zürich, Switzerland

The fungal genus *Mycosphaerella* is the largest genus of plant pathogenic fungi with species affecting a wide range of plants, including many tree species. Typical symptoms include chlorotic and necrotic leaf lesions containing darkly pigmented asexual fruiting bodies called pycnidia. A high throughput phenotyping method based on automated digital image analysis was developed to accurately measure the percentage of leaf area covered by lesions (PLACL) as well as the number, size and pigmentation of pycnidia for the wheat pathogen *M. graminicola* (aka *Zymoseptoria tritici*) (Stewart & McDonald, 2014). This method allowed us to accurately measure phenotypic differences in two different *M. graminicola* mapping populations. Measuring the density and size of pycnidia on a leaf gives a direct measure of the reproductive output of the pathogen which has not been feasible until now. Pycnidia size, density and pigmentation were found to be quantitative traits that showed a continuous distribution in both mapping populations. Using the next generation sequencing technology RADseq, ~8500 informative SNP genetic markers were generated in each mapping population and were used in conjunction with the phenotype data to carry out a QTL mapping study. QTLs underlying all measured virulence traits were identified. In some cases, the same QTLs were associated with PLACL and pycnidia production, but in other cases they mapped as separate characters. Different QTLs were found between different mapping populations and different wheat cultivars. Based on the QTL mapping results, several candidate genes associated with virulence were identified. Whilst the current study was focused on a wheat pathogen, the methods would be equally suited to screening tree species for *Mycospherella* spp. disease levels as the symptoms are consistent across the genus. The method is freely available and also has the potential to be adapted to other plant diseases.

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Keywords

Image analysis, QTL mapping, plant pathology.

Genome-wide analysis of parasitic fitness in the Dutch elm disease fungi

Louis BERNIER, NIGG M, NARUZAWA ES, CORNET A

Centre d'étude de la forêt (CEF) and Institut de biologie intégrative et des systèmes (IBIS),
Université Laval, 1030 avenue de la Médecine, Québec (QC) G1V 0A6, Canada

Two successive pandemics of Dutch elm disease (DED) have been impacting elm populations during the last 100 years. The first pandemic was caused by the moderately aggressive ascomycete fungus *Ophiostoma ulmi* which has since been displaced by the more aggressive *O. novo-ulmi*, the species responsible for current pandemic. The nuclear genomes of *O. ulmi* and *O. novo-ulmi* were recently sequenced and annotated, thus providing new opportunities for deciphering the complex basis of parasitic fitness in these pathogens. Comparative analyses of the two genomes support previous reports that *O. novo-ulmi* has acquired new, useful alleles through interspecific crosses with *O. ulmi*. In silico annotation has confirmed that the DED pathogens possess several hundred orthologs of genes encoding putative pathogenicity/virulence factors, carbohydrate-active enzymes, cytochrome P450s and secondary metabolites. These genes are obvious candidates for functional analyses, including the recovery and phenotyping of knockout- and knockdown mutants. Ophiostoma reference genomes also provide a template in ongoing de novo sequencing projects for identifying cryptic mutations resulting in attenuated virulence in *O. novo-ulmi*. In addition, molecular, genomic and transcriptomic approaches are now being used in the study of yeast-mycelium dimorphism, a trait that the DED fungi share with other pathogens of plants and mammals. This has resulted in the identification of nuclear genes that play a role in dimorphism and whose products may also be involved in cross-talk with other metabolic pathways including those underlying reproduction and pathogenicity.

Keywords

Dutch elm disease, pathogenicity, virulence, dimorphism, genomics, transcriptomics

Using direct amplification and next-generation sequencing technology to explore foliar endophyte communities in experimentally inoculated western white pines

Lorinda S. BULLINGTON, LARKIN G

MPG Operations, LLC, 1001 S Higgins Suite A3, Missoula, MT 59801, USA

Fungal endophytes can influence survivability and disease severity of trees. Here we characterized the endophyte community in *Pinus monticola* (western white pine), an important species in the northwest USA, largely decimated by pathogenic fungi. We also assessed the ability to successfully inoculate seedlings with desirable endophytes, with the long-term goal of providing a protective microbiome and added defense from pathogens. We inoculated *P. monticola* seedlings in the field with potential pathogen antagonists and fungi isolated from healthy mature trees. Following inoculations we used direct amplification and next generation sequencing to characterize fungal endophyte communities, and explore interspecific competition, diversity, and co-occurrence patterns in needle tissues. Negative co-occurrence patterns between inoculated fungi and potential pathogens, as well as many other species, indicate early competitive interactions. Our results support the role of competitive interactions in early endophyte community assemblage and show that inoculations influence endophyte community development and tree health.

Keywords

Fungal inoculations, western white pine, next-generation sequencing

SESSION 6: From genetics to INNOVATIVE strategies

SubtiTree: an approach of synthetic biology in antifungal treatment for plants

Mathieu FOURNIE and the Toulouse iGEM 2014 team, Gilles TRUAN (instructor)

Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés (LISBP), 135 Avenue de Rangueil, 31400 Toulouse, FRANCE

A threat, caused by the *Ceratocystis platani* fungus, affects the gorgeous plane trees in one of the most beautiful sites in Southern France named “Canal du Midi”. A Toulouse student team (INSA, UPS) was committed to protect this UNESCO World Heritage site and has designed a bacterial cure named SubtiTree during the iGEM competition. Using a bacterium vector (*Bacillus subtilis*) naturally presents in the trees, our team offers an alternative solution issued from a synthetic biology approach. Using different genetic modules, we engineered a bacterium (SubtiTree) capable of swimming towards the pathogen, binding to its cell wall and finally delivering different fungicides to save the tree from its invaders. Besides, our team also began to imagine different strategies to prevent any accidental spreading of the optimized microorganism, thus limiting the ecological and ethical footprints of our synthetic bacterium. The first step of our project was to have a proof of concept of the three functions required to fight against the fungus disease. This work has received two prestigious distinctions during the iGEM competition: a gold medal and the Best Prize Innovation in measurement. Although our project originated from a very local and specific tree disease, SubtiTree opens new perspectives to vanquish many fungal associated diseases in plants

References

http://2014.igem.org/Team:Toulouse

Keywords

Synthetic biology, fungal diseases, plane trees, engineered bacterium, student

Plant tolerance as a component of *Ganoderma philippii* management in *Acacia mangium* plantations

Abdul GAFUR, SYAFFIARY S, NUGROHO A, WONG CY, SHARMA M

Group AAA Research and Development, Pangkalan Kerinci 28300, Riau, Indonesia

Plantation forests of fast-growing tree species, including *Acacia mangium* Willd., are established on a wide scale in South East Asia in anticipation of the ever-increasing global demand for wood. The effort was initiated to sustain the supply of forest products while preserving the natural forests. Disease infection, however, has occurred since establishment of the plantation forests. Red root rot caused by *Ganoderma philippii* (Bres. et Henn. ex Sacc.) Bres. is one of the most economically important diseases in the region. Research on field management had in the past focused on inoculum reduction, silviculture practices and application of biological control agents. Incorporation of tolerant genotypes into the effort was not adequately explored, mainly because of the lack of reliable and quick screening protocols. Recently we developed a faster and more consistent shade house screening method for red root rot tolerance in *A. mangium*. Data obtained from field experiments and commercial plantations indicate that tolerant materials previously selected in the nursery screening have less root rot incidence. This should allow progressive reduction in disease incidence, opening up an opportunity to using plant tolerance in integrated management of red root rot disease in tropical acacia plantations.

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Keywords

Acacia, disease management, plantation forest, red root rot, resistance

Timing interventions to sustain host populations and ecosystems threatened by non-native pests and pathogens

Anna W. SCHOETTLE[1], BURNS KS[2], CLEAVER CM[3], SNIEZKO RA[4], CONNOR JJ[5]

[1] USDA Forest Service, Rocky Mountain Research Station, 240 W. Prospect Rd, Fort Collins, CO 80526 USA; aschoettle@fs.fed.us
[2] USDA Forest Service, Forest Health Protection, Golden, CO USA
[3] Colorado State University and USDA Forest Service, Rocky Mountain Research Station, Fort Collins, CO USA
[4] USDA Forest Service, Dorena Genetic Resource Center, Cottage Grove, OR USA
[5] USDI National Park Service, Rocky Mountain National Park, Estes Park, CO USA

Restoration of forests devastated by non-native invaders often dominates the attention of forest managers and their actions. However, taking a broader view of the invasion beyond the crisis areas reveals opportunities where proactive management can alter the outcome of the invasion in threatened areas. Proactive management moves beyond the idea of protecting the hosts from exposure to the established non-native invader and shifts toward facilitating naturalization by preparing the landscape to sustain critical ecosystem function into the future in the presence of the invader.Increasing the frequencies of durable resistance traits within tree populations is accepted as a promising avenue for the co-existence of native tree species and non-native pathogens. For timely development and deployment of resistant material or promoting natural selection in the field, research on resistance mechanisms and their frequencies must be complimented by research on host genetic structure and adaptive variation, population dynamics, ecological interactions, and pest epidemiology. Developing the science foundation is especially critical when the host species is of ecological, but not commercial, interest and may therefore have little research history. Information on both qualitative and quantitative resistance and their frequencies may help guide management to attempt to slow pathogen co-evolution and sustain durability. The early timing of management interventions in natural populations can significantly reduce the ecological consequences of mortality during naturalization.Early attention to threatened but not-yet-severely impacted species or stands also provides opportunity for gene conservation. Seed, pollen or tissue collections can be made from the full genetic diversity of the host population before the potential bottleneck caused by high pathogen-induced mortality.This talk will present the approach and discuss its application in the western US for the white pine blister rust pathosystem. Aspects of the proactive strategy have been adopted for *Pinus flexilis*, *P. aristata*, *P. longevea*, *P. strobiformis* in the Southern Rocky Mountains, the Southwest and portions of the Great Basin. This strategy has also been incorporated into the US National Strategic Framework for Invasive Species Management (2013) (http://www.fs.fed.us/foresthealth/publications/Framework_for_Invasive_Species_FS-1017.pdf).

Keywords

Cronartium ribicola, *Pinus flexilis*, *Pinus aristata*, *Pinus longevea*, *Pinus strobiformis*, resistance deployment, qualitative resistance, quantitative resistance, management

A Systems Approach to Investigating Acute Oak Decline in Britain

Sandra DENMAN

Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK.
sandra.denman@forestry.gsi.gov.uk

Oak Decline is considered a complex disease syndrome of native oak, which has received particular, episodic attention in Europe from the 1900s onwards. In the 1980s in Britain, native oak trees with distinctive stem weeping symptoms were first noticed and increasing reports over time raised concerns about cause and effects on these iconic trees. Symptomology studies revealed bark necrosis and vascular cambial tissue degradation underlying the weeping patches suggesting a microbial component, as well as >90% co-occurrence of larval galleries of the bark boring buprestid, *Agrilus biguttatus* (Coleoptera: *Buprestidae*) implicating it in the condition. The study confirmed that it is a distinctive disease now called Acute Oak Decline (AOD), with four diagnostic symptoms. To investigate possible causes of AOD spatial epidemiology studies were initiated to determine spatiotemporal patterns of outbreaks, and conventional isolation and 454 NGS targeted metagenomic studies on healthy and symptomatic trees were carried out to detect putative microbial elements. The spatial studies recorded important epidemiological information and demonstrated local clustering of affected trees. Overall results of microbial studies demonstrated corroboration of the different approaches and highlighted two novel species of bacteria, *Brenneria goodwinii* (Bg) and *Gibbsiella quercinecans* (Gq) consistently associated with necrotic tissue, suggesting a role in lesion formation. Recognition of the possible involvement of multiple agents led to the hypotheses that (i) Bg and Gq have roles in causing necrosis and degradation of oak phloem and sapwood, (ii) interaction between *A. biguttatus* and these bacteria will lead to typical AOD symptoms, (iii) AOD is a complex Decline disease (as opposed to a primary disease) dependent upon the interaction of multiple factors for disease establishment. To address these hypotheses Systems research approaches are being adopted at different scales from molecule to landscape, cross-linking disciplines and results to produce integrated knowledge and contribute to deciphering key elements, their function and effects of interactions in this complex disease. The overall research plan will be outlined demonstrating that as far as finances allow, a holistic view of the problem and how to tackle it is being followed.

Keywords

Keywords: Acute Oak Decline, *Agrilus biguttatus*, *Brenneria*, *Gibbsiella*, Oak Decline, Systems approach

Influence of the genetically different types of *Cryphonectria Hypovirus 1* (CHV1) found in Europe on the virulence of *Cryphonectria parasitica*

Franziska PETERS[1], BUßKAMP J[2], NAKOU A[1], METZLER B[1]

[1] Forstliche Versuchs- und Forschungsanstalt Baden-Württemberg, Wonnhaldestr. 4, 79100 Freiburg, Germany;
[2] Nordwestdeutsche Forstliche Versuchsanstalt, Grätzelstr. 2, 37079 Göttingen, Germany;

Cryphonectria parasitica, the fungus causing chestnut blight, is spreading with an increasing genetic diversity throughout south-western Germany. Virulence of the pathogen is decreased by infection with a hypovirus. Several *C. parasitica* strains infected with CHV1 have been isolated from Baden-Württemberg. Genetically, these German CHV1 differ distinctly from hypoviruses found in Italy (CHV1_I) and France (CHV1_F1 and CHV1_F2, (Peters et al. 2014)). The latter are characterized by a difference in ecological fitness. Growth and sporulation of *C. parasitica* infected with CHV1_F1 and CHV1_F2 are much debilitated, so that trees are nearly cured, but further dispersal of the hypovirus is limited. On the other hand, infection with CHV1_I generally only impairs the pathogen with a limited effect, so that infected chestnut trees survive infection and timber is not damaged, while dissemination of the hypovirus-carrying fungus is nevertheless ensured (Robin et al. 2010, Bryner and Rigling 2011).The German hypovirus was compared to those from France and Italy by inoculating dormant chestnut stems. Additionally, we compared lesion size after inoculation of a chestnut stem with both the virulent and hypovirulent form of the pathogen with inoculation of only one form of the pathogen. A fast transmission of the hypovirus to the virulent form of *C. parasitica* was indicated. The type of hypovirus was the essential factor which determined lesion size on a chestnut stem. Results from *C. parasitica* strains infected with the German hypovirus were similar to those *C. parasitica* strains infected with CHV1_I, which has been described as having good ecological fitness. Accordingly, in the last few years, a distinct spread of hypovirulence has been recorded in the chestnut forests of Baden-Württemberg (Peters et al. 2012, Peters et al. 2014).

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Keywords

Cryphonectria parasitica, hypovirulence, ecological fitness, genetic diversity

Development of Real-time PCR assays for the detection of *Brenneria goodwinii* and *Gibbsiella quercinecans*

Sarah PLUMMER, BARRETT G, KACZMAREK M, HUNTER G, KIRK S, DENMAN S

Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK

Acute Oak Decline (AOD) is a comparatively new complex, Decline disease affecting native oaks in the UK. Some trees with symptoms of AOD can die within 4 years of the initial onset of symptoms while others appear to be able to overcome the attack and callus over the necrotic areas. Typical symptoms and signs of AOD include stem bleeding from longitudinal cracks in the bark, tissue necrosis beneath the weeping patches, D-shaped exit holes and larval galleries of the buprestid beetle *Agrilus biguttatus*.Through extensive sampling, two novel species of bacteria, Brenneria goodwinii and Gibbsiella quercinecans, have been consistently found on diseased tissue and have been shown to cause tissue necrosis and are therefore considered causal components of AOD. Conventional sampling methods are time consuming and costly. As a large number of samples are processed every year there is the requirement for a cost-efficient rapid molecular diagnostic (q-PCR assays) as a tool for the detection of these two key bacterial species on oak trees with symptoms of AOD. Oligonucleotide primers and fluorescently labelled hydrolysis probes were developed using species specific regions within the gyraseB gene. The *Gibbsiella* assay was developed in house and the *Brenneria* assay using a commercial company (PrimerDesign). Specificity tests were performed on a LightCycler® real-time PCR platform using DNA from a number of species within the *Brenneria* and *Gibbsiella* genera. Since the development of these primers and probes a number of novel, closely related species in both genera have been published with the consequence of some species cross reactivity within both genera. This necessitates more specificity testing and refinement of the primers and probes. Results of this work will be presented.

Keywords

Brenneria, *Gibbsiella*, molecular diagnostics, q-PCR

Development of biological control agents to manage *Ganoderma philippii* in tropical *Acacia mangium* plantations

Abdul GAFUR, NASUTION A, WONG CY, SHARMA M

Group AAA Research and Development, Pangkalan Kerinci 28300, Riau, Indonesia

The ever increasing global demand for wood is anticipated in some South East Asian countries through reforestation programs. Plantation forests, both industrial and community-based, have been developed to maintain sustainability of forest products while preserving the natural forests. This way, not only the economic importance of the forests, but their environmental and social roles are also preserved. Plantation forest productivity in the humid tropic areas is always challenged by pests and diseases. With the introduction of new plant species such as acacias and eucalypts for the development of industrial plantation forests, new pests and diseases become emerging threats. As a component of integrated pest management, some biocontrol agents have been developed to manage pests and diseases in plantation forests. This presentation discusses development of endophytic Trichoderma to manage red root rot disease caused by *Ganoderma philippii* in tropical *Acacia mangium* plantation forests. Results of some trials are elaborated. Future works should focus on continuous isolation of locally more adapted and stable antagonists. Endophytic microbes should also be introduced into the effort.

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Keywords

Biological control, disease, endophyte, *Ganoderma philippii*, plantation forest, red root rot, Trichoderma

Building the science foundation for threatened *Pinus aristata*, *P. flexilis*, and *P. longaeva*: opportunities for proactive intervention and studying consequences of invasion by *Cronartium ribicola* in real time

Anna W. Schoettle

USDA Forest Service, Rocky Mountain Research Station, 240 W. Prospect Rd, Fort Collins, CO 80526 USA; aschoettle@fs.fed.us

Species that are ecologically important yet generally unmanaged are being challenged by non-native pests and pathogens. Pre-invasion ecological and genetics research is often lacking for these species hindering the development of strategies to mitigate impacts. A framework and integrated research program was initiated in 2001 for high elevation five-needle pines threatened by white pine blister rust (WPBR) to provide the science foundation. The program includes studies of:
(1) Host genetics (e.g. genetic structure (Schoettle et al. 2012), adaptive trait variation (Borgman et al. 2014, 2015)),
(2) Genetic disease resistance (e.g. qualitative/quantitative resistance (Schoettle et al. 2014, Schoettle et al. 2011), DNA marker development (Liu et al., in progress)),
(3) Ecology (e.g. disturbance dynamics (Brown and Schoettle 2008; Schoettle 2004), regeneration (Coop and Schoettle 2009), species interactions (Tomback et al. 2005), planting methods (Casper et al. 2015)), and
(4) Areas at the intersections of these disciplines (e.g. physiological costs of resistance (Vogan and Schoettle 2015), host population-disease-genetic resistance modeling (Field et al. 2011, Schoettle et al. 2012; Schoettle/Antolin in prep), gene conservation, and seed transfer).
This poster summarizes the program's progress for P. aristata, P. longaeva, and P. flexilis and how it has been integrated to inform management (Schoettle and Sniezko 2007; Burns et al. 2008; Keane and Schoettle 2011; Schoettle et al. 2013; Schoettle et al. 2015). This research foundation and associate archived samples also offers further opportunities to research in real time the genetic, population, and ecological consequences of WPBR invasion in natural forest ecosystems.
Cooperators include: Kelly Burns, Richard Sniezko, Amy Angert, Michael Antolin, Erin Borgman, Peter Brown, Christy Cleaver, Jeff Connor, Jonathan Coop, William Jacobi, Robert Keane, Angelia Kegley, Jun-Jun Liu, Christopher Richards, Diana Tomback, Patrick Vogan, Detlev Vogler, and others.

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PlantaComp: Forestry genetic experimental network of the French National Institute for Agricultural Research

Christel ANGER

Department of Forest, Grassland and FreshWater Ecology, National Institute for Agricultural Research, UE GBFOR 995, Orléans, France

PlantaComp network aggregates about one thousand comparative trials installed through France. These experiments allow to compare several genetic units (provenances, progenies, clones) in varied environmental conditions. Installed by INRA genetic breeders since the 60s for the oldest, and currently handled by several INRA units, these trials aim to study the natural diversity of the main French forestry species and to create improved materials of reforestation. Several teams of INRA use PlantaComp network with this approach, but it can also be used as a tool to answer other scientific questions. Indeed, some comparative trials have already been used to study the behaviors of several genetic units facing new pathogen attack.

Keywords

Experimental network, comparative trial, genetic resources

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