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Proceedings of the Sixth International Workshop on the Genetics of Host-Parasite Interactions in Forestry

Tree Resistance to Insects and Diseases: Putting Promise into Practice

Mt. Sterling, Ohio
August 5–10, 2018

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Cover Photos

Front:

Top left and top right—Several provenances of North American and European ash trees are evaluated in field trials for resistance to the invasive emerald ash borer (*Agrilus planipennis*) at the U.S. Forest Service Northern Research Station, Delaware, OH. (photo courtesy of Ignazio Graziosi)

Bottom left—Emerald Ash Borer on an ash tree that has visible bark cracks from larval feeding.

Bottom center—The extensive emerald ash borer (*Agrilus planipennis*) feeding galleries visible on this European narrow leaf ash (*Fraxinus angustifolia*), part of a field trial along with other North American ash trees at the U.S. Forest Service Northern Research Station, Delaware, OH indicates that it is susceptible. (photo courtesy of Ignazio Graziosi)

Bottom right—Several tree species are grown in common gardens to be evaluated for resistance to various insect pests and pathogens at the U.S. Forest Service Northern Research Station, Delaware, OH. (photo courtesy of Ignazio Graziosi)

Back:

Top left—Dead Port-Orford-cedar (*Chamaecyparis lawsoniana*) in Redwood National Park, California, killed by the non-native pathogen *Phytophthora lateralis*. (photo by Richard Sniezko, U.S. Forest Service)

Top right—Dead Port-Orford-cedar along Highway 101 in southern Oregon. (photo by Richard Sniezko, U.S. Forest Service)

Second row left—Resistant parent tree surrounded by Port-Orford-cedar killed by *P. lateralis* in northern California. (photo courtesy of Chuck Frank)

Second row right—Breeding Port-Orford-cedar for resistance in containerized orchard at Dorena Genetic Resource Center. (photo by Richard Sniezko, U.S. Forest Service)

Third row center—Port-Orford-cedar (dead and alive) in field trial. (photo by Richard Sniezko, U.S. Forest Service)

Third row right—Resistance trial planted underneath dead Port-Orford-cedar at South Slough NERR in Oregon. (photo by Richard Sniezko, U.S. Forest Service)

Bottom left—Port-Orford-cedar field trial near Oregon coast. (photo by Richard Sniezko, U.S. Forest Service)

Disclaimer

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Editors: C. Dana Nelson, Jennifer L. Koch, and Richard A. Sniezko

ABSTRACT

The Sixth International Workshop on the Genetics of Host-Parasite Interactions in Forestry—Tree Resistance to Insects and Diseases: Putting Promise into Practice was held the first week of August 2018 at Deer Creek State Park in Mt. Sterling, Ohio. The workshop provided a continuing forum for researchers, tree breeders, and forest managers to focus on perhaps the most salient action society can take to restore tree species imperiled by invasive pests and pathogens—developing and deploying resistant tree populations. The 95 presentations (oral and poster) covered a wide range of tree species and associated pathogens and pests from throughout the world. Ash species (genus *Fraxinus*) throughout Europe and North America face relatively new perils (ash dieback disease and emerald ash borer) and a special session was devoted to this genus. Screening for resistance is a fundamental component of resistance development and the topic of another full session. Additional presentations detailed efforts to develop genomics and biotechnology tools and resources that have the potential to increase the efficiencies of applied resistance programs. Other presentations provided inputs on collaborative breeding approaches, citizen science in forest health, the potential role of endophytes in managed forests, threats to agroforestry species, prioritization of species in need of resistance programs, considerations necessary for the use of new biotechnologies, and concepts for integrating genetic, phenotypic and environmental data across host-parasite systems. Several presentations provided updates on "the ultimate goal"—progress of applied resistance programs and actual restoration efforts—leading into discussions on key topics such as the durability, stability, and usability of resistance in forest tree species. Taken together the presentations and discussions provided ample evidence that developing resistant populations is a viable (and in some cases necessary) approach for society to use to ensure healthy forests for future generations. These proceedings document the presentations given as lightly reviewed full papers, extended abstracts, and standard abstracts. In concluding the workshop, the attendees approved Spain by acclamation as the host of the Seventh Tree Resistance Workshop to be held in 2020[1].

Keywords: Durable resistance, ecosystem restoration, forest health, genetics of resistance, insect and disease mitigation, invasive pests and pathogens, resistance breeding.

[1] This workshop has been postponed until 2021 due to the Covid-19 pandemic.

ACKNOWLEDGMENTS

Many people and organizations helped to make this a successful workshop, including the workshop organizing and technical committees (listed below). We especially thank the local organizing committee and volunteers and the field tour presenters and guides (see listed below). We also thank Ellen Crocker, Reneé Williams, Leslie Queary, Briana Fortunato, Hannah Hollowell, and Sarah Hayes (University of Kentucky, Department of Forestry and Natural Resources) for administrative and logistic support including workshop program and abstract book preparation. We thank Monica Schwalbach and her colleagues (U.S. Forest Service, Southern Research Station) for coordinating the USFS's meetings management program for the workshop. A special thank you to our Keynote speaker Dr. Stephen Woodward (University of Aberdeen), Banquet speaker Dr. Kim Steiner (Pennsylvania State University), and the Western Forestry and Conservation Association for on-line registration. Our sponsors were key to making the workshop happen and included: USDA Forest Service; INRA, France; Forest Health Research and Education Center (University of Kentucky); Departments of Plant Pathology and Entomology (The Ohio State University); New Phytologist Trust (and their Plants, People, Planet journal for publishing the special issue); the International Union of Forestry Research Organizations (IUFRO) Groups: 7.03.11—Resistance to Insects and Pathogens, 2.02.15—Breeding and Genetic Resources of Five-needle Pines, Task Force on Forests and Biological Invasions; and the American Phytopathological Society (APS), Forest Pathology Committee. We also gratefully acknowledge the support provided by the Foundational Program on Pests and Beneficial Species in Agricultural Production Systems, grant no. 2018-67013-28487/project accession no. 1016633, USDA National Institute of Food and Agriculture (NIFA).

Finally, we thank all the participants of the workshop for presenting their work and serving as moderators, discussion leaders, poster session organizers, and closing summary commenters; and we are grateful for the many interactions that made this workshop so successful and helping to keep resistance work in a prominent place in maintaining forest health into the future.

Workshop Technical Committee:

- **Enrico Bonello**, Ohio State University, USA
- **Stephen Woodward**, University of Aberdeen, UK
- **Acelino Couto Alfenas**, Federal University of Viçosa, Brazil
- **Caterina Villari**, University of Georgia, USA
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- **Jared LeBoldus**, Oregon State University, USA
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Local Organizing Committee: Kathleen Knight, Mary Mason, Leila Pinchot, Dave Carey, Aletta Doran

Local volunteers: Kathi Combs, Andrea Watros, Terri Herd

Field tour presenters: Mary Mason, Dave Carey, Kathleen Knight, Leila Pinchot, Charlie Flower, Jim Slavicek

Field tour guides: Aletta Doran, Kelly Rusin, Kirsten Lehtoma, Tim Fox, Stephen Kelleher, Ruby Stathers, Alan Coburn

Workshop website: https://treeresistance2018.ca.uky.edu/.

CONTENTS

PREFACE

In 2018, an engaging and energizing workshop on Resistance to Pests and Pathogens in Forest Trees (also known as the Sixth International Workshop on the Genetics of Host-Parasite Interactions in Forestry) was held at the Deer Creek State Park Lodge in Mt. Sterling, Ohio. This was the third workshop in this series since 2011. The 2011 workshop re-initiated the series that had started in the 1960s and last convened in 1980 (information and the rationale for the pre-2011 workshops are included in the Preface to the proceedings of the 2011 workshop. We compiled and edited these proceedings to document the research presented and discussed at the "Sixth Workshop" and to encourage attendees and our readers to continue their efforts to develop forest trees that are resistant to pests and pathogens. Information about the Fourth, Fifth and Sixth Workshops can be found at the following web sites:

Fourth (2011)—https://ucanr.edu/sites/tree_resistance_2011conference/,

Fifth (2015)—https://colloque.inra.fr/tree-parasite-interactions2015/, and

Sixth (2018)—https://treeresistance2018.ca.uky.edu/.

The Fourth Workshop (2011) workshop inspired several of its participants to call further attention to an underlying problem in forest health with a paper published in Frontiers in Ecology and the Environment. In the same manner, discussions at the Fifth Workshop (2015) helped crystallize some thoughts that led directly to a publication in Frontiers in Ecology and Evolution focused on the potential of tree resistance to mitigate invasive pathogens and pests. Following this, an IUFRO Task Force on Forests and Biological Invasions recognized the importance of including the resistance topic, and produced a special issue in Biological Invasions which included a paper that provided recent success stories in breeding for resistance.

For the Sixth Workshop (2018), we partnered with New Phytologist Trust to produce a special issue on Tree Resistance in its journal Plants, People and Planet (PPP). This special issue (PPP, volume 2, issue 1) includes full papers that are directly related to four of the abstracts presented at the Sixth Workshop. The four abstracts as submitted to the workshop are published in these proceedings and we annotated them with their reference in the special issue of PPP. A fifth presented abstract was published as a paper in New Phytologist and its reference is provided. In addition, the special issue includes three other papers that are related to various presentations given at the workshop.[1] In total, the proceedings contain abstracts, extended abstracts or full papers on all 59 talks and 37 posters offered at the meeting. Of these, 14 are extended abstracts or full papers.

It is notable that Dr. Norman Borlaug (Nobel Peace Prize winner in 1970 for his world-wide contributions in agriculture) participated in the first two workshops (in 1964 and 1969). The proceedings and book resulting from the earlier workshops inspired both the re-initiation of this workshop series, and the format for the meeting—with an emphasis on discussion, debate, and the absence of concurrent sessions. This workshop series was envisioned as a melding of research

[1] Showalter, D.N., et al. Resistance of European ash (*Fraxinus excelsior*) saplings to larval feeding by the emerald ash borer (*Agrilus planipennis*); Plumb, W.J., et al. The viability of a breeding programme for ash in the British Isles in the face of ash dieback; Buggs, R.A. Changing perceptions of tree resistance research.

with application, that is, the delivery of effective pest and pathogen resistance for restoration and reforestation. To do this, it was realized that we needed to retain the focus of the end point—the development and use of resistance—and bridge the divide that exists between researchers, tree breeders and forest managers. To facilitate this goal, we held a discussion session with a panel of forest health managers that specifically addressed this topic. Furthermore, we felt it was important to emphasize success stories to help dislodge both the myths around resistance and the fallacies that other management activities by themselves could save our forests. Success stories on the development of resistance are starting to emerge and this should facilitate support for ongoing and future efforts. Developing resistance is a concrete, attainable goal that can lead to substantial societal benefits.

The need for action on efficiently developing genetic resistance to the excessively damaging pests and pathogens in forest trees is more apparent now than ever, and several examples presented at the Sixth Workshop and published in these proceedings show that it can be done. As new tools in genomics and biotechnology are developed in an integrated fashion with ongoing resistance breeding programs, there is evidence that they can increase efficiency and effectiveness of the production of resistant trees. The key is keeping the focus on the breeding objectives and integrating tools that can expedite progress. Success in developing resistance will require communication and partnerships between researchers, tree breeders, resource managers and the public, as well as a sharp and steady focus on the "ultimate goal"—restoration or reforestation of species of concern for forest health and human benefit. To fully succeed we also need to be aware of other key ecological factors that may play a role in the health of forest trees.

These workshops have been well received and interest in the topic continues to grow. As an example, groups were volunteering to host the next two workshops before the 2018 workshop began. The 2018 attendees approved by consensus a proposal to host the Seventh Workshop in Spain in September 2020[2]. Furthermore, the time between workshops has been reduced as both the urgency of action and the enthusiasm of the many visionaries in this field have coalesced. We are grateful that IUFRO and other groups that have supported these meetings and optimistic that the next generation of scientists and resource professionals will continue this important work for the benefit of our planet's forests. In addition, we thank the participants and sponsors of the Sixth Workshop for their generous support and for adding to the vision that with Tree Resistance we are truly on the cusp of a paradigm change for restoring forest health. Finally, we thank those that came before us and organized and hosted the prior five workshops. To succeed, we need to be a community, all committed to a common cause – forest health, globally.

Richard Sniezko, USDA Forest Service, Cottage Grove, Oregon

Jennifer Koch, USDA Forest Service, Delaware, Ohio

C. Dana Nelson, USDA Forest Service, Lexington, Kentucky

March 10, 2020

[2] This workshop has been postponed until 2021 due to the Covid-19 pandemic.

Participants in The Sixth International Workshop on the Genetics of Host-Parasite Interactions in Forestry. Mt. Sterling, Ohio, August 5–10, 2018.

Tree Resistance Workshop Organizing Committee. From left to right: Ellen Crocker, Mark Coggeshall, Dana Nelson, Jeanne Romero-Severson, Jennifer Koch, Richard Sniezko

A SHORT HISTORY OF TREE RESISTANCE SELECTION AND BREEDING THROUGH THE JOURNAL *FOREST PATHOLOGY*

Steve Woodward[1], Stuart Fraser[1,4], Tuğba Doğmuş[2], and Alberto Santini[3]

Forest Pathology was first published in 1971, then under the title *European Journal of Forest Pathology*. The first editor-in-chief, Peter Schütt, assembled a board of like-minded individuals based in several European countries to present the progress of research on tree and forest health, initially focused on Europe itself. Within a short time, however, the journal was recognized internationally and attracted manuscripts from all forested continents. In 2000, under the second editor-in-chief, Ottmar Holdenrieder, the inclusion of increasing quantities of global science in the journal led to a change in the name to *Forest Pathology*, which is maintained to this day.

Since the outset, the journal has published papers on the potential use of resistance to pathogens in the management and control of tree diseases. Using "resistance" as the search term on the journal home page produces 1,131 "hits" for individual papers, although the precise nature of the "resistance" referred to is obscure in some cases. Host-pathogen systems reported on vary widely, from the expected (e.g., white pine blister rust, Dutch elm disease, chestnut canker) to less commonly known interactions (e.g., *Microcyclus* on *Hevea*; *Ralstonia* on *Casuarina*). More recently, the focus has, to some extent, shifted to potential resistance against recently detected invasive pathogens, such as ash dieback and species of *Phytophthora*, but other longer-term problems still receive plenty of attention.

Subjects for examination have also varied from the traditional aspects of selection and breeding through the physiological mechanisms contributing to resistance, to, more recently, the molecular biology of tree-pathogen interactions.

In this review, we will present an analysis of the work on tree resistance published in *Forest Pathology* since the journal's first issue, including data on the numbers of papers published on different host-pathogen interaction. We will evaluate, as far as is possible, the overall contribution of papers in this journal to our current understanding of the potential for host resistance in contributing to the production of trees resistant to pathogens.

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Session 1

BREEDING FOR RESISTANCE TO INSECTS

SCREENING FOR RESISTANCE AGAINST INSECT HERBIVORES USING CHEMICAL AND ANATOMICAL DEFENSES IN LODGEPOLE PINE AND WHITE SPRUCE

Jennifer G. Klutsch[1], Chen. X. Kee[2], and Nadir Erbilgin[2]

Insect outbreaks and drought have increasingly caused widespread tree mortality worldwide. Host defenses are important factors that determine tree resistance to these insect disturbances. However, trade-offs between growth and defense may occur and vary with life history strategy of the host tree. We measured defense and growth patterns from mature lodgepole pine (*Pinus contorta* var. *latifolia*) and white spruce (*Picea glauca*) in two progeny trials in Alberta, Canada. These trees have different life history strategies, with lodgepole pine being shade intolerant and white spruce being shade tolerant. Furthermore, the major insect pests for these trees differ; the bark beetle *Dendroctonus ponderosa* is a tree-killing insect on lodgepole pine and defoliation from *Choristoneura fumiferana* is the main disturbance for boreal white spruce. We measured anatomical defenses found in the xylem and monoterpene concentrations within phloem of the stem, which have been associated with resistance to bark beetle attack. Lodgepole pine and white spruce were found to differ in their growth-defense relationships. In addition, we did not find evidence of a trade-off between anatomical defenses and radial growth. Furthermore, we found an effect of the presence of the disease caused by *Endocronartium harknessii* on lodgepole pine on both anatomical and chemical defense traits. Our results preliminarily suggest that the major insect herbivore associated with tree species may influence their relative investment in defense and that understanding the variation in constitutive defense capabilities and the existence of genetic trade-offs between growth and defenses can be important factors for pest resistance screening.

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ADULT HOST SELECTION TRIALS FOR SPRUCE BUDWORM RESISTANCE IN DOUGLAS-FIR: A CAUTIONARY TALE

Ward Strong[1]

Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, is a major commercial forestry species in British Columbia, Canada. Its range includes wet coastal areas and dryer Interior regions (Hermann and Lavender 1990) (fig. 1). In the interior range, it is subject to attack by the western spruce budworm, *Choristoneura occidentalis* Freeman (Lepidoptera: Tortricidae). The larvae of this moth can cause up to 100 percent foliage loss, and tree death can occur after successive years of defoliation (Maclauchlan et al. 2006).

During the latest outbreak, which peaked in 2007, over 800 000 ha were infested (British Columbia Forest Service 2007). Current means of controlling *C. occidentalis* include aerial sprays of *Bacillus thuringiensis*, a bacterium toxic to Lepidoptera larvae. In 2012, 116 012 ha were sprayed to attempt control of this insect (British Columbia Forest Service 2012), at an estimated cost of CDN $2.51M (Personal communication. 2018. Lorraine Maclauchlan, BC Ministry of Forests, Lands, Natural Resource Operations,

Figure 1—Range of Douglas-fir (*Pseudotsuga menziesii*) in British Columbia. (courtesy photo by Province of British Columbia)

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and Rural Development, Kamloops, BC Canada). A more cost-effective means of control might be to develop genetically-based resistance to *C. occidentalis*.

The British Columbia Forest Service has been conducting genetics studies and breeding programs for commercially important trees since the 1960s (e.g., Roche 1969). Selective traits of importance have historically been volume, tree form, and wood quality, and more recently pest resistance has become a focus. One region of BC, where we have an advanced breeding program, is from the Thompson-Okanagan region, which suffered widespread *C. occidentalis* infestation during the 2007 outbreak. This breeding program is composed of 78 parent genotypes, and clones of these parents are held in a breeding arboretum in Vernon, BC, with 3 ramets per clone. We decided to examine these trees for *C. occidentalis* resistance, with the objective of incorporating resistance into our breeding program, for eventual deployment across the landscape if gains can be made for this trait.

We started in 2012 by purchasing *C. occidentalis* pupae from an insectary (NRCan Insect Production services, Sault Ste-Marie, Ontario), derived from British Columbia stock. These were reared at 22 °C and 40 percent RH until eclosion. Moths were allowed to mate and females oviposited on wax paper. Paper tabs supporting 10 eggs were cut with scissors and affixed with a paper clip to new shoots of Douglas-fir, shortly after budburst. One shoot on each ramet of each parent genotype was thus infested. Over each shoot a 53 x 63 cm Organza mesh bag was installed (Creative Bag, Toronto, Canada) to keep *C. occidentalis* in and predators or parasites out. In late June the bags were removed, remaining moths, pupae or larvae within counted, and foliage rated for proportion consumed.

There was a wide range in variation in feeding performance (fig. 2), ranging from 0 percent of foliage consumed to over 90 percent. No larvae remained, but the number of pupae and moths that came out also varied, with generally the lowest numbers coming from the least palatable parents, and the highest numbers from the most palatable parents. This is a result typical of other studies (Cates 1983, McDonald 1981), though no other studies specifically measured the influence of genotype. However, the usefulness of these results might be limited, because while the larvae cause the damage, it is their parents, which are strong

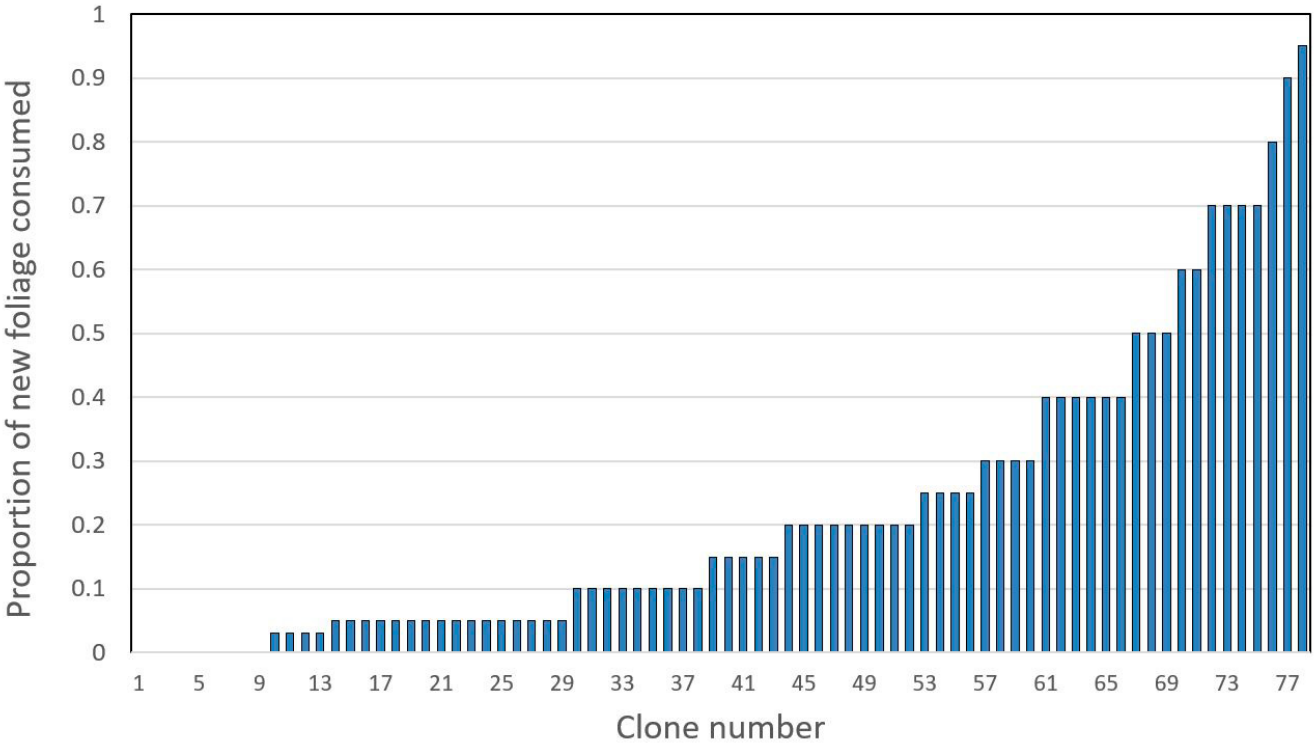

Figure 2—Proportion of new foliage consumed by captive spruce budworm larvae on 78 different Douglas-fir genotypes.

flyers that choose the host tree that the larvae must then feed upon. We therefore decided to investigate adult host selection of *C. occidentalis* on Douglas-fir.

Surprisingly, only two papers were found on adult budworm host selection: Städler (1974) described host plant characteristics, presumably under genetic control, that influence oviposition choice in eastern spruce budworm, *C. fumiferana*; and Leyva et al. (2000) investigated adult host preference of *C. occidentalis* among 3 host species, without reference to genotype within species. Another similar system in which adult host preference has been investigated is in fruit trees with other members of the same moth family, Tortricidae. Oriental fruit moth *Grapholyta molesta* females show oviposition preference between fruit tree species (Myers et al. 2006a), while obliquebanded leafroller *Choristoneura rosaceana* adult females prefer some host species over others (Carrier et al. 1995), both again without reference to genotype within species. Only one paper was found investigating within-species host preference: Myers et al. (2006b) found that *C. rosaceana* oviposited preferentially on certain apple cultivars. Discussion with Spruce Budworm researchers in Canada revealed no knowledge of adult host selection, within host species, in any *Choristoneura* in Canada. This is apparently a wide-open field.

In 2013, we collected shoots from each of the 78 parent genotypes, in early July when moths emerge, fly, and oviposit in the wild. We installed these shoots in water troughs in a small greenhouse, arranged in a randomized complete block design with 5 replicates. Insect mesh was installed inside the glass to contain adult moths. Into this enclosure we introduced 500 *C. occidentalis* pupae, again purchased from the Canadian insectary, spread among 10 petri dishes. By the next morning, all pupae had been consumed by mice. Because the insectary had no more pupae available, we were shut down for the year.

In 2014, we tried again with a similar experimental design and physical facilities, but with a mouse-proof release device. We also purchased an extra 500 pupae as a backup. The moths emerged within 5 days and immediately flew to the ground and laid their eggs on the concrete floor. The backup pupae produced moths that behaved in exactly the same manner. Our moths were highly confused.

In discussion with several budworm experts in Canada, we identified three potential issues.

- The cuttings were removed from the parent trees, they were not a part of the natural tree. In other systems, as soon as a shoot is removed from the parent plant, its volatiles change dramatically, potentially losing attractiveness, or even repelling adult *C. occidentalis.*
- The greenhouse environment might confuse the moths, by altering the light, temperature, humidity, or wind flow characteristics that moths outdoors use to detect potential hosts.
- The *C. occidentalis* colony might have a highly restricted genetic makeup, having been selected through several generations for freedom from disease and good performance in a laboratory colony on artificial diet, ovipositing on wax paper. Their normal host-finding systems may have been affected, and even their ability to fly may have been impacted.

We decided to deal with these issues by grafting scion from each of the 78 parents onto rootstock growing in an outdoors compound. Four hundred and fifty Douglas-fir seedlings of random genetic origin were planted in 2015. Growth was not as expected by 2016, so we waited until 2017 to collect scion and make the grafts. Grafts were arranged in a 13 x 6 alpha-design (Fu et al. 1998) with five linearized replicates. In 2018, grafts were growing well, so one-half the rootstock foliage was removed. The objective is to remove all the rootstock by 2019 so that the only foliage remaining is from the parent genotype.

In 2019, a screenhouse will be constructed over the outdoor trees. This light structure, covered with a large-mesh insect screen, will provide very little shade or other light disturbance, and allow maximum airflow through the grafts, but contain the moths once they have emerged.

We plan to release wild-collected pupae. Wild-collected pupae might be more adapted to host-finding in their native habitat and will have a broader genetic background. Though the greater genetic variation of the budworm pupae might add experimental error, any inferences will be more broadly applicable.

Though we have no data yet in the 4th year of our *C. occidentalis* adult host selection study, we have answered one question: why this has not been done before. It's hard! This sort of study is not amenable to a graduate student program, so it remains to government researchers like our group to conduct it. We hope to have some results by the end of 2019.

Addendum, April 2020. Results from the year 2019. We constructed a screenhouse as planned over the grafted outdoor trees. We collected about 1,800 pupae from the wild in late June. These were placed in brass soil screens (20.3 cm diameter, 5 cm rim, 8–12 mesh) suspended by wire from the screenhouse structure. Soil screens were used so that rain would not accumulate and drown the pupae; they were suspended by wires, and not allowed to touch any trees, in order to prevent ants from discovering and preying upon the pupae. Pupal emergence was only 13.8 percent, and the moths that emerged died within 1 or 2 days of emergence, without laying any eggs. We determined later that the brass soil screens heated in the sun to temperatures in excess of 45 ºC, which likely killed most of the pupae. Those that did emerge encountered very dry conditions with no dew formation or wildflowers to provide moisture, so moths likely died of dehydration. We will try one last kick at the can in 2020, modifying the release devices to provide shade (so they do not heat up in the sun) and misting the plants daily to provide moisture. Maybe after 7 years of trying we will finally get some results.

LITERATURE CITED

British Columbia Forest Service. 2007. 2007 Overview of Forest Health in the Southern Interior Forest Region. Government of British Columbia, Ministry of Forests and Range.

British Columbia Forest Service. 2012. 2012 Overview of Forest Health in the Southern Interior Forest Region. Government of British Columbia, Ministry of Forests, Lands, and Natural Resource Operations.

Carrier, Y.; Paré, S.; Roitberg, B.D. 1995. Oviposition preference of a polyphagous moth, the Obliquebanded Leafroller, Choristoneura rosaceana (Harris) (Lepidoptera: Tortricidae). The Canadian Entomologist. 127: 577–586.

Cates, R.G.; Redak, R.; Henderson, C.B. 1983. Natural product defensive chemistry of Douglas-fir, Western Spruce Budworm success, and forest management practices. Journal of Applied Entomology. 96: 173–182.

Fu, Yong-Bi.; Peter, G.; Clarke, Y. [et al.]. 1998. Incomplete block designs for genetic testing: statistical efficiencies of estimating family means. Canadian Journal of Forest Research. 28: 977–986.

Hermann, R.K.; Lavender, D.P. 1990. Pseudotsuga menziesii (Mirb.) Franco Douglas-fir. In: Silvics of North America vol 1: Conifers. USDA Handbook 654. U.S. Department of Agriculture Forest Service, Washington, DC: 527–540.

Leyva, K.J.; Clancy, K.M.; Price, P.W. 2000. Oviposition preference and larval performance of the Western Spruce Budworm (Lepidoptera: Tortricidae). Environmental Entomology. 29: 281–289.

Maclauchlan, L.E.; Brooks, J.E.; Hodge, J.C. 2006. Analysis of historic western spruce budworm defoliation in south central British Columbia. Forest Ecology and Management. 226: 351–356.

McDonald, G.I. 1981. Differential defoliation of neighboring Douglas-fir trees by Western Spruce Budworm. Research Note INT-306. Ogden, UT: U.S. Department of Agriculture Forest Service. Intermountain Forest and Range Experiment Station. 306 p.

Myers, C.T.; Hull, L.A.; Krawczyk, G. 2006a. Effects of orchard host plants on the oviposition preference of the oriental fruit moth (Lepidoptera: Tortricidae). Journal of Economic Entomology. 99: 1176–1183.

Myers, C.T.; Hull, L.A.; Krawczyk, G. 2006b. Seasonal and cultivar-associated variation in oviposition preference of oriental fruit moth (Lepidoptera: Tortricidae) adults and feeding behavior of neonate larvae in apples. Journal of Economic Entomology. 99: 349–358.

Roche, L. 1969. A genecological study of the genus Picea in British Columbia. New Phytologist. 68: 505–554.

Städler, E. 1974. Host plant stimuli affecting oviposition behaviour of the Eastern Spruce Budworm. Entomologia Experimentalis et Applicata. 17: 176–188.

SCREENING FOR GENETIC RESISTANCE TO THE HEMLOCK WOOLLY ADELGID

Ben C. Smith[1], Scott A. Merkle[2], and Fred P. Hain[3]

The exotic hemlock woolly adelgid (HWA) (*Adelges tsugae*) has caused widespread decline and mortality in natural-occurring and ornamental eastern (*Tsuga canadensis*) and Carolina (*T. caroliniana*) hemlocks native to the Eastern United States. The Forest Restoration Alliance (FRA) seeks to develop and produce hemlocks resistant to or tolerant of HWA that are suitable for both species restoration and ornamental uses. FRA strategies include the identification of resistant and tolerant genotypes of native hemlocks for inclusion in a resistance breeding program, and creation of interspecific hybrids between native and HWA-resistant or tolerant exotic hemlocks. In June 2017, 2-year-old cuttings propagated from 24 candidate trees were intentionally infested with HWA using a rain-down technique of crawlers hatching from suspended HWA-infested cut branches. Rooting success among clones was highly variable, so the number of ramets per clone ranged from 1 to 16 in the infested treatment (n = 271 plants) and 1 to 20 in the non-infested control treatment (n = 118 plants). We evaluated the trees in July 2018 for adelgid presence, total height, growth of the dominant terminal branch, branch tip dieback, and overall plant vigor. Height and growth differed significantly among clones, but no measured trait was significantly affected by infestation. HWA survival and reproduction was poor over time, so additional artificial infestation and evaluation are needed to accurately assess levels of resistance. Through the *in vitro* propagation technique of somatic embryogenesis (SE), we have successfully produced somatic seedlings from eastern, Carolina, and hybrids between Carolina and Chinese (*T. chinensis*) hemlock and Carolina and southern Japanese (*T. sieboldii*) hemlock, verified by chloroplast DNA markers. We are utilizing SE to accelerate clonal replication for resistance/tolerance screening and deployment of tested genotypes. We have also attempted, without success yet, to create novel interspecific hybrids incorporating eastern hemlock using embryo rescue.

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A COLLABORATIVE MULTI-AGENCY PROGRAM TO DEVELOP RESISTANCE TO BEECH BARK DISEASE IN AMERICAN BEECH (*FAGUS GRANDIFOLIA*)

Title presented at workshop:
BEECH BARK DISEASE RESISTANCE BREEDING PROGRAM IN AMERICAN BEECH

David W. Carey[1], Marjorie Allmaras[2], Paul Bloese[3], David Burke[4], Paul Berrang[5], Lindsay Dalton[6], Roger Gettig[4], Tom Hall[7], Andrea Hille[8], Jeff D. Kochenderfer[9], Scott Lint[10], Mary Mason[11], James McKenna[12], Scott Rogers[13], Jill Rose[14], Carol Young[15], Keith Konen[16], and Jennifer L. Koch[1]

American beech is typically not a desired timber species, but it is among the most ecologically important trees of the Eastern United States. Beech nuts, a common component of mature forests, provide an important source of food for many birds and mammals. Beech bark disease (BBD) has been killing American beech trees since the late 1890's, but it is only over the last 15 years that significant progress has been made in developing techniques to identify, screen, propagate and breed resistant beech trees. Beech bark disease is caused when feeding of the invasive beech scale insect (*Cryptococcus fagisuga*) creates cracks in the bark, providing entry points for spores of *Neonectria* species of fungi. Although it is the growth of the fungus that weakens and may kill the tree, beech trees that remain disease-free despite long-term exposure to BBD have been shown to have heritable resistance to the scale insect. Genetic studies have shown that when two resistant parents are bred, about 50 percent of the progeny will be resistant. Working collaboratively with State and national forest managers, we have taken a participatory breeding approach to develop regional seed orchards of genetically diverse, grafted, BBD-resistant genotypes. Workshops are conducted to provide partner guidelines for the identification of resistant beech trees, training on the use of scale eggs to artificially inoculate candidate trees to confirm resistance, and instructions on the collection of beechnuts (to be used as root stock) and scion. After identifying and confirming scale-resistant trees, partners ship scion to U.S. Forest Service researchers in Ohio, or Region 9 personnel in Wisconsin, for grafting. A total of nine grafted regional beech seed orchards have been established or are underway in seven different States. Prior to seed orchards becoming productive, grafted containerized seed orchards are being used to accelerate seed production for genetic studies and restoration plantings.

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Session 2

GENUS FRAXINUS—THREATENED BY EMERALD ASH BORER AND ASH DIEBACK DISEASE

PHOENIX FROM THE ASHES: EXPLOITING HOST RESISTANCE TO CONSERVE AND RESTORE POPULATIONS OF EUROPEAN ASH

Michelle Cleary[1], Lars-Göran Stener[2], Pierluigi Bonello[3], and Caterina Villari[4]

The invasive ascomycete fungus (*Hymenoscyphus fraxineus*) has caused a serious, steady population decline of common ash (*Fraxinus excelsior*) across Europe. In Sweden, ash is an important species among the noble broad-leaved trees available to forest management. Since 2010, ash is a Red-listed species and its status has worsened during recent years to become critically endangered. This is concerning not only for this economically and culturally important species but also for its associated biodiversity given its role as a keystone species for natural communities throughout Europe. Several studies have shown large genotypic variation in the level of disease susceptibility among different individuals, and that a small proportion (1–5 percent) of the natural population of *F. excelsior* exhibits disease tolerance. Here we report on several activities aimed at selecting and testing *F. excelsior* genotypes including: (1) broad-scale mapping, identifying and selecting more than 500 disease-tolerant *F. excelsior* with respect to ash dieback damage in wild populations for further monitoring and testing, (2) screening resistance in *F. excelsior* genotypes in clonal trials, and (3) early results of progeny testing from known susceptible and tolerant families. In addition, phenomics technologies show great promise to advance breeding efforts. Fourier-transform Infrared (FT-IR) spectroscopy coupled with chemometric model was able to successfully discriminate between resistant and susceptible ash genotypes. Collectively, these activities and the implementation of rapid phenotyping technologies in practice will help support the development of a more resistant population of *F. excelsior* restoration activities aimed at repopulating Swedish forests, cities, and landscapes.

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RESTORING GREEN ASH (*FRAXINUS PENNSYLVANICA*): BREEDING FOR RESISTANCE TO THE EMERALD ASH BORER (*AGRILUS PLANIPENNIS*)

Jennifer L. Koch[1], David W. Carey[1], Mary E. Mason[2], Therese M. Poland[3], Kathleen S. Knight[1], Jeanne Romero-Severson[4], Charles Tubesing[5], and Roger Gettig[5]

The invasion of emerald ash borer (EAB) (*Agrilus planipennis*) threatens the survival of green ash (*Fraxinus pennsylvanica*) in the United States, where it is extensively used for soil conservation, rural water management, and in urban green spaces. It is also one of the most common hardwood species in riparian forests of eastern North America. Long term monitoring of permanent plots in EAB infested natural forests identified surviving green ash trees, or "lingering" ash trees, that had maintained healthy canopies for at least 2 years after all other ash trees (>10 cm DBH) had died. EAB egg bioassay experiments confirmed that these trees possess an increased level of resistance due to multiple types of host defense responses, including mortality of early instar larvae, development of larvae having significantly lower weights, and reduced adult feeding preference of foliage. Fifty-five lingering green ash trees have been accessioned and a replicated clone test has been established to assess field performance of 42 of these. We are employing a polycross breeding strategy and to date, 16 full-sibling families resulting from crosses between 8 mother trees and 15 different father trees have been produced. Additional families will be added as select parent trees begin to flower. Bioassay evaluation of seedling progeny from 7 different lingering x lingering families, 2 susceptible x susceptible families, 1 susceptible x lingering family and 1 susceptible open-pollinated family demonstrated variation both within and between families. Between 15 to 40 percent of lingering x lingering progeny had a more effective defensive response to EAB than either parent, a result expected if the two parents employ different mechanisms based on allelic variants at more than one gene. Polycross progeny are being further evaluated in field trials that also include progeny from susceptible x susceptible and susceptible x lingering families as reference populations to allow for analysis of genetic gain.

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EVALUATION OF RESISTANCE OF ASIAN AND EUROPEAN ASH SPECIES AND CULTIVARS TO EMERALD ASH BORER (*AGRILUS PLANIPENNIS*)

Frederic Miller[1], Erin McMahan[1], and Kim Shearer[1]

Since its discovery in Michigan in 2002, the emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) has been a devastating exotic invasive pest of North American ash trees (*Fraxinus* spp.), killing millions of urban and rural ash trees. Many communities have lost or risk losing many valuable ash trees, and as part of reforestation efforts, it is imperative that new *Fraxinus* taxa less susceptible to EAB be developed. To identify possible *Fraxinus* taxa less susceptible to EAB, we performed a series of no-choice (NC) feeding bioassays with 24 different species and cultivars of Asian and European ash between 2009 and 2017. Ash studies included laboratory NC adult leaf feeding studies, and laboratory and field larval colonization studies. The Asian ash taxa *F. chinensis*, *F. chinensis* var. *rhynchophylla*, *F. mandschurica*, and *F. mandschurica* var. *japonica* were the least preferred in the NC leaf feeding studies, with similar results found in the larval colonization studies. Of the European ash taxa tested, *F. angustifolia*, *F. excelsior*, *F. pallisae*, and *F. longicuspus* were the least preferred by adult EABs. Field evaluation of these same Asian and European taxa, exposed to a natural EAB population in an arboretum tree breeding nursery, was consistent with the aforementioned studies. In addition, since elm (*Ulmus*) is mentioned in the literature as a potential EAB host, we conducted adult NC leaf-feeding and larval colonization studies to determine the relative susceptibility and preference for elm taxa by EAB. Field and laboratory studies revealed that none of the 11 elm taxa tested were susceptible to feeding by adult EABs and were not colonized by larvae. To date, overall, six Asian and three European species appear to be less suitable and/or preferred for adult feeding and larval colonization by EAB and may have potential for use in future ash breeding programs.

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THE PAST IS PROLOGUE: UNDERSTANDING THE CURRENT STATE OF TREE IMPROVEMENT IN THE UNITED STATES

Kim Steiner[1]

Forest genetics research and related tree improvement R& D in the United States has followed a long arc of interest and investment since pioneering efforts by the USDA Forest Service in the first decades of the last century. The scope and intensity of this work exploded between 1950 and 1985 as public agencies, private industry, and universities shifted resources to capitalize on optimism about the application of genetics to forestry and forest productivity. In the latter part of this period, there was a genetics or breeding specialist in every Forest Service experiment station, every National Forest Region, many individual National Forests, about half of all State forestry agencies, most large timber companies, and almost every forestry school. As the century ended, however, there followed a period of rapid, almost precipitous retrenchment in tree improvement work due to a "perfect storm" of circumstances that are described in the presentation. Forest tree improvement has contracted to natural core of work focused on productivity gains in species that are planted on a large scale. As a research discipline, forest genetics survives in a healthy, if somewhat diminished state, but the focus has shifted to lab-based genomic studies, and the scientists are often disengaged from real-world problems. Unfortunately, institutional disengagement from tree improvement has left a lingering reluctance to invest in long-term projects that may be essential for the protection and rescue of species threatened by invasive diseases or insects. Permanent solutions to some of these problems will require long-term breeding programs coupled with silvicultural opportunities to implement gains.

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VARIATION AND GENOMIC BASIS OF *FRAXINUS EXCELSIOR* (COMMON ASH) SUSCEPTIBILITY TO *HYMENOSCYPHUS FRAXINEUS* (ASH DIEBACK) THROUGHOUT BRITAIN

Jonathan J. Stocks[1], Steve J. Lee[2], and Richard Buggs[3]

Ash dieback (ADB), caused by *Hymenoscyphus fraxineus*, has severely damaged a large proportion of ash trees (*Fraxinus excelsior*) in continental Europe. In Britain, the disease was found only 6 years ago in the southeast, and is still spreading. A large-scale screening trial to evaluate ADB damage to provenances of *F. excelsior* sourced from throughout the British Isles was planted in 2013 in the southeast of England. Trees were scored in 2015 and 2016 for their level of ADB damage observed in field. Significant differences were found in average ADB damage among planting sites and seed source provenances in 2016. All provenances contained some healthy trees, so a breeding program to produce genetically variable native ash tree populations with lower ADB susceptibility may be feasible. In 2015, we undertook a pilot project, using Restriction Site Associated DNA sequencing (RADseq) on 95 samples, to search for alleles associated with low susceptibility to ash dieback. We are now sequencing whole genomes from 1,400 individuals sampled across all provenances: 700 high-susceptibility and 700 low-susceptibility trees. Of these 1,250 are placed in 31 pools with 80X coverage per pool, and 150 are being sequenced as individuals with 20X coverage each. This provides data for a genome wide association study, searching for loci associated with low susceptibility, and genomic prediction of trees with low susceptibility.

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Session 3

BREEDING FOR RESISTANCE TO PATHOGENS

PROGRESS OF AN APPLIED DISEASE SCREENING AND SELECTION PROGRAM FOR RESISTANCE TO VASCULAR WILT IN HAWAIIAN *ACACIA KOA*

Nick Dudley[1], Tyler Jones[1], Aileen Yeh[1], Richard Sniezko[2], and Phil Cannon[3]

Koa (*Acacia koa* A.Gray) is a valuable tree species economically, ecologically, and culturally in Hawaii. A vascular wilt disease of koa caused by the fungal pathogen *Fusarium oxysporum* f. sp. *koae* (FOXY) causes high rates of mortality in field plantings and threatens native koa forests in Hawaii. The Hawaii Agriculture Research Center (HARC), with both public and private partners, operates a tree improvement program to develop koa wilt resistant populations in Hawaii. This applied program was started in 2003 and has made progress including the establishment of first generation seed orchards, delineation of 11 provisional seed zones, and release of the first seed with confirmed levels of genetic resistance for reforestation and restoration. Data from seedling inoculation trials and the first field trials suggest survival on infected sites may be expected to exceed 60 percent in planting using seed from the best parents compared to 30 percent or less survival in unimproved seedlings. One clonal and six seedling field trials were established between 2012–2016 using selections based on the inoculation trials. The screening method serves as a powerful tool to rapidly evaluate koa families prior to outplanting. Additional seedlings screening is on-going to identify parent trees for seed zones not yet established. This rapidly developing resistance program will need to continue the monitoring of field trials to further evaluate the durability of resistance.

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THE DEVELOPMENT OF A RESISTANCE SCREENING PROGRAM OF OHIA TO *CERATOCYSTIS* PATHOGENS CAUSING WIDESPREAD MORTALITY ON HAWAII ISLAND

Marc Hughes[1], Blaine Luiz[2], Lisa Keith[3], and Christian Giardina[4]

Ohia (*Metrosideros polymorpha*) is Hawaii's most abundant and ecologically important native tree. Present on all major Hawaiian Islands, ohia trees account for roughly 50 percent of the stems and 50 percent of total basal area of all forests (native or non-native) across the Hawaii Island. In 2014, high and expanding levels of ohia mortality were observed in the Puna district on the east side of Hawaii Island, spurring investigations to its cause. Pathological investigations revealed a complex of two xylem-affecting diseases caused by two novel fungal species, *Ceratocystis lukuohia* and *C. huliohia*. The mortality caused by these diseases, called Rapid Ohia Death (ROD), has quickly spread to most districts of the island affecting over 54 600 ha of forests. ROD is a primary concern to the health of ohia populations, forests, and the vast assemblages of organisms that rely on this keystone tree. To combat the expanding mortality associated with ROD, a resistance screening program is in development to screen the various varieties and ecotypes of ohia from Hawaii. Initial screening results of *M. polymorpha* varieties (*glaberrima*, *incana*, *newellii*, and *polymorpha*) found on the Hawaii Island show promise of differential susceptibility and the potential for host resistance. Selection will involve collection of seed and vegetative material for propagation and later screened for resistance by inoculation trials. In addition to targeting surviving *M. polymorpha* individuals both within and adjacent to sites of intense ROD mortality, we will also screen a diverse collection of *M. polymorpha* genotypes from a State-wide seed drive as well as four other *Metrosideros* species from Hawaii.

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SCREENING RANGE-WIDE BLACK WALNUT SEED FAMILIES FOR THOUSAND CANKERS DISEASE (TCD) RESISTANCE AND MEASURING THE REGIONAL ADAPTABILITY OF RESISTANT SEED SOURCES

James R. McKenna[1], Racheal A. Sitz[2], Ned Tisserat[3], Don Carlson[3], and Paul Bloese[4]

Black walnut (*Juglans nigra*), an economically important native species in the Eastern United States is threatened by a new disease thousand cankers disease (TCD) caused by a fungus (*Geosmithia morbida*) vectored by a bark beetle (*Pityophthorus juglandis*). Both organisms originate in the Southwestern United States but have spread to become endemic throughout the Western United States and the disease was first found in the eastern native range in 2010. We challenged a range-wide collection of native black walnut (*Juglans nigra*) families, collected in 2012, to artificial stem inoculation of *Geosmithi morbida* (*Gm*) to determine the variation among regions and families of black walnut, to test the regional adaptability of any sources of resistance and to capture resistant material for future breeding and new seed orchards. In addition, we included a number of our select timber families from Indiana as an improved check-lot.

Up to 9 seedlings per family were shipped to Colorado State University (CSU) and planted in three complete blocks in the field. Families with more than 9 seedlings were planted into the common garden (CG) plots in Arkansas, Indiana, and Michigan. Each plot was planted on a 2.2 meter grid with 24 mixed families per provenance per block and 12 seedlings of three oak species (bur, northern red, and cherry bark) per block (36 trees total). Each plot was fenced to exclude deer and weeds were controlled for the first 2 years with herbicides and cultivation.

Trees were inoculated with two *Gm* strains first in 2014 the summer after planting, and then again in the summer of 2016 at CSU. Plugs of two *Gm* isolates and a sterile control were placed into live stems with a 6-mm cork-borer and the branches cut 90 days later and stored in a cooler until we could scrape off the outer bark and measure the length and width of cankers. The first year screen at CSU showed *Gm* isolate significantly affected canker size, but provenance did not. In the repeat screen of 2016, stem diameter significantly affected canker size as did family and provenance. Breeding values were used to estimate the percent difference among provenances in canker size relative to all trees in the study and we found western provenances had from -5.3 to -5.7 percent smaller *Gm* cankers while the eastern provenances had +5.5 percent to +5.8 percent larger *Gm* cankers. The center provenance had cankers midway between both western and eastern provenances (+0.3 percent). The improved walnut families, selected for increased growth and timber quality, had the most variation and as a group produced the largest cankers +6.8 percent.

First year survival was poorest for both the SW provenance and the Arkansas CG plot. HTI families survived the best in all 3 CG plots and there was no significant plot × provenance effect. Three year height growth of the provenances in the CG plots shows that the selected HTI walnut outgrew all wild provenances in Indiana, growing nearly 70 percent taller than wild CTR families.

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We had difficulties keeping deer out of the MI CG plot but were successful at eliminating deer browse in AR and IN. Thus, meaningful growth differences among the CG plots is not possible yet.

Previous studies have demonstrated variation among black walnut genotypes by this type of screening which suggests that black walnut has co-evolved with *Gm* and hence variation in resistance exists. Our results suggest that western black walnut possesses more resistance than eastern black walnut. Ranking families by State show NC, PA, and TN families produced the largest *Gm* cankers and those States were the first three where TCD was found in the East after 2010. Whether or not this is a coincidence will require further study. Finally, black walnut selected for faster growth may unwittingly reduce resistance to TCD and should be addressed by improvement programs.

THE SEARCH FOR RESISTANCE IN LODGEPOLE PINE TO COMANDRA BLISTER RUST IN THE CENTRAL INTERIOR OF BRITISH COLUMBIA

Richard W. Reich[1]

Comandra blister rust (*Cronartium comandrae* Peck) although indigenous to North America may result in severe epidemic levels of infection on lodgepole pine (*Pinus contorta* var. *latifolia* Engelm. ex. S. Watson). Proximity to the alternate host plant, bastard toadflax [*Geocaulon lividum* (Richardson) Fern] may act as a significant confounding factor in the evaluation of resistance in field trials. Incidence and severity of comandra was assessed at age 15 in a 130 seedlot (family) progeny trial (referred to as the Chief Lake site) designed to identify families with superior growth and wood quality traits within the tree improvement program in British Columbia, Canada. The distribution of the alternate host was mapped and individual plant stems counted to evaluate the degree of local infection gradients. The incidence and severity of other pine stem rust pathogens and Elytroderma foliage and shoot disease [*Elytroderma deformans* (Weir) Darker] was assessed to evaluate the degree of cross resistance to other pathogens. The cumulative incidence of comandra was 52 percent, with cumulative family incidence ranging between 2 and 95 percent. The cumulative severity of infection was 2.37 infections per tree, and the mean family severity ranged between 0.02 and 11.95 infections per tree (a difference of over 500 times between least susceptible and most susceptible family). The results of the Chief Lake site was contrasted with a previously reported upon assessment of a different 130 seedlot (family) comandra screening trial, which was replicated on three sites. Although the previous study showed that the majority of families in that trial appeared to be highly susceptible, the set of families tested at the Chief Lake site appeared to contain a considerably higher proportion of families with low susceptibility. As a result, it may now be possible to identify a sufficient number of families from this subsequent trial to establish a comandra resistant orchard.

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GENETIC RESISTANCE TO *PHYTOPHTHORA LATERALIS* IN PORT-ORFORD-CEDAR (*CHAMAECYAPRIS LAWSONIANA*)

Richard A. Sniezko[1], Douglas P. Savin[1], and Paul Reeser[2]

Native forest ecosystems as well as reforestation and restoration efforts in forest trees can be severely impacted by pathogens and insects, especially non-native ones. In many of these cases, the future viability and utility of a species may depend heavily upon efforts to harness any naturally occurring genetic resistance. Port-Orford-cedar (POC), *Chamaecyparis lawsoniana*, a long-lived conifer native to northwest California and southwest Oregon, and also used horticulturally internationally, is highly susceptible to a non-native root disease, caused by the oomycete pathogen *Phytophthora lateralis*. An applied program to develop populations of POC with genetic resistance has identified both qualitative and quantitative resistance based on seedling inoculation trials. Families from some parent trees show high survival, consistent with the expectation of resistance from a single major dominant gene. Seedlings from families with quantitative resistance show a differential rate of mortality within a family over 3 years. The level of quantitative resistance varies by family with some families reaching 100 percent mortality by the end of 3 years, while other families still have low to moderate survival. Parents (or their seedling progeny) with each type of resistance are being incorporated into containerized seed orchards for each breeding zone, and breeding to increase the level of quantitative resistance is underway. Early data from field trials is encouraging, and restoration and reforestation with resistant seedlings is underway. The applied resistance program in POC can offer some guidance to other programs contemplating the development of resistant populations to help maintain viable populations of forest tree species affected by non-native pathogens or insects.

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GENETIC DIVERSITY IN THE LAUREL WILT PATHOGEN, *RAFFAELEA LAURICOLA*, AND THE CONSEQUENCES FOR RESISTANCE BREEDING

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Laurel wilt is caused by *Raffaelea lauricola*, a fungal nutritional symbiont of its vector, *Xyleborus glabratus*, the redbay ambrosia beetle. Both the fungus and beetle are native to Asia but were found in Georgia in the early 2000s. The disease has spread to much of the Southeastern United States killing >300 million host trees in the Lauraceae family. The objectives of this research were to elucidate the genetic structure of populations of *R. lauricola*, examine its reproductive strategy, and determine the number of USA introductions. A panel of 12 simple sequence repeat (SSR) markers identified 15 multilocus genotypes (MLGs) in a collection of 59 isolates from the USA (34 isolates), Myanmar (18), Taiwan (6) and Japan (1). Limited diversity in the USA isolates and the presence of one MAT idiotype (mating type locus) indicated that *R. lauricola* was probably introduced into the country once. MLG diversity was far greater in Asia than the USA. Only three closely related MLGs were detected in the USA, the most prevalent of which (30 of 34 isolates) was also found in Taiwan. Although more work is needed, the present results suggest that a Taiwanese origin is possible for the population of *R. lauricola* in the USA. Isolates of *R. lauricola* from Myanmar were distinct from those from Japan, Taiwan and the USA. Although both MAT idiotypes were present in Myanmar and Taiwan, only the population from Taiwan had the genetic structure of a sexually reproducing population. The results highlight the need to prevent the introduction of additional genotypes and the second mating type into the USA because this could allow the pathogen to rapidly overcome the resistance that has been developed. The pathogen population needs to be monitored so that new genotypes can be identified and incorporated into resistance screening trials if they are found.

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FUSIFORM RUST RESISTANCE IN AN ELITE POPULATION OF LOBLOLLY PINE POPULATION

April Meeks[1], Steve McKeand[1], Ross Whetten[1], and Fikret Isik[1]

Fusiform rust (caused by the fungus *Cronartium quercuum* f. sp. *fusiforme*) is the most critical disease affecting the health and productivity of loblolly pine (*Pinus taeda* L.), the most commercially important pine in the Southeastern United States. An Atlantic Coastal Elite (ACE) breeding population was developed in the N.C. State University Cooperative Tree Improvement Program to assess short-term genetic gain for the Coastal regions of the Southeast. Twenty-four elite Atlantic Coastal parents were mated to produce 76 crosses and were screened at the U.S. Forest Service Resistance Screening Center (RSC) in Asheville, NC. About 9,775 progeny of the 76 crosses were challenged with a broad-based inoculum from across the expected deployment region. A spore density of 50,000 spores per milliliter was used, and seedlings were assessed for gall presence or absence after 6 months. The overall rust incidence was 0.48, and full-sibling family means ranged from 0.11 to 0.83. The narrow-sense heritability of half-sibling family means was 0.95 (± 0.003), suggesting strong genetic differences among families. Broad-sense heritability of full-sibling family means was 0.93 (± 0.03).

Based on the 6-month results, all seedlings with rust galls as well as some entire full-sibling families were discarded based on the expectation they would also be susceptible in the field. The remaining 2,362 seedling progeny of the 51 crosses (~45 per family) were clonally propagated via rooted cuttings, and ramets of the clones were planted across eight test sites. A half-sibling family that was not tested for rust resistance at the RSC was included in the test design as a checklot, and fusiform rust disease incidence was recorded for these seedlings in the field tests. Incidence of fusiform rust galls, survival, height, diameter at breast height, straightness, forking and ramicorn branching were all assessed at ages four and six. At age 6 years, the overall rust incidence was 0.06. The checklot family had 0.19 rust incidence both at ages four and six. Two sites in Colleton county South Carolina had 56 percent and 71 percent rust incidence in the non-screened checklot compared to 0.11 and 0.15 in the screened rooted cuttings. These results indicate screening at the RSC with subsequent field-testing of survivors was effective.

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GENETIC PARAMETER ESTIMATES FOR FUSIFORM RUST DISEASE FROM A MULTI-ENVIRONMENT TRIAL OF LOBLOLLY PINE (*PINUS TAEDA* L.)

Eddie Lauer[1], Steve McKeand[1], and Fikret Isik[1]

Fusiform rust, caused by the fungus *Cronartium quercuum* f. sp. *fusiforme*, is an important disease of loblolly pine (*Pinus taeda*) plantations in the Southern United States, causing multimillion dollar losses to landowners. In controlled seedling inoculation trials, fusiform rust resistance segregates within families like a traditional Mendelian gene. However, at the population level in the field, rust resistance appears more quantitative in nature. This is due to the presence of multiple R genes segregating among different families, conferring resistance to different races of the pathogen. In this study, a dataset of 11,987 trees from 68 half-sib families of loblolly pine tested across 10 environments was analyzed to understand the inheritance and patterns of GxE interactions for fusiform rust resistance. Heritability of family means was 0.91, indicating that a substantial amount of phenotypic variation in the field can be explained by genetic differences between families. The type B genetic correlation was 0.77, indicating a small amount of GxE. When the rust resistance breeding values for each family within each environment were ranked, the most resistant and most susceptible genotypes tended to have the same rank across environments and families with moderate levels of resistance showed frequent changes in rank. The results of this study indicate that substantial gain can be made through traditional family selection by exploiting the additive genetic variance component.

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USDA ADVANCES IN PLANT BREEDING AND INNOVATION: RESISTANCE BREEDING, GERMPLASM COLLECTIONS, AND GENETIC DIVERSITY

Sarah Federman[1] and Paul Zankowski[1]

The Office of the Chief Scientist (OCS) assists in providing strategic coordination of the science that informs Departmental policies by facilitating discourse and the identification of areas of synergy. Major focal areas in OCS include plant health, production and plant products; and agricultural systems and technology. This talk presents a series of vignettes on plant breeding and innovation throughout the Department, with a focus on breeding for diversity and resilience; resistance breeding programs; the national plant germplasm database; and finally, examples of recent plant breeding coordination work at OCS to introduce the office as a resource and partner for facilitating the increased visibility of ongoing research and initiatives.

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Session 4

GENOMIC AND METABOLOMIC APPROACHES TO IDENTIFY RESISTANCE GENES

GENOMICS OF *FRAXINUS* (OLEACEAE): A GENUS UNDER SEVERE THREAT

Laura J. Kelly[1], Steve Lee[2], Jennifer Koch[3], Stephen J. Rossiter[1], and Richard J.A. Buggs[1,4]

Fraxinus (Oleaceae; olive family) is a genus of c. 48 tree and shrub species, including taxa of ecological and economic importance. *Fraxinus* species face severe threats from an invasive beetle species, emerald ash borer (EAB) in North America and a disease caused by an invasive fungal pathogen, ash dieback (ADB) in Europe. Susceptibility to these threats varies among ash species, and seems to be low in at least some Asiatic species. We have therefore sequenced and assembled whole genomes of 28 species or sub-species of *Fraxinus* (http://www.ashgenome.org/worldwide). We are taking a new approach for identifying genes conferring resistance to threats, based on detecting evidence of convergent molecular evolution among host species' genomes. We are currently analyzing patterns of gene evolution between species highly susceptible to EAB (*F. americana*, *F. latifolia*, *F. pennsylvanica*, *F. ornus* and *F. velutina*) and species with low susceptibility (*F. baroniana*, *F. chinensis*, *F. floribunda*, *F. mandshurica*, and *F. platyploda*). Genes showing patterns of convergent sequence similarity within low susceptibility species are identified as candidate genes roles in susceptibility. We will soon extend these studies to further species and to the analysis of ADB susceptibility.

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EXPRESSION PROFILING OF CANDIDATE GENES OF RESISTANCE TO *PHYTOPHTHORA CINNAMOMI* DETERMINED IN DIFERENTE GENOTYPES OF *CASTANEA* SPP

Carmen Santos[1,2], Sofia Duarte[1], Sara Tedesco[1], Pedro Fevereiro[2,3], and Rita L. Costa,[1,4]

Phytophthora cinnamomi is a hemibiotrophic that causes root rot, also known as ink disease. Little information has been acquired in chestnut on the molecular defense strategies against this pathogen. The expression of eight candidate genes potentially involved in the defense to *P. cinnamomi* was quantified by digital PCR in *Castanea* genotypes showing different susceptibility to the pathogen. Seven of the eight candidate genes displayed differentially expressed levels depending on genotype and time-point after inoculation. Cast_Gnk2-like revealed to be the most expressed gene across all experiments and the one that best discriminates between susceptible and resistant genotypes. Our data suggest that the pre-formed defenses are crucial for the resistance of *Castanea crenata* to *P. cinnamomi*. A lower and delayed expression of the eight studied genes was found in the susceptible *Castanea sativa*, which may be related with the establishment and spread of the disease in this species. A working model integrating the obtained results is presented.

INTRODUCTION

The aim of this study was to evaluate the early expression of candidate resistance genes to *P. cinnamomi* infection (0, 24, and 48 h) in *C. sativa* and a *C. crenata*, as well as in four hybrids (three *C. sativa* × *C. crenata* genotypes and a *C. sativa* × *C. mollissima*) with different susceptiblities to *P. cinnamomi*, produced from the breeding program on course, for resistance to *P. cinnamomi*, the causal agent of root rot, to understanding of the molecular mechanisms underlying the resistance that Asian species (*C. crenata* and *C. mollissima*) show to this pathogen. Among the different methods available to quantify gene expression in plants, digital PCR (dPCR) is emerging as an absolute quantification method with high precision, sensitivity and specificity (Majumdar et al. 2015). This new technology has been mainly used for biomedicine research, however, some studies in plant science using dPCR have also been recently released (Bahder et al. 2016; Ge et al. 2016; Stevanato and Biscarini 2016).

MATERIAL AND METHODS

Six chestnut genotypes showing different levels of resistance after inoculation with pathogen were used in this work. *C. crenata* (resistant), *C. sativa* (susceptible) and four hybrid genotypes, selected from the on-going chestnut breeding program (Costa et al. 2011): three *C. sativa* × *C. crenata* hybrids (SC55 – resistant, SC914 – intermediate, and SC903 – susceptible) and one *C. sativa* × *C. mollissima* hybrid (SM904), selected as a resistance control. All plant material used in this study was multiplied by micropropagation. *P. cinnamomi* root inoculation was performed 80 days after plant acclimatization, under controlled conditions according to Santos et al. (2015). Genes were selected from the 283 *C. crenata* differentially expressed genes (DEGs), previously identified by Serrazina et al. (2015). In this study, gene selection parameters were: (1) DEGs with the log2 of the ratio between *C. crenata* inoculated (Cci) and non-inoculated (Ccn) reads higher than 1.5(Log2Cci/Ccn >1.5); (2) The correspondent

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DEGs in *C. sativa* transcriptomes with Log2Csi/Csn <1.5 or absent; (3) DEGs not involved in general biological processes, such as oxidative, metabolic and transporter activities; (4) DEGs involved in defense response and categorized in pathogen recognition which usually triggers resistance signalling pathways, anti-pathogen proteins, cell wall modification proteins, and transcription factors involved in the regulation of other defense related processes. QS3D digital PCR System (Life Technologies) was used to quantify gene expression of eight *P. cinnamomi* resistance candidate genes in the roots of the six chestnut genotypes under study.

RESULTS AND DISCUSSION

Physical and Chemical Barriers to *P. cinnamomi* Infection

Using the gene selection parameters defined, eight candidate genes were identified. These genes codify proteins potentially involved in the three layers of defense to *P. cinnamomi* infection, previously described (Freeman and Beattie 2008) two pathogen recognition proteins (Cast_LRR-RLK and Cast_C2CD) which trigger resistance signaling pathways; three transcription factors (Cast_WRKY 31, Cast_ABR1, and Cast_Myb4) involved in the regulation of other defense processes; a ubiquitination regulator (Cast_RNF5); a cell wall modification enzyme (Cast_PE-2) and an antifungal protein (Cast_Gnk2-like). All genes selected were up-regulated after inoculation in *C. crenata* root transcriptomes (Serrazina et al. 2015). The *P. cinnamomi* resistance candidate genes, their respective contig name (Serrazina et al., 2015), primers and TaqManR - Probes sequences are listed in Santos et al. (2017).

Before inoculation there is a clear differentiation in gene expression between *C. sativa* and *C. crenata*. Except for Cast_ABR1 and Cast_RINF5, the pre-inoculated expression of all other genes is significantly higher in *C. crenata*. This pattern, with some variation, holds for the two most resistant hybrids tested (SM904 and SC55). The secretion of toxic compounds is an effective defense mechanism against pathogens in plants (Montesinos 2007, Wittstock and Gershenzon 2002). Ginkbilobin-2 (Gnk2) is a protein secreted by *Ginkgo biloba* seeds that exhibits an antifungal activity (Sawano et al. 2007, Wang and Ng 2000). Gnk2 has a plant-specific cysteine-rich motif DUF26 (domain of unknown function 26, also known as stress-antifungal domain: PF01657) which belongs to cysteine-rich receptor-like kinases (CRKs) (Miyakawa et al. 2014) not showing any similarity with other known antimicrobial proteins (Miyakawa et al. 2014, Sawano et al. 2007). It was recently shown that Gnk2 can also activate actin-dependent cell death (Gao et al. 2016). Therefore, Cast_Gnk2-like may prevent pathogen growth either by its chemical properties or by inducing HR-related cell death. The highest Cast_Gnk2-like expression registered in non-inoculation conditions suggests that *C. crenata* root surroundings may be a hostile environment for fungal and fungal-like pathogens, such as *P. cinnamomi*. On the other hand, *C. sativa* showed a very low Cast_Gnk2-like expression level, even after pathogen inoculation. Considering the whole experiment, Cast_Gnk2-like was the most expressed gene and that best discriminates between susceptible and resistant genotypes (Santos et al. 2017). The isolation and purification of Cast_Gnk2- like protein may have biotechnological applications, such as the development of an antimicrobial phytopharmaceutical against *P. cinnamomi*.

A crucial constitutive defense is the formation of wall appositions that comprise a physical barrier to pathogen growth (Hardham and Blackman 2010). The reinforcement of plant cell walls by calcium-pectate gel apposition with the involvement of pectinesterases have been shown to confer resistance to *Phytophthora* species (Kieffer 2000, Wiethölter et al. 2003). In this study, expression levels of Cast_PE-2 show that this enzyme may have a role on *P. cinnamomi* resistance in chestnut. Compared with *C. sativa*, *C. crenata* exhibited higher Cast_PE-2 expression levels in all time points, mainly in the non-inoculated samples (about 10x more), suggesting that their

cell walls may be more resistant to pathogen penetration. After the first pathogen contact, Cast_PE-2 expression increases, suggesting a possible continuing apposition of pectates in cell walls, probably to inhibit further colonization. This seems to be more important in a late stage of infection (48 hpi) except for the *C. sativa* × *C. mollissima* hybrid. Possibly, other resistance mechanisms may be activated earlier in this hybrid and control the infection.

Pathogen Recognition and Successive Host Response Regulation

Generally, during pathogen infection, PAMPs are recognized by pattern-recognition receptors (PRRs) at the plant's cell surface. The best-studied class of plant PRRs are receptor-like kinases (RLKs), which have an ectodomain of leucine-rich repeats (LRRs) involved in PAMP perception (Boller and Felix 2009; Jones and Dangl 2006; tenHove et al. 2011). Resistance related LRR proteins have been shown to be differentially expressed in global transcript profiling studies in *Phytophthora* spp. infection response (Ballvora et al. 2002, Boava et al. 2011, Coelho et al. 2011, Gao et al. 2005, Mahomed and Berg 2011, van der Vossen et al. 2003). Contrasting to *C. sativa*, *C. crenata* has a much higher (about 10x more) Cast_LRR-RLK expression before inoculation (Santos et al. 2017), which may mediate a fast and effective response against *P. cinnamomi*, suggesting that this earlier recognition is part of the resistance phenotype. Furthermore, Cast_LRRRLK expression increased after *P. cinnamomi* inoculation for all *Castanea* genotypes. Considering the previous studies on LRR biological functions in Fagaceae, Cast_LRR-RLK may recognize and interact with PAMPs molecules, secreted by *P. cinnamomi*, activating downstream signaling responses (Coelho et al. 2011).

Cast_WRKY 31 may have a role in the response of chestnut to *P. cinnamomi* infection, since its expression increased in inoculated samples when compared with non-inoculated ones, probably regulating SA-responsive genes expression. This increase seems more consistent in the more resistant hybrids.

The overexpression of WRKY 31 in rice seedlings after treatment with a hemibiotrophic fungus (*Magnaporthe grisea*) was associated with blockade of pathogen invasion (Zhang et al. 2008). The balance between SA and other phytohormones is increasingly recognized as central to the outcome of plant– pathogen interactions (de Torres-Zabala et al. 2009). Abcisic acid (ABA) disrupts SA-mediated response and suppresses the expression of many defense-related genes. The ethylene responsive transcription factor ABR1 is a negative regulator of ABA signaling pathway in *Arabidopsis thaliana* (Pandey et al. 2005) and its expression allows SA and lignin accumulation (Boatwright and Pajerowska-Mukhtar 2013; de Torres-Zabala et al. 2009; Mohr and Cahill 2007). Cast_ABR1 expression was triggered after *P. cinnamomi* inoculation, earlier in the more resistant genotypes, suggesting that ABA may be repressed after pathogen perception. In the resistant *C. crenata* genotype the relatively low increase of Cast_ABR1 expression may be due to the efficiency of other resistant mechanisms that avoid pathogen colonization, or by independence of ABA suppression for SA signalling activation. Genes of the MYB transcription factor family are involved in the control of specific processes including responses to biotic stresses (Dubos et al. 2010). The expression balance of Cast_Myb4 in *Castanea* genotypes may regulate SA accumulation vs. synthesis of phenylpropanoids. The ratio of Cast_Myb4 expression between 24/48 hpi decreased progressively from the resistant *C. crenata*, to *C. sativa* × *C. crenata* hybrids (the most resistant to the most susceptible) to the susceptible *C. sativa*. This indicates that SA signaling may be faster (24 hpi) in resistant genotypes than in susceptible ones. As mentioned before, elevated concentrations of endogenous SA will induce expression of Cast_Gnk2-like and Cast_WRKY31. For resistant genotypes, (*C. crenata* and SC55) after a probable early induction of SA pathways, expression of Cast_Myb4 decreases at 48 hpi, which may allow the synthesis of lignin and other defense molecules.

Hypothetical *P. cinnamomi* Response Mechanism in *Castanea*

The expression profiles obtained suggest that susceptible and resistant plants may share the same response mechanisms. Despite, resistant plants show a much higher constitutive expression of the tested candidate genes before inoculation. A working model describing part of the molecular interaction of *Castanea* spp. to *P. cinnamomi* infection was presented in Santos et al. (2017): Physiochemical barriers, antifungal proteins secretion (Cast_Gnk2-like) and stronger cell walls (by action of Cast_PE-2, Cast_ABR1) respectively, may inhibit *P. cinnamomi* growth and infection. If *P. cinnamomi* overcome those barriers, specific pathogen recognition may occur, by Cast_LRR-RLK. Hence, host transcription is reprogrammed via MAPK cascades and SA signaling. Cast_WRKY 31 should activate transcription of LRR-RLK. Cast_ABR1 regulate SA accumulation via ABA suppression. HR could be activated by many mechanisms: SA or calcium signaling, via Cast_Gnk2-like (actin-dependent) or by vital protein degradation (by Cast_RNF5). Cell walls not infected may be reinforced and antifungal proteins may be secreted in more abundance, inhibiting further colonization.

Resistant genotypes present a higher expression of genes in non-inoculation conditions that may be part of a constitutive defense mechanism that prepare and protect the plant in advance to *P. cinnamomi* infection by secreting antifungal proteins and having stronger cell walls even before the contact with the pathogen. If *P. cinnamomi* overcomes those chemical and physical barriers, specific pathogen recognition proteins are earlier and more expressed in the resistant genotypes when compared to the susceptible ones. Thereafter, the transcription of the host will probably be reprogrammed via signal transduction and SA signaling. HR-related cell death is probably activated and cell walls may be reinforced in non-infected tissues, preventing further colonization.

In conclusion, the first layer of defense seems to be active and decisive in the resistance of *C. crenata* to *P. cinnamomi*. A lower and delayed expression of the eight studied genes was found in *C. sativa*, which may be related with the sensitivity of this species towards the disease. One probable explanation for this difference can be the allelic variation of the genes or gene-promoters that in *C. sativa* may condition the levels of gene expression before inoculation. *C. mollissima*, also a resistant species, may share with *C. crenata* some of the allelic variants that allow an efficient level of resistance against *P. cinnamomi*. This will be the object of further research. Natural selection could have had an active role in keeping those allelic variants, since Asian species have evolved in contact with *P. cinnamomi*. This study is part of an ongoing Portuguese breeding program to introduce resistance to *P. cinnamomi* in *C. sativa*. This knowledge may contribute for the development of strategies to control ink disease in chestnut and other woody plants, which may include early selection of resistant genotypes.

REFERENCES

Bahder, B.W.; Zalom, F.G.; Jayanth, M. [et al.]. 2016. Phylogeny of geminivirus coat protein sequences and digital PCR aid in identifying Spissistilus festinus as a vector of Grapevine red blotch-associated virus. Phytopathology. 10: 1223–1230. doi: 10.1094/PHYTO-03-16-0125-FI.

Ballvora, A.; Ercolano, M.R;, Weiss, J. [et al.]. 2002. The R1 gene for potato resistance to late blight (Phytophthora infestans) belongs to the leucine zipper/NBS/LRR class of plant resistance genes. Plant Journal. 30: 361–371. doi: 10.1046/j.1365-313X.2001.01292.x.

Boatwright, J.L.; Pajerowska-Mukhtar, K. 2013. Salicylic acid: an old hormone up to new tricks. Molecular Plant Pathology. 14: 623–634. doi: 10.1111/mpp.12035.

Boava, L.P.; Cristofani-Yaly, M.; Mafra, V.S. [et al.]. 2011. Global gene expression of *Poncirus trifoliata*, *Citrus sunki* and their hybrids under infection of *Phytophthora parasitica*. BMC Genomics. 12: 1–13. doi: 10.1186/1471-2164-12-39.

Boller, T.; Felix, G. 2009. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annuual Review Plant Biology. 60: 379–406. doi: 10.1146/annurev.arplant.57.032905.105346.

Bormann, C.A.; Oberhagemann, P. [et al.]. 2002. The R1 gene for potato resistance to late blight (Phytophthora infestans) belongs to the leucine zipper/NBS/LRR class of plant resistance genes. Plant Journal. 30: 361–371. doi: 10.1046/j.1365-313X.2001.01292.

Coelho, A.C.; Ebadzad, G.; Cravador, A. 2011. Quercus suber–P. cinnamomi interaction: hypothetical molecular mechanism model. New Zealand Journal of Forest Science. 41: 143–157.

Costa, R.; Santos, C.; Tavares, F. [et al.]. 2011. Mapping and transcriptomic approaches implemented for understanding disease resistance to Phytophthora cinnamomi in Castanea sp. BMC Proceedings. 5: O18. doi: 10.1186/1753-6561-5-S7-O18.

de Torres-Zabala, M.; Bennett, M.H.; Truman, W.H.; Grant, M.R. 2009. Antagonism between salicylic and abscisic acid reflects early hostpathogen conflict and moulds plant defence responses. Plant Journal. 59: 375–386. doi: 10.1111/j.1365-313X.2009.03875.

Dubos, C.; Stracke, R.; Grotewold, E. [et al.]. 2010. MYB transcription factors in Arabidopsis. Trends Plant Science. 15: 573–581. doi: 10.1016/j.tplants.2010.06.005.

Freeman, B.; Beattie, G. 2008. An overview of plant defenses against pathogens and herbivores. Plant Health Instructor. doi: 10.1094/PHI-I-2008- 0226-01.

Gao, N.; Wadhwani, P.; Mühlhäuser, P. [et al.]. 2005. An antifungal protein from Ginkgo biloba binds actin and can trigger cell death. Protoplasma. 253: 1159–1174. doi: 10.1007/s00709-015-0876-4.

Ge, X.; Deng, W.; Lee, Z.Z. 2016. Tempered mlo broad-spectrum resistance to barley powdery mildew in an Ethiopian landrace. International Journal of Scientific Reports. 6: 29558. doi: 10.1038/srep29558.

Hardham, A.; Blackman, L. 2010. Molecular cytology of Phytophthora-plant interactions. Australas. Plant Pathology. 39: 29–35. doi: 10.1071/AP09062.

Jones, J.; Dangl, J. 2006. The plant immune system. Nature. 444: 323–329. doi: 10.1038/nature05286.

Kieffer, F. 2000. The fungal elicitor cryptogein induces cell wall modifications on tobacco cell suspension. Journal of Experimental Botany. 51: 1799–1811. doi: 10.1093/jexbot/51.352.1799.

Mahomed, W.; van den Berg, N. 2011. EST sequencing and gene expression profiling of defence-related genes from Persea americana infected with Phytophthora cinnamomi. BMC Plant Biology. 11: 167. doi: 10.1186/1471.

Majumdar, N.; Wessel, T.; Marks, J. 2015. Digital PCR modeling for maximal sensitivity, dynamic range and measurement precision. PLoS ONE. 10: e0118833. doi: 10.1371/journal.pone.0118833.

Miyakawa, T.; Hatano, K.; Miyauchi, Y. [et al.]. 2014. A secreted protein with plant-specific cysteine-rich motif functions as a mannose-binding lectin that exhibits antifungal activity. Plant Physiology. 166: 766–778. doi: 10.1104/pp.114.242636.

Mohr, P.G.; Cahill, D.M. 2007. Suppression by ABA of salicylic acid and lignin accumulation and the expression of multiple genes, in Arabidopsisinfected with Pseudomonas syringae pv. tomato. Functional & Integrative Genomics. 7: 181–191. doi: 10.1007/s10142-006-0041-4.

Montesinos, E. 2007. Antimicrobial peptides and plant disease control. FEMS Microbiology Letters. 270: 1–11. doi: 10.1111/j.1574-6968.2007.00683.

Pandey, G.K.; Grant, J.J.; Cheong, Y.H. [et al.]. 2005. ABR1, an APETALA2-domain transcription factor that functions as a repressor of ABA response in Arabidopsis. Plant Physiology. 139: 1185–1193. doi: 10.1104/pp.105.066324. Phytopathology. 10: 1223–1230. Doi: 10.1094/PHYTO-03-16-0125-FI.

Santos, C.; Duarte, S.; Tedesco, S. [et al.]. 2017. Expression profiling of Castanea genes during resistant and susceptible interactions with the oomycete pathogen Phytophthora cinnamomi reveal possible mechanisms of immunity. Frontiers in Plant Science. 8: 515. doi: 10.3389/fpls.2017.00515.

Santos, C.; Machado, H.; Correia, I. [et al.]. 2015. Phenotyping Castanea hybrids for Phytophthora cinnamomi resistance. Plant Pathology. 64: 901–910. doi: 10.1111/ppa.12313.

Sawano, Y.; Miyakawa, T.; Hiroshi, Y. [et al.]. 2007. Purification, characterization, and molecular gene cloning of an antifungal protein from Ginkgo biloba seeds. Journal of Biological Chemistry. 388: 273–280. doi: 10.1515/BC.2007.030.

Serrazina, S.; Santos, C.; Machado, H. [et al.]. 2015. Castanea root transcriptome in response to Phytophthora cinnamomi challenge. Tree Genetics & Genomes. 11: 1–19. doi: 10.1007/s11295-014-0829-7.

Stevanato, P.; Biscarini, F. 2016. Digital PCR as new approach to SNP genotyping in sugar beet. Sugar Tech. 18: 429–432. doi: 10.1007/s12355-015-0408-8.

ten Hove, C.A.; Bochdanovits, Z.; Jansweijer, V.M.A. [et al.]. 2011. Probing the roles of LRR RLK genes in Arabidopsis thaliana roots using a customT-DNA insertion set. Plant Molecular Biology. 76(1-2): 69-83.

van der Vossen, E.; Sikkema, A.; Hekkert, B. [et al.]. 2003. An ancient R gene from the wild potato species Solanum bulbocastanum confers broad-spectrum resistance to Phytophthora infestans in cultivated potato and tomato. Plant Journal. 36: 867–882. doi: 10.1046/j.1365-313X.2003.01934.

Wang, H.; Ng, T.B. 2000. Ginkbilobin, a novel antifungal protein from Ginkgo biloba seeds with sequence similarity to embryo-abundant protein. Biochemical and Biophysical Research Communications. 279: 407–411. doi: 10.1006/bbrc.2000.3929.

Wiethölter, N.; Graessner, B.; Mierau, M. [et al.]. 2003. Differences in the methyl ester distribution of homogalacturonans from near-isogenic wheat lines resistant and susceptible to the wheat stem rust fungus. Molecular Plant Microbe Interactions. 16: 945–952. doi: 10.1094/MPMI.2003.16.10.945.

Wittstock, U.; Gershenzon, J. 2002. Constitutive plant toxins and their role in defense against herbivores and pathogens. Current Opinion in Plant Biology. 5: 300–307. doi: 10.1016/S1369-5266(02)00264-9.

Zhang, J.; Peng, Y.; Guo, Z. 2008. Constitutive expression of pathogeninducible OsWRKY31 enhances disease resistance and affects root growth and auxin response in transgenic rice plants. Cell Research. 18: 508–521. doi: 10.1038/cr.2007.104.

EXPLOITING NATURAL VARIATION IN A TREE SPECIES TO COMBAT AN INVASIVE FOREST PATHOGEN

Jared M. LeBoldus[1]

Invasive microbes, causing diseases such as sudden oak death negatively affect ecosystems and economies around the world. The deployment of resistant genotypes for combating introduced diseases typically relies on breeding programs that can take decades to complete. To demonstrate how this process can be accelerated, we employed a genome-wide association mapping of ca. 1,000 re-sequenced *Populus trichocarpa* trees individually challenged with *Sphaerulina musiva*, an invasive fungal pathogen. Among significant associations, three resistance loci were identified and predicted to encode a putative membrane-bound L-type receptor-like kinase and two receptor-like proteins. A susceptibility-associated locus was predicted to encode a putative G-type D-mannose-binding receptor-like kinase. Multiple lines of evidence including allele analysis, transcriptomics, binding assays, and overexpression support the function of these genes in mediating this interaction. Herein we demonstrated how the power of population-wide re-sequencing of undomesticated, non-model plant species could accelerate the mitigation of emerging diseases in native ecosystems.

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GENOME-WIDE EXON-CAPTURE APPROACH IDENTIFIES GENETIC VARIANTS ASSOCIATED WITH SUSCEPTIBILITY OF NORWAY SPRUCE TREES TO *HETEROBASIDION PARVIPORUM* INFECTION

Mukrimin Mukrimin[1,2], Andriy Kovalchuk[1], Leandro G. Neves[3], Emad Jaber[1], Matti Haapanen[4], Matias Kirst[3], and Fred O. Asiegbu[1]

In boreal forests the root and butt rot caused by members of the *Heterobasidion annosum* species complex is the most economically important disease of conifer trees. In the infected trees, the wood decay dramatically decreases their value and causes considerable losses to forest owners. Trees vary in their susceptibility to *Heterobasidion* infection, but the genetic determinants underlying the variation in the susceptibility are not well understood. We performed the identification of Norway spruce genes associated with the resistance to *Heterobasidion parviporum* infection using genome-wide exon-capture approach. Sixty-four clonal Norway spruce lines were phenotyped, and their responses to *H. parviporum* inoculation were determined by lesion length measurements. Afterwards, the spruce lines were genotyped by targeted resequencing and identification of genetic variants (SNPs). Genome-wide association analysis identified 36 SNPs located within 34 genes as significantly associated with the larger necrotic lesions in response to *H. parviporum* inoculation. The genetic variants identified in our analysis are potential marker candidates for future breeding programs.

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METABOLOMIC COMPARISON OF EUROPEAN ASH GENOTYPES WITH DIFFERENT TOLERANCE LEVELS AGAINST ASH DIEBACK

Miguel Nemesio-Gorriz[1], Christian Paetz[2], Riya C. Menezes[2], Almuth Hammerbacher[3], Jonathan Gershenzon[2], and Gerry Douglas[1]

Ash dieback, a tree disease caused by the ascomycete *Hymenoscyphus fraxineus*, is currently decimating ash populations in Europe and poses a threat to other ash species in North America. Despite its severity, between 1 percent and 3 percent of the European ash trees show moderate to high tolerance to the disease. This study compares the biochemical profile of 20 highly tolerant and 20 highly susceptible *Fraxinus excelsior* genotypes from 16 populations in 7 European countries using metabolomics. In terms of intraspecific diversity, phenotyping, and number of individuals, this is the most complete metabolomics study in ash that has been ever attempted. Results show a very different biochemical profile for both groups and focus on specific compounds that may play a major role in determining the tolerance or susceptibility of ash genotypes.

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Session 5

USE OF GENOMICS TO DISSECT MECHANISMS OF HOST-PARASITE INTERACTION

CANDIDATE GENES FROM GWAS AND RNASEQ FOR BEECH BARK DISEASE RESISTANCE IN AMERICAN BEECH

John E. Carlson[1], Irina Ćalić[2,3], Jennifer Koch[4], David Carey[4], Charles Addo-Quaye[5], Donghwan Shim[1], and David B. Neale[3]

SUMMARY

American beech (*Fagus grandifolia* Ehrh.) is an aesthetically, ecologically, and economically important native component of the North American eastern hardwood forest. American beech is susceptible to beech bark disease (BBD), however, and has suffered high rates of mortality as the disease complex spreads. The invasive sap-feeding woolly beech scale insect (*Cryptococcus fagisuga*) is the pre-disposing factor for infection by introducing either *Neonectria faginata* or *N. ditissima* fungus species that result in extensive cankering of American Beech trees. A small percentage of trees survive BBD attack, and many of these show signs of natural resistance to the insect vector in the egg-inoculation tests used to assay for resistance. We have developed and applied genomics resources to learn more about the molecular genetic basis of gene expression and gene sequence variation associated with cases of natural resistance to the insect. Initially, transcriptome resources were developed, and differential gene expression analyses conducted from which candidate genes were selected. From the transcriptome, an SNP chip assay was developed to genotype an association population of 506 individuals from across the American beech range, 249 of which were resistant and 257 susceptible to BBD. We also constructed a genetic linkage map based on SNPs with a full-sib family of 115 individuals to locate BBD-resistance QTL. The GWAS project revealed four highly significant SNPs on Linkage Group 5 for a single gene encoding a metallothionein-like protein. Metallothioneins are cysteine-rich metal chelator proteins that can moderate oxidative stress by coordinating metal atoms, which may provide a resistance mechanism against the woolly beech scale insect.

OBJECTIVES

The primary goal of this research was to gain a better understanding of the molecular mechanisms underlying resistance and susceptibility to beech bark disease (BBD) in American beech. Our objectives towards this goal were to:

1. Develop genomics tools for molecular genetic studies in American beech.
2. Conduct differential gene expression analyses to identify candidate genes for future research on BBD resistance in beech.
3. Conduct a genome-wide association study (GWAS) to identify which genes or alleles have the greatest contribution to BBD resistance in natural stands of American beech.

METHODS AND RESULTS

Development of Genomics Tools for American Beech

Our development of genomic resources for American beech began with support from the National Science Foundation's Plant Genome Resources Program for the "Fagaceae Genomic Tool Development Project", which ran from 2006 to 2009. This project was led by Ronald Sederoff (North Carolina State University), with participants from Pennsylvania State University (John Carlson, Haiying Liang, Abdelali Barakat, Stephan Schuster), SUNY ESF (William Powell, Kathleen Baier, Charles Maynard), Clemson University (Albert Abbott, Margaret Staton, Jeff Tomkins,

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Steven Ficklin, Barbara Blackman, Eric Fang), North Carolina State University (Nick Wheeler, Chris Smith, Dahlia Nielsen, Ron Sederoff), The Connecticut Agricultural Experiment Station (Sandra Anagnostakis, Lila Pinchot), USDA Forest Service (Tom Kubisiak, Dana Nelson), The American Chestnut Foundation (Fred Hebard, Paul Sisco), and Science Advisory Board members (Doug Cook of UC-Davis, Jennifer Koch of USDA Forest Service, and Jeanne Romero-Severson of Notre Dame University).

The "Fagaceae Project" focused on Chinese chestnut (*Castanea mollissima*), American chestnut (*C. dentata*), Northern red oak (*Quercus rubra*), White oak (*Q. alba*), and American beech. For these five species we developed expressed sequence tag (EST) databases by next generation sequencing (NGS) with 454 technology (Roche) for use in gene discovery, DNA markers for genetic mapping, and in some species BAC libraries for gene cloning and physical mapping. In addition, work on high density genetic mapping and physical mapping was initiated. In total, 16 cDNA libraries were constructed and sequenced, including two for American beech. The beech libraries were prepared from poly-A RNA isolations from tissue collections designed to represent gene expression plant-wide, including roots, bark, buds, twigs, leaves, petioles, and flowers. The tissues were collected from USDA Forest Service genotype 1504—a healthy American beech tree, and genotype 1506—a BBD-infected American beech tree. For American beech, a total of 14.6 million bases of RNA sequence data (ESTs) were generated and assembled into 8,319 transcripts. From the assembled transcripts, 2,383 proteins could be predicted based on protein databases (such as InterProScan) of which 2,231 were assigned putative annotations and functions based on similarities to known genes in the NCBI databases. The EST sequences for each of the American beech trees 1504 and 1506 were deposited at the NCBI Short Read Database under accession numbers SRX001797 and SRX001798, respectively. The American beech EST sequences, transcripts, annotations, and DNA markers from the Fagaceae Project are also available for download and query at the Hardwood Genomics Database entry for *Fagus grandifolia* (https://www.hardwoodgenomics.org/organism/Fagus/grandifolia) that also includes a Gene Ontology Browser of 629 biological, 242 cellular, and 830 molecular functions predicted from the transcript annotations, as well as a KEGG Brower with the placement of the beech transcripts into enzymatic pathways.

After completion of the Fagaceae Project, genomic resources targeted to BBD-resistance in American beech were greatly expanded through a grant to Jennifer Koch from the USDA Forest Service Forest Health Protection's Special Technology Development Program. The project "Development of DNA-based markers to identify beech bark disease (BBD) - resistant trees in natural stands" ran from 2009 to 2012, with Jennifer Koch as Principal Investigator and participants from Pennsylvania State University (John Carlson, Donghwan Shim, Charles Addo-Quaye, Tyler Wagner, Lynn Tomsho), UC-Davis (David Neale, Irina Ćalić, Randi Famula, Mirko Ledda, Christopher Campbell), and USDA Forest Service Northern Research Station lab in Delaware, OH (David Carey).

The "BBD-resistance project" focused on gaining a better understanding of the molecular mechanisms underlying BBD-resistance in American beech. We studied a set of BBD-resistant and BBD-susceptible trees identified in natural stands by Forest Service researchers, some of which were accessioned as part of the beech bark disease genetic resistance improvement program (Koch et al. 2010; Houston and Houston 2000). RNA sequence data was produced by 454 NGS technology from each of 10 cDNA libraries prepared from poly-A RNA isolated from bark tissues of 5 BBD-resistant trees (USDA Forest Service accessions 1228, 1208, 2692, 1504, 2776) and from 5 BBD-susceptible trees (accessions 726, 3128, 1973, 2143, "Holden") following treatment of clonal replicates with larvae of the woolly beech scale under greenhouse conditions. The relative resistance of nine of the 10 clonal accessions (genotypes) (fig. 1) was assayed by determining the number of adult scale insects produced from genotypes following egg inoculations (the Holden genotype did not survive the inoculation test).

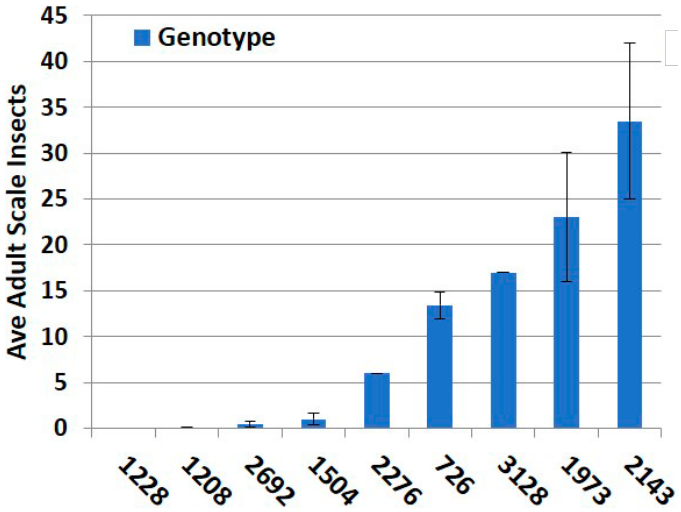

Figure 1—Average number of scale insects produced from clones of American beech genotypes following egg inoculation under greenhouse conditions. A range of resistance and susceptibility to BBD are demonstrated.

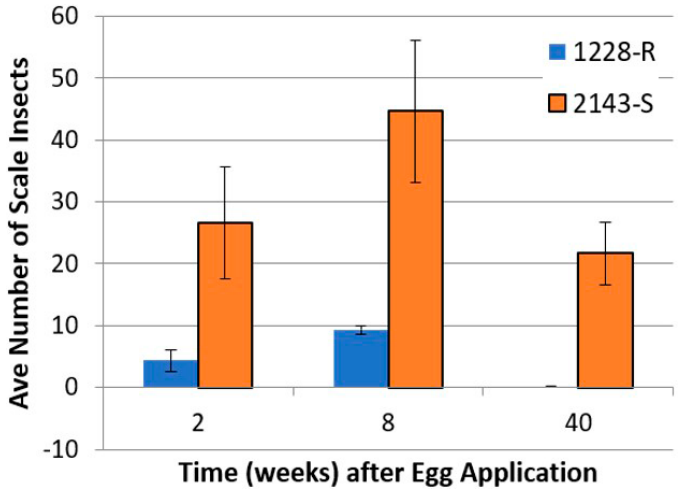

Figure 2—Comparison of scale insect development on BBD-resistant vs. BBD-susceptible genotypes 1228R and 2143S.

In total we obtained 257.5 Mb of RNA sequence data from the BBD-resistant trees and 508.7 Mb from the susceptible trees. The RNA sequences from the BBD-resistant trees were pooled as were the sequences from the BBD-susceptible trees, and then assembled into two sets of transcripts from which over 26,000 Single Nucleotide Polymorphism (SNP) DNA markers were identified for use in genetic linkage mapping and genome-wide association (GWAS) studies (Ćalić et al. 2017). The sequences from this study are available in the Short Read Archive at the National Center for Biotechnology Information, genotype numbers SRX1781388 to SRX1781397 (NCBI BioProject genotype PRJNA321730; http://www.ncbi.nlm.nih.gov).

From the 10 beech tree genotypes, we chose genotypes 1228 ('1228R') and 2143 ('2143S') to represent the putatively most BBD-resistant and BBD-susceptible (fig. 1) for an initial study of the response of gene expression in beech to attack by the scale insect. The results of a time course comparing insect viability at 2, 8, and 40 weeks, confirmed the pronounced differences in susceptibility between genotypes 1228R and 2143S (fig. 2).

The workflow devised for analysis and comparison of gene expression in genotypes 1228R and 2143S is presented in figure 3. Steps completed during this project are indicated by solid lines, while dotted lines suggest steps that should be taken in future projects, based on results gained in the BBD-resistance project.

The RNA sequence yields and statistics from the cDNA libraries from genotypes 1228R and 2143S are shown in table 1. A total of 1,021,530 sequence reads corresponding to 403,362,357 bases were obtained from the two outer bark tissue RNA samples. The RNA sequences from the two libraries were assembled separately and after pooling (using Trinity software). A total of 22,463 transcripts were assembled for genotype 1228R and 21,957 for genotype 2143S, and 31,525 from the pooled RNA sequence data.

Of the 31,525 transcripts in the reference set of transcripts from pooling the two sets of RNA sequences prior to assembly (table 1 "Both"), 26,784 transcripts were for unique genes (table 1 "Total trinity components"), while 4,741 were predicted to be isoforms from RNA-splicing. Of the 26,527 unique transcripts, 22,015 genes were expressed in both genotypes 1228R and 2143S, while 2,352 transcripts could only be detected in genotype 1228R and 2,160 transcripts were unique to genotype 2143S (fig. 4A). The combined assembly served as a reference against which the number of individual sequence reads for each transcript from each library was aligned

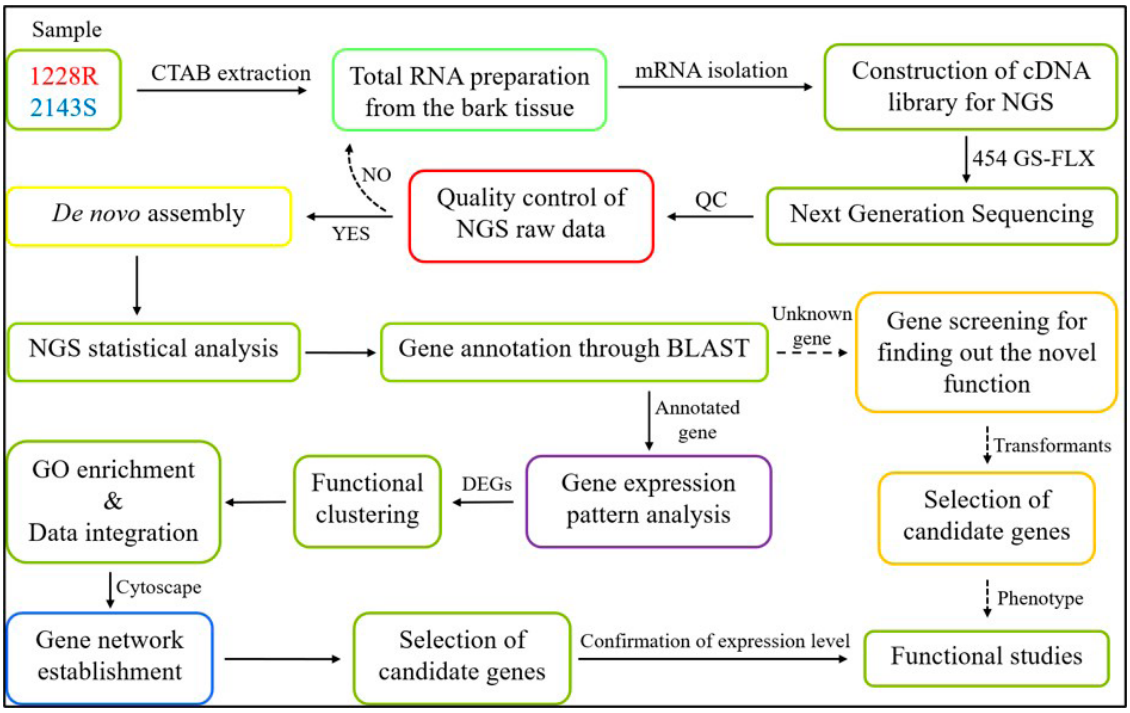

Figure 3—RNA sequence analysis workflow. Dashed arrows are steps which are recommended for future research projects.

Table 1—Statistics of 454 pyrosequencing reads and assembled contigs derived for BBD-resistant and BBD-susceptible *F. grandifolia* trees

		Resistant tree (1228R)	Susceptible tree (2143S)	
Raw reads				
	Num. sequences	530,622	493,807	
	Total nucleotides	211,316,501	191,666,186	
	Min. length	27	27	
	Max. length	1,568	1,558	
	Ave. read length	398.24	388.14	
	Q20	85.07	84.99	
	Q30	57.82	57.02	
	Ave. quality score	30.47	30.33	
Cleaned reads				
	Num. sequences	408,108	380,425	
	Total nucleotides	83,041,684	77,164,051	
	Min. length	50	50	
	Max. length	620	603	
	Ave. read length	203.48	202.84	
	Q20	98.1	98.15	
	Q30	87.62	87.11	
	Ave. quality score	36.29	36.14	
		Resistant tree (1228R)	**Susceptible tree (2143S)**	**Both**
Assembled contigs				
	Total trinity transcripts	22,463	21,957	31,525
	Total trinity components	20,574	20,238	26,784
	Percent GC	43.23	43.35	42.8
	Contig N50	754	735	921
	Average contig	606.89	599.17	688.78
	Total assembled bases	13,632,611	13,155,905	21,713,754

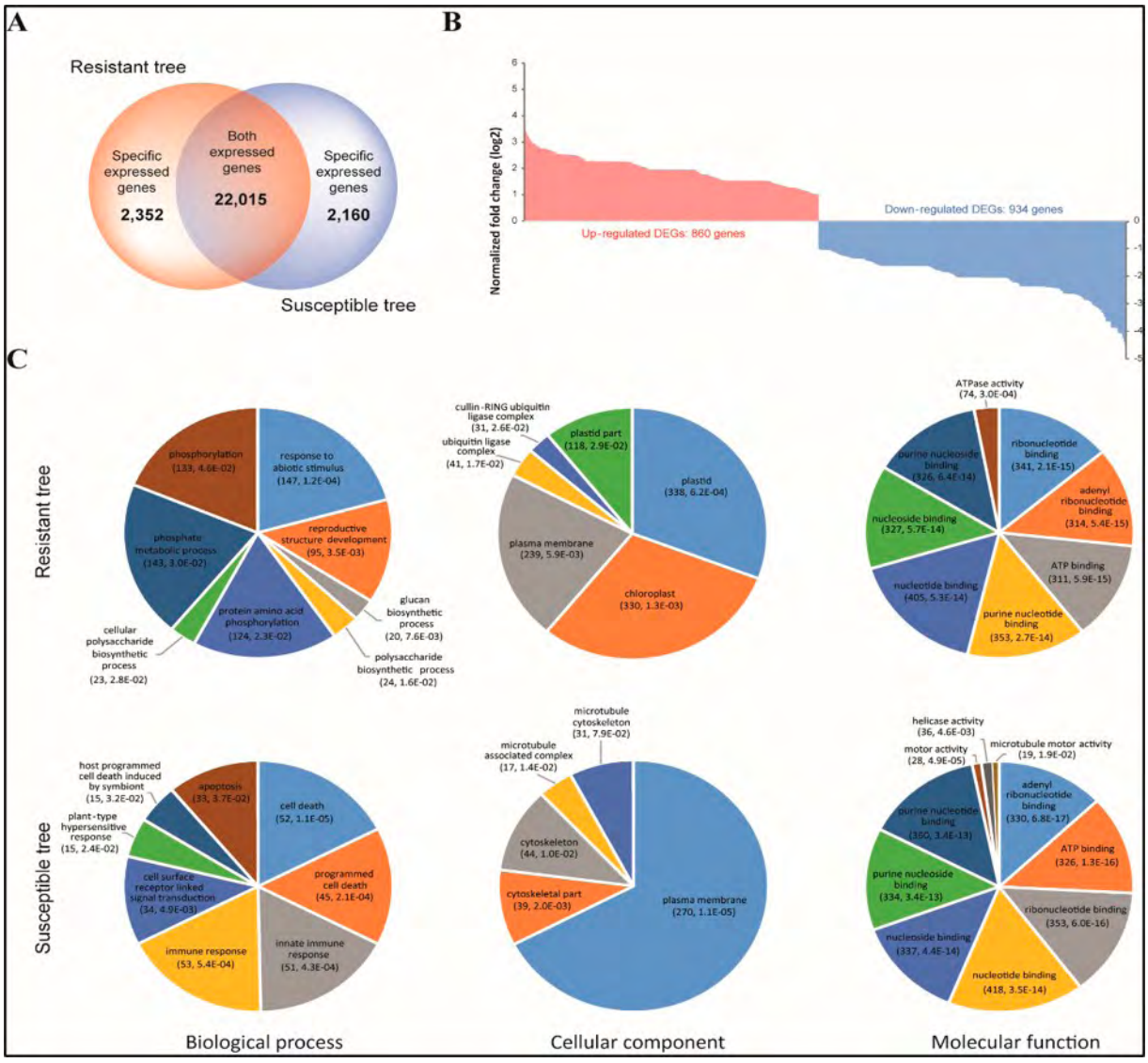

Figure 4—Distribution of differentially expressed genes (DEGs) in the BBD-resistant (1228R) and BBD-susceptible (2143S) American beech trees. (A) Venn diagram showing proportions of DEGs expressed in common and expressed uniquely in 1228R and 2143S trees; (B) Log-fold changes observed in up-regulated (induced) and down-regulated (suppressed) genes overall; (C) Venn diagrams showing numbers and proportions of Gene Ontology determined functional categories of DEGs in biological processes, cell components, and molecular functions.

and counted (using Qiagen/CLCBio Genomics Workbench software). Expression analysis of the RNA-Seq data revealed a total of 860 genes that were upregulated (induced) and 934 that were downregulated (suppressed) across the two genotypes, 1228R and 2143S (fig. 4B). Gene ontology (GO) analysis of putative functions showed a wide range of functional classifications represented in the transcriptomes of each genotype (fig. 4C). Twenty-two candidate genes, for research on BBD resistance in American beech, were selected based on the magnitudes of upregulation of expression in 1228R and alignment scores to known proteins (table 2).

To determine which of the 4,458 uniquely expressed transcripts were significantly different in expression between genotypes 1228R and 2143S, we conducted a differential gene expression analysis using the combined transcriptome referenced as above (using Qiagen/CLCBio Genomics Workbench software). A total of 608 genes were identified as significantly differentially expressed between genotypes 1228R and 2143S (at P-value < 0.01, unique reads > 5), of which 303 were up-regulated at least 2-fold and 305 were down-regulated by 2-fold or more. Among these differentially expressed genes (DEGs), 313 transcripts could be putatively identified by

Table 2—Candidate genes for BBD resistance in American beech based on relative strengths (fold change) of BBD-induced gene expression differences, rather than GO functional classifications. Only genes with the highest BLAST sequence alignment scores (E-values) to known genes in the model plants Arabidopsis and poplar in the NCBI Database are shown

Transcript ID	Lowest E-value	Accession (E-value)	AGI	NCBI annotation	Fold Change
AB_contig_394	8.732E-67	POPTR_0001s20780.1	AT2G04865.1	Aminotransferase-like, plant mobile domain family	8.165
AB_contig_1849	2.844E-130	POPTR_0016s14560.1	AT3G51550.1	Malectin/receptor-like protein kinase family protein	4.869
AB_contig_661	3.776E-156	POPTR_0010s18600.1	AT5G36890.1	Beta-glucosidase 42	4.217
AB_contig_2533	1.001E-124	POPTR_0002s03020.1	AT4G39090.1	Papain family cysteine protease	4.115
AB_contig_123	7.883E-27	POPTR_0001s03100.1	AT2G28910.1	CAX interacting protein 4	3.724
AB_contig_4199	1.516E-159	POPTR_0001s11360.1	AT2G46660.1	Cytochrome P450, family 78, subfamily A, polypeptide 6	3.5
AB_contig_5292	0	POPTR_0002s19480.1	AT3G62360.1	Carbohydrate-binding-like fold	3.335
AB_contig_4874	0	POPTR_0001s00330.1	AT1G63370.1	Flavin-binding monooxygenase family protein	3.126
AB_contig_25862	2.136E-22	POPTR_0001s20400.1	AT1G17720.2	Protein phosphatase 2A, regulatory subunit PR55	3.091
AB_contig_6118	4.385E-94	POPTR_0010s12440.1	AT1G23010.1	Cupredoxin superfamily protein	3.07
AB_contig_320	3.339E-109	POPTR_0001s17020.1	AT1G72650.2	TRF-like 6	2.923
AB_contig_2593	1.167E-106	POPTR_0002s07190.1	AT1G21360.1	Glycolipid transfer protein 2	2.885
AB_contig_5610	0	POPTR_0001s11860.2	AT5G49730.1	Ferric reduction oxidase 6	2.815
AB_contig_6174	1.285E-104	POPTR_0019s01650.1	AT5G17680.1	Disease resistance protein (TIR-NBS-LRR class), putative	2.759
AB_contig_8613	0	POPTR_0010s05170.1	AT1G60470.1	Galactinol synthase 4	2.38
AB_contig_2023	7.681E-67	POPTR_0011s12120.1	AT3G15360.1	Thioredoxin M-type 4	2.346
AB_contig_10163	7.492E-96	POPTR_0005s20870.1	AT1G17950.1	MYB domain protein 52	2.33
AB_contig_5378	9.648E-131	POPTR_0008s09140.1	AT1G13960.1	WRKY DNA-binding protein 4	2.154
AB_contig_9926	0	POPTR_0001s04500.1	AT2G42500.1	Protein phosphatase 2A-3	2.099
AB_contig_3528	2.117E-54	POPTR_0007s03840.1	AT5G09330.1	NAC domain containing protein 82	2.092
AB_contig_2070	1.343E-127	POPTR_0015s14600.1	AT4G22680.1	MYB domain protein 85	2.088
AB_contig_1218	0	POPTR_0003s21690.1	AT5G13000.1	Glucan synthase-like 12	2.003

homology to genes in the NCBI non-redundant protein database, while 295 transcripts were "unknown", i.e. with no sequence matches in the database.

The number of annotated (putative protein function) DEGs in the major GO functional classifications observed between the BBD-resistant vs. BBD-susceptible genotypes following greenhouse inoculations is shown by histogram comparisons in figure 5. In the BBD-resistant genotype 1228R, most DEGs were observed in functional categories related to stress-response - "response to abiotic stress" (Heidel-Fischer et al. 2018, Marwal et al. 2019, Tajima et al. 2020, Waterman et al. 2019), "polysaccharide and glucan related responses" (Piršelová and Matušíková 2013; Pogorelko et al 2013), "programmed cell death" (Locato and De Gara 2018), and "potassium ion transport" (Wang et al. 2013). In sharp contrast, the DEGs observed in the BBD-susceptible genotype 2143S were classified into GO functional categories that were in general more related to normal metabolic and developmental processes. These stark differences in gene expression in response to the scale insect indicate that genotype 1228R has the ability to recognize and mount a strong molecular defense against BBD, while genotype 2143S is susceptible due to lack of response at the gene expression level to BBD. We selected 17 candidate genes (table 3) for future studies, based on the four GO functional classifications and the putative gene assignments of DEGs observed in BBD-response by the resistant genotype.

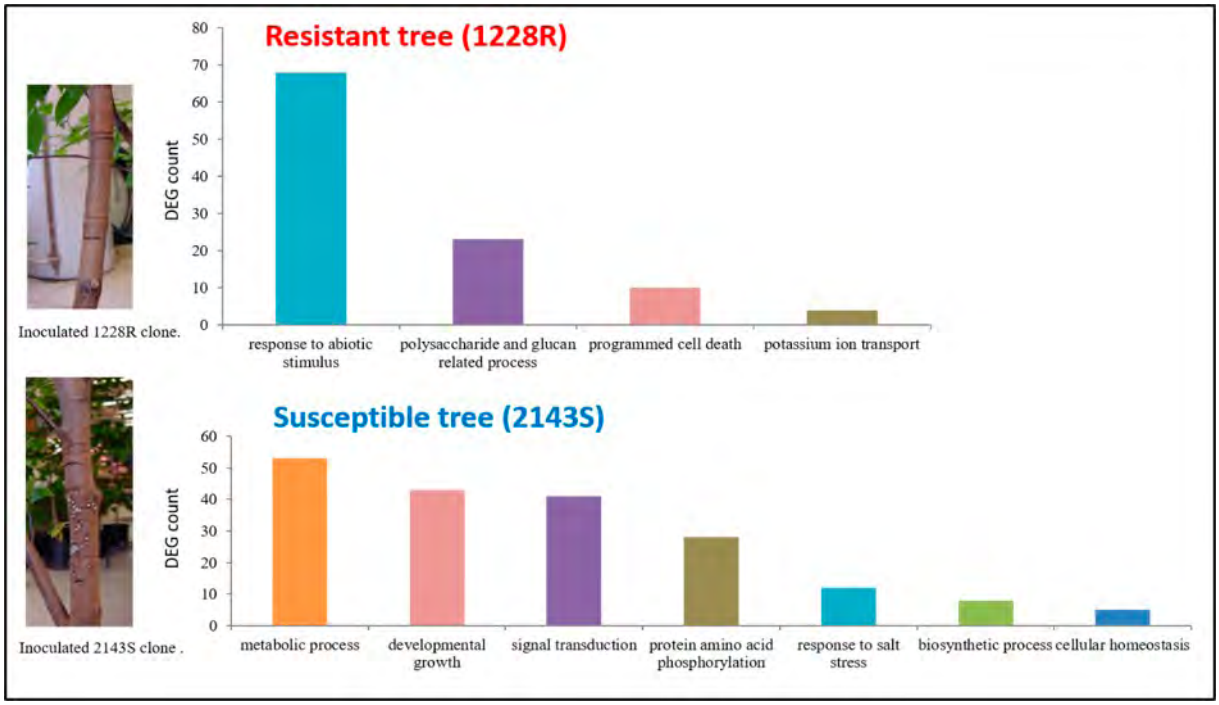

Figure 5—Gene Ontology functional categories of differentially expressed genes.

Table 3—Gene Ontology predicted descriptions for differentially expressed candidate genes in the stress response and apoptosis functional categories

Term	Description	Count	P-value
GO:0009628	response to abiotic stimulus	34	2.21E-04
GO:0048585	negative regulation of response to stimulus	6	2.79E-03
GO:0055114	oxidation reduction	29	6.53E-03
GO:0009266	response to temperature stimulus	11	3.29E-02
GO:0009416	response to light stimulus	13	3.80E-02
GO:0009414	response to water deprivation	7	4.29E-02
GO:0009314	response to radiation	13	4.70E-02
GO:0005976	polysaccharide metabolic process	10	4.55E-03
GO:0000272	polysaccharide catabolic process	5	2.21E-02
GO:0044042	glucan metabolic process	7	2.23E-02
GO:0009250	glucan biosynthetic process	5	2.31E-02
GO:0006073	cellular glucan metabolic process	6	3.38E-02
GO:0012501	programmed cell death	10	5.12E-03
GO:0016265	cell death	10	1.17E-02
GO:0008219	cell death	10	1.17E-02
GO:0006915	Apoptosis	8	1.26E-02
GO:0006813	potassium ion transport	4	4.29E-02

Genome-Wise Association Study

The GWAS revealed four highly significant SNPs on Linkage Group 5 for a single gene encoding a metallothionein-like protein (Ćalić et al. 2017). Metallothioneins are cysteine-rich metal-chelating proteins. Metallothioneins and metallothionein-like proteins respond to abiotic and biotic stresses in plants, including heavy metals, insect herbivory and fungal infections (reviewed by Leszczyszyn et al. 2013). A major role of metallothioneins and metallothionein-like proteins is their anti-oxidant effects that moderate damage from oxidative stress through scavenging of cofactors needed by reactive oxygen generating enzymes. One of the largest groups of pathogenesis-related proteins are also upregulated after heavy metal treatment, suggesting cross-talk between the heavy metal (abiotic) and biotic stress responses in plants (Joshi et al. 2019). Metallothionein-like class II proteins have previously been identified in *Fagus sylvatica* (NCBI accession number CAA10232) and *Picea abies* (Yakovlev et al. 2006). Type 4 metallothionein-like protein genes were reported to be expressed in inner bark tissues of *Cryptomeria japonica* (Leszczyszyn et al. 2013).

CONCLUSIONS, CAVEATS AND FUTURE RESEARCH

It is important to point out that certain caveats existed with the GWAS approach taken in this study. First, at the time the GWAS study (Ćalić et al. 2017) was undertaken, and still at the time of this presentation and manuscript, a reference genome for American beech was not available. Thus, a SNP chip was used as the GWAS reference, based on the combined bark transcriptomes from BBD-resistant and BBD-susceptible trees, to target genes in the tissue and biotic stress of interest. However, unlike a full genome sequence, transcriptomes never include all possible genes of interest nor all the genome-wide variation that exists for a trait, especially outside of coding regions. For example, transcriptomes are limited to the specific time points selected to sample after treatment, which for practical reasons are always limited and cannot encompass all the cascades of gene expression that are qualitatively and quantitatively important in accomplishing resistance. In addition, when dealing with a complex disease such as BBD that involves an insect vector and multiple fungal pathogens, it cannot necessarily be presupposed that using a transcriptome reference based on only the insect establishment, feeding, and reproduction will provide any markers for resistance mechanisms to the fungal infection process. Furthermore, other loci or alleles that fall below a selected significance threshold may also collectively (quantitatively) be important in BBD-resistance, or important in specific populations but not across the range of populations sampled.

Validations of our GWAS results still need to be completed. Ideally, validation populations should be collected from the same or similar populations so that both resistant and susceptible individuals are included, but without advance knowledge of resistance prior to conducting the GWAS and not including samples used in the original study. This is a difficult criterion to meet when dealing with natural populations that are already experiencing mortality from BBD, and/or that are under other biotic or abiotic stresses affecting population structure.

Another powerful approach to validating GWAS results is to test whether the loci with significantly associated alleles map in or near QTL of the trait on genetic linkage maps. The Forest Service's beech bark disease genetic resistance improvement program is evaluating 118 individuals from the 1505 x 1504 full-sib family used to construct the SNP-based genetic linkage map in the GWAS study (Ćalić et al. 2017). At the time of this presentation, only part (46 trees) of the mapping population had so far been evaluated, for which two QTL for resistance were found at regions other than the metallothionein locus detected by GWAS. This negative result, if it holds up in the whole mapping population, does not necessarily invalidate the GWAS result which derived from the strongest genetic signal that could be detected in 327 genetically distinct individuals sampled from several populations, rather than inheritance from only two genotypes (parents of the mapping family which could be fixed at the GWAS locus). It could however suggest that multiple mechanisms may result in BBD-resistance, for

example constitutive resistance traits versus induced-resistance traits, and differences in the natural histories of population exposures to past environmental stresses.

The metallothionein-like protein gene detected in the GWAS study was not one of the candidate genes selected from our transcriptome studies following BBD treatments. Changes in expression of the gene are not necessarily expected from variants in the coding sequence. However, the transcriptome overall did contain 12 transcript contigs which aligned well by BLAST with two metallothionein protein genes in the plant model *Arabidopsis thaliana*—a metallothionein 2A gene (NCBI accession AT3G09390.1) and metallothionein 3 (NCBI accession AT3G15353.1). It would be interesting to conduct a qRT-PCR study of expression of metallothionein or metallothionein-like genes, and other candidate genes, during a detailed time course of response to inoculations of well-established resistant and susceptible genotypes. In the study on differential gene expression reported here, we directly compared only the two extreme genotypes. A detailed gene network analysis study including the 8 genotypes with intermediate resistance/susceptibility phenotypes might reveal additional candidate genes, perhaps of smaller individual effects, which might collectively still be quantitatively important. However, the ultimate validation of the metallothionein-like protein detected by GWAS will require functional genomics studies to confirm its role in BBD-resistance. That awaits development of a gene transfer system for American beech with which knock-out, suppression, or gene editing studies can be conducted.

Beyond basic knowledge of how beech trees respond to BBD, such transcriptome and GWAS studies aim to contribute to the restoration of the species by providing molecular tools for selection and breeding. Markers that exhibit a significant association with the resistance phenotype can provide the basis of efficient indirect selection techniques such as marker assisted selection (MAS) and genomic selection (GS). For simply inherited, single-gene resistance traits, having several DNA markers within the gene increases the power of MAS, as individuals within a population may vary as to how many or which of the markers they carry for the gene. The presence of several markers and thus possibly several alleles for resistance may also slow the breakdown of resistance that can be a concern with single-gene traits in long-lived organisms like American beech. It should be acknowledged, however, that individual markers or alleles for a trait obtained from QTL mapping or transcriptome studies with one family or a small number of genotypes may contribute to only a small amount of phenotypic variation within and between large natural populations (Korte and Fallow 2013). Thus, MAS conducted with a few markers may not accomplish substantial advancements of selection and breeding goals, if not incorporated into genome-wide approaches. Genome-wide selection models that aggregate the small effects of many markers or alleles for a wider range of traits have proven to be powerful in representing a substantial fraction of genetic and phenotypic variation in tree breeding programs (Resende et al. 2017a, 2017b; Müller et al. 2017; Tan et al. 2017). The costs and benefits should be carefully considered prior to undertaking such approaches, as costs (real and opportunity) may be prohibitive, particularly in trees of ecological value that lack commercial value.

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REFERENCES

Ćalić, I.; Koch, J.; Carey, D. [et al.]. 2017. Genome-wide association study identifies a major gene for beech bark disease resistance in American beech (*Fagus grandifolia* Ehrh.). BMC Genomics. 18(1): 547.

Heidel-Fischer, H.M.; Musser, R.O.; Vogel, H. 2018. Plant transcriptomic responses to herbivory. Annual Plant Reviews: 155–196.

Houston, D.B.; Houston, D.R. 2000. Allozyme genetic diversity among *Fagus grandifolia* trees resistant or susceptible to beech bark disease in natural populations. Canadian Journal of Forest Research. 30(5): 778–789. doi:1O.1139/cjfr-30-5-778.

Joshi, R.; Dkhar, J.; Singla-Pareek, S.L.; Pareek, A. 2019. Molecular mechanism and signaling response of heavy metal stress tolerance in plants. In: Srivastava S.; Srivastava A.; Suprasanna P., eds. Plant-Metal Interactions. Springer, Cham: 29–47.

Koch, J.L.; Carey, D.W.; Mason, M.E.; Nelson, C.D. 2010. Assessment of beech scale resistance in full- and half-sibling American beech families. Canadian Journal of Forest Research. 40: 265–272.

Korte, A.; Farlow, A. 2013. The advantages and limitations of trait analysis with GWAS - a review. Plant Methods. 9: 29.

Leszczyszyn, O.I.; Imam, H.T.; Blindauer, C.A. 2013. Diversity and distribution of plant metallothioneins: a review of structure, properties and functions. Metallomics. 5(9): 1146–1169.

Locato, V.; De Gara, L. 2018. Programmed cell death in plants: an overview. New York: In: Plant Programmed Cell Death. Humana Press: 1–8.

Marwal, A.; Kumar, R.; Verma, R.K. [et al.]. 2019. Genomics and molecular mechanisms of plant's response to abiotic and biotic stresses. In: Plant Biotechnology: Progress in Genomic Era. Springer, Singapore: 131–146.

Müller, B.S.; Neves, L.G.; de Almeida Filho, J.E. [et al.]. 2017. Genomic prediction in contrast to a genome-wide association study in explaining heritable variation of complex growth traits in breeding populations of Eucalyptus. BMC Genomics. 18(1): 524.

Piršelová, B.; Matušíková, I. 2013. Callose: the plant cell wall polysaccharide with multiple biological functions. Acta Physiologiae Plantarum. 35(3): 635–644.

Pogorelko, G.; Lionetti, V.; Bellincampi, D.; Zabotina, O. 2013. Cell wall integrity: targeted post-synthetic modifications to reveal its role in plant growth and defense against pathogens. Plant Signaling and Behavior. 8(9): e25435.

Resende, R.T.; Resende, M.D.V.; Silva, F.F. [et al.]. 2017a. Assessing the expected response to genomic selection of individuals and families in Eucalyptus breeding with an additive-dominant model. Heredity. 119(4): 245–255.

Resende, R.T.; Resende, M.D.V.; Silva, F.F. [et al.]. 2017b. Regional heritability mapping and genome-wide association identify loci for complex growth, wood and disease resistance traits in Eucalyptus. New Phytologist. 213(3): 1287–1300.

Tajima, Y.; Loo, E.P.I.; Saijo, Y. 2020. Plant physiological and molecular mechanisms in cross-regulation of biotic-abiotic stress responses. In: Priming-Mediated Stress and Cross-Stress Tolerance in Crop Plants. Academic Press: 21–34.

Tan, B.; Grattapaglia, D.; Martins, G.S. [et al.]. 2017. Evaluating the accuracy of genomic prediction of growth and wood traits in two Eucalyptus species and their F 1 hybrids. BMC Plant Biology. 17(1): 110.

Wang, M.; Zheng, Q.; Shen, Q; Guo, S. 2013. The critical role of potassium in plant stress response. International Journal of Molecular Sciences. 14(4): 7370–7390.

Waterman, J.M.; Cazzonelli, C.I.; Hartley, S.E.; Johnson, S.N. 2019. Simulated herbivory: the key to disentangling plant defense responses. Trends in Ecology Evolution. 34(5): 447–458.

Yakovlev, I.A.; Carl-Gunnar, F.; Johnsen, Ø. [et al.]. 2006. Analysis of gene expression during bud burst initiation in Norway spruce via ESTs from subtracted cDNA libraries. Tree Genetics and Genomes. 2: 39–52.

FACTORS UNDERPINNING RESISTANCE AGAINST THE GALLING PEST, *LEPTOCYBE INVASA* IN *EUCALYPTUS GRANDIS*

Sanushka Naidoo[1], Caryn Oates[1], Lorraine Mhoswa[1], Marja 'O Neil[1], Juan Acosta[2], Nanette Christie[1], Mmoledi Mphahlele[3], Kitt Payn[3], Alexander Myburg[1], Bernard Slippers[1], and Carsten Külheim[4]

The blue gum chalcid, *Leptocybe invasa* is considered a serious threat to the production of Eucalyptus species and clones in plantations. This pest induces galls on the midrib, petiole, and shoot tip of young Eucalyptus leaves leading to deformation, stunting and in severe cases, tree death. We adopted a multi-pronged approach to uncover factors contributing to resistance in *E. grandis*. We performed a genome wide association study for resistance against the insect pest, in a breeding population of *E. grandis*, and identified seven genomic regions. Transcriptomics in resistant and susceptible interactions with the pest showed the expression of several candidate genes within 50Kb windows of the significantly associated SNP markers, including putative R-genes. We also investigated whether plant specialized metabolites such as the terpenes would be associated with resistance against the insect pest. Based on near infra-red spectral models we could predict specific terpene content with a prediction ability of 0.67. The terpenes α-pinene, γ-terpinene and iso-pinocarveol were important for predicting *L. invasa* infestation. Susceptibility was associated with increased γ-terpinene and α-pinene. Resistance was associated with iso-pinocarveol. The attractant or repellent properties of these terpenes remain to be tested in *E. grandis*. In the next phase of our study, the markers will be translated to other *E. grandis* populations for breeding purposes and we will focus on characterizing the functional role of the candidate genes. Together, these findings contribute to improving resistance against *L. invasa*.

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IDENTIFICATION OF GENES INVOLVED IN THE RESISTANCE RESPONSE OF *PINUS PINASTER* TO THE PINEWOOD NEMATODE INFECTION

Inês Modesto[1,2,3], Lieven Sterck[3,4], Isabel Carrasquinho[5,6], Yves Van de Peer[3,4,7], and Célia Miguell,[2,8]

Pine wilt disease (PWD), caused by the pathogenic nematode *Bursaphelenchus xypophilus*, is a serious threat to conifer forests in Asia and Europe. In the Iberian Peninsula, *Pinus pinaster* is the main species affected by this disease, and trees start to wilt and die soon after the infection. Despite the high susceptibility of most *P. pinaster* trees, a previous study has shown that some individuals display a resistance phenotype after inoculation and that this trait is heritable. Understanding the genetic basis of this resistance to PWD can be of extreme relevance for future programs aiming at reducing PWD in *P. pinaster* forests. In order to test if contrasting phenotypes are consistent with differential responses of gene expression after infection with *B. xypophilus*, RNA-seq was used to compare transcriptional changes between resistant and susceptible seedlings. Our analysis showed a more intense defense response in plants resistant to *B. xypophilus* infection, with a higher number of differential expressed genes (DEGs) in resistant plants (1281) than in susceptible ones (773). Although part of the defense response is shared between resistant and susceptible plants, gene set enrichment analysis highlighted biological processes and molecular functions that may interfere with nematode feeding, growth, and reproduction. This is the first work that enlightens the mechanisms involved in *P. pinaster* resistance to PWD.

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IDENTIFYING AND VALIDATING NECROTROPHIC EFFECTORS IN THE *POPULUS TRICHOCARPA-SPHAERULINA MUSIVA* PATHOSYSTEM

Kelsey L. Søndreli[1], Robert Brueggeman[2], Jonathan Richards[2], Timothy Friesen[3], and Jared M. LeBoldus[1,4]

*Sphaerulina musiv*a causes stem cankers on *Populus* species in North America. The literature indicates that the use of resistance genotypes is the best approach to manage *S. musiva*, so it is imperative that we know more about the mechanisms that underlie resistance/susceptibility in the *S. musiva–Populus* pathosystem. RNA-seq was used to identify potential *S. musiva* necrotrophic effectors involved in stem canker formation at two and three weeks post-inoculation. Approximately 20 percent of the total reads were aligned to the *S. musiva* reference genome with the remaining reads aligning to the reference genome of *Populus trichocarpa*. There were 70 genes identified at two weeks post-inoculation and 110 genes identified at three weeks post-inoculation differentially expressed between the inoculated trees and the control. The candidate genes were selected for transformation into *Pichia pastoris*, subsequent protein expression, and infiltration of the proteins into *P. trichocarpa* leaves. This study provides the first evidence that low coverage RNA-seq can be used to identify putative necrotrophic effectors facilitating the study of host-pathogen interactions in the *P. trichocarpa*-*S. musiva* pathosystem.

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HOST-PATHOGEN INTERACTION IN LEAVES OF LAUREL WILT HOSTS

Junli Zhang[1], Stephanie M. Adams[2], and Jason A. Smith[3]

Laurel wilt is devastating to trees in the Lauraceae family. Since 2003, laurel wilt has caused death to over 30 percent of all the redbay (*Persea borbonia*) trees in the Southeastern United States. And in 2012, laurel wilt on Avocado (*P. americana* Miller) was discovered. The disease is caused by the fungus *Raffaelea lauricola* and is transmitted by its ambrosia beetle symbiont (*Xyleborus glabratus*) or root grafting. It has been reported that the accumulation of gel and tyloses induced by *R. lauricola* in the xylem was related to disease symptom development. However, movement of *R. lauricola* inside host trees has not been clearly elucidated. Whether the fungal pathogen moves to host leaves and reversibly move from leaves to other parts of a host tree is still unknown. We demonstrated that the fungus moves to tree leaves from stems, which is evidenced by the fact that the fungus was isolated from the leaves of stem-inoculated trees. At the same time, we also discovered that *R. lauricola* is able to be translocated from leaf to leaf and further to the entire tree. We isolated the fungus from non-inoculated leaves and induced systemic wilt in trees after inoculation of *R. lauricola* into tree leaves. This is the first report on bilateral movement of *R. lauricola* inside host trees. This study further sheds light on mechanisms of laurel wilt disease development.

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Session 6

GENETIC MAP-BASED APPROACHES TO IDENTIFY RESISTANCE GENES

RESISTANCE TO *HETEROBASIDION ANNOSUM* S.L. IN *PICEA ABIES* IS ASSOCIATED WITH A MORE ACTIVE ALLELE OF PaLAR3, A LECUANTHOCYANIDIN REDUCTASE GENE

Title presented at workshop:
DO ALLELE-SPECIFIC EXPRESSION PATTERNS CONTROL RESISTANCE TO *HETEROBASIDION ANNOSUM* IN *PICEA ABIES*?

Miguel Nemesio-Gorriz[1,7], Almuth Hammerbacher[2], Kerstin Dalman[1], Julia Wind[3], Åke Olson[1], Martin Lascoux[4], Bo Karlsson[5], Jan Stenlid[1], Ines Ezcurra[3], Jonathan Gershenzon[6], and Malin Elfstrand[1]

Norway spruce [*Picea abies* (L.) Karst.] wood is economically important to the European forest industry and infections by the *Heterobasidion annosum* s.l. species complex, and particularly *Heterobasidion parviporum* the major forest pathogen on Norway spruce cause substantial losses. The economic losses to this pathogen amounts to approximately 200 0000 USD every day, only in Sweden. One major route for infection is carryover between rotations on already infected forest sites. Thus, replantation material with improved resistance could be a valuable resource in the management of *H. annosum* s.l. There is sufficient genetic variation in resistance against *H. annosum* s.l in Norway spruce for substantial progress to be made through genetic selection. The variation in resistance in Norway spruce is quantitative in its nature, and the genetic component explains 10–46 percent of the variation (Arnerup et al. 2010; Chen et al. 2018). No adverse correlations have been found between growth and resistance which makes resistance an attractive trait to implement in breeding programs (Chen et al. 2018). Resistance of *P. abies* to *H. annosum* s.l., is linked to a number quantitative trait loci (QTL) in the host (Lind et al. 2014).

We have examined one of the QTLs for variation in fungal growth in sapwood (FGS) reported by Lind et al. (2014) in 14 unrelated Norway spruce families from Northern Europe. The QTL includes *PaLAR3*, a *leucoanthocyanidin reductase* gene, (Nemesio-Gorriz et al. 2016). We found two conserved *PaLAR3* allelic lineages in Norway spruce and higher resistance to *H. parviporum* was associated with the minor allele *PaLAR3B* in all studied families. Trees carrying at least one copy of *PaLAR3B* showed a significant reduction in FGS after inoculation with *H. parviporum* compared to their half-siblings homozygous for the major allele. Progenies homozygous for *PaLAR3B* have significantly higher levels of (+)-catechin, the catalytic product of *PaLAR3*, in their bark than their siblings homozygous for *PaLAR3A*. Differences in transcriptional regulation between alleles is a likely explanation for the resistance associated with *PaLAR3B*. The two isoforms of the *PaLAR3* protein showed similar in vitro catalytic properties, but allele-specific transcript levels were significantly higher for *PaLAR3B* in the heterozygous progenies (Nemesio-Gorriz et al. 2016). Expression profiling suggests that *PaLAR3* is part of a wider transcriptional network responding to *H. annosum* s.l. infection in Norway spruce involving a number of transcription factors possibly interacting differently with promoters of the two *PaLAR3* allelic lineages in Norway spruce (Dalman et al. 2017, Nemesio-Gorriz et al. 2017).

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LITERATURE CITED

Arnerup, J.; Swedjemark, G.; Elfstrand, M. [et al.]. 2010. Variation in growth of *Heterobasidion parviporum* in a full-sib family of *Picea abies*. Scandinavian Journal of Forest Research. 25: 106–110.

Chen, Z-Q.; Lundén, K.; Karlsson, B. [et al.]. 2018. Early selection for resistance to *Heterobasidion parviporum* in Norway spruce is not likely to adversely affect growth and wood quality traits in late-age performance. European Journal of Forest Research. 137: 517–525.

Dalman, K.; Wind, J.J.; Nemesio-Gorriz, M. [et al.]. 2017. Overexpression of *PaNAC03*, a stress induced NAC gene family transcription factor in Norway spruce leads to reduced flavonol biosynthesis and aberrant embryo development. BMC Plant Biology. 17: 6.

Lind, M.; Källman, T.; Chen, J. [et al.]. 2014. A *Picea abies* linkage map based on SNP markers identifies QTLs for four aspects of resistance to *heterobasidion parviporum* Infection. PLoS ONE. 9: e101049.

Nemesio-Gorriz, M.; Hammerbacher, A.; Ihrmark, K. [et al.]. 2016. Different alleles of a gene encoding Leucoanthocyanidin Reductase (PaLAR3) influence resistance against the fungus *Heterobasidion parviporum* in *Picea abies*. Plant Physiology. 171: 2671–2681.

Nemesio-Gorriz, M.; Blair, P.B.; Dalman, K. [et al.]. 2017. Identification of Norway Spruce MYB-bHLH-WDR transcription factor complex members linked to regulation of the flavonoid pathway. Frontiers in Plant Science. 8: 305.

DEVELOPMENT AND APPLICATION OF HIGH DENSITY GENETIC MAP OF LIMBER PINE (*PINUS FLEXILIS* JAMES) FOR GENOMICS-BASED BREEDING

Jun-Jun Liu[1], Anna W. Schoettle[2], and Richard A. Sniezko[3]

Development and adaptation of genomics-based tools, such as high-throughput genotyping, high-density genetic maps, marker-assisted selection, genome-wide association of genotype versus phenotype, breeding by design, breeding without breeding, and genomic selection is moving conventional breeding into a new era. Next-generation sequencing (NGS)-based RNA-seq, exome-seq, and related technologies provide very effective approaches for detection of genome-wide variations and identification of DNA markers for marker-assisted selection in breeding of forest resistance against pathogens and pests. Genome-wide mapping of functional genes enhances our understanding of genetic resistance and local adaptation to changing climates in living organisms. Limber pine is one of the keystone conifer species of high elevation forest ecosystems in North America, and its populations in many regions are seriously impacted by the invasive, exotic fungal pathogen *Cronartium ribicola*, which causes white pine blister rust (WPBR) on five-needle pines of the subgenus Strobus. In the present study, we constructed limber pine high-density genetic maps by exome-seq, and developed TaqMan arrays for marker-assisted selection for breeding programs and field applications. Base on a limber pine reference transcriptome *de novo* assembled previously (Liu et al. 2016), hybridization probes were designed to enrich the exome from the complex genome. Genetic variations and genotypes were explored by exome-seq in two seed families with phenotypic segregation of major gene (*Cr4*) resistance. Single nucleotide polymorphism (SNP) markers were used to construct genetic maps. A total of 9.5K expressed genes, including > 600 NBS-LRR genes and > 200 RLK genes, were mapped on 12 linkage groups for a consensus genetic map. Based on SNP markers at the *Cr4* locus, TaqMan SNP arrays were developed for marker-assisted selection. Using these genomic tools, we revealed genetic relationships of major gene resistance between limber pine seed families originated in Canada and the United States, as well as between limber pine (*Cr4*) and other five-needle pines (including southwestern white pine *Cr3* and western white pine *Cr2*). Genomic resource and practical tools developed here will benefit breeding and genetic conservation programs and facilitate the genome-wide association study and assembly of the full-length genomes in limber pine and related *Pinus* species.

LITERATURE CITED

Liu J-J; Schoettle, A.W.; Sniezko, R.A. [et al]. 2016. Genetic mapping of Pinus flexilis major gene (*Cr4*) for resistance to white pine blister rust using transcriptome-based SNP genotyping. BMC Genomics. 17: 753.

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GENETIC MAPPING AND FUNCTIONAL GENOMICS ANALYSES OF THE RESISTANCE/SUSCEPTIBLE RESPONSE IN CHESTNUT SEEDLINGS TO *PHYTOPHTHORA CINNAMOMI* INFECTION

Tetyana Zhebentyayeva[1,2], Andrew Gitto[3], Steven Jeffers[3], Paul Sisco[4], Taylor Perkins[5], Hill Craddock[5], Christopher Saski[1,2], Jared Westbrook[6], Frederick Hebard[6], Laura Georgi[6], Joseph James[7], Margaret Staton[8], C. Dana Nelson[9,10], and Albert Abbott[10]

Due to availability of resistant Asian and susceptible American and European chestnut species, *Castanea* is ideally suited for studying plant- *Phytophthora cinnamomi* (*Pc*) interactions. We employed a genetic approach to map resistance to *Pc* in interspecific crosses using resistant Chinese chestnut, *Castanea mollissima* ("Mahogany" and "Nanking" resistance donors) and susceptible American chestnut *C. dentata* (multiple parents). Five BC1, BC3 and F2 populations (1,435 individuals in total) were phenotyped multiple years for root rot severity and genotyped by reduced genome-representative sequencing. Using extensive sets of markers anchored to the *C. mollissima* genome v1.0 assembly, we constructed genetic maps and identified genomic regions underlying resistance to *Pc* in multiple crosses. The most consistent QTL signals in crosses with both Mahogany and Nanking backgrounds were detected in the lower part of LG E. Based on performance in years 2015 and 2016, we selected Nanking-derived F_2 reciprocal crosses NK5 and NK6 segregating for healthy:unhealthy:dead trees in a ratio 1:1:1 ($P < 0.05$) for further studies of plant-*Pc* interaction using transcriptomics and metabolomics approaches. In a pilot experiment we challenged 1-year old NK5 progeny with *Pc* and harvested root tissue at 7 time-points (0, 1, 2, 3, 5, 7 and 14 days) post inoculation. Non-inoculated plants were used as controls along with inoculated Chinese and American chestnut seedlings. Inoculated plants were monitored for progression of root rot symptoms and the three most resistant and three most susceptible plants were chosen for RNAseq analysis and metabolite profiling. Results of these experiments and the initial mapping experiments will be presented.

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MULTIPLE APPROACHES TO DISSECT FUSIFORM RUST RESISTANCE IN *PINUS TAEDA* L.

Daniel Ence[1], Katherine E. Smith[1,2], Robin Paul[4], Matias Kirst[1], Jill L. Wegrzyn[3], C. Dana Nelson[2], and John M. Davis[1]

Mapping the specific loci that regulate phenotypic traits in conifers is a major undertaking because of their very large genomes. However, the release of the annotated loblolly pine (*Pinus taeda* L.) genome may allow fine-mapping of Mendelian traits that are economically critical, such as for disease-resistance. Here we present the results of work mapping Fusiform rust resistance locus 1 (*Fr1*) in elite rust resistant loblolly pine trees. Fusiform rust is a disease incited by the fungus *Cronartium quercuum* f.sp. *fusiforme* (Cqf) on southern pines (where it causes galls on stems and branches) and on oaks (where it causes minimal leaf damage). Fusiform rust is a major disease threat to the timber industry in the United States. Rust galls cause yield losses that exceed US$100M/year. During the genome annotation process, an expressed sequence tag (EST) was identified that contains a single nucleotide polymorphism (SNP) mapping to the locus (*Fr1*) that interacts with the fungal avirulence gene, *Avr1*. This EST aligns to a transcript from RNA-sequencing data and a TIR-NB-LRR protein, thus identifying it as a candidate *Fr1* gene. In order to further characterize the *Fr1* locus, we assembled the transcriptomes of 92 elite rust-resistant loblolly pine genotypes from five pine-growing regions, identifying candidate resistance genes in the process. Next we aligned these transcripts to the loblolly pine genome and calculated population genetic parameters. These results enable analysis of the diversity and conservation of resistance genes that interact with Cqf and present a foundation for further characterization of *Fr1* and other resistance loci.

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Session 7

INTEGRATING GENOMICS/BIOTECHNOLOGY WITH TRADITIONAL TREE BREEDING

THE POTENTIAL FOR BIOTECHNOLOGY TO ADDRESS FOREST HEALTH

Title presented at workshop:

OVERVIEW ON THE POTENTIAL FOR BIOTECHNOLOGY TO ADDRESS FOREST HEALTH—BASED ON PENDING NATIONAL ACADEMY OF SCIENCE, ENGINEERING AND MEDICINE REPORT

Kara N. Laney[1]

The National Academies of Sciences, Engineering, and Medicine is conducting a consensus study on the potential for biotechnology to address forest health. A committee of 13 experts from diverse disciplines have collaborated to examine the potential use of biotechnology to mitigate threats to forest tree health; identify the ecological, ethical, and social implications of deploying biotechnology in forests; and develop a research agenda to address knowledge gaps about its application. In particular, the committee has considered the use of biotechnology to prevent the extirpation of a tree species by an insect or disease that could have negative consequences for forest health. The study includes the committee's definition of forest health, a review of the state of the science for tree biotechnology and other tools for improving forest health, and an overview of the unique challenges and opportunities of using biotechnology to address forest health. To accomplish the goals of the study, the committee has held information-gathering meetings on a wide range of topics, including the ethics of using biotechnology in conservation, Native American perspectives on using biotechnology in forests, and how forest trees modified with biotechnology are handled in the U.S. regulatory system. The study will culminate in a report, *Forest Health and Biotechnology: Possibilities and Considerations*, published in early 2019. The final report can be found at https://doi.org/10.17226/25221. Funding for the study was provided by the U.S. Department of Agriculture, the U.S. Environmental Protection Agency, and the U.S. Endowment for Forestry and Communities. More information can be found at nas.edu/forest biotech.

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RESTORATION OF AMERICAN CHESTNUT: A MARRIAGE OF BREEDING AND BIOTECHNOLOGY

Jared W. Westbrook[1], Jason A. Holliday[2], and William A. Powell[3]

Efforts to restore the American chestnut are approaching major milestones. Selection of the most blight-resistant backcross trees in The American Chestnut Foundation's (TACF's) seed orchards at Meadowview Research Farms is expected to be complete between 2020 and 2025. Progeny testing with small stem assays and genomic selection are being used to increase the speed and accuracy of selection within seed orchards. The offspring from selected parents are predicted to have blight resistance that is, on average, intermediate between American chestnut and Chinese chestnut. Meanwhile, collaborators at the State University of New York have demonstrated that transgenic American chestnut containing the oxalate oxidase (OxO) gene has blight resistance that equals that of Chinese chestnut. Preparation for federal regulatory review for the release of transgenic American chestnut is ongoing. Beyond these milestones, blight resistance in backcross populations will be improved through two additional generations intercrossing and selection. Pending regulatory approval, the transgenic founder tree will be outcrossed to pure American chestnut and backcross trees over three generations with the objectives of (1) stacking blight resistance alleles from backcross trees with OxO, (2) combining OxO with backcross resistance to Phytophthora root rot (PRR), (3) creating a restoration population with an effective population size > 500. American chestnuts will be conserved in orchards for eventual outcrossing with transgenic trees by collecting seeds and transplanting from regions that are most genetically diverse and underrepresented by TACF's breeding program. A diversified American chestnut population in which transgenic blight resistance is combined with backcross blight and PRR resistance is expected to be available for restoration by 2050. While backcross seed from selected seed orchards and first generation transgenic progeny are expected to be available for restoration trials in the next 5 years, long-term commitment to continued breeding and selection is required for full-scale restoration.

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NEW FRONTIERS IN FORESTRY: COMBINING PHENOMICS, COMMON GARDENS, AND LANDSCAPE GENOMICS TO ADDRESS DISEASE RESISTANCE AND CLIMATE CHANGE

Jeremy S. Johnson[1,2], Sam Cushman[3], Andrew Eckert[4], Lluvia Flores-Rentería[5], Richard Sniezko[2], Christopher Still[6], Christian Wehenkel[7], Amy Whipple[8], and Michael Wing[6], Justin Bagley[4], Emily Boes[2], Ethan Bucholz[1], Jonathan Burnett[6], Antonio Castilla[2], Jessica DaBell[1], Cory Garms[6], Marja Haagsma[9], Evan Heck[2], Erin Landguth[10], Alejandro Leal Sáenz[7], Jared Swenson[1], Brianna McTeague[2], Mitra Menon[4], Ehren Moler[8], Gerald Page[6], Andrew Shirk[11], and Kristen Waring[1]

A collaborative team of researchers from the United States and Mexico have begun a project funded by The National Science Foundation's Macrosystems Biology program. The project is to study ecological and evolutionary processes affecting the distribution of southwestern white pine, an important tree species of mixed conifer forests in the Southwest United States and Mexico. Southwestern white pine (*Pinus strobiformis*) sustainability is threatened by changing climate, and an invasive non-native tree disease, white pine blister rust. White pine blister rust causes extensive tree decline and mortality where it occurs in North America, including where it overlaps with southwestern white pine, an ever-expanding area. Climate may advance too rapidly for southwestern white pine to move or adapt. The dual threats of climate change and an invasive species make forecasting future tree distributions across continental scales an urgent challenge. The goal of this project is to determine how gene movement among populations, adaptation to disease and drought, epigenetic changes, and a changing environment interact to govern the success of southwestern white pine. This project is developing tools to help forecast and manage the future of the species, including genomics, common gardens, disease resistance testing, phenomics, and computer modeling in landscape ecology and genomics. We show how the project is combining genomics and traditional common garden approaches and its potential management applications for southwestern white pine.

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WISE USE OF GENOMICS IN IMPROVING FOREST HEALTH

Jeanne Romero-Severson[1]

Over the last several hundred years, colonialism and international trade have had the unintended consequence of introducing destructive insects, microbial pathogens and invasive weeds into native forests throughout the world. Programs intended to save native forest species from destructive pests are often led by single discipline investigators who focus first on the insect or the pathogen, then on methods of external control (i.e., containment, pesticides and biocontrol agents). Investigation of genetic mechanisms using structured populations may be dismissed as "too expensive" while genomics costing at least as much if not more may be embraced without serious evaluation of the likelihood of success. The interdisciplinary approach to pests and pathogens used by the most successful private sector companies frequently succeeds and not for the reason you might think. These successful approaches include cost-benefit analysis of all strategies, including traditional breeding and genomics.

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Session 8

SCREENING FOR RESISTANCE TO PATHOGENS: METHODS DEVELOPMENT

VIRULENCE OF *SPHAERULINA MUSIVA* ISOLATES DETERMINED BY GENOME-WIDE ASSOCIATION MAPPING, HOST INFECTION, AND COLONIZATION

Susanna Keriö[1], Kelsey L. Søndreli[1], and Jared M. LeBoldus[1,2]

The ascomycete *Sphaerulina musiva* causes Septoria leaf spot and stem canker of *Populus*, and is considered to be the most serious pathogen affecting poplar cultivation in North America. Knowledge of *S. musiva* virulence factors can promote the development of efficient control strategies against the disease, and improve the accuracy of risk analysis. In this project, we characterized the virulence of 120 *S. musiva* isolates from geographically distinct populations in a non-wounding spray inoculation experiment. The isolates were sequenced (Illumina 150bp Paired End HiSeq 3000) and used for genome-wide association mapping. In a second set of experiments, we selected a subset of six *S. musiva* isolates with either high or low virulence, which were used to inoculate two genotypes (GW9823 and GW11026) of black cottonwood (*Populus trichocarpa*). The degree of host colonization was estimated by qPCR, and qualitative differences in host colonization were analyzed by microscopic inspection. We will report the genes associated with the observed virulence differences, and discuss the significance of the results from the second set of experiments in the context of disease resistance breeding.

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EARLY SCREENING POTENTIALLY BLIGHT-RESISTANT AMERICAN CHESTNUT USING SMALL STEM ASSAYS

Thomas Saielli[1] and Jared Westbrook[2]

Restoration of the American chestnut (*Castanea dentata*) depends on producing a founder population of trees that are sufficiently disease-resistant to provide self-sustaining populations that have the timber-type growth and form of American chestnut. For 30 years, The American Chestnut Foundation (TACF) has pursued backcross breeding to introgress genetic resistance to chestnut blight (caused by the fungal pathogen *Cryphonectria parasitica*) from Chinese chestnut (*C. mollissima*) into a genetically diverse population of American chestnut. The final step in TACF's backcross breeding program, prior to species restoration, is to establish multiple, large-scale seed orchards throughout the Eastern United States. Although many thousands of seedlings are planted in each seed orchard, once seedlings are artificially inoculated and assessed, only the few hundred trees with demonstrated high levels of resistance are kept. Initial screening may be performed by visual inspection of cankers, but the final candidates must be progeny tested in order to select the best trees in the orchards. The small stem assay (SSA) is one method being tested to progeny test large numbers of chestnut seedlings for resistance, which, when compared to orchard progeny tests, may allow for a greater number of seedlings to be tested in less time. In the small stem assay, the stems of chestnut seedlings > 3 mm in diameter are inoculated with *C. parasitica* and canker length is measured every 6 to 8 weeks for several months. In 2017, TACF tested approximately 5,180 seedlings among 95 hybrid families. Results indicate that canker length was heritable among test families ($h^2 = 0.30 \pm 0.09$) and variation in canker length was significantly correlated between SSA tests and orchard progeny tests ($r_{genetic} = 0.84 \pm 0.74$). TACF will continue to experiment with SSA techniques, as many tactical questions remain and results were not consistent throughout all tests.

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DEFENSE RESPONSES OF AUSTRIAN PINE TO TWO OPPORTUNISTIC PATHOGENS OF CONTRASTING AGGRESSIVENESS UNDER COMBINED DROUGHT AND TEMPERATURE STRESS

Pierluigi Bonello[1], Anna Conrad[1], Jason Slot[1],
Erik Andrei Visser[2], and Sanushka Naidoo[2]

Understanding the effects of climate change on host pathogen relationships is key to managing diseases in new weather extremes expected in the future, especially among long-lived perennials like trees. For example, trees under drought stress become more susceptible to pathogens as resources are allocated towards basic survival rather than chemical defenses, thus creating more favorable conditions for disease development. Our study investigates the defense mechanisms underlying the responses of Austrian pine (*Pinus nigra*) to the tip blight and canker pathogens *Diplodia sapinea* and *D. scrobiculata* under combined, elevated temperature, and drought; two conditions that are projected to become the norm in many areas of the world. Among the defense mechanisms likely to be affected by temperature and water restrictions are those involving specialized metabolites, specifically phenolics, which are modulated, in part, by the particular amino acid pathways associated with the response to drought. We are analyzing gene expression and amino acid metabolism, as well as accumulation/depletion of soluble phenolics and lignin. Increased understanding of the interactions between hosts and pathogens undergoing climate stress will contribute to the development of integrated management strategies, such as implementing updated monitoring/detection programs, creating predictive models, and furthering our understanding of host resistance mechanisms.

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Session 9

MECHANISMS OF RESISTANCE TO INSECTS

ARE NOVEL PLANT CHEMICALS FRIENDS OR FOES OF NATIVE INVASIVE INSECT HERBIVORES? EXPLAINING SUCCESSFUL HOST RANGE EXPANSION OF MOUNTAIN PINE BEETLE IN CANADA'S BOREAL FORESTS

Nadir Erbilgin[1]

Mountain pine beetle has recently breached the geo-climatic barrier of the northern Rocky Mountains and invaded in the jack pine (*Pinus banksiana*) forests in Canada. The jack pine forest ecosystem is considered a novel habitat. However, the mechanism underlying this successful host expansion is unknown, but likely involves phytochemicals that play significant roles in the acceptance and colonization of hosts by insect herbivores and their microbial symbionts. We have focused on whether compatibility of jack pine chemicals with beetles and their symbiotic fungi has facilitated the colonization of this novel host and identified five mechanisms that have likely facilitated the beetle host range expansion. First, jack pine trees appeared to have less pronounced chemical defenses than a historical host of MPB (lodgepole pine, *P. contorta*). Second, prior to the arrival to the jack pine forests, invasion of a zone of hybrids of jack and lodgepole pine trees in Alberta by beetles likely improved their success in the jack pine as hybrids show chemical characteristics of both novel and historical hosts. Third, fungal and bacterial symbionts of beetles likely contributed beetle performance in the jack pine by detoxifying toxic secondary compounds as jack pine was compatible for the growth of both the fungi and bacteria. Fourth, similarity of secondary compounds and fatty acids between the novel and historical hosts may have facilitated the host expansion of beetles because of compatibility of these chemical compounds for pheromone production, aggregation on the host trees, larval development, and the growth of its fungal symbionts. Finally, jack pine contained low concentrations of defense and inhibitory compounds, and high concentrations of precursor and synergistic compounds that make historical host trees susceptible to beetles. I conclude that compatibility of primary and secondary chemical composition of jack pine to MPB and its symbiotic fungi has likely facilitated the host range expansion.

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VOLATILES RELEASED BY *PINUS PINASTER* HALF-SIB GENETICALLY CONTRASTING FAMILIES FOR PINE WILT DISEASE SUSCEPTIBILITY BEFORE AND DURING *MONOCHAMUS GALLOPROVINCIALIS* INSECT-VECTOR FEEDING

Elsa Gonçalves[1], A. Cristina Figueiredo[1], Isabel Carrasquinho[2,3], José G. Barroso[1], Maria l. Inácio[2], Joana Henriques[2], Edmundo Sousa[2], and Luís Bonifácio[2]

The pine wilt disease (pwd), caused by the pinewood nematode (PWN) *bursaphelenchus xylophilus*, is a major threat to conifer trees worldwide. The PWN is transmitted from infected to healthy trees by insect vectors in the cerambycid beetle genus *monochamus*. The PWD cycle involves the movement of the nematode between the insect vector and the host tree. PWN leaves the insect and enters the host through feeding wounds made by the insect. This study analyzed the volatiles released by genetically contrasting maritime pine half-sib families with susceptibility to PWD, before and during insect vector feeding activity, which can act as signals for the nematode to move into healthy trees. Thirty-eight pine trees from four half-sib families were selected among 96 different families. The canopy of each of the 7 year old pines was covered by a metallic net and foliage was enclosed in a plastic bag to collect the headspace volatiles, before and during 24 h exposure to feeding by a pair of newly emerged *m. galloprovincialis* males and females. Volatiles were collected by solid phase microextraction and analyzed by gas chromatography-mass spectrometry for component identification, and by gas chromatography for component quantification. For comparing the different families, the non-parametric kruskal-wallis test and dunn's test with bonferroni-type adjustment were used. Seventeen volatile compounds were detected both before and during feeding. Before insect feeding, β-pinene, β-myrcene, β-caryophyllene, phenethyl 2-methylbutyrate, and phenethyl 3-methylbutyrate showed significant differences among the contrasting families. During feeding, only β-myrcene and germacrene d showed different emission patterns among them. In this case, the most pwn susceptible family released significantly higher amounts of β-pinene and β-caryophyllene, and lower amounts of β-myrcene than the resistant cultivars.

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HEMLOCK AND HEMLOCK WOOLLY ADELGID: PAST RESEARCH, CURRENT FINDINGS, AND FUTURE DIRECTIONS IN A TROUBLED NORTH AMERICAN TREE-PEST SYSTEM

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Hemlock woolly adelgid (HWA) (*Adelges tsugae*) was first introduced to the East Coast of North America in the 1950's and has since spread throughout roughly half of the native range of its primary host, eastern hemlock (hemlock; *Tsuga canadensis*), which is under threat of extirpation due to this insect. Here, we summarize past and current work that our research group has undertaken and address future research directions and management concerns in this system. Over the past several years, one aspect of our research has been investigating HWA-hemlock interactions and HWA interactions with other hemlock herbivores. Adelgid infestation results in a "hypersensitive-like" response in hemlock foliage, with the accumulation of reactive oxygen species, certain amino acids, hormones, defensive metabolites and enzymes, and increased volatile emissions, a response that more resembles pathogen infection than herbivore attack. The infestation of HWA additionally results in deleterious phenological, growth, and belowground effects on hemlock. Adelgid interacts with other native and exotic hemlock herbivores, presumably due to the manipulation of host physiology, and appears to make hemlock more attractive to certain herbivores. Our research group has also led efforts to develop HWA-resistance in hemlock. Unfortunately, decades-long efforts to develop effective biological controls have not yet proven effective on highly susceptible eastern hemlocks. However, hundreds of hemlock trees have been found persisting in HWA-decimated forests throughout the East Coast, and some have remained healthy for decades. We are leading efforts to screen persisting trees for elevated levels of resistance and to propagate trees to supply stakeholders, tree breeders, and scientists with resistant germplasm. We also utilize these trees to develop phenotyping assays for the rapid identification of resistance in the field. We strongly advocate for the development of resistance and the breeding of resistant hemlocks as part of a long-term management strategy for hemlock.

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A HINT LEFT BY MOUNTAIN PINE BEETLE ON ANATOMICAL DEFENSES OF LODGEPOLE PINE TREES: LARGER RESIN DUCTS ENHANCE TREE RESISTANCE

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Mountain pine beetle (MPB) [*Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae)] populations have increased in Alberta since they have crossed the Rocky Mountains and colonized pine forests in western Alberta in 2006. Millions of mature lodgepole pine (*Pinus contorta* var. *latifolia*) trees have been killed, leaving only a low number of residual overstory mature lodgepole pine trees and non-host tree species, such as spruce and *Populus*, remaining in beetle-killed stands (Taylor et al. 2006, Dhar et al. 2016). The reason behind the survival of residual trees remains unknown. The change in nutrient cycling, underground soil communities, forest structure, and other factors in post-MPB stands can certainly affect the health condition of residual pine trees and potentially change their suitability to other insects and tree pathogens (McIntosh and Macdonald 2013b, Cigan et al. 2015, Karst et al. 2015, Pec et al. 2015). Considering the low pine recruitment and regeneration in MPB-affected stands (Astrup et al. 2008, McIntosh and Macdonald 2013a), the residual overstory pine trees might be the only seed source for the future pine regeneration in Alberta. Thus, studies on the future health conditions of residual pine trees in these disturbed landscapes are urgently needed. While concentration of chemical compounds in tree phloem can vary between resistant and susceptible lodgepole pine trees (Erbilgin et al. 2017), anatomical defense represents tree defense capacities over a longer time. Here we analyzed patterns in anatomical defenses and tree increment growth to understand (1) how residual trees survived the MPB outbreak, (2) whether the outbreak altered growth/defense relationships, and (3) identify relationships with current health conditions of residual trees.

We selected 31 sites in post-MPB stands in western Alberta, Canada. At most of the selected sites we established 2 plots (n = 61 plots in total). In plots, we sampled the wood from 140 beetle-killed trees using wedges and 210 residual trees using increment cores at breast height (1.4 m). Samples were collected in 2016. All trees had a diameter at breast height (DBH) over 15 cm and had a crown class of intermediate, codominant, or dominant. We confirmed MPB-killed tree by the presence of MPB attack signs such as beetle entrance holes (pitch tubes) and extensive beetle galleries under bark. The sampled residual trees were classified into three groups based on tree health conditions and included 76 healthy, 62 declining due to biotic agents other than MPB, and 72 trees that survived MPB with signs of attack but appeared vigorous.

We measured ring width (mm yr^{-1}) from bark to pith on all samples by using WinDendro™ (Regent Instruments 2008). A master chronology was developed based on the ring width series of cores from healthy residual trees. The strength of cross-dating was confirmed by COFECHA (Grissino-Mayer 2001). This master chronology was used to justify any missing or false rings on cores before any calendar year was assigned to each ring. The year of death for beetle-killed trees was adjusted by visual cross-dating due to the low number of years sampled on wedges. Since most sites experienced beetle mortality at multiple years, the start of an outbreak was considered as the year of the earliest death caused by MPB that occurred in a site.

A sampling area of 0.9 mm for cores and wedges was used to count and measure resin ducts. The resin duct characteristics that were measured

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from the sampling area included annual resin duct production (no. year^{-1}) and individual resin duct area (mm^2 year^{-1}), which were determined by ImageJ (version 1.50i, National institutes of Health, USA). Two standardized variables of resin duct characteristics were also calculated on each core or wedge: resin duct density (no. mm^{-2} year^{-1}) and relative resin duct area (percent year^{-1}).

There were no differences on mean growth rate between beetle-killed and residual trees within the first 10-year preceding outbreak. However, beetle-killed trees produced 127–131 percent more xylem axial resin ducts than residual trees, while the size of individual duct in residual trees was 18–27 percent larger than those in MPB-killed trees. A logistic model was built to predict the survival probability of lodgepole pine trees during the MPB outbreak based on the resin duct production and resin duct size within 10 years before the MPB outbreak. The survival probability increased with larger resin ducts, but less numbers of resin ducts. Meanwhile, trees that survived attack responded to MPB outbreaks in the stand with a lower mean growth rate in the first 3- and 5-year following outbreak, and higher mean resin duct production for up to 10 years after outbreak, while healthy trees only showed increased resin duct production in the total growth period after the MPB outbreak. Furthermore, we found that healthy trees had larger individual resin ducts than declining trees in the recent 20 years (1996 to 2015), while survived trees also had larger resin ducts than declining trees most of the time from 1996 to 2015.

Our results indicated that anatomical defenses were critical components of lodgepole pine survival during beetle attacks as well as they might continue to play major roles in tree defense to bark beetles in post-outbreak stands. Thus, pine anatomical defenses appear to be important traits for understanding tree resistance to bark beetles, supporting earlier studies (Kane and Kolb 2010, Hood and Sala 2015). Although it is not clear whether resin duct size is heritable, but if so, we expect that the next generation of lodgepole pine forests in western Alberta would be resistant to future bark beetle attacks. Using the logistic model generated in the current study, the survival probability of pine trees during MPB attacks could be calculated by determining resin duct production and resin duct size. Thus, keeping, and not harvesting, these residual trees should be the highest priority for land managers to assure the future sustainability of lodgepole pine forests in western North America.

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LITERATURE CITED

Astrup, R.K.; Coates, K.D.; Hall, E. 2008. Recruitment limitation in forests: Lessons from an unprecedented mountain pine beetle epidemic. Forest Ecology and Management. 256: 1743–1750.

Cigan, P.W.; Karst, J.; Cahill, J.F. [et al.]. 2015. Influence of bark beetle outbreaks on nutrient cycling in native pine stands in western Canada. Plant Soil. 390: 29–47.

Dhar, A.; Parrott, L.; Hawkins, C.D.B. 2016. Aftermath of mountain pine beetle outbreak in British Columbia: stand dynamics, management response and ecosystem resilience. Forests. 7: 1–19.

Erbilgin, N.; Cale, J.A.; Hussain, A. [et al.]. 2017. Weathering the storm: How lodgepole pine trees survive mountain pine beetle outbreaks. Oecologia. 184: 469–78.

Grissino-Mayer, H.D. 2001. Evaluating crossdating accuracy: a manual and tutorial for the computer program COFECHA. Tree-Ring Research. 57: 205–221.

Hood, S.; Sala, A. 2015. Ponderosa pine resin defenses and growth: Metrics matter. Tree Physiology. 35: 1223–1235.

Kane, J.M.; Kolb, T.E. 2010. Importance of resin ducts in reducing ponderosa pine mortality from bark beetle attack. Oecologia. 164: 601–609.

Karst, J.; Erbilgin, N.; Pec, G.J. [et al.]. 2015. Ectomycorrhizal fungi mediate indirect effects of a bark beetle outbreak on secondary chemistry and establishment of pine seedlings. New Phytologist. 208: 904–914.

McIntosh, A.C.S.; Macdonald, S.E. 2013a. Potential for lodgepole pine regeneration after mountain pine beetle attack in newly invaded Alberta stands. Forest Ecology and Management. 295: 11–19.

McIntosh, A.C.S.; Macdonald, S.E. 2013b. Short-term resistance of ecosystem properties and processes to simulated mountain pine beetle attack in a novel region. Ecosphere. 4: 1–28.

Pec, G.J.; Karst, J.; Sywenky, N. [et al.]. 2015. Rapid increases in forest understory diversity and productivity following a mountain pine beetle (*Dendroctonus ponderosae*) outbreak in pine forests. PLoS One. 10: 1–16.

Regent Instruments. 2008. WinDendro. Quebec, Canada: Regent Instruments.

Taylor, S.W.; Carroll, A.L.; Alfaro, R.I. [et al.]. 2006. Forest, climate and mountain pine beetle outbreak dynamics in western Canada. In Safranyik, L.; Wilson, B., eds. Mountain pine beetle a synthesis: biological management impacts lodgepole pine. Victoria, British Columbia, Canada: Pacific Forestry Centre: 67–94.

VARIATION ACROSS POPULATIONS IN RESISTANCE TO A KEY HERBIVORE AND ITS RELATIONSHIP WITH CONSTITUTIVE AND INDUCED SECONDARY METABOLITES IN A MEDITERRANEAN PINE TREE

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Resistance to herbivores and pathogens has been recognized as an adaptive key plant trait (Futuyma and Agrawal 2009), particularly at early stages of plant development (Goodger et al. 2013). In long-lived organisms like trees, resistance against enemies usually relies on high concentrations of a diverse array of plant secondary metabolites (PSM) (Wiggins et al. 2016). These compounds reduce enemy performance and/or plant damage in a dose-dependent manner (Zhao and others 2011). PSM production is highly plastic, involving substantial changes in the profile and concentrations of PSM upon enemy attack (Heil 2009, Karban et al. 1999). This biochemical plasticity, referred to as inducibility, is a source of functional phenotypic variation in resistance and is commonly considered a trait under selection (Agrawal et al. 2015). However, very few studies have addressed the relationship between inducibility of defences with effective resistance against herbivores (Bingham and Agrawal 2010, Rasmann et al. 2015), and much less in long-lived plants (Moreira et al. 2014, Ward et al. 2012). One of the main reasons for this scarcity in the literature is that exploring relationships between inducibility of PSM and the effective resistance against herbivory is challenging. First, it requires knowing the concentration of PSM before and after exposure to herbivory when measuring plant damage or insect performance. Moreover, the mere fact of sampling plant tissues also induces plant defences, biasing inducibility estimation (Moreira et al. 2012). For small plants that require destructive sampling to evaluate defensive traits, this issue can only be solved using clonal replicates of the same genotype. Second, most studies on this topic have reported positive associations between few defensive traits and resistance (Pratt and Mooney 2013, Stevens et al. 2007), while plant resistance are expected to be multivariate (Agrawal 2005, Lason et al. 2011, O'Reilly-Wapstra et al. 2014).

In this study, we aimed at investigating whether intraspecific variation in effective resistance of maritime pine (*Pinus pinaster*) to the pine weevil *Hylobius abietis* (a harmful insect herbivore) may be predicted by variation in inducibility of PSM. To do so, we used clonally replicated genotypes with known family structure from 10 populations representative of the main distribution range of maritime pine, and that belonged to the clonal collection 'CLONAPIN Bank 1' [10 populations × 5 families per population × 5 genotypes per family = 250 genotypes in total] (López-Goldar et al. 2018). We performed a greenhouse experiment where 2-years old pines were distributed in a split-plot design replicated in 5 blocks, with population as the whole plot factor and the factorial combination of family (3–5 families per population) and the induction treatment [2 levels: control and induced] as the split factor. For the induced treatment, we exogenously applied 25 mM methyl jasmonate (MJ) to half of the plants, where the other half were left untreated. MJ is a plant hormone involved in herbivore damage signalling that has been widely accepted as a chemical elicitor (Sampedro et al. 2011, Zas et al. 2014). One month after induction, two clonal replicates not subjected to herbivory (one control and one MJ-induced) were used to measure the concentration of constitutive terpenes and phenolics in the stem phloem, as well as their inducibility, by gas chromatography-mass

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spectrometry (GC-MS) and GC-flame ionization detection (GC-FID), and by ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) and UHPLC-diode array detection (UHPLC-DAD). We identified and quantified (in $mg \cdot g^{-1}$ dry weight of stem tissue) 98 chemical compounds that were classified into eight chemical groups [monoterpenes, sesquiterpenes, diterpenes, flavonoids, hydroxycinnamic acids (HCAs), lignans, eugenols, and fatty acids]. Another control and MJ-induced clonal plants were exposed to a couple of insects for 48 hours to measure plant damage (in mm^2) as a proxy of resistance (i.e., the greater the damage, the lower the resistance). Prior to herbivory treatment, insects were starved for 48 hours and then weighed, and plant diameter was measured.

Variation in *H. abietis* damage was analyzed by fitting a mixed model using the PROC MIXED procedure in SAS v9.4 (Littell et al. 2006). For the statistical analyses, the induction treatment (MJ), pine population (P), pine family within population [F(P)] and MJ×P and MJ×F(P) interactions were considered as fixed factors. Block (B) and the B×P interaction were considered as random factors to analyze the main effect of P with the appropriate error term (Littell et al. 2006). The genotype was also included as a random factor to account for the dependence between the two clonal replicates subjected to herbivory within each block. Weevil weight and stem diameter of the plant were included as covariates. Because structured genetic variation among populations was previously found for this species (Jaramillo-Correa et al. 2015, Serra-Varela et al. 2015), the same model was fit to explore the genetic variation among genetic clusters of populations (Atlantic France, Atlantic Spain, Central Spain, South Spain, and Morocco) and among families within clusters. In order to estimate the variance contribution of each factor to the genetic variation among populations and families within populations in resistance to the pine weevil, the previous mixed model was run again assuming all factors as random factors.

To explore the relationships between plant resistance and PSM in the stem for the constitutive and inducibility states, we performed pairwise correlations and stepwise multiple regression analyses between the weevil damage and PSM concentration at the genotypic level. Prior the analyses, we accounted for the non-independence among genotypes within families and populations by incorporating the population structure (Q) and kinship (K) matrices in mixed models (López-Goldar et al. 2018). We fitted a mixed model for constitutive (C) and for the inducibility (MJ – C) of each total and individual PSM and weevil damage. For each defensive state, population (P) and family within population [F(P)] were considered as fixed factors. Block (B) and the B×P interaction were considered random factors as above. The predicted values of PSM variables and weevil damage from each plant were extracted from each mixed model and used for the analyses.

Plant resistance to weevil herbivory in the stem significantly varied among plant populations (fig. 1) and genetic groups of populations ($F_{4, 140} = 4.73$, $P = 0.001$), but not between families within populations (fig. 1) or within genetic groups of populations ($F_{43, 140} = 1.36$, $P = 0.096$). Spanish Atlantic populations showed the greatest constitutive resistance (61.5 ± 7.3 mm^2 of damage; figure 2), whereas the French Atlantic populations were the most susceptible (94.9 ± 12.3 mm^2 of damage; fig. 2). The MJ-induction treatment significantly increased plant resistance to weevil herbivory in the stem (figs. 1 and 2). The inducibility of resistance was similar for all plant populations and families [no significant MJ×P and MJ×F(P) interactions, fig. 1], but differed among genetic groups (MJ×GP interaction, $F_{4, 140} = 3.36$, $P = 0.012$). In fact, greater inducibility of resistance after induction was found in both Atlantic genetic groups (71 and 67 percent in Atlantic France and Atlantic Spain, respectively) than in the other genetic groups (Central Spain, 37 percent; South Spain, 34 percent; and Morocco, 45 percent). Among the main effects of our study, that accumulated an explained variance of 36 percent of the total variance in resistance, MJ-induction effect accounted for > 80 percent of that explained variance in resistance, with the contribution of all remaining factors being comparatively much lower (fig. 1).

When exploring pairwise relationships at the genotypic level, we found that neither total nor individual PSM at the constitutive level

Fixed effect	*DF (n, d)*	*F*	*P-value*
MJ-Induction (MJ)	1, 140	104	**<0.0001**
Population (P)	9, 36	2.32	**0.0353**
Family [F(P)]	38, 140	1.33	0.1205
MJ×P	9, 140	1.62	0.1159
MJ×F(P)	38, 140	0.92	0.6032
Weevil weight	1, 140	18.5	**<0.0001**
Plant diameter	1, 140	4.66	**0.0326**

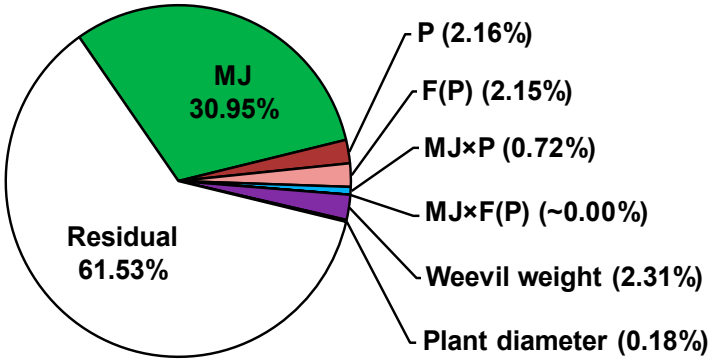

Figure 1—Summary of the mixed model testing the effects of the induction treatment with methyl-jasmonate (MJ), population (P), family within population [F (P)] and the MJ×P and MJ×F(P) interactions on the early resistance to herbivory by the pine weevil on plants from 10 maritime pine populations. Variance components for each effect and the residual are shown in the companion pie chart. Weevil weight and plant diameter were included as covariates. Significant *p*-values ($p < 0.05$) are highlighted in bold.

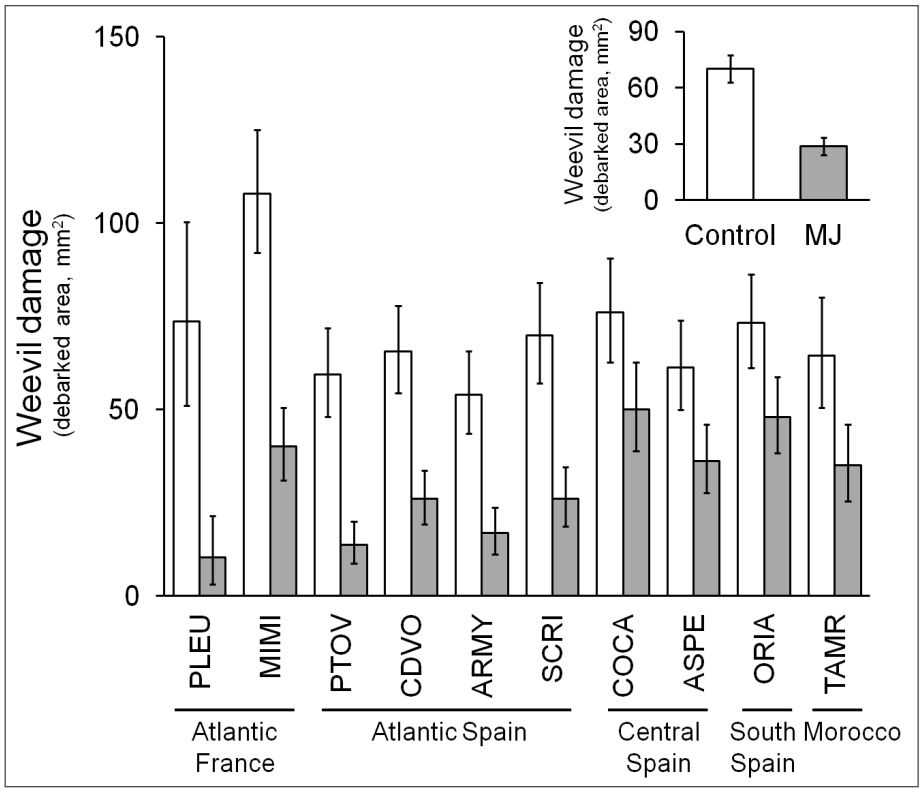

Figure 2—Intraspecific genetic variation in constitutive resistance (white bars) and MJ-induced resistance (grey bars) to the pine weevil for 10 maritime pine populations along its main distribution range, grouped by the main genetic groups in the species. The inset panel shows the main effect of the MJ-induction on the resistance to weevil herbivory. Bars represent the least square mean ± SE (N = 8 to 25 plants for each population; N = 197 to 205 plants for each induction treatment in the small panel).

were significantly associated with weevil damage. Contrarily, we found significant negative relationships between inducibility of total flavonoids and total lignans with weevil damage (r = -0.33, $P = 0.001$ and r = -0.36, $P < 0.001$, respectively). When exploring individual PSMs, we found significant negative relationships between the inducibility of lignan hexoside derivative 1 and 2 with weevil damage (r = -0.30, $P = 0.003$; r = -0.33, $P = 0.001$, respectively). In the multiple regression model, concentration of constitutive PSM significantly predicted 70 percent of the total variation in weevil damage, but only 6 PSM were negatively related to weevil damage (table 1). Inducibility of PSM significantly predicted 68 percent of the

total variation of weevil damage, and 8 PSM were negatively related to weevil damage after MJ-induction (table 1).

This study represents a good example of the relevance of using clonal replicates of genotypes, belonging to families within populations along the distribution range of a single conifer species, to evaluate the role of inducibility of PSM in resistance. Moreover, our findings are based on a rigorous framework by accounting for spurious associations due to genetic relatedness, often overlooked in intraspecific studies. Here we provide evidences that multivariate analyses of PSM, rather than bivariate correlations, provide more realistic information about the potentially causal relationships between PSM and resistance to herbivory in pine trees.

Table 1—Summary of the multiple regression analysis explaining the resistance to the pine weevil using the concentration of individual PSM as predictor variables in constitutive (upper part of the table) and in inducibility (lower part of the table) defensive states in 102 genotypes from 10 maritime pine populations. The regression coefficients (β) and partial R^2 of PSM included in the model after stepwise selection method are shown

Defensive mode	Plant secondary chemicals	β	Partial R^2
Constitutive PSM	**β-Phellandrene + Limonene**	**-0.98**	**0.150**
(N = 87)	**Lignan hexoside derivative 1**	**-1.58**	**0.077**
Intercept = 7.908	**Ferulic acid**	**-0.33**	**0.028**
Model adjusted R^2 = 0.70	**α-Copaene**	**-1.89**	**0.025**
$F_{18,62}$ = 18.97	**Unk P11**	**-0.35**	**0.024**
P < 0.001	**α-Cubebene + α-Longipinene**	**-0.76**	**0.016**
	Coumaric acid hexoside	0.60	0.079
	Methyl thymyl ether	0.57	0.054
	α-Phellandrene	0.52	0.040
	Methyl eugenol	0.87	0.035
	Elemol	0.75	0.031
	Eugenol	0.36	0.029
	Bicyclosesquiphellandrene	1.31	0.028
	Myrcene	0.56	0.022
	Lignan xyloside derivative 2	0.40	0.021
	Citronellyl propionate	0.46	0.018
	Sabinene	0.53	0.015
	Unk P6	0.39	0.011
Inducibility of PSM	**Unk P5**	**-22.46**	**0.234**
(N = 93)	**Lignan hexoside derivative 2**	**-19.17**	**0.060**
Intercept = -36.03	**Isopimaric acid**	**-10.05**	**0.060**
Model adjusted R^2 = 0.68	**Elemol**	**-12.11**	**0.058**
$F_{12,78}$ = 17.32	***cis*-β-Ocimene**	**-9.04**	**0.048**
P < 0.001	**Methyl thymyl ether**	**-10.36**	**0.040**
	Pimaric acid	**-11.76**	**0.029**
	trans-Pinocamphone	**-10.15**	**0.022**
	Oleic acid C18:1	10.03	0.048
	Abietic acid	31.24	0.039
	Ferulic acid hexoside	8.03	0.024
	trans-β-Ocimene	4.78	0.019

PSM associated with resistance to weevil damage (negative β) are typed in bold. Linear regression model at each defensive mode is significant at $p < 0.05$. Genotypic sample size of each defensive state is indicated in parentheses. Given that some clonal replicates (control or MJ-induced) were not available for several genotypes, sample size for the inducibility dataset was slightly lower than the original number of genotypes used. Unk P# = unidentified phenolic compound.

LITERATURE CITED

Agrawal, A.A. 2005. Natural selection on common milkweed by a community of specialized insect herbivores. Evolutionary Ecological Research. 7: 17.

Agrawal, A.A.; Hastings, A.P.; Bradburd, G.S. [et al.]. 2015. Evolution of plant growth and defense in a continental introduction. The American Naturalist. 186(1): E1–E15.

Bingham, R.A.; Agrawal, A.A. 2010. Specificity and trade-offs in the induced plant defence of common milkweed *Asclepias syriaca* to two lepidopteran herbivores. Journal of Ecology. 98(5): 1014–1022.

Futuyma, D.J.; Agrawal, A.A. 2009. Macroevolution and the biological diversity of plants and herbivores. Proceedings of the National Academy of Sciences U S A. 106(43): 18054–18061.

Goodger, J.Q.; Heskes, A.M.; Woodrow, I.E. 2013. Contrasting ontogenetic trajectories for phenolic and terpenoid defences in *Eucalyptus froggattii*. Annals of Botany. 112(4): 651–659.

Heil, M. 2009. Plastic defence expression in plants. Evolutionary Ecology Research. 24(3): 555–569.

Iason, G.R.; O'Reilly-Wapstra, J.M.; Brewer, M.J. [et al.]. 2011. Do multiple herbivores maintain chemical diversity of Scots pine monoterpenes? Philosophical Transactions of the Royal Society :B: Biological Sciences. 366(1569): 1337–1345.

Jaramillo-Correa, J.P.; Rodriguez-Quilon, I.; Grivet, D. [et al.]. 2015. Molecular proxies for climate maladaptation in a long-lived tree (*Pinus pinaster* Aiton, Pinaceae). Genetics. 199(3): 793–807.

Karban, R.; Agrawal, A.A.; Thaler, J.S.; Adler, L.S. 1999. Induced plant responses and information content about risk of herbivory. Trends in Ecology & Evolution. 14(11): 443–447.

Littell, R.C.; Milliken, G.A.; Stroup, W.W. [et al.]. 2006. SAS System for Mixed Models. 2nd ed. Cary, NC.: SAS Institute Inc.

López-Goldar, X.; Villari, C.; Bonello, P. [et al.]. 2018. Inducibility of plant secondary metabolites in the stem predicts genetic variation in resistance against a key insect herbivore in maritime pine. Frontiers in Plant Science. 9: 1651.

Moreira, X.; Mooney, K.A.; Rasmann, S. [et al.]. 2014. Trade-offs between constitutive and induced defences drive geographical and climatic clines in pine chemical defences. Ecology Letters. 17(5): 537–546.

Moreira, X.; Zas, R.; Sampedro, L. 2012. Quantitative comparison of chemical, biological and mechanical induction of secondary compounds in *Pinus pinaster* seedlings. Trees. 26(2): 677–683.

O'Reilly-Wapstra, J.M.; Hamilton, M.; Gosney, B. [et al.]. 2014. Genetic correlations in multi-species plant/herbivore interactions at multiple genetic scales. Advances in Ecological Research. 50: 267–295.

Pratt, J.D.; Mooney, K.A. 2013. Clinal adaptation and adaptive plasticity in *Artemisia californica*: implications for the response of a foundation species to predicted climate change. Global Change Biology. 19(8): 2454–2466.

Rasmann, S.; Chassin, E.; Bilat, J. [et al.]. 2015. Trade-off between constitutive and inducible resistance against herbivores is only partially explained by gene expression and glucosinolate production. Journal of Experimental Botany. 66(9): 2527–2534.

Sampedro, L.; Moreira, X.; Zas, R. 2011. Resistance and response of *Pinus pinaster* seedlings to *Hylobius abietis* after induction with methyl jasmonate. Plant Ecology. 212(3): 397–401.

Serra-Varela, M.J.; Grivet, D.; Vincenot, L. [et al.]. 2015. Does phylogeographical structure relate to climatic niche divergence? A test using maritime pine (*Pinus pinaster* Ait.). Global Ecology and Biogeography. 24(11): 1302–1313.

Stevens, M.T.; Waller, D.M.; Lindroth, R.L. 2007. Resistance and tolerance in *Populus tremuloides*: genetic variation, costs, and environmental dependency. Evolutionary Ecology Research. 21(6): 829–847.

Ward, D.; Shrestha, M.K.; Golan-Goldhirsh, A. 2012. Evolution and ecology meet molecular genetics: adaptive phenotypic plasticity in two isolated Negev desert populations of Acacia raddiana at either end of a rainfall gradient. Annals of Botany. 109(1): 247–255.

Wiggins, N.L.; Forrister, D.L.; Endara, M.J. [et al.]. 2016. Quantitative and qualitative shifts in defensive metabolites define chemical defense investment during leaf development in *Inga*, a genus of tropical trees. Ecology and Evolution. 6(2): 478–492.

Zas, R.; Bjorklund, N.; Nordlander, G. [et al.]. 2014. Exploiting jasmonate-induced responses for field protection of conifer seedlings against a major forest pest, *Hylobius abietis*. Forest Ecology and Management. 313: 212–223.

Zhao, T.; Krokene, P.; Hu, J. [et al.]. 2011. Induced terpene accumulation in Norway spruce inhibits bark beetle colonization in a dose-dependent manner. PLoS One. 6(10): e26649.

Session 10

PRIORITIZING WORK AND PATHS TO SUCCESS

BETTER FOREST HEALTH THROUGH TREE RESISTANCE—COLLABORATIVE APPROACHES

C. Dana Nelson[1], Jennifer Koch[2], Kevin Potter[3], Susan McCord[4], Adam Costanza[4], Mark Coggeshall[5], Margaret Staton[6], and Albert Abbott[7]

Exotic pests and pathogens are causing wide-spread decline and possible extirpation of several of our foundational forest tree species. The decline of such species has large-scale effects on their associated ecosystems and services they provide. A steady barrage of new threats to important forest species is anticipated to continue as a by-product of increasing global trade. The Forest Health Initiative (FHI) was conceived in recognition of these issues and the challenges they present to biodiversity and ecosystem sustainability. The FHI concept is a new paradigm that engages social, regulatory, and biological science experts and various stakeholders in developing and evaluating options for solving our most critical forest health problems. Blight resistance in American chestnut was selected as a test case for FHI, and although not completely solved, significant progress is being made and the promise of effective resistance and chestnut restoration has been advanced. Clearly additional work remains for American chestnut as well as the many other severely threatened forest tree species. To address these additional species, we are building on the FHI experience and developing two complementary initiatives designed to improve the ability of the research community to identify, produce, and deploy effective tree resistance. A project-oriented initiative, Forest.Health, will prioritize the most seriously threatened species, bring researchers and stakeholders together to reach consensus on a science-based solution, and seek collaborators and funding to carry out the work. At the same time, a network-based participatory tree breeding consortium is proposed to ensure long-term development of publically available, genetically improved forest trees.

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TREEGENES: INTEGRATING GENOTYPIC, ENVIRONMENTAL, AND PHENOTYPIC DATA FOR FOREST HEALTH

Emily Grau[1], Nic Herndon[1], Sean Buehler[1], Taylor Falk[1], Peter Richter[1], Sumaira Zaman[1], Stephen Ficklin[2], Margaret Staton[3], Doreen Main[2], and Jill Wegrzyn[1]

TreeGenes (https://treegenesdb.org/) is a web-based information resource designed to serve the diverse needs of the forest tree genomics research community by uniting information resources with visualization and analysis tools. TreeGenes has recently undergone a complete redesign using Tripal, a tool to create and manage genomic database websites. An open source project, Tripal allows developers the flexibility to create and share their own tools (modules) as well as open communication among Tripal supported repositories.

TreeGenes hosts a range of modules that have expanded functionality to deliver genomic and phenotypic data on >1,700 forest tree species. The new Tripal Galaxy module allows users to execute analytical workflows with next generation sequencing datasets on high performance computing (HPC) resources with the click of a button. The Tripal Elasticsearch module allows flexible searching and data retrieval between sites such that TreeGenes can share data with Hardwood Genomics Project and the Genome Database for Roseaceae.

TreeGenes is also developing new Tripal modules including the Tripal Plant PopGen Submit (TPPS) module, the TreeGenes TSeq module, TreeGenes OrthoQuery, and Cartogratree. TPPS is a pipeline for accepting direct submissions from researchers. This module collects relevant metadata/data for studies focused on the interactions between genotype, phenotype, and the environment while reducing the burden on the researcher for submission. The new Tripal Sequence Similarity (TSeq) Module module offers speed improvements over BLAST via DIAMOND and allows sequence similarity searches across numerous pre-indexed genomes and transcriptomes. TreeGenes OrthoQuery permits gene family and phylogenetic analysis for single sequences and/or entire proteomes with a robust and interactive visualization.

CartograTree (https://cartogratree.org) is a map-based open-source analytic module integrates across data that is curated through TPPS to facilitate association mapping and landscape genetics analysis. It uses genotypic and phenotypic data provided by two clade organism databases: TreeGenes via TPPS and Hardwood Genomics. Environmental data currently includes climate variables, land cover, canopy density, atmospheric metrics, and soil types. CartograTree leverages Galaxy workflows to support analysis in the web framework with High Performance Computing (HPC) resources.

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A NATIONAL PRIORITIZATION OF UNITED STATES TREE SPECIES THREATENED BY INSECT AND DISEASE INFESTATION

Kevin M. Potter[1], Maria E. Escanferla[1], Barbara S. Crane[2], Robert M. Jetton[1], and Gary Man[3]

Insect and disease infestations pose major threats to several North American forest tree species. Scientists and managers from throughout the U.S. Forest Service developed a conservation priority-setting framework for forest tree species at risk from insects and disease and other threats. The Project CAPTURE (Conservation Assessment and Prioritization of Forest Trees Under Risk of Extirpation) framework is data-driven and guided by expert opinion, allowing the quantitative grouping of species into vulnerability classes that may require different management and conservation strategies. We applied this framework to categorize and prioritize 419 native North American tree species for conservation, monitoring, and management using trait data and insect and disease threat data for each host tree species. The categorization is based on vulnerability factors relating to each tree species' (1) insect and disease threat severity, (2) sensitivity to insect and disease infestation, and (3) capacity to adapt to insect and disease infestation. We used K-means clustering to group species into 11 classes based on these vulnerability dimensions. The three most vulnerable classes encompassed 15 species which require the most immediate conservation intervention. Two additional classes face less severe insect and disease threats and may be good candidates for resistance breeding efforts. Other groups had traits associated with high sensitivity and/or low adaptive capacity to potential future insect and disease threats, suggesting that these species need close monitoring. This assessment tool should be valuable for decisionmakers determining which species and populations to target for monitoring efforts and for pro-active gene conservation and management activities.

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SEARCHING, DOWNLOADING AND ANALYZING TREE GENETIC AND GENOMIC DATA WITH THE HARDWOOD GENOMICS WEBSITE

Margaret E. Staton[1], Abdullah Almsaeed[1], Bradford Condon[1], Ming Chen[1], Jill L. Wegrzyn[2], Doreen Main[3], Stephen P. Ficklin[3], Albert G. Abbott[4], C. Dana Nelson[4], and John E. Carlson[5]

The Hardwood Genomics Website (HWG) provides access to genetic and genomic data from woody plant species, including many ecologically important and threatened forest trees such as American chestnut, green ash, black walnut, and American beech. The site has tree genomes and transcriptomes that are not housed elsewhere or lack publicly available annotation. It provides searchable annotation for all genes and transcripts, including BLAST results to curated plant protein databases, protein domains, and gene ontology terms. To further characterize gene sequences, HWG houses expression values from high throughput RNASeq studies. We identify simple sequence repeats (SSRs) and flanking primers from genome and gene sequences and make these available on HWG for use as genetic markers. Where available, HWG also provides access to published microsatellite markers. To facilitate access to these datasets, we have a number of tools for researchers, including a powerful keyword search, JBrowse, and BLAST. Most recently we have added access to bioinformatics analysis workflows powered by Galaxy software. Users can select from a number of common analysis types, including mapping DNA or RNA reads, performing differential gene expression analysis, calling genetic variants from reads, etc. A user can upload their own data for analysis, select HWG site data, or use both as input to a workflow. To meet the growing need for forest tree breeding programs and genetic research, we are beginning a new effort to add additional genetic markers, genetic maps including association and QTL studies, and high throughput genotyping and phenotyping data. The site will also be adding descriptions of physical collections such as germplasm or mapping populations with contact information for the maintainer. The HWG is a growing resource for tree scientists, and we welcome feedback, data submissions, or new partnerships to continue site development. HWG is supported by the National Science Foundation (NSF) awards numbers 1443040 and 1444573.

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DURABILITY OF RESISTANCE AND "USEFUL RESISTANCE" IN FOREST TREES TO NON-NATIVE PATHOGENS

Richard A. Sniezko[1], Douglas P. Savin[1], Angelia Kegley[1], and Robert Danchok[1]

Many tree species are extremely susceptible to one or more non-native pathogens or insects. For some, the level of susceptibility can be so severe that they are essentially extirpated from many forest ecosystems, and their use for restoration, reforestation, or urban forestry is lost. Applied programs to find, enhance, and utilize genetic resistance in some tree species are well underway, and more are being considered. Some people equate genetic resistance with 100 percent immunity in planting stock, but in most forest situations this will not be the case. Resistance can also refer to having fewer, less severe, or incompatible infections, or being able to survive and reproduce, and it may be present in only a portion of the planting stock. Trees are long-lived organisms, and resistance has to be effective for decades (in managed plantations) to centuries (in native forests) to be utilitarian. Resistance must not only be durable and stable but also at a useful level, which may vary by species and application. A seedlot with 'useful resistance' (UR) is expected to meet management objectives for long-term deployment, often over many sites and for decades after planting. Field plantings provide the best knowledge of durability of resistance and its stability across a range of environments. We examine the durability, stability, and resistance levels of two white pine species (western white pine, *Pinus monticola* and sugar pine, *P. lambertiana*) from some of the oldest existing white pine blister rust (caused by the fungal pathogen *Cronartium ribicola*) resistance field trials. Genetic resistance will be key to retaining many prominent species in forest ecosystems as well as for use in reforestation and urban forestry. The examples provided here can provide guidance to managers on early expectations of resistance in application to a range of other non-native pathogens or insects in forest trees.

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Session 11

NOVEL TOOLS FOR IDENTIFYING RESISTANCE

TREESNAP: A CITIZEN SCIENCE PROJECT AND MOBILE APP TO TAG TREES FOR SCIENCE

Bradford Condon[1], Abdullah Almsaeed[1], Ellen Crocker[2], Albert Abbott[2], C. Dana Nelson[3], and Margaret Staton[1]

To help scientists find forest trees of particular species and characteristics, we have built a new mobile app and citizen science outreach project, TreeSnap. TreeSnap uses the ubiquity of smartphones in our society to engage the public in scouting for trees affected by invasive insects and diseases, including North American ashes, American chestnut, eastern and Carolina hemlocks, and American elm. This strategy provides tree locations, images, and characteristics to tree research programs while also engaging citizens and promoting public awareness of forest health threats and the benefits of forest health. The TreeSnap mobile app is available for free on both iOS and Android. The list of highlighted tree species may be updated to reflect current needs across many forest health research programs. To increase the utility of the collected data for scientists, we ask a set of customized questions for each species, such as a list of signs of Emerald Ash Borer for ash trees and presence of cones for hemlocks. This information is paired with photos taken by the user and GPS coordinates automatically detected by the mobile device. TreeSnap has an accompanying website where users can view their own and others' tagged trees. The website also serves as a data curation and outreach workspace for scientists. They can login to sort and filter trees, download user observations, and contact individual users for more information. Also, scientists can set up an email alert to be notified when new observations fitting their criteria are submitted (i.e., American Chestnuts in Tennessee with a diameter of at least 10 inches). We envision TreeSnap as a crucial long term research tool for threatened forest trees, which often lack a large pool of lingering genotypes to study. We are actively seeking new partner scientists to expand the trees available in TreeSnap and partner forest outreach groups that can promote the App to interested citizens, or incorporate it into their existing activities.

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DEVELOPMENT OF A TOOL FOR RAPID IDENTIFICATION OF RESISTANT TREES IN SPECIES AFFECTED BY ALIEN INVASIVE PATHOGENS

Anna O. Conrad[1], Caterina Villari[2], Richard A. Sniezko[3], Luis Rodriguez-Saona[4], and Pierluigi Bonello[1]

Alien invasive pathogens continue to threaten the health of forests across the United States and globally. Identifying and breeding resistant trees is a viable strategy for disease management and may be used as part of efforts aimed at restoring disturbed habitats. However, identifying disease resistant trees requires artificial inoculation or natural infection with a pathogen, and time needed for symptoms to develop. This often comes after significant resources have already been invested in collecting seeds and growing seedlings. Therefore, the objective of our work is to develop a more rapid phenotyping method for disease resistance using Fourier transform infrared (FT-IR) and Raman spectroscopy combined with chemometrics. Currently, we are focusing our efforts on two forest pathosystems: root rot of Port-Orford-cedar (*Chamaecyparis lawsoniana*) caused by *Phytophthora lateralis* and white pine blister rust on whitebark pine (*Pinus albicaulis*) caused by *Cronartium ribicola*. In Port-Orford-cedar, we are analyzing material with origins across the geographical range of the species, while with whitebark pine our efforts are focused on populations of the Pacific Northwest. Our goals include evaluating whether the tool can be used to predict resistance within and between families, and the impact of geographical origin on model predictions. We ultimately aim to develop protocols for rapid, in-field analysis of intact plant materials that can be used to facilitate and expedite current breeding and restoration efforts.

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DETECTION OF NEAR INFRARED SPECTRAL DIFFERENCES IN *PINUS RADIATA*

Judith S. Nantongo[1], Thomas Rodemann[2], Hugh Fitzgerald[1],
Brad M. Potts[1], and Julianne O'Reilly-Wapstra[1]

Selective bark stripping of *P .radiata* by wallabies and pademelons may be explained by differences in constitutive or induced chemistry (Miller et al. 2014). The potential of near infra-red spectroscopy (NIRS) to detect differences between the less and more susceptible genotypes was evaluated. Nine families classified as less susceptible (R) and more susceptible (S) were selected. Three families of each were treated with stress inducing Methyl jasmonate (MJ), mechanical stripping (strip) or no treatment (control) in a randomised block design. Physio-chemical changes were monitored by NIRS for 4 weeks. Partial least squares (PLS) regression was used to group the principle components (PC) of the spectra. The results showed clearly that NIRS distinguished less and more susceptible genotypes. NIRS also separated individuals subjected to different treatments. In conclusion, there is evidence of differences in bark physio-chemical attributes of the less and more resistant genotypes. NIRS provides a powerful tool for detecting physio-chemical differences in *P. radiata*.

REFERENCES

Miller, A.; O'Reilly-Wapstra, J.; Potts, B. 2014. Genetic variation in bark stripping among *Pinus radiata*. Internal Report. Submitted to the National Centre for Future Forest Industries (NCFFI) and Timberlands Pacific Pty Ltd.

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Session 12

RESISTANCE AND INTEGRATED PEST MANAGEMENT

ENDOPHYTE ENHANCEMENT OF SPRUCE AND EASTERN WHITE PINE SEEDLINGS TO IMPROVE TOLERANCE TO INSECTS AND DISEASE

Gregory W. Adams[1], Andrew McCartney[2], J. David Miller[3], and Dan Quiring[4]

Research over the past two decades has focused on endophytic fungi isolated from the needles of conifer trees, forming mutualistic associations with their hosts. We have identified native strains of endophytic fungi that produce secondary metabolites which act antagonistically towards certain forest pests, namely eastern spruce budworm (*Choristoneura fumiferana*) and white pine blister rust (*Chronatium ribicola*). Spruce budworm, which is the most significant insect pest in Canada and parts of the United States, defoliates balsam fir and white spruce and to a lesser extent red spruce and black spruce. In 2017, 7 million ha of forests in the Province of Québec experienced defoliation from the current outbreak. White spruce is the most widely planted tree species in Canada and was the subject of early studies on potentially useful foliar endophytes. Research was initiated in 1998 to determine if white spruce seedlings could be inoculated with selected endophyte strains. Field tests were established to study the persistence of the endophytes in trees, to understand the transmission of the endophyte, and to provide trees for challenging experiments with the spruce budworm. Challenging studies with spruce budworm have demonstrated a reduction of spruce budworm survival through to adulthood and a reduction of defoliation in the range of 10–30 percent. Based on positive results of this work, research was initiated in 2008 to test the same approach on white pine blister rust, a devastating non-native disease affecting all five-needle pines in North America. Through screening many strains, endophytes have been selected which produce potent anti-fungal secondary metabolites and inoculation procedures have been developed. Lab assays using the disease pathogen and secondary metabolites at relevant concentrations have demonstrated inhibition of the disease and field testing is underway. Twenty years of research has demonstrated that selected endophytes can play a role in improving tolerance of planted trees to pests as one component of integrated pest management. J.D. Irving, Limited has implemented endophyte enhancement in nursery seedling production at a large scale since 2008.

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MONITORING OF ASH MORTALITY PATTERNS INFORMS EMERALD ASH BORER (EAB) RESISTANCE BREEDING EFFORTS: INTEGRATED PEST MANAGEMENT FOR EAB

Kathleen Knight[1], Rachel H. Kappler[2], Karen V. Root[2], Jennifer L. Koch[1], and Charles E. Flower[1]

Integrated pest management (IPM) is a framework that combines multiple different strategies to reduce the impacts of pests and pathogens. Strategies include preventative measures; cultural, biological, and chemical control; monitoring of host and pest populations; and mitigation of impacts. Pest- or pathogen-resistant trees are cultural control techniques that, even standing alone, can reduce impacts by increasing survival and health of the host tree species. By combining tree resistance breeding with other strategies within an integrated pest management framework, even greater reductions in pest and pathogen impacts may be achieved and may last over a much longer term. We present a case study of the insect pest emerald ash borer (*Agrilus planipennis*) (EAB) and its impacts on ash trees (*Fraxinus* spp.) in the United States. Ten years of monitoring data on ash and EAB populations has clearly shown the typical trajectory of ash mortality and subsequent crash in EAB populations, and has also shown the factors that affect the rate of mortality of ash trees. Intensive monitoring of "lingering" ash populations after the EAB mortality wave has revealed how these factors that affect mortality may change in this post-infestation landscape. While areas with higher ash density exhibit slower mortality during the initial mortality wave, ash trees with neighboring ash trees exhibit greater decline and mortality in a post-infestation time period. This finding suggests that cultural control methods, while not useful prior to or during the initial wave of EAB, may be helpful in maintaining lingering ash populations after EAB has killed most of the trees. The remaining healthy trees, many exhibiting resistance to EAB, may benefit from reductions in susceptible declining neighbors. Consideration of natural patterns of mortality may inform resistance breeding programs: not only which trees to choose for breeding programs and the appropriate timing to choose them, but how to deploy them using appropriate cultural control measures that may influence their success.

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EMERGING THREATS OF PRIORITY AGROFORESTRY TREES IN AFRICA: CHALLENGES AND OPPORTUNITIES

Jane Njuguna[1], Eston Mutitu[1], Ely Mwanza[1], Alice Muchugi[2], and Ignazio Graziosi[2]

Agroforestry has a strategic role for Africa's smallholders, as it builds resilient agro-ecosystems and provides enduring benefits to farmers. The World Agroforestry Centre is conserving over 18,000 germplasm accessions of more than 200 tree species in globally established field genebanks and seed banks. Emerging biotic threats are targeting the cultivation of crucial tree species in Sub-Saharan Africa, thus frustrating local benefits of agroforestry and forestry. A stem canker disease caused by Botryosphaeriaceae pathogens is triggering extensive dieback to the Australian silk oak *Grevillea robusta*, widely used in Africa as highly adaptable, multi-purpose, and fast growing tree. Botryosphaeriaceae disease complexes are also involved in the newly detected dieback of native baobab *Adansonia digitata*, and, along with Teratosphaeriaceae species, in cankers of *Eucalyptus* spp. In addition, *Eucalyptus* planted forests in Southern and Eastern African countries are under attack from non-native bronze bug *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae), blue gum chalcid *Leptocybe invasa* (Hymenoptera: Eulophidae), and red gum lerp psyllid *Glycaspis brimblecombei* (Hemiptera: Psyllidae). We developed laboratory protocols to detect fungal pathogens affecting *G. robusta* and *A. digitata* seeds and seedlings with the aim of distributing clean agroforestry material and reducing further spread of these organisms. We also implemented a countrywide survey to assess distribution, incidence, and population levels of *Eucalyptus* insect pests in Kenya. As the next step, we plan to determine variation in disease resistance within germplasm collections for *G. robusta* (40 accessions from Australia and Kenya) and *A. digitata* (297 accessions from Tanzania Burkina Faso, Kenya, Mali), and investigate the occurrence of natural resistance to insect pests in Kenya's *Eucalyptus* planted forests. Developing management strategies that include selecting for resistance to pests and diseases may be the key to mitigate biotic threats to agroforestry in Sub-Saharan Africa.

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Session 13

INTERACTIONS BETWEEN RESISTANCE AND ENVIRONMENT

THE EFFECT OF SITE QUALITY ON PERFORMANCE OF AMERICAN CHESTNUT (*CASTANEA DENTATA*) SEEDLINGS BRED FOR BLIGHT (*CRYPHONECTRIA PARASITICA*) RESISTANCE

Title presented at workshop:
INFLUENCE OF SITE QUALITY ON BLIGHT RESISTANCE AND GROWTH OF PLANTED HYBRID CHESTNUT SEEDLINGS

Cornelia C. Pinchot[1], Alejandro A. Royo[2], Scott E. Schlarbaum[3], Matthew P. Peters[1], Ami M. Sharp[3], and Sandra L. Anagnostakis[4]

ABSTRACT

Efforts to produce American chestnuts (*Castanea dentata*) resistant to chestnut blight fungus (*Cryphonectria parasitica*) have spurred an interest in reintroducing the species to managed forests. Understanding how site characteristics impact chestnut performance will inform appropriate site selection. This study was designed to evaluate the impact of site quality on survival, growth, competitive ability, and blight resistance durability of American, Chinese, and three families of backcross hybrid chestnuts. Chestnuts were planted in xeric, intermediate, and sub-mesic sites in Pennsylvania. Three years after planting, survival was 86 percent across all treatments. American chestnuts were taller on intermediate than xeric sites, and two backcross hybrid families were taller on intermediate as compared to mesic sites. Intermediate sites may offer enough soil moisture to optimize growth, without the intense competition characteristic of more mesic sites. Incidence of blight infection was too low to assess differences among treatments, though is expected to increase over time.

INTRODUCTION

Introductions of non-native invasive pests and pathogens have caused the loss or decline of an increasing number of tree species globally (Campbell and Schlarbaum 2014, Loo 2008, Santini et al. 2013). These losses can cause significant alterations to ecosystem processes and functions and threaten forest resilience to future pressures (Ellison et al. 2005, Flower et al. 2013). In response, there is a growing interest to identify or breed populations of tree species resistant to their respective pests and pathogens (Sniezko 2006), with the goal of reintroducing these species to managed landscapes. Reintroducing extirpated tree species may restore altered ecosystem dynamics, functions, and services, thereby enhancing resilience, and contributing to larger ecosystem restoration goals (Knight et al. 2011, 2017). Understanding the durability of resistance (Sniezko, 2006) and performance of improved genotypes in field settings (Clark et al. 2014, Seddon 2010) is essential for successful species reintroduction, however long term studies on these considerations are limited (Thompson et al. 2006).

Extensive efforts have gone into developing American chestnuts (*Castanea dentata*) that are resistant to chestnut blight disease (caused by the fungus *Cryphonectria parasitica*), and more recently ink disease (caused by the oomycete, (*Phytophthora cinnamomi*),(Anagnostakis 2012, Steiner et al. 2017). The principal strategy has involved hybridizing American chestnuts with blight resistant chestnut species (primarily Chinese chestnut, *C. mollissima*), followed by repeated backcrossing and intercrossing to recover American chestnut traits (Anagnostakis 2012, Hebard 2005). More recently, The American Chestnut Foundation, one of the primary organizations working to develop blight-resistant American chestnuts, has incorporated the use of genomic selection to accelerate the breeding program (Steiner et al. 2017).

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Once blight-resistant American chestnuts are available, planting them into managed forests will be the next step toward restoring the species. Understanding the interacting effects of genotype and environment on long-term performance and durability of resistance of planted chestnuts will inform operational reintroduction of the species, including site selection. Selecting sites for American chestnut reintroduction that optimize growth, competitive ability, and blight control will help maximize limited resources available for restoration efforts. Site characteristics may affect these metrics of establishment success differently, however (Griffin et al. 2006). For example, Rhoades et al. (2009) found that planted American chestnut had better growth rates and lower incidence of blight on mesic than xeric sites 2 years after planting. McNab (2003), however, found that planted chestnuts were outcompeted by faster growing species on mesic sites, a trend that Griffin et al. (1991) also noted for naturally occurring chestnut sprouts growing on mesic sites. Bauman et al. (2014) found that site preparation treatments that led to greater growth of planted chestnut also yielded higher blight infection rates, by decreasing the time required for bark splits and subsequent infection. Gao and Shain (1995) found that chestnut blight canker expansion on American chestnut stem segments was negatively related to moisture availability, which suggests blight resistance may be influenced by soil moisture availability in situ. Here we present early results from a study to evaluate the long-term impact of site quality on field performance and resistance durability of hybrid backcross American chestnuts planted in recent shelterwood harvests.

METHODS

Study Area

We planted chestnut seedlings at 15 northern hardwood forest sites distributed throughout a 6500-km^2 area of northern Pennsylvania, USA, spanning from Warren County to the west and Potter County to the east. Major tree species found at the sites include red maple (*Acer rubrum*), sugar maple (*Acer saccharum*), black cherry (*Prunus serotina*), and American beech (*Fagus grandifolia*), with northern red oak (*Quercus rubra*), eastern hemlock (*Tsuga canadensis*), birch (*Betula* spp.), and white ash (*Fraxinus americana*) found in lesser abundance. The 15 sites used in this study were selected from a larger set of 25 sites (Royo et al. 2016, 2017) to capture variation in soil moisture availability. To do this we calculated the integrated moisture index (IMI), (Iverson et al. 1997) for all 25 available sites. The integrated moisture index combines GIS-derived topographic and soil features of the landscape into a single index that models potential soil moisture capacity (Iverson et al. 1997). We then ran a cluster analysis to group sites based on their IMI values (PROC CLUSTER, SAS Institute Inc. 2011). The analysis distributed the 25 sites among four clusters, one containing only one site with the highest IMI value (most mesic), which we merged with the cluster with the next highest IMI values to produce three clusters representing xeric, intermediate and sub-mesic site types. We chose five sites within each cluster to maximize variability across treatments. The integrated moisture index for the selected xeric sites averaged 31.7 (range 29.4–34.7), 40.3 for the intermediate sites (range 39.5–40.8), and 46.9 for the sub-mesic sites (range 43.6–55.6).

In 14 of the 15 sites, managers conducted the initial cut of a shelterwood sequence to reduce stand relative density (the residual relative density was 31–61.5 percent and the residual basal area was 13.9–26.1 m^2/ha) and applied broadcast herbicides (tank mix of glyphosate and sulphometuron methyl), (Marquis et al. 1992) to control interfering plant species in the mid- and understory layers 3–6 years prior to planting. The site that did not receive a harvest treatment was dropped from the analysis, leaving four sites in the intermediate treatment and five each in the xeric and sub-mesic treatments.

Experimental Materials

Four-hundred and eighty seven chestnut seedlings; 83 American, 84 Chinese, and 320 backcross hybrid chestnuts (Anagnostakis 2012, Hebard 2005) from three families were used in this study (table 1). The backcross hybrid chestnuts were from the BC_3F_2 generation—second generation of the third backcross —or were crosses between

Table 1—Chestnut species/generations and families used in this study

Chestnut species/hybrid generation	Family	N
American chestnut	American	83
Chinese chestnut	Chinese	84
BC_3F_2 backcross hybrid	W1-100	83
$BC_2F_1xBC_3F_1$ backcross hybrid[a]	W3-20	84
$BC_2F_1xBC_3F_1$ backcross hybrid[a]	W4-75	153
Total	—	487

[a] All chestnuts in the Windsor orchard except for one family are male-sterile, therefore, the father family is known, even though the crosses were open-pollinated.

BC_3F_1 and BC_2F_1 parents. All chestnuts were from open-pollinated seed collections made in the fall of 2013 and grown for 1 year at commercial or State tree nurseries. The American chestnuts were collected from one mother tree in Maryland and grown at the Kentucky State tree nursery (Grassy Creek, KY). The Chinese chestnuts were collected from multiple mothers from one orchard and grown at the Forrest Keeling Nursery (Elsberry, MO). The backcross hybrid American chestnuts (three families, table 1) were collected at the Windsor Locks orchard of the Connecticut Agricultural Experiment Station (Anagnostakis 2012) and grown at the Vallonia Nursery in Vallonia, IN (fig. 1A). All seedlings were lifted as 1-0 bare-root seedlings in the early spring of 2015 and stored in a cold room (~1° C). The seedlings were processed for planting in March, with roots trimmed to 15 cm from the main tap root to facilitate planting (fig. 1B). Seedlings were planted April 10–14, 2015 with a Jim Gem KBC© bar, modified by adding 5 cm to each side of the blade, creating a blade 20 cm at the top, tapering to the tip.

Experimental Design and Data Collection

Site type (xeric, intermediate, sub-mesic) was analyzed as a completely randomized design. Chestnut species and hybrids were analyzed as one treatment factor, called "family", with five total treatment levels (table 1). Within each site type replicate, the chestnut seedlings were arranged using an incomplete block design, each

Figure 1—(A) backcross hybrid chestnuts growing at Vallonia Nursery (Vallonia, IN), and (B) Trimming the roots of and tagging the lifted seedlings at the Tennessee Tree Improvement Center (Knoxville, TN). (Forest Service photo by Cornelia Pinchot)

block containing four seedlings from different family treatments. At each site, between 34 and 36 chestnut seedlings were planted within a 0.42 ha deer exclosure. Seedlings were planted in grids, 3.7 m spacing within and 6 m spacing between rows.

Seedling height and ground-level diameter (GLD) were measured at the time of planting and towards the end of the first three growing seasons (August or September, once the buds were set) since planting. Mortality and incidence of chestnut blight was also assessed at this time.

A camera equipped with a hemispherical lens was used to evaluate canopy openness above each planted chestnut seedling during the 2017 (year 3) field season. To evaluate competing vegetation, a 2.6 m diameter competition plot was centered on each chestnut seedling and competition data on height and species of the tallest understory woody plants (DBH <10 cm) collected towards the 2017 growing season.

All analyses for this study were processed using SAS 9.3 software (SAS Institute 2011). Seedling height and GLD were analyzed using a mixed-model analysis of covariance (ANACOVA) to determine significant differences among the fixed effects of site type, chestnut family, and their interactions for year 3 after planting. Initial height, GLD, and percent canopy openness were tested as covariates in the ANACOVA models for height and GLD, respectively. Generalized linear mixed model with binomial distribution was used to analyze third year survival (1 = alive, 0 = dead) and dominance of the seedlings. Seedlings that attained at least 80 percent of the height of the tallest competitor within the competition plot were defined as dominant (Spetich et al. 2002). Data were checked for homogeneity of variance and normality. Unequal variance was added to the model if the Akaike Information Criterion (AICc) was significantly improved. Least-significant-difference tests were performed to identify differences among means ($\alpha = 0.05$). Incidence of blight was too low to analyze for statistically significant differences among treatments.

RESULTS

Survival

Three growing seasons after planting, 86 percent (±2) of the chestnut seedlings were alive. There was no difference in survival among site types ($P = 0.99$), while family did differ ($P = 0.01$). Chestnuts in the W4-75 backcross family had greater survival (99 percent ± 2) than all other chestnuts except those in the W1-100 backcross family (97 percent ± 2). All other chestnuts were statistically similar in survival (and did not differ from the W1-100 backcross family); 87 percent ± 4 for the W3-20 backcross family; 84 percent ± 5 for Chinese, and 83 percent ± 5 for American chestnuts. The interaction between site and family treatments was not significant ($P = 0.24$).

Height and Ground Level Diameter

Height after three growing seasons did not differ among site or family treatments ($P = 0.20$, $P = 0.19$, respectively) (fig. 2), and averaged 162 cm across all treatments. However, the interaction between the two treatment factors was significant ($P = 0.03$) (fig. 3). American chestnuts were taller on intermediate than xeric sites; while W1-100 and W4-75 backcross hybrid families were taller on intermediate than sub-mesic sites. There were no differences in height for W3-20 backcross hybrid or Chinese chestnuts across the site types. Height at planting and percent canopy openness were both significant covariates in this model ($P < 0.0001$ and $P = 0.001$, respectively). Ground level diameter after three growing seasons was similar across the site types ($P = 0.41$), but differed among families ($P < 0.0001$). Diameter was greatest for backcross hybrid families W1-100 and W4-75 (17.5 mm ± 0.8 and 17.4 mm 0.7, respectively). Diameter was similar among the remaining families; 14.7 mm ± 0.8 for W3-20, 14.6 mm ± 0.8 for American, and 13.7 mm ± 0.8 for Chinese chestnuts. The interaction between site and family treatments was not significant ($P = 0.23$). Ground level diameter at planting and percent canopy openness were both significant covariates in the model ($P < 0.0001$ for each).

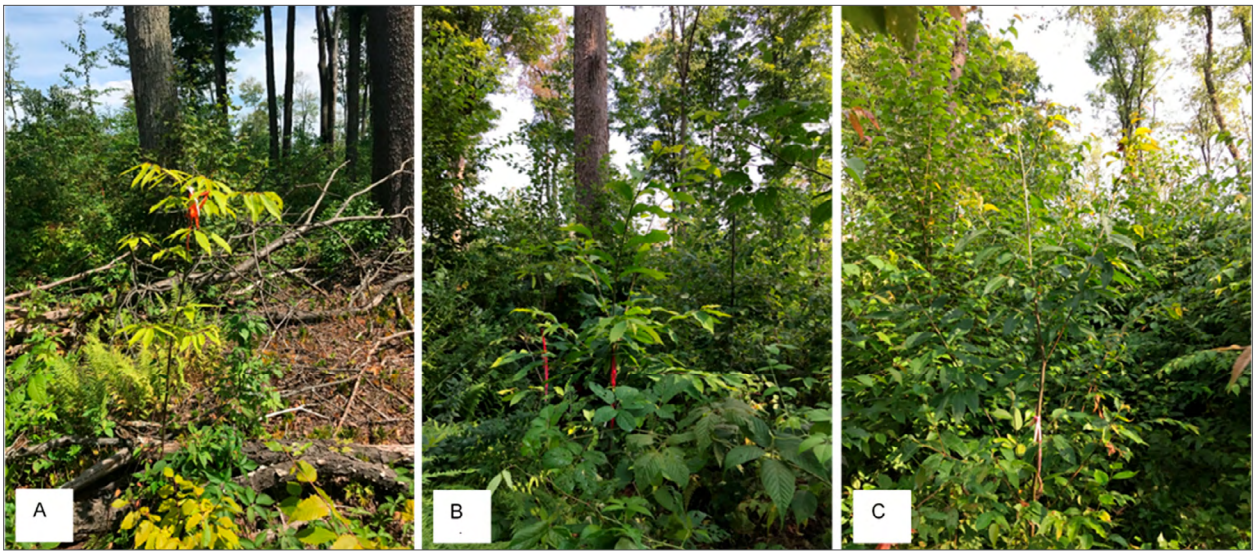

Figure 2—Chestnut seedlings in a xeric (A), intermediate (B), and sub-mesic (C) sites 3 years after planting. (Forest Service photo by Cornelia Pinchot)

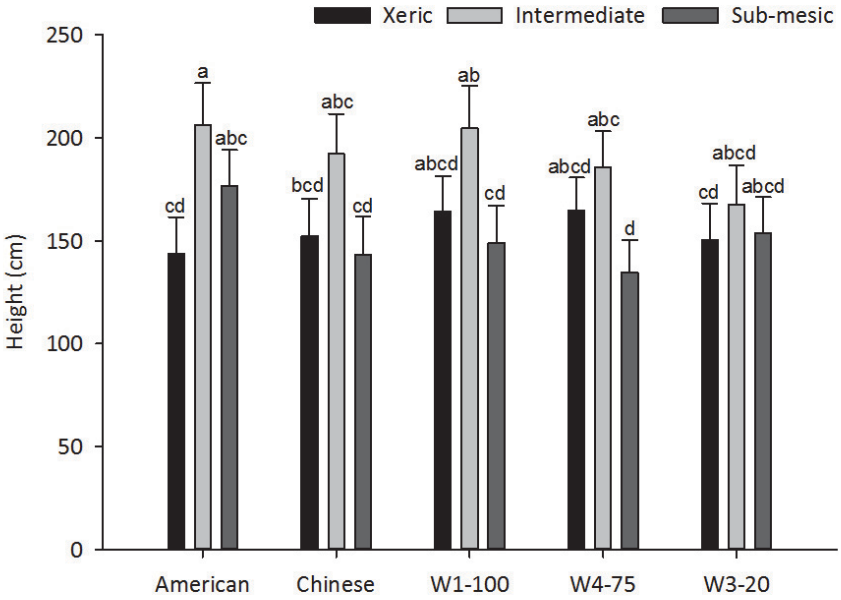

Figure 3—Mean height (± standard error) of chestnut families among site types. Different letters indicate height differences among treatments ($\alpha = 0.05$).

Dominance

Dominance was statistically similar across the site types (P = 0.33) see figure 4 for examples of dominant and suppressed seedlings), but differed both among families (P = 0.004) and the interaction between site and family treatments (P = 0.04). Dominance was greatest for backcross family W4-75 (42 percent) and family W1-100 (36 percent) and lowest for Chinese chestnuts (17 percent) (table 2). Family W4-75 was the only family to differ in dominance among site types;

Table 2—Dominance probability among chestnut families

Chestnut family	Dominance after 3 years (%)
American	22 ± 7 BC
Chinese	17 ± 6 C
W1-100	36 ± 9 AB
W3-20	27 ± 7 BC
W4-75	42 ± 9 A

Note: Means followed by the same letter are not statistically different.

Figure 4—(A) dominant and (B) suppressed chestnut seedlings, 3 years after planting. Arrows point to the top of each seedling. (Forest Service photo by Cornelia Pinchot)

54 percent of W4-75 seedlings growing in xeric sites were dominant, compared with 60 percent (± 15) in intermediate sites, and 18 percent (± 9) in sub-mesic sites.

Blight Incidence

Chestnut blight was identified on 23 individual chestnut seedlings throughout the first three growing seasons; nine in xeric, and seven each in intermediate and sub-mesic sites. Blight was identified in three of five xeric, three of four intermediate, and four of five sub-mesic sites. Nine of the seedlings with blight symptoms were American chestnut, one Chinese, five W1-100, five W4-75, and three were W3-20 backcross hybrid chestnuts.

DISCUSSION

Early survival of planted chestnut seedlings was high across site and family treatments. Two of the backcross families (W1-100 and W4-75) demonstrated superior results in at least one performance metric (diameter, survival, and/or dominance). Differences in performance among chestnut families has been found in other studies evaluating outplanting performance in forested settings (Clark et al. 2016, Pinchot et al. 2017, Thomas-Van Gundy et al. 2017). Of greater interest is the significant interaction between site and family treatments for height and dominance. American chestnuts demonstrated inferior height in xeric, compared to intermediate sites, while two of the backcross hybrid families demonstrated inferior growth in sub-mesic sites. Intermediate sites may offer enough soil moisture to optimize growth, without the intense competition characteristic of more mesic sites (Loftis 1983). Continued monitoring of the seedlings over time is necessary to determine if differences across sites will become more pronounced across all families, or if there is indeed a genotype by environment interaction for these variables. While incidence of blight was too low for statistical analysis, more American chestnuts were infected than Chinese or backcross hybrid chestnuts. Incidence and severity

of blight infection will likely increase over time, as Hebard (1982) and Griffin (1989) have found for natural American chestnut sprout populations following canopy disturbance. Understanding if site and family treatments effect blight severity, particularly in the context of growth and competitive ability among treatments, will help inform a holistic American chestnut reintroduction strategy.

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LITERATURE CITED

Anagnostakis, S.L. 2012. Chestnut breeding in the United States for disease and insect resistance. Plant Disease. 96(10):1392–1403. https://doi.org/10.1094/PDIS-04-12-0350-FE.

Bauman, J.M.; Keiffer, C.H.; McCarthy, B.C. 2014. Growth performance and chestnut blight incidence (*Cryphonectria parasitica*) of backcrossed chestnut seedlings in surface mine restoration. New Forests. 45(6): 813–828. https://doi.org/10.1007/s11056-014-9439-3.

Campbell, F.T.; Schlarbaum, S.E. 2014. Fading forests III: American forests. What choice will we make? The Nature Conservancy and The University of Tennessee. 155 p. http://treeimprovement.utk.edu/pdfs/Fading_Forests_III.pdf. [Date accessed: January 24 2017].

Clark, S.L.; Schlarbaum, S.E.; Pinchot, C.C. [et al.]. 2014. Reintroduction of American Chestnut in the National Forest System. Journal of Forestry. 112(5): 502–512. https://doi.org/10.5849/jof.13-106.

Clark, S.L.; Schlarbaum, S.E.; Saxton, A.M.; Hebard, F.V. 2016. Establishment of American chestnuts (*Castanea dentata*) bred for blight (*Cryphonectria parasitica*) resistance: influence of breeding and nursery grading. New Forests. 47(2): 243–270. https://doi.org/10.1007/s11056-015-9512-6.

Ellison, A.M.; Bank, M.S.; Clinton, B.D. [et al.]. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. Frontiers in Ecology and the Environment. 3(9): 479–486. https://doi.org/10.1890/1540-9295(2005)003[0479:lofscf]2.0.co;2.

Flower, C.E.; Knight, K.S.; Gonzalez-Meler, M.A. 2013. Impacts of the emerald ash borer (*Agrilus planipennis* Fairmaire) induced ash (*Fraxinus* spp.) mortality on forest carbon cycling and successional dynamics in the eastern United States. Biological Invasions. 15(4): 931–944. https://doi.org/10.1007/s10530-012-0341-7.

Gao, S.; Shain, L. 1995. Effects of water stress on chestnut blight. Canadian Journal of Forest Research. 25(6): 1030–1035. https://doi.org/10.1139/x95-112.

Griffin, G.J.; Elkins, J.R.; McCurdy, D.; Griffin, S.L. 2006. Integrated use of resistance, hypovirulence, and forest management to control blight on American chestnut. In: Steiner, K.C.; Carlson, J.E, eds. Restoration of American Chesntut to Forest Lands - Proceedings of a conference and workshop. Natural Resources Report NPS/NCR/CUE/NRR - 2006/001. Washington, DC: National Park Service: 97–105.

Griffin, G.J. 1989. Incidence of chestnut blight and survival of American chestnut in forest clearcut and neighboring understory sites. Plant Disease. 73: 123–127. https://doi.org/http://dx.doi.org/10.1094/PD-73-0123.

Griffin, G.J.; Smith, H.C.; Dietz, A.; Elkins, J.R. 1991. Importance of hardwood competition to American chestnut survival, growth, and blight development in forest clearcuts. Canadian Journal of Botany. 69(8): 1804–1809. https://doi.org/10.1139/b91-229.

Hebard, F.V. 1982. Biology of virulent and hypovirulent *Endothia parasitica* on American chestnut (*Castanea dentata*). Blacksburg, VA: Virginia Polytechnic Institute and State University. 295 p. Ph.D. dissertation.

Hebard, F.V. 2005. The Backcross breeding program of The American Chestnut Foundation. Journal of the American Chestnut Foundation. 19(2): 55–78. https://doi.org/10.17660/ActaHortic.2014.1019.20.

Iverson, L.R.; Dale, M.E.; Scott, C.T.; Prasad, A. 1997. A GIS-derived integrated moisture index to predict forest composition and productivity of Ohio forests (U.S.A.). Landscape Ecology. 12(5): 331–348. https://doi.org/10.1023/A:1007989813501.

Knight, K.S.; Haugen, L.M.; Pinchot, C.C.; Schaberg, P.G. 2017. American elm (*Ulmus americana*) in restoration plantings: a review. In: Pinchot, C.C.; Knight, K.S.; Haugen, L.M.; Flower, C. E.; Slavicek, J.M. eds. Proceedings of the American elm restoration workshop. Gen. Tech. Rep. NRS-P-174. Newtown Square, PA: U.S. Department of Agriculture Forest Service, Northern Research Station: 133–140.

Knight, K.S.; Slavicek, J.M.; Kappler, R. [et al.] 2011. Using dutch elm disease-tolerant elm to restore floodplains impacted by Emerald Ash Borer. In: Sniezko, R.A.; Yanchuk, A.D.; Kliejunas, J.T. [et al.], tech. coords. Proceedings of the fourth international workshop on the genetics of host-parasite interactions in forestry: Disease and insect resistance in forest trees. Gen. Tech. Rep. PSW-GTR-240. Albany, CA: U.S. Department of Agriculture Forest Service, Pacific Southwest Research Station: 317–323.

Loftis, D.L. 1983. Regenerating red oak on productive sites in the Southern Appalachians: a research approach. In: Jones, E.P., Jr., ed. Proceedings of the second biennial southern silvicultural Research Station conference. Gen. Tech. Rep. SE-24. Asheville, NC: U.S. Department of Agriculture Forest Service, Southeastern Forest Experiment Station: 144–150.

Loo, J.A. 2008. Ecological impacts of non-indigenous invasive fungi as forest pathogens. Biological Invasions. 11(1): 81–96. https://doi.org/10.1007/s10530-008-9321-3.

Marquis, D.A.; Ernst, R.L.; Stout, S. 1992. Prescribing silvicultural treatments in hardwood stands of the Alleghenies (revised). Gen. Tech. Rep. NE-96. Broomall, PA: U.S. Department of Agriculture Forest Service, Northeastern Forest Experimental Station. 101 p.

McNab, H.W. 2003. Early results from a pilot test of American chestnut seedlings under a forest canopy. Journal of the American Chestnut Foundation. 16(2): 32–41.

Pinchot, C.C.; Schlarbaum, S.E.; Clark, S.L. [et al.]. 2017. Growth, survival, and competitive ability of chestnut (Castanea Mill.) seedlings planted across a gradient of light levels. New Forests. 48: 491–512. https://doi.org/10.1007/s11056-017-9577-5.

Rhoades, C.; Loftis, D.; Lewis, J.; Clark, S. 2009. The influence of silvicultural treatments and site conditions on American chestnut (*Castanea dentata*) seedling establishment in eastern Kentucky, USA. Forest Ecology and Management. 258(7): 1211–1218. https://doi.org/10.1016/j.foreco.2009.06.014.

Royo, A.A.; Kramer, D.W.; Miller, K.V. [et al.]. 2017. Spatio-temporal variation in foodscapes modifies deer browsing impact on vegetation. Landscape Ecology. 32(12): 2281–2295. https://doi.org/10.1007/s10980-017-0568-x.

Royo, A.A.; Kramer, D.W.; Miller, K.V. [et al.]. 2016. The canary in the coal mine: Sprouts as a rapid indicator of browse impact in managed forests. Ecological Indicators. 69: 269–275. https://doi.org/10.1016/j.ecolind.2016.04.030.

Santini, A.; Ghelardini, L.; De Pace, C. [et al.]. 2013. Biogeographical patterns and determinants of invasion by forest pathogens in Europe. New Phytologist. 197(1): 238–250. https://doi.org/10.1111/j.1469-8137.2012.04364.x.

SAS Institute Inc., 2011. SAS 9.3 Help and documentation. Cary, NC: SAS Institute Inc.

Seddon, P.J. 2010. From reintroduction to assisted colonization: Moving along the conservation translocation spectrum. Restoration Ecology. 18(6): 796–802. https://doi.org/10.1111/j.1526-100X.2010.00724.x.

Sniezko, R.A. 2006. Resistance breeding against nonnative pathogens in forest trees—current successes in North America. Canadian Journal of Plant Pathology. 28(S1): S270–S279. https://doi.org/10.1080/07060660609507384.

Spetich, M.A.; Dey, D.C.; Johnson, P.S.; Graney, D.L. 2002. Competitive capacity of *Quercus rubra* L. planted in Arkansas' Boston Mountains. Forest Science. 48(3): 504–517.

Steiner, K.C.; Westbrook, J.W.; Hebard, F.V. [et al.]. 2017. Rescue of American chestnut with extraspecific genes following its destruction by a naturalized pathogen. New Forests. 48(2): 317–336. https://doi.org/10.1007/s11056-016-9561-5.

Thomas-Van Gundy, M.; Bard, J.; Kochenderfer, J.; Berrang, P. 2017. Mortality, early growth, and blight occurrence in hybrid, Chinese, and American chestnut seedlings in West Virginia. In: Kabrick, J.M.; Dey, D.C.; Knapp, B.O.; Larsen, D.R. [et al.], eds. Proceedings of the 20th Central Hardwood Forest Conference. Columbia, MO: U.S. Department of Agriculture Forest Service, Northern Research Station: 222–239.

Thompson, L.M.; van Manen, F.T.; Schlarbaum, S.E.; DePoy, M. 2006. A spatial modeling approach to identify potential butternut restoration sites in Mammoth Cave National Park. Restoration Ecology. 14(2): 289–296. https://doi.org/10.1111/j.1526-100X.2006.00131.x.

DRIVERS OF DISEASE EMERGENCE IN BOREAL CONIFER FORESTS, IMPORTANCE OF PHENOTYPIC BALANCE

Jan Stenlid[1], Laura Brodde[1], J. Julio Camarero[2], Ángela Sánchez-Miranda[2], and Jonàs Oliva[1,3]

We consider host–tree imbalances from a phenotypic perspective, the lack of co-evolutionary and evolutionary history with the pathogen and the environment, respectively. Phenotypic plastic responses to environmental shifts may become maladaptive when hosts are faced with novel pathogens. Complex interactions involving the interaction with the environment (host or pathogen phenotype) are in theory possible, but are still understudied. Following an increase of records in Northern Europe, the first large scale outbreak of *Diplodia pinea* was discovered in Southern Sweden in 2016. By microsatellite markers, we saw that Diplodia strains from the outbreak area did not differ from strains belonging to the pre-existing innocuous Diplodia population, rejecting the hypothesis of disease driven by a new strain of the pathogen. Disease increased steadily over time, but new infections were more frequent in anomalously dry years. Tree-ring and isotope (δ C) analyses showed that highly infected trees produced more latewood and had lower water-use efficiency than their non-infected counterparts prior to the outbreak, pointing to a phenotypic predisposition and increased susceptibility. However, following disease outbreak, the highly infected trees produced practically no latewood while more healthy trees maintained latewood formation. We speculate that infected trees, by forming more wood in the late growing-season show a less conservative water use and may have experienced stronger summer drought stress, making them more susceptible to the pathogen. Host phenotype plays a relevant role driving disease emergence in boreal forests. Since current climatic conditions in Sweden are suitable for Diplodia survival, management efforts should aim to minimize the spread of the pathogen. Trees with a conservative use of water may be more suitable for areas in which Diplodia is present and where climate models forecast warmer and possibly drier conditions.

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INTRASPECIFIC GENETIC VARIATION IN DEFENSIVE INVESTMENT OF A MODEL MEDITERRANEAN PINE: A DENDROECOLOGICAL ANALYSIS OF RESIN DUCTS

Title presented at workshop:
INTRASPECIFIC GENETIC VARIATION IN DEFENSIVE INVESTMENT VARIES ALONG THE ONTOGENY OF A MODEL MEDITERRANEAN PINE: A DENDROECOLOGICAL ANALYSIS OF RESIN DUCTS

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Conifers, the largest and longest-lived organisms on the planet, have evolved a sophisticated defensive system which has contributed to their colonization of diverse habitats (Franceschi et al. 2005). The defensive system of conifer trees heavily relies on the production of oleoresin, a terpenoid-based viscous fluid which is toxic for insect herbivores and pathogens (Phillips and Croteau 1999). Oleoresin is produced and accumulated in structures of different specialization degree, from single cells to a complex network of resin ducts in some genera such as *Picea* or *Pinus* (Bannan 1936, Wu and Hu 1997). Resin ducts protect the tree against pests by flushing out resin upon wounding, but also by acting as a mechanical barrier that prevents invasion by tree enemies (Franceschi et al. 2005). Importantly, resin duct characteristics (e.g., abundance and size) have been reported to be strongly associated with tree resistance against insect pests and pathogens in several conifer species. For instance, previous studies in different *Pinus* species have shown trees that survived after bark beetle attacks had more and/or larger xylem resin ducts than trees that died (Ferrenberg et al. 2014, Hood et al. 2015, Kane and Kolb 2010).

As other plant defensive traits, genetic and environmental factors mediate resin duct production. On the one hand, genetic variation in resin duct production has been reported in several conifer species (Esteban et al. 2012, Martin et al. 2010, Zas et al. 2015). Such differences among populations are expected to be, at least partially, the result of local adaptation. Supporting this idea, Esteban et al. (2012) found that geographic and climatic variables at population origin explained intraspecific genetic variation in resin duct characteristics in *P. nigra*. On the other hand, environmental conditions that limit plant growth may enhance resource allocation to defences in trees due to the existence of trade-offs between both plant functions (Endara and Coley 2011, Herms and Mattson 1992). In this sense, previous studies have shown that trees growing under low resource (e.g., nutrients, light, water) conditions tend to growth less and increase their resin duct production (Moreira et al. 2015, Moreira et al. 2008). In addition to the individual effects of plant genotype (G) and environment (E), there may also be interactive effects of these two major sources of variation (G×E interactions). An increasing number of studies have addressed G×E effects on resin duct production with contrasting results (Hannrup et al. 2004, Moreira et al. 2015, Rosner and Hannrup 2004, Westbrook et al. 2013). Such interactions may have important implications for tree breeding, such as unstable selection for resistance when considering global warming scenarios. More research is thus needed to derive general patterns.

Plant defence allocation can be also determined by plant ontogeny (Barton and Koricheva 2010, Boege and Marquis 2005). In particular, previous studies have reported both positive and negative trends in defensive investment with tree age (Boege and Marquis 2005). However, none of

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these previous studies have investigated the effect of plant ontogeny on resin duct production. Axial resin ducts are recorded in growth rings, representing thus an annually resolved archive of plant defences. This special feature allows their quantification by standard dendrochronological procedures (Fritts 2012) to retrospectively assess the existence of age-related effects on defensive investment.

In this study, we aimed at disentangling the independent and interactive effects of plant genotype and environment on annual resin duct production across the last 30 years of growth of a model Mediterranean pine (*P. pinaster*). Additionally, we explored the adaptive origin of this anatomical defensive trait by testing whether climatic gradients at the population origin explain intraspecific genetic variation in resin duct production.

We sampled *P. pinaster* trees from two replicated genetic trials established in 1967 located in two different sites (Cabañeros and Riofrío). Cabañeros was located on a plain top-hill while Riofrío was on a mid-hill with irregular topography and stonier soil. These contrasting abiotic conditions caused that tree growth was significantly higher in Cabañeros than in Riofrío. In each genetic trial, we sampled stem cores from trees belonging to nine different populations (N = 10 trees per population) covering most of the natural distribution of this species. Stem cores were extracted at breast height with a Pressler borer, obtaining four cores per tree, each from a cardinal point. Wood cores were progressively sanded and visually crossdated following standard dendrochronological procedures (Fritts 2012). We measured tree-ring width and statistically validated the crossdating applying a cross-correlation analysis in COFECHA software (Grissino-Mayer 2001). We counted axial resin ducts (RD) in each annual growth ring in the latewood (LW), the earlywood (EW) and the total ring (TR) for the last 30 years of growth. Annual RD density was calculated as the number of RD per squared millimetre of wood and year (RD mm^{-2} yr^{-1}). We ran linear mixed models to test for the effects of site, population and their interaction (all fixed factors) on annual RD density. To test for ontogenetic effects on resin duct production during three 10-year periods along the tree lifespan (10–20, 20–30, 30–40 years old), we also included the effect of year as a fixed categorical factor in the model. Block within site and population by block within site were included as random factors. Finally, to assess the existence of climatic clines explaining among population variation, we performed correlations between climatic variables at the population origin and annual RD density. The climatic data was obtained according to a climatic model detailed in Gonzalo (2008). All statistical procedures were performed in *R* software version 3.4.3 (RStudio Team 2016).

Our results showed no significant differences in mean annual RD density in the TR among pine populations and sites (table 1). However, when we separately analysed EW and LW, we found significant variation in annual RD density in the EW and LW among pine populations (table 1). A number of studies have also reported intraspecific genetic variation in RD production among populations in different *Pinus* species (Esteban et al. 2012, Martin et al. 2010, Zas et al. 2015). However, none of the previous studies have measured RD production separately in both ring compartments. Similarly, we found significant differences in RD density in the EW across sites (table 1), being such RD density markedly higher in the low quality site (Riofrío). These findings agree with a long-standing ecological paradigm which predicts that trees growing under poor-resource environments tend to invest less in growth and more in defence (Endara and Coley 2011, Herms and Mattson 1992).

Our results also showed that the G×E interaction did not significantly affect RD density (fig. 1, table 1), indicating that the relative performance of the populations regarding resin duct differentiation is stable across environments. Our results agree with those reported previously in *Picea abies* under drought conditions (Hannrup et al. 2004, Rosner and Hannrup 2004). Contrary to our findings, Moreira et al. (2015) found that *P. pinaster* genotypes markedly differed in the phenotypic plasticity of resin duct production in response to soil nutrient availability (i.e., a

Table 1—Effect of Population (Pop), site (S), year and their interactions on *Pinus pinaster* resin duct (RD) density (RD mm^{-2} yr^{-1}) in total ring, the earlywood and the latewood, F-ratio and associated p-values are shown

	Total ring		Earlywood		Latewood	
	F	*p-value*	*F*	*p-value*	*F*	*p-value*
Pop	2.1	0.06	2.9	**0.011**	4.40	**< 0.01**
S	4.2	0.09	18.8	**< 0.01**	5.14	0.06
Year	39.1	**< 0.01**	74.2	**< 0.01**	68.49	**< 0.01**
Pop × *S*	0.7	0.72	0.7	0.71	0.55	0.81
Pop × *Year*	2.6	**< 0.01**	1.4	**< 0.01**	1.50	**0.01**
S × *Year*	10.7	**< 0.01**	12.6	**< 0.01**	10.93	**< 0.01**

Linear mixed model results. Significant p-values are indicated in bold.

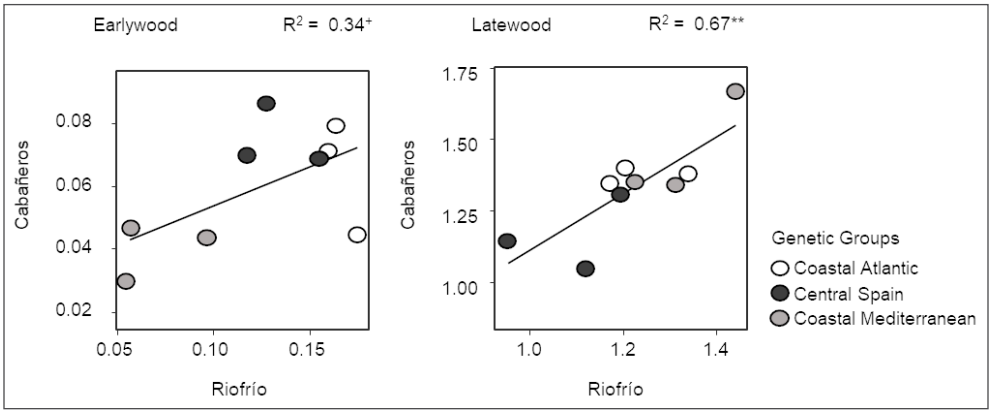

Figure 1—Relationship between annual RD density in earlywood and latewood in both common gardens (Cabañeros and Riofrío). Each dot represents a *Pinus pinaster* population (N = 9). R-squared coefficients (R^2) are shown. Asterisks indicate significant ($^+P < 0.1$, $^*P < 0.05$, $^{**}P < 0.01$) *P*-values.

significant G×E interaction). However, these contrasting findings could be explained by ontogenic effects. In particular, these autors measured resin duct production in juvenile trees (Moreira et al. 2015), whereas we focused our study in adult (reproductive) trees. Supporting this idea, we found that plant ontogeny significantly affected resin duct production (significant effect of year; table 1), where RD density in the EW was significantly greater at younger stages and RD density in LW was significantly greater at older stages (fig. 2). Previous studies have commonly reported increased resin production with tree age in other tree species (Bryant and Julkunen-Tiitto 1995), but to our best knowledge this is the first study reporting plant ontogenetic effects on resin duct production.

Finally, our results showed that local climate at the population origin significantly explained resin duct differences among populations. Specifically, RD density in EW was negatively correlated with summer temperature, whereas RD density in LW was positively correlated with spring temperature ($r = -0.89$ and $r = 0.72$ respectively; $p < 0.05$). Accordingly, our results suggest that pine trees from populations at warmer regions produce more RD in the late growing season, while resin duct development takes place earlier in trees from populations at colder regions. Supporting our findings, Esteban et al. (2012) found that temperature at the population origin negatively affected resin duct investment among *P. nigra* populations.

Despite the implications of G×E interactions for tree breeding, to date most breeding programs have been developed based on genetic or environmental factors, disregarding the effect of their interaction. Our results indicate that, despite we observed significant effects of plant ontogeny and environment on resin duct production,

Figure 2—Mean annual RD density (RD mm^{-2} yr^{-1}) in the earlywood and latewood of *Pinus pinaster* trees in both common gardens (white dots for Cabañeros and black dots for Riofrío). Values represent mean annual RD density in three different 10-year periods along tree lifespan.

intraspecific genetic variation in this anatomical defence can be exploited in breeding programs for stable resistance, due to the absence of G×E effects. Moreover, the contribution of climatic clines explaining among population differentiation highlights the importance and putatively adaptive value of this trait. However, other processes that generate non-adaptive intraspecific genetic variation (e.g., genetic drift and demographic or migratory patterns) can also contribute to explain phenotypic differences among populations (Grivet et al. 2010). For this reason, in order to fully understand the adaptive value of this anatomical defensive trait, further work should also take into account the effect of neutral genetic variation.

LITERATURE CITED

Bannan, M. 1936. Vertical resin ducts in the secondary wood of the Abietineae. New Phytologist. 35: 11–46.

Barton, K.E.; Koricheva, J. 2010. The ontogeny of plant defense and herbivory: characterizing general patterns using meta-analysis. The American Naturalist. 175: 481–493.

Boege, K.; Marquis, R.J. 2005. Facing herbivory as you grow up: the ontogeny of resistance in plants. Trends in Ecology & Evolution. 20: 441–448.

Bryant, J.P.; Julkunen-Tiitto, R. 1995. Ontogenic development of chemical defense by seedling resin birch: energy cost of defense production. Journal of Chemical Ecology. 21(7): 883–896.

Endara, M.J.; Coley, P.D, 2011. The resource availability hypothesis revisited: a meta-analysis. Functional Ecology. 25: 389–398.

Esteban, L.G.; Martin, J.A.; de Palacios, P.; Fernandez, F.G. 2012. Influence of region of provenance and climate factors on wood anatomical traits of *Pinus nigra* Arn. subsp *salzmannii*. European Journal of Forest Research. 131: 633–645.

Ferrenberg, S.; Kane, J.M.; Mitton, J.B. 2014. Resin duct characteristics associated with tree resistance to bark beetles across lodgepole and limber pines. Oecologia. 174: 1283–1292.

Franceschi, V.R.; Krokene, P.; Christiansen, E.; Krekling; T. 2005. Anatomical and chemical defenses of conifer bark against bark beetles and other pests. New Phytologist. 167(2): 353–376.

Fritts, H. 2012. Tree rings and climate. Elsevier. 582 p.

Gonzalo, J. 2008. Phytoclimatic analysis of the Spanish Peninsula: update and geostatistical analysis. University of Valladolid; Palencia; Spain. Ph.D. dissertation.

Grissino-Mayer, H.D. 2001. Evaluating crossdating accuracy: a manual and tutorial for the computer program COFECHA. Tree-Ring Research.

Grivet, D.; Sebastiani, F.; Alía, R. [et al.]. 2010. Molecular footprints of local adaptation in two Mediterranean conifers. Molecular Biology and Evolution. 28: 101–116.

Hannrup, B.; Cahalan, C.; Chantre, G. [et al.]. 2004. Genetic parameters of growth and wood quality traits in *Picea abies*. Scandinavian Journal of Forest Research. 19: 14–29.

Martin, J.A.; Esteban, L.G.; de Palacios, P.; Fernandez, F.G. 2010. Variation in wood anatomical traits of *Pinus sylvestris* L. between Spanish regions of provenance. Trees-Structure and Function. 24: 1017–1028.

Moreira, X.; Zas, R.; Solla, A.; Sampedro, L. 2015. Differentiation of persistent anatomical defensive structures is costly and determined by nutrient availability and genetic growth-defence constraints. Tree Physiology. 35: 112–123.

Moreira, X. Q.; Sampedro, L.; Zas, R.; Solla, A. 2008. Alterations of the resin canal system of *Pinus pinaster* seedlings after fertilization of a healthy and of a *Hylobius abietis* attacked stand. Trees-Structure and Function. 22: 771–777.

Phillips, M.A.; Croteau, R.B. 1999. Resin-based defenses in conifers. Trends in Plant Science. 4: 184–190.

Rosner, S.; Hannrup, B. 2004. Resin canal traits relevant for constitutive resistance of Norway spruce against bark beetles: environmental and genetic variability. Forest Ecology and Management. 200: 7–87.

R Studio Team. 2016. R Studio: Integrated Development for R. Boston: R Studio, Inc. http://www.rstudio.com/. [Date accessed: 5/1/20].

Westbrook, J.W.; Resende, M.F.R.; Munoz, P. [et al.]. 2013. Association genetics of oleoresin flow in loblolly pine: discovering genes and predicting phenotype for improved resistance to bark beetles and bioenergy potential. New Phytologist. 199: 89–100.

Wu, H.; Hu, Z.H. 1997. Comparative anatomy of resin ducts of the Pinaceae. Trees. 11: 135–143.

Zas, R.; Moreira, X.; Ramos, M. [et al.]. 2015. Intraspecific variation of anatomical and chemical defensive traits in Maritime pine (*Pinus pinaster*) as factors in susceptibility to the pinewood nematode (*Bursaphelenchus xylophilus*). Trees-Structure and Function. 29: 663–673.

Poster Session

AN ANSWER AWAITING A QUESTION: THE UNITED KINGDOM NATIONAL TREE SEED PROJECT COLLECTIONS

Alice R. Hudson[1], Richard J.A. Buggs[2,3], and Clare Trivedi[4]

Forest trees are facing an increasing range of threats from pests and pathogens globally. Because of this, research into both natural and induced resistance is more important than ever to provide a solid foundation for informed management decisions. Despite this, there are a wide range of challenges researchers face including easy access to suitable native plant material. Here we present a forest tree resource developed by the Royal Botanic Gardens (RBG) Kew Millennium Seed Bank Partnership. The UK National Tree Seed Project (UKNTSP) was established in 2013 with the aim of collecting and storing seeds from all UK trees across their native UK distribution to capture as much the genetic diversity as possible. Now in its fifth year, this collection totals over 9.6 million seeds, with multi-provenance collections for 68 native UK species. For 22 species, we have between 8 and 53 seed collections banked by individual maternal trees many of which are georeferenced. This broad suite of species covers many facing particular plant health threats in the UK and elsewhere such as *Fraxinus excelsior*, *Juniperus communis*, and *Ulmus glabra*. This unique resource offers the potential to study natural variability in pest and pathogen resistance, pest-host relationships, as well as the possibility to test control mechanisms. This resource is ready and available for research via the Millennium Seed Bank (MSB) Seed List along with accompanying field data and herbarium specimens.

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GENOTYPIC DIVERSITY AND REPRODUCTIVE BIOLOGY OF *THEKOPSORA AREOLATA*, THE CAUSAL AGENT OF CHERRY SPRUCE RUST IN NORWAY SPRUCE SEED ORCHARDS

Hernán Capador[1], Berit Samils[1], and Åke Olson[1]

Swedish forestry is sustained by planting about 200 million Norway spruce (*Picea abies*) seedlings every year. Most of these seedlings come from seed orchards, which have been established to transfer the genetic gain in growth and yield achieved through plant breeding since the 1940s. However, today there is a deficit of seeds coming from Swedish seed orchards mainly caused by irregular flowering, but also due to pest and pathogen infections such as *Thekopsora areolata*—the causal agent of cherry spruce rust, a fungus that significantly reduces seed production in Fennoscandia. Here, we aim to investigate the reproduction mode and population structure of the pathogen using a newly developed microsatellite marker and a hierarchical sampling strategy. Sampling was done at different locations: one in Norway, one in Finland and five in Sweden. For each location, one aecium per infected cone was analyzed. In addition, multiple aecia per scale and cone were sampled at two locations in Sweden. The results show an overall high genetic diversity in *T. areolata* at all hierarchical levels with no genetic structure, an indication of high gene flow and random mating. However, at the cone/scale level non-random mating was observed. These results suggest that *T. areolata* has long distance spore dispersal in Fennoscandia with common recombination events and vegetative spread in cones and scales.

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AN IMPROVED CHINESE CHESTNUT GENOME

John E. Carlson[1], Margaret E. Staton[2], Charles Addo-Quaye[1,3], Nathaniel Cannon[1], Tetyana Zhebentyayeva[4], Nurul Islam-Faridi[5], Jiali Yu[2], Matthew Huff[2], Shenghua Fan[6], Anna O. Conrad[6], Stephan C. Schuster[1,7], Albert G. Abbott[6], Jared Westbrook[8], Jason Holliday[9], C. Dana Nelson[10], Laura Georgi[11], and Frederick V. Hebard[11]

SUMMARY

The introduction of the chestnut blight fungus (*Cryphonectria parasitica*) to North America in 1904 devastated American chestnut populations. Asian Chestnut species, which evolved resistance to the sympatric chestnut blight fungus, are being used as donor species for the transfer of resistance genes to *C. dentata* and *C. sativa* via hybridization. In the United States, Chinese chestnut (*Castanea mollissima*) genotypes are the source of blight-resistance genes for introgression into American chestnut by backcross breeding. To better understand the genetic basis of blight resistance and to provide tools for chestnut breeding, we sequenced the genome of Chinese chestnut, with a particular focus on the major blight resistance quantitative trait locus (QTL). A draft genome (v.1.1) covering app. 90 percent of the genome of The American Chestnut Foundation's Chinese chestnut cultivar "Vanuxem" was released to the public in January 2014. Recombinant DNA clones covering the three major blight resistance QTL were also sequenced to great depth. Over 780 genes were identified in the 3 blight resistance QTLs, including 15 known "defense response" genes. We are developing a new version of the Chinese chestnut genome with chromosome-scale assemblies of genome scaffolds anchored to the 12 chestnut linkage groups. This will serve as a reference for genome-wide selection in advanced generations of backcross breeding programs, and for basic research on genome structure and function in woody plants.

GOALS

The overarching goals of the public Chinese chestnut genome project were to:

(1) Construct a complete genome sequence for *Castanea mollissima*

(2) Identify candidate genes for *Chryphonectria parasitica* (chestnut blight) resistance.

(3) Provide tools to accelerate breeding and restoration of *C. dentata* (American chestnut)

PROGRESS

Version 1 of the Chinese Chestnut Genome

The first version of the genome assembly was produced for the TACF cultivar Vanuxem (fig. 1). Derived from 60 Gb of 454 and Illumina NGS sequence data, the assembly comprised 724.4 Mb in 41,270 scaffolds which averaged app. 40,000 bp in length (table 1). This represented 91 percent of the predicted size of the Chinese chestnut genome, based on estimates from previous

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Figure 1—Fred Hebard and Laura Georgi with a clone of the cultivar "Vanuxem" at Meadowview, VA.

Table 1—Sequencing and assembly statistics for the first genome assembly, v1.1

METRIC	Assembly 1.1, 2014 (available at hardwoodgenomics.org)
genomic DNA sequence	**60 Billion nucleotides** of (75X depth) • 454 sequence of 500 bp DNA fragments • Illumina paired-end sequence (2Kb & 8Kb)
Assembly of contigs:	• 323,611 contigs (2 to 1.87 Kb) • **41,270 scaffolds** • 724 Mbases assembled • 90.5% genome coverage • Scaffolds 2K to 429K bases
Gene models:	• **36,478 genes** predicted
Resistance QTL	• Three blight-resistance QTL sequenced, assembled, and genes identified
Physical Map Coverage:	• 92% of BAC sequences mapped to scaffolds • Ns (gaps) in scaffolds = 2 %

cytometry studies. A total of 36,146 likely genes were identified. The quality and contiguity of the version 1.1 genome for Chinese chestnut proved suitable for genome-wide studies of genetic diversity, gene expression, DNA marker development, and evolutionary studies. LaBonte et al. (2018) used the version 1.1 genome to detect signatures of selection for genes related to flower phenology and development, fruit maturation, and secondary metabolism, and genes potentially impacted by domestication in the Chinese Chestnut genome. We conducted initial genome-wide comparative genome structure and evolution analyses, which revealed the levels of similarities with genes from other plants, and the extent to which large blocks of syntenies are shared between chestnut and model genome such as peach, grape, and poplar (fig. 2).

Version v1.1 of the Chinese chestnut genome was released to the public as a genome browser in January 2014 at the website https://hardwoodgenomics.org/chinese-chestnut-genome, developed and curated by Margaret Staton, along with browsers for the three chestnut-blight resistance QTL. For the blight resistance QTL, sets of overlapping BAC recombinant DNA clones that physically spanned each QTL region were separately sequenced and assembled into a total of 395 scaffolds covering 13.8 Mb (table 2). Over 1,900 genes were found in the QTLs, including 194 known stress-response genes, from which 15 candidate genes for blight resistance were selected for further study (Fang et al. 2013). Annotations of the QTL gene sequences were used to conduct GO functional category analyses. From

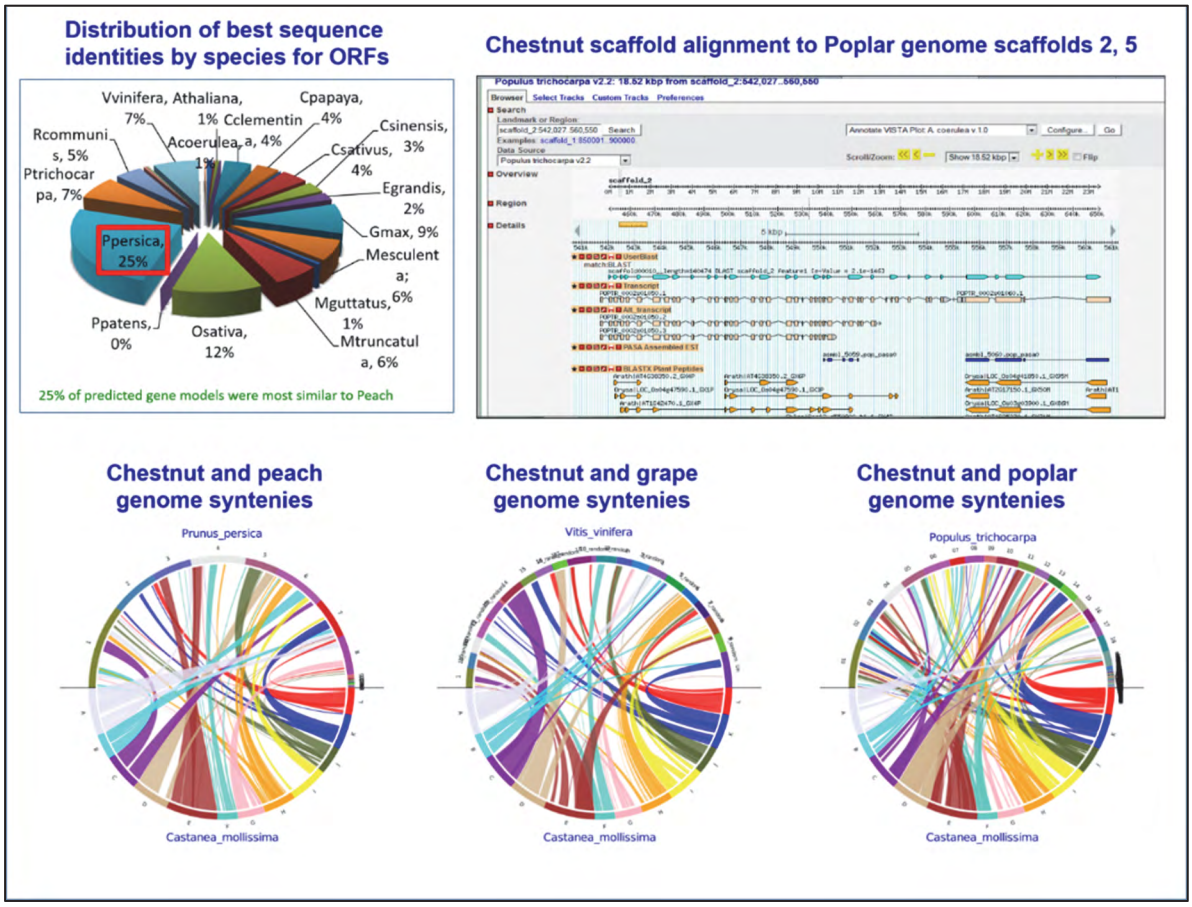

Figure 2—Examples of synteny analyses to model plant genomes and plant gene sequence databases.

Table 2—Chestnut blight-resistance QTL assembly and gene content information

QTL	LG	# scaffolds	# bases / QTL	Total # Genes	# Genes / Mbases	# Stress-response genes
cbr1	**B**	214	6.77Mb	994	151	98
cbr2	**F**	128	4.12Mb	548	137	58
cbr3	**G**	53	2.99Mb	410	139	38

the genes classified as disease- or stress-response, 15 of the most likely candidate genes for chestnut blight resistance were identified (table 3) for future transgenic studies. The genome and the QTL browsers have had thousands of visits from across the globe, for gene searches and downloads of genome and QTL scaffolds, genes, transcripts, and predicted protein data.

Improvements to the Chinese Chestnut Genome

For application of the chestnut reference genome in Genome-Wide-Selection to advance back-cross breeding and disease resistance introgression programs, such as the TACF is conducting, chromosome-scale sequences assemblies of scaffolds are required. For the past 4 years,

Table 3—Fifteen candidate disease resistance genes in Chinese chestnut blight-resistance QTL selected for future functional studies

Seq. Name	Seq. Description	Closest matching NCBI nr protein
cbr1_scaffold114-gene-0.3-mRNA-1	transcription factor tga1	Transcription factor TGA1 (Vitis vinifera)
cbr1_scaffold134-gene-0.0-mRNA-1	cc-nbs-lrr resistance protein	Putative disease resistance protein RGA3 (Vitis vinifera)
cbr1_scaffold16-gene-0.12-mRNA-1	rna recognition motif-containing protein	PREDICTED: DAZ-associated protein 1-like (Vitis vinifera)
cbr1_scaffold17-gene-0.29-mRNA-1	beta-hydroxyacyl-acp dehydratase	predicted protein (Populus trichocarpa)
cbr1_scaffold28-gene-0.12-mRNA-1	transcription factor tga1	TGA transcription factor 1 (Populus tremula x Populus alba)
cbr1_scaffold32-gene-0.28-mRNA-1	14-3-3-like protein gf14 lambda	hypothetical protein ARALYDRAFT_496774 [Arabidopsis lyrata subsp. lyrata)
cbr1_scaffold4-gene-0.38-mRNA-1	multicatalytic endopeptidase complex	proteasome subunit alpha type-7 (Vitis vinifera)
cbr1_scaffold61-gene-0.11-mRNA-1	disease resistance protein at4g27190-like	PREDICTED: disease resistance protein At4g27190-like (Vitis vinifera)
cbr2_scaffold29-gene-0.6-mRNA-1	cc-nbs-lrr resistance protein	cc-nbs-lrr resistance protein (Populus trichocarpa)
cbr2_scaffold34-gene-0.3-mRNA-1	feronia receptor-like kinase	Serine/threonine-protein kinase PBS1, putative (Ricinus communis)
cbr2_scaffold3-gene-0.42-mRNA-1	Protein	PREDICTED: MLO protein homolog 1-like (Glycine max)
cbr2_scaffold5-gene-0.9-mRNA-1	transferring glycosyl	transferase, transferring glycosyl groups, putative (Ricinus communis)
cbr3_scaffold1-gene-1.1-mRNA-1	histone-lysine n-methyltransferase ashh2-like	PREDICTED: uncharacterized protein LOC100245350 (Vitis vinifera)
cbr3_scaffold1-gene-1.19-mRNA-1	set domain protein	PREDICTED: uncharacterized protein LOC100245350 (Vitis vinifera)
cbr3_scaffold28-gene-0.8-mRNA-1	cysteine proteinase rd19a	Cysteine proteinase RD19a (Arabidopsis thaliana)

we worked on producing such an improved, chromosome-scale version of the Chinese chestnut genome. The approach taken to accomplish this involved merging assembled sequence contigs and scaffolds from the v1.1 genome using longer genome sequences, followed by the anchoring of the larger scaffolds to positions on genetic linkage maps based on alignment to DNA marker sequences defining loci on the genetic maps (fig. 3). Taking this approach, we used long PACBio sequences (over 10 Kb in length) to bridge contigs into scaffolds and close gaps within scaffolds, reducing the number of genome scaffolds to 12,684, covering 784 Mb of genome sequence (table 4). By aligning sequences from BAC clones distributed across the physical length of the genome (Fang et al 2013), we estimated that app. 98 percent of the estimated genome size was included in the new set of scaffolds. Margaret Staton's group used our RNA sequence resources for chestnut to find and annotate 30,832 high quality gene models in the new assembly. Pseudo-chromosome sequences were assembled by anchoring 4,099 of the scaffolds to DNA markers in the reference genetic linkage map for chestnut (Kubisiak et al. 2013) (fig. 3D), and the integrated genetic-physical map for Chinese chestnut (Fang et al., 2013) (fig. 3A). The pseudo-chromosome sequences accounted for 421.3Mb, representing about 60 percent of the full genome.

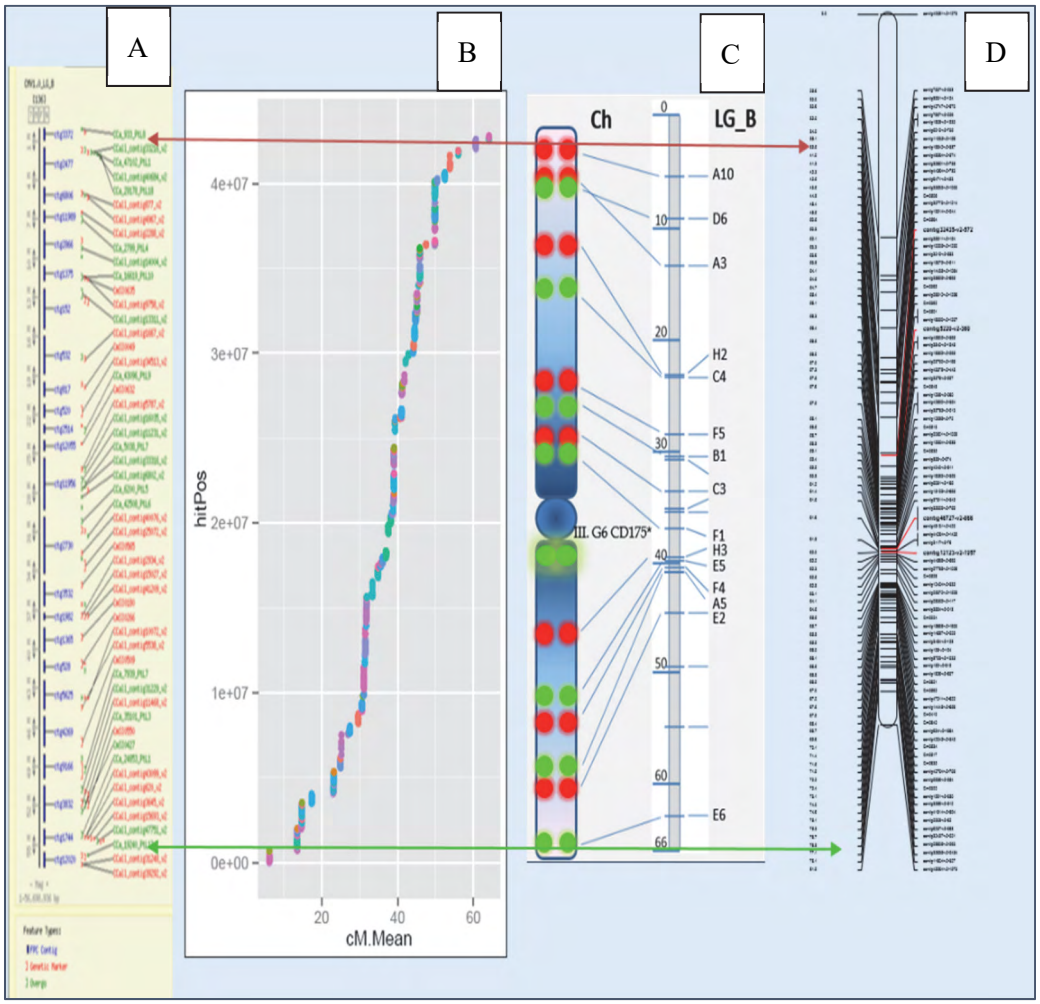

Figure 3—Illustration of approach taken in building pseudo-chromosome sequences by alignment and anchoring to genetic, physical, and cytology maps. (A) Example of physical map integrated with linkage group, (B) a chromosome-scale assembly of scaffolds, (C) cytogenetic map of linkage group DNA markers, and (D) chestnut genetic linkage map alignment example.

Table 4—Statistics for assembly improvements and initial anchoring of scaffolds to genetic maps

METRIC	Chromosome-scale assembly of the Chinese chestnut genome
Contig Assembly	• **421.3Mb (**4,099 contigs) assembly of scaffolds anchored to *C. mollissima* chromosomes (genetic linkage groups) • Anchored sequence per chromosome ranged from 25.9Mb (LG_L) to 60.2 Mb (LG_A) • 303.9Mb (10,011 contigs) unanchored contig sequences • 57.8Mb estimated gaps (based on estimated genome size of 794Mb)
Gene models:	• 30,832 high quality gene models identified in V3.2 • 20,770 high quality gene models in anchored contigs
Validations:	• BUSCO reported 1,355 of 1,440 expected single-copy genes are complete and present within the *C. mollissima* genome • Alignments of BAC end sequences from the *C. mollissima* physical map confirmed order of contigs in the pseudochromosomes

Gene positions and overall genome organization of the assembly were determined and compared to the genomes of other related trees and model plant systems. A manuscript on this chromosome-scale assembly (table 4) was published as a preprint on April 22, 2019 (Staton et al. 2019). Submission of a peer-reviewed manuscript is planned, following efforts to anchor more scaffold to increase gene and genome coverage in the pseudo-chromosomes.

FUTURE RESEARCH DIRECTIONS

The current assembly, although an incomplete draft, does by virtue of chromosome-scale sequences provide a significant advancement in our ability to investigate genome organization and the evolution and genetic structure of important traits such as disease resistance, as well as applications such as genome-wide selection. Future improvement of the assembly may be achieved through the use of more recent long-read technologies, such as Nanopore (Madoui et al. 2015), and/or scaffolding with chromatin-interaction data, such as Hi-C (Jiao and Schneeberger 2017). However even these approaches may result in less than full-genome assembly given the challenges of high heterozygosity levels and an inability to generate dihaploid individuals in Chinese chestnut. The recently published *Quercus robur* genome (Plomion et al. 2018) utilized synteny with the *Prunus persica* genome to order incorporate contigs and scaffolds that had not been assembled *de novo* nor scaffolded with oak genetic map markers. This approach assumes that micro-level syntenies follow known macro-syntenies based on genetic maps, which may not always hold true. However, the hybrid synteny approach could also complement long-read technologies in future Chinese chestnut genome improvements.

Data Availability

The contigs, scaffolds, and pseudochromosome sequences are available at the NCBI BioProject No. PRJNA46687, and are also available for download and query at the Hardwood Genomics Project website (https://www.hardwoodgenomics.org/genomes).

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REFERENCES

Fang, G.C.; Blackmon, B.P.; Staton, M.E. [et al.]. 2013. A physical map of the Chinese chestnut (*Castanea mollissima*) genome and its integration with the genetic map. Tree Genetics & Genomes. 9(2): 525–537.

Jiao, W.B.; Schneeberger, K. 2017. The impact of third generation genomic technologies on plant genome assembly. Current Opinion in Plant Biology. 36: 64–70.

Kubisiak, T.L.; Nelson, C.D.; Staton, M.E. [et al.]. 2013. A transcriptome-based genetic map of Chinese chestnut (Castanea mollissima) and identification of regions of segmental homology with peach (Prunus persica). Tree Genetics and Genomes. 9(2): 557–571.

LaBonte, N.R.; Zhao, P.; Woeste, K. 2018. Signatures of selection in the genomes of Chinese chestnut (Castanea mollissima Blume): the roots of nut tree domestication. Frontiers in Plant Science. 9: 810.

Madoui, M.A.; Engelen, S.; Cruaud, C. [et al.]. 2015. Genome assembly using Nanopore-guided long and error-free DNA reads. BMC Genomics. 16(1): 327.

Plomion, C.; Aury, J.M.; Amselem, J. [et al.]. 2018. Oak genome reveals facets of long lifespan. Nature Plants. 4(7): 440–452.

Staton, M.; Addo-Quaye, C.; Cannon, N. [et al.]. 2019. The Chinese chestnut genome: a reference for species restoration. BioRxiv. 615047.

ADVANCED PHENOTYPING USING FT-IR DISTINGUISHES DISEASE RESISTANCE IN *FRAXINUS EXCELSIOR* AGAINST *HYMENOSCYPHUS FRAXINEUS*

Caterina Villari[1], Arnaud Dowkiw[2], Rasmus Enderle[3], Marjan Ghasemkhani[4], Thomas Kirisits[5], Erik Kjaer[6], Diana Marčiulynienė[7], Lea McKinney[6], Berthold Metzler[3], Facundo Muñoz[2], Lene Rostgaard Nielsen[6], Alfas Pliūra[7], Lars-Göran Stener[8], Vytautas Suchockas[7], Luis Rodriguez-Saona[9], Pierluigi Bonello[10], and Michelle Cleary[4]

The invasive ascomycete fungus *Hymenoscyphus fraxineus* has caused large scale population decline of European ash (*Fraxinus excelsior*) throughout much of its distribution range in Europe. Large genotypic variation in susceptibility to the pathogen exists in the natural population, albeit resistance occurs at a low level. Damage traits associated with the pathogen are strongly genetically controlled, which suggests that considerable gain can be achieved through selection and breeding. Large potential exists for utilizing modern approaches or tools that can quantitatively identify phenotypes and help explain the genetic basis of important plant traits, such as disease resistance. One main obstacle to making such advances is the lack of viable tools for rapid resistance phenotyping. Vibrational spectroscopy is one approach that has been used successfully for rapid phenotyping for plant resistance. In this study, we used Fourier-transform Infrared (FT-IR) spectroscopy coupled with a chemometric model to discriminate between resistant and susceptible European ash genotypes. Non-infected leaves and stem samples from known susceptible and resistant *F. excelsior* genotypes were collected from six European countries. Purified phenolic extracts were analyzed on a FT-IR spectrometer and soft independent modeling of class analogy (SIMCA) was used to discriminate between resistant and susceptible trees. The model built with stem samples, and subsequently validated using random blind samples, gives powerful evidence that FT-IR can clearly discriminate between susceptible and resistant genotypes. These results suggest that spectroscopic phenotyping tools may allow for superior genotypes to be quickly identified and employed in restoration efforts.

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DELPHI EXPERT OPINION SURVEY TO ASSESS THREATS TO OAKS IN THE EASTERN UNITED STATES

Anna O. Conrad[1], Ellen V. Crocker[1], Xiaoshu Li[1], Billy Thomas[1], Thomas Ochuodho[1], and C. Dana Nelson[2]

Oaks are important fixtures of many Eastern United States forests, providing both ecological and economic benefits. While regeneration is a major issue impacting oaks currently, biotic (e.g., pests and pathogens) and abiotic (e.g., abnormal weather and climate change) stressors, may also threaten oaks in this region. The goal of our Delphi expert opinion survey is to identify the most significant threats (biotic and abiotic) to oaks in the Eastern United States (as defined by the eastern and southern regions of the U.S. Forest Service), and to gauge the potential impact of these threats on oaks. To accomplish this, we initiated a three-part Delphi expert opinion survey. The iterative Delphi approach is useful for evaluating consensus (or lack thereof) among experts on a specific topic. In the course of this survey series, we asked experts to identify current and future biotic and abiotic threats to oaks, and then based on expert opinions, gauged the current and potential impact of these threats by asking a series of questions concerning, for example, their spatial and temporal manifestation. Data collected as part of this Delphi survey series will be used to support subsequent analyses aimed at assessing the economic impact of these threats, and may be useful for prioritizing the management of these threats within the Eastern United States.

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TREESNAP: A CITIZEN SCIENCE APP CONNECTING TREE ENTHUSIASTS AND FOREST SCIENTISTS

Title presented at workshop:
TREESNAP: A MOBILE APP TO HELP YOU ENGAGE THE PUBLIC IN RESEARCH

Bradford Condon[1], Ellen Crocker[2], Abdullah Almsaeed[3], C. Dana Nelson[3], Albert G. Abbott[2], and Margaret Staton[1]

SUMMARY
(from *Plants, People, Planet* PPP article)

- TreeSnap is a citizen science project that enables members of the public to observe and report trees of interest to scientists with a focus on tree species impacted by invasive pests and pathogens.
- TreeSnap includes a free mobile app that guides users in creating an observation, including answering questions customized for each tree species, taking relevant photos, and automatically collecting the GPS coordinates.
- This utility of a mobile app and online database for tree observations has been successful beyond the initial project plan, facilitating scientific data collection by both citizen scientists and professional scientists.
- Technology designed to be intuitive and comprehensive for the needs of both professional and nonprofessional scientists, as demonstrated by TreeSnap, can greatly expand the reach of tree research programs and engage the public in forest health research.

Note: For complete abstract, please see paper 49 (page 92).

The full paper was published in *Plants, People, Planet*
https://nph.onlinelibrary.wiley.com/toc/25722611/2020/2/1.

The reference for the published paper is:

Crocker, E.; Condon, B.; Almsaeed, A.; Jarret, B.; Nelson, C.D.; Abbott, A.G.; Main, D.; Staton, M. 2019. TreeSnap: A citizen science app connecting tree enthusiasts and forest scientists. People, Plants, Planet. 2(1): 47–52. DOI: https://10.1002/ppp3.41.

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PHYTOPHTHORA RAMORUM IN LARCH: FROM EPIDEMIOLOGY TO HOST RESISTANCE

Title presented at workshop:
UNDERSTANDING SUDDEN LARCH DEATH—FROM EPIDEMIOLOGY TO HOST RESISTANCE

Heather Dun[1,2], John Mackay[1], and Sarah Green[2]

Phytophthora ramorum is a forest pathogen known for two major epidemics, sudden oak death in the USA, which began in the 1990s (Rizzo et al. 2002) and, more recently, sudden larch death in the United Kingdom in which *P. ramorum* was first found infecting Japanese larch in south-west England in 2009. This was the first record of *P. ramorum* infecting a conifer species in a natural situation (Webber et al. 2010). In spring 2013 there was an observed dramatic increase in the spread and severity of *P. ramorum* in larch stands across western Britain, including in the Galloway Forest. The Galloway Forest is the largest forest in the UK and provides a particularly interesting field site for studying the epidemiology of *P. ramorum* in larch as disease control legislation for the 2013 epidemic resulted in a management zone where infected stands could be retained for research rather than being immediately felled. The Galloway Forest outbreak was caused by the EU2 lineage of *P. ramorum* which is particularly virulent on Japanese larch (*Larix kaempferi*) and European larch (*Larix decidua*) (King et al. 2014), two important timber species in the UK accounting for 10 percent of all UK conifer plantations.

A better understanding of the epidemiology of sudden larch death is needed to inform management decisions and maintain larches as commercial timber species. Survivor trees within high mortality stands in the Galloway Forest suggest the possibility of natural resistance within the Japanese larch population. Understanding how the pathogen spreads within and between individuals will help to predict if survivor trees have escaped infection by chance or may actually be resistant.

In order to understand disease spread, 21 individual larch trees in each of 21 plots located in larch stands of varying ages were surveyed for disease symptoms each May and September from 2016 to 2018. In addition to recording symptoms on needles, shoots, branches, and main stem, samples of lesion material were taken from central trees in each plot for pathogen isolation by selective media and qPCR to confirm infection by *P. ramorum*. In September 2017 and 2018 symptomatic foliage was collected and 20 needles were stained with lactophenol blue and the abaxial and adaxial surfaces examined for *P. ramorum* sporulation.

Climate records from the Met Office Hadley Centre Observations provide a homogeneity-adjusted series of averaged precipitation across areas of the UK. Our study sites lie within the South Scotland area, which uses records from eight weather stations. Three of these stations are located within 50 km of our study sites.

Between the 2013 epidemic and the start of our surveys in September 2016 disease levels had been stable and spread both within and between stands was limited. Observations in our plots between 2016–2018 showed that disease spread varied between years. In the first survey in September 2016 all disease symptoms were recorded at low levels, with on average < 5 percent of each tree being affected. The main stem mortality was on average higher at 8 percent as this included the trees that had died and were recorded as 100 percent mortality (fig. 1).

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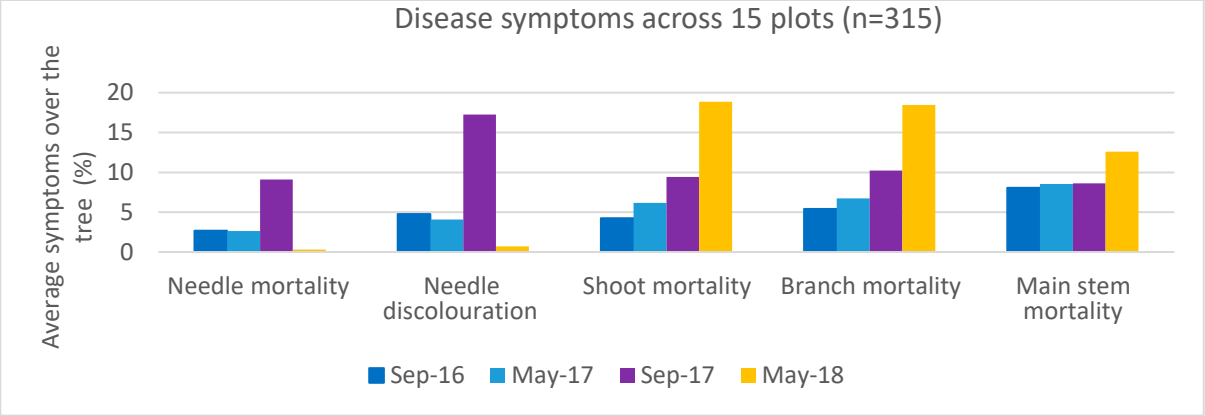

Figure 1—Results of field surveys of *P. ramorum* disease symptoms in Japanese larch in the Galloway Forest. (n = 315) between September 2016 and May 2018. Main stem mortality is a measure of mortality of the main trunk from the apex of the tree down.

Needle mortality and discolouration were similar between September 2016 and May 2017 but fine shoot and branch mortality increased slightly. Needle discolouration was notably higher in September 2017 compared with the previous September, this was followed by a doubling in the average shoot and branch mortality in May 2018 and we infer that this discoloration may have been caused by needle infection by *P. ramorum*. The adaxial and abaxial sides of sample sets of 40 needles from 5 plots were examined for sporangia production but no sporangia were observed on the needles at that assessment time. Successful isolations from lesion material have been made from trees in each plot thus confirming that *P. ramorum* is present across all the plots.

The May 2018 survey found a large increase in *P. ramorum* infection within and between stands (which was also apparent in larch across the wider region) with many trees showing symptoms for the first time in this study and stands that were previously uninfected becoming infected.

The most obvious symptom across a range of plots was a frequently occurring dieback of the fine shoots; these shoots had not flushed at all since spring and were prominent across the crown (fig. 2). These aerial infections appeared to have largely started in the fine, outer shoots of the upper crown and extended down branches towards the main stem. Suggesting significant aerial spread of infection, possibly through windborne inoculum.

There were similarities in disease expansion in the outbreaks in 2013 and 2018 but subsequent lulls in infection in the intervening years. The increase in infection seen in May 2018 brought up the question of why disease spread was much slower between the May 2013 epidemic and May 2018. Comparing our survey results over time with climate records also allows us to consider the possible links of climatic conditions to epidemic spread of *P. ramorum*.

Figure 2—Fine shoot and branch dieback on Japanese larch in Galloway Forest in May 2018. (Courtesy photo by Heather Dun)

Comparison to climate records suggests that the disease expansions observed in 2013 and 2018 were preceded by record wet summers (fig. 3). Monthly rainfall between 2012 and 2018 is highly variable compared to the 18-year average rainfall between 2000 and 2018.

Of particular interest is June rainfall in 2012 and 2017, with double average precipitation preceding both the 2013 and 2018 epidemics whereas the intervening years, in which disease expansion was much slower, had lower than average June rainfall. Less obvious but also worth noting is the higher than average rainfall in July, August, and September in both 2012 and 2017.

We observed an increase in infection of larch characterized by shoot mortality in our field sites in the spring of 2018 and this was also observed across the wider region (Forestry Commission Scotland 2018). Our observations of infection spread over the scale of individual trees allowed us to develop a more detailed understanding of how disease progresses. Previous reports of infection in larch focused on main stem lesions that caused girdling leading to whole tree mortality together with sporangia production on needles (Webber et al. 2010). Shoot dieback after infection with *P. ramorum* has been recorded in multiple woody species, including both deciduous and evergreens in the USA (Hansen et al. 2005). Our observations indicate that fine shoot infection is a mechanism of rapid disease progression in *P. ramorum* in larch.

We propose that high summer rainfall might influence the levels of infection observed at flushing in the following season. It is possible that high summer humidity would facilitate the prolific needle sporulation needed for subsequent epidemic spread. The positive relationship between infection and humidity has been well documented in *P. infestans* (Harrison and Lowe 1989) (Hirst and Stedman 1960). In sudden oak death in the USA sporangia in soil remained infectious over summer for 3 months after a rain event (Fichtner et al. 2007). It is possible that summer rainfall induces sporangia production on needles which then infect shoots over winter, becoming obvious with lack of spring flush. Alternatively the sporangia, if able to overwinter, might infect fine shoots directly in spring.

This study proposes that disease spread involves fine shoot infections and the influence of rainfall in the preceding season. Inoculation experiments would be a useful approach to further test this infection pathway of *P. ramorum* in larch and investigate the effects of climate on epidemiology.

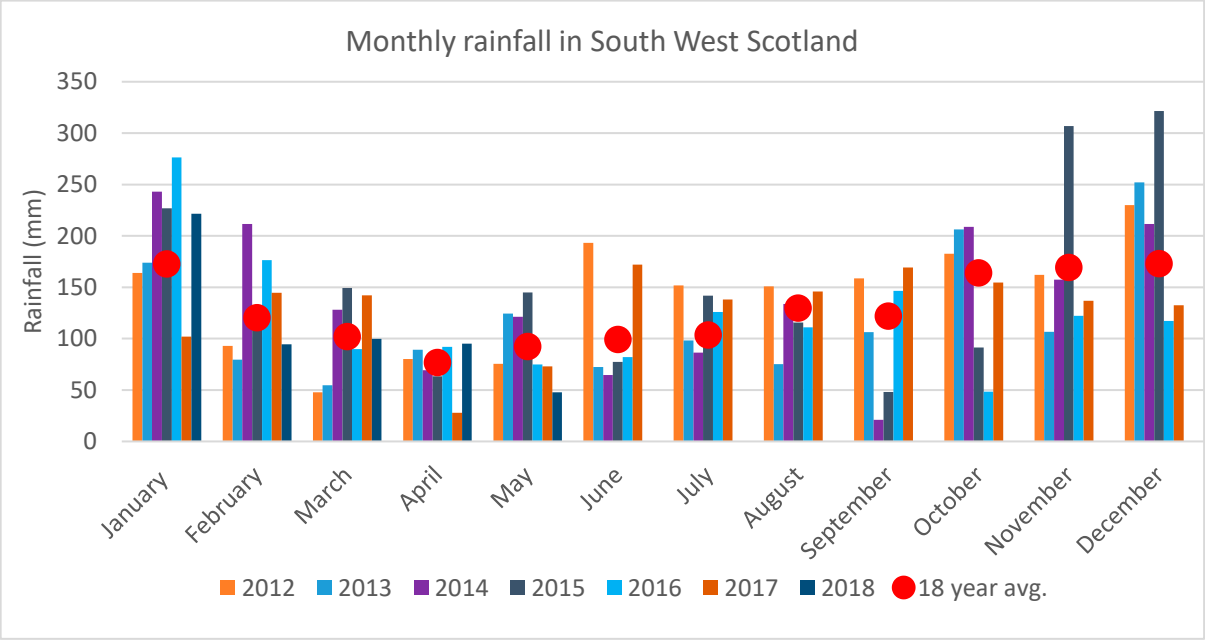

Figure 3—Monthly precipitation in south-west Scotland between 2012–2018 and 18-year average precipitation for each month.

LITERATURE CITED

Fichtner, E.J.; Lynch, S.C.; Rizzo, D.M. 2007. Ecology and epidemiology detection, distribution, sporulation, and survival of Phytophthora ramorum in a California Redwood-Tanoak forest soil. Phytopathology. 10: 1366–1375. https://doi.org/10.1094/PHYTO-97-10-1366.

Forestry Commission Scotland. 2018. 04 December 2018 statutory plant health notices (SPHNs) served for Phytophthora ramorum on Larch sites in woodland settings. https://scotland.forestry.gov.uk/images/corporate/pdf/Phytophthora-ramorum-in-Scotland-map-4-December-2018.pdf. [Date accessed: December 19, 2018].

Hansen, E.M.; Parke, J.L.; Sutton, W. 2005. Susceptibility of Oregon forest trees and shrubs to *Phytophthora ramorum* : A comparison of artificial inoculation and natural infection. Plant Disease. 89(1): 63–70. https://doi.org/10.1094/PD-89-0063.

Harrison, J.G.; Lowe, R. 1989. Effects of humidity and air speed on sporulation of Phytophthora infestans on potato leaves. Plant Pathology. 38: 585–591. https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1365-3059.1989.tb01455.x.

Hirst, J.M.; Stedman, 0.J. 1960. Annals of Applied Biology. 48(3): 471–488.

King, K.; Harris, A.; Webber, J. 2014. Current distribution of the new EU2 lineage of Phytophthora ramorum (PDF Download Available). In: Proceedings of the Sudden Oak Death symposium. https://www.researchgate.net/publication/265468027_Current_distribution_of_the_new_EU2_lineage_of_Phytophthora_ramorum. [Date accessed: April 7, 2020].

Rizzo, D.M.; Garbelotto, M.; Davidson, J.M. [et al.]. 2002. *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and *Lithocarpus densiflorus* in California. Plant Disease. 86(3): 205–214. https://doi.org/10.1094/PDIS.2002.86.3.205.

Webber, J.F.; Mullett, M.; Brasier, C.M. 2010. Dieback and mortality of plantation Japanese larch (Larix kaempferi) associated with infection by Phytophthora ramorum. New Disease Reports. 22(19): 2044–0588. https://doi.org/10.5197/j.2044-0588.2010.022.019.

GEOGRAPHIC AND LOCAL GENETIC VARIATION IN PACIFIC MADRONE LEAF BLIGHT

Laura E. DeWald[1], Marianne Elliott[2], Richard Sniezko[3], and Gary A Chastagner[2]

Pacific madrone (*Arbutus menziesii*) is an evergreen hardwood species in western North America that occurs from British Columbia to southern California. The species has high cultural and specialty wood values, and is also ecologically important for wildlife habitat, wildfire revegetation, and creating midstory canopy biodiversity in mixed conifer forests. Pacific madrone is experiencing unsustainable mortality (Lintz et al. 2016), but reasons for the decline are unknown. Foliar pathogens such as described by Zeller (1934) have been implicated because of their high visibility and association with defoliation. However, very little is known about the genetics of this species, including variation in resistance to pathogens and other adaptive traits.

Range-wide common garden tests were established to examine patterns of genetic variation in traits such as height growth and survival, to identify relative contributions of abiotic, biotic and genetic causes of health declines, and for identifying resistant seed sources. The Washington State University Pacific madrone seed collection contains seed from 320 families in seven ecoregions. Using this seed collection, common gardens were planted at seven locations in 2011 in California (1 site), Oregon (2), Washington (2), and in 2013 in British Columbia (2). These common garden sites are located in four of the seven ecoregions where seed was collected (fig. 1) and consist of 105 half-sib families representing 42 seed sources. Survival and performance data were collected annually and leaf blight, caused by a complex of fungal pathogens, was also rated annually for the first 3 to 5 years, depending on site. In addition to leaf blight, assessments have been made of growth, other diseases, cold damage, flowering, and phenology. Here we summarize variation in leaf blight severity in the OR and WA common gardens.

Across all years and common gardens, average blight severity was slight to moderate (< 25 to 25–50 percent of current leaves affected) with an average incidence of 25–50 percent. Blight symptoms were more severe in years where a common garden test was particularly stressed by drought and/or cold winter temperatures and tended to be more severe at the WA sites (PH, PV) compared to the OR sites (SF, SO). Patterns of blight severity among sites might be related to site conditions such as moisture and temperature. Typically precipitation decreases and temperatures increase from north to south latitude, thus the WA sites would be cooler and wetter than the SF site, which would be cooler and wetter than the SO site. However, the size of the differences in blight symptoms may be magnified since the OR sites were generally assessed in late fall/early winter while the WA sites were assessed in late winter. Subsequent observations in another year on a subset of trees confirm an increasing degree of blight over this time period.

Fungi associated with severe leaf blight, such as *Phacidiopycnis washingtonensis* (Elliott et al. 2014) and *Phomopsis* sp., were not found at the southernmost site (SO) in 2018 samples (fig. 2).

Blight severity within sites varied significantly ($p < 0.01$) among seed sources for all years at all sites except for SO where sources only differed in 2012 (data not shown). Blight severity among half-sib families within sources also varied significantly ($p < 0.01$) for all years and at all sites except at SF where families only

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Figure 1—Locations of madrone common garden sites, seed sources, and ecoregions. (Insert: full range of Pacific madrone). Sites used in this study: PV, PH (WSU Puyallup), SF (Starker Forest), and SO (Sprague Orchard).

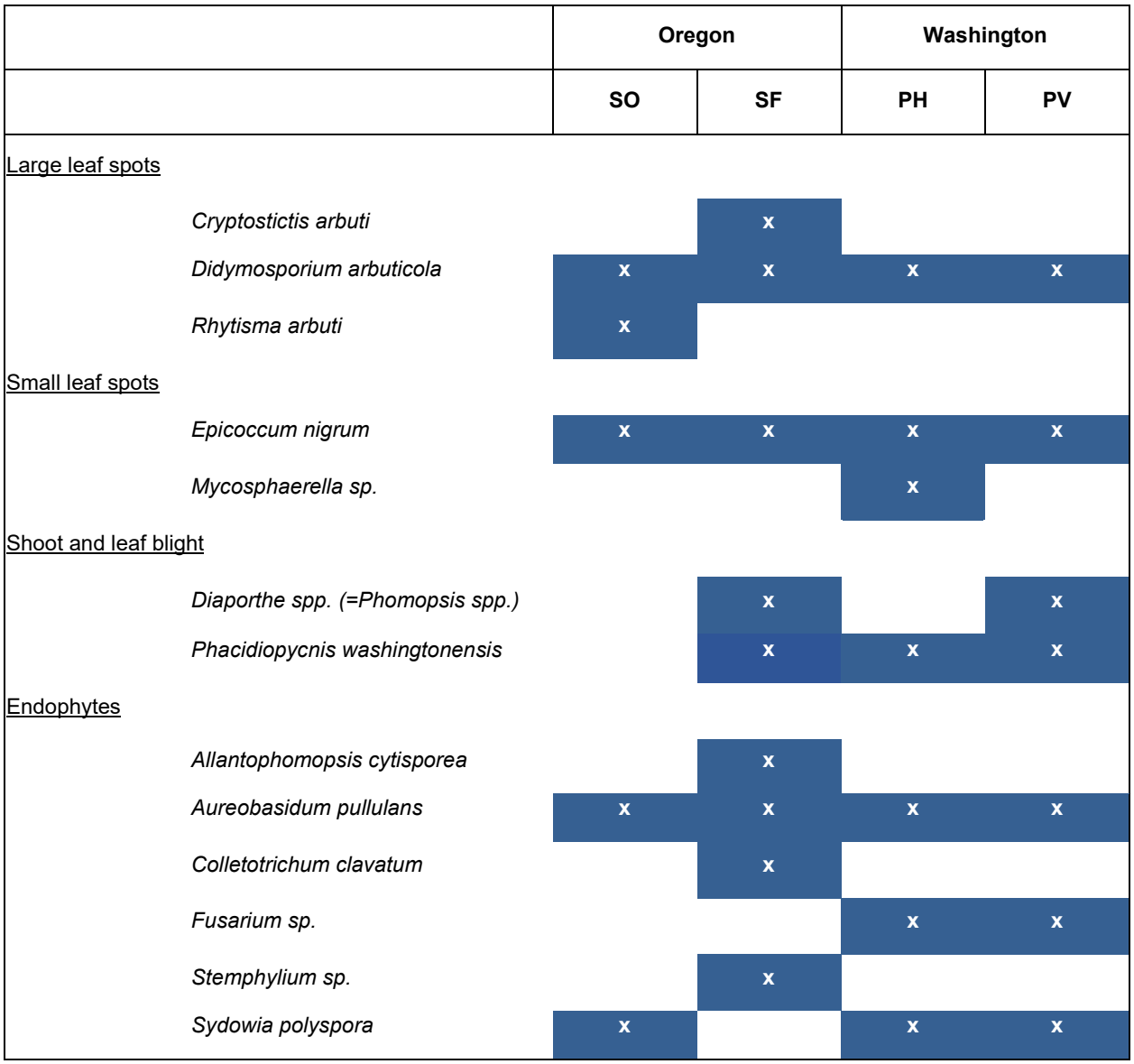

Figure 2—Fungi associated with leaf blight. Fungi were identified from madrone foliage samples at the common garden sites using morphological and molecular techniques, from south (SO) to north (PV) in 2018.

differed in 2015. Averaged across all sites, variation in blight severity among sources was not related to longitude of origin but was significantly ($p < 0.01$) related to latitude of origin, ($R^2 = 0.09$- 0.69), and elevation of origin ($R^2 = 0.13$-0.16). When analyzed separately by site, variation in blight severity among sources was not related to longitude at any site, and at SO variation was also not related to latitude or elevation. Relationships with latitude were strongest for PH ($R^2 = 0.48$-0.65) but at PV latitude was only significantly related in 2014.

The strongest relationships between seed source origin and site were at the most northern common gardens (PH & PV). Averaged across sites, trends in table 1 show that in general, southern sources had higher blight severity than northern sources. Sources with the lowest blight severity were from the north: OH, CL (Oak Harbor and Clinton, WA), OR (Cornelius, OR). Sources with the highest blight severity were from the south: e.g., HC, HP, LA (Humboldt County, Hopland, Los Altos, CA). However, some northern sources such as PA, DE (Port Angeles, WA , Detroit, OR) had relatively

Table 1—Blight severity score averaged across common garden sites for each Pacific madrone seed source for each year

Source	State or Province	Latitude	2012	2013	2014	2015	Mean, all years
SA	California	37.02010	14.1	37.0	34.5	26.5	28.1
SC	California	37.21705	16.2	28.2	26.9	21.2	23.1
LA	California	37.29174	18.2	31.3	31.1	42.3	30.7
CC	California	38.00579	14.6	26.3	30.4	38.2	27.4
SR	California	38.01276	11.8	29.8	32.9	26.0	25.1
CA	California	38.60876	12.8	24.0	22.7	29.2	22.2
PL	California	38.75026	13.2	22.2	16.5	38.0	22.5
HP	California	39.00000	22.3	31.9	31.5	44.0	32.4
BL	California	39.16684	17.5	33.1	26.1	40.2	29.2
RV	California	39.25356	17.1	29.0	30.7	42.7	29.9
SN	California	39.35246	14.1	27.7	21.0	37.9	25.2
GA	California	40.08990	18.2	31.0	26.7	41.4	29.3
HC	California	40.29190	17.2	37.7	31.9	44.4	32.8
KL	California	41.69240	16.2	27.3	18.6	40.7	25.7
RA	Oregon	42.19585	15.4	25.1	18.7	40.6	24.9
BK	Oregon	42.21448	12.2	23.3	25.4	37.3	24.6
CP	Oregon	42.26213	21.0	33.6	17.1	41.6	28.3
GP	Oregon	42.47669	18.0	28.1	17.0	40.5	25.9
CY	Oregon	42.91553	15.4	35.5	16.6	40.4	27.0
BA	Oregon	43.01794	12.4	20.5	17.7	33.1	20.9
OK	Oregon	43.67947	13.3	27.9	15.0	40.0	24.1
VE	Oregon	44.03646	11.6	21.2	12.3	32.2	19.3
DE	Oregon	44.69452	20.3	30.3	17.4	44.4	28.1
AK	Oregon	44.82509	13.4	22.5	14.0	38.0	22.0
ZE	Oregon	45.01889	10.2	19.0	11.8	31.2	18.0
WL	Oregon	45.35004	7.2	23.8	10.4	32.6	18.5
WV	Oregon	45.36323	15.5	24.8	12.4	33.0	21.4
OR	Oregon	45.44143	10.8	18.6	10.5	28.0	17.0
CR	Washington	46.06361	14.4	20.1	13.2	29.6	19.3
PF	Washington	46.84067	15.7	24.4	13.2	37.7	22.7
OL	Washington	47.05435	10.5	15.7	10.9	33.6	17.7
FW	Washington	47.30014	16.1	23.9	13.6	33.2	21.7
KM	Washington	47.30751	14.0	23.1	16.0	35.7	22.2
BR	Washington	47.55823	14.2	23.3	13.6	33.8	21.2
CL	Washington	47.97990	8.6	17.5	10.7	28.6	16.4
LC	Washington	48.06585	14.9	22.4	15.8	40.5	23.4
PA	Washington	48.10687	20.2	29.2	15.8	37.8	25.8
OH	Washington	48.30342	8.4	16.7	10.8	28.5	16.1
FB	Washington	48.48699	8.7	22.1	14.0	31.1	19.0
VI	British Columbia	48.51078	16.5	26.4	16.7	29.0	22.1
CB	Washington	48.64940	14.7	31.1	13.7	32.4	23.0
BC	British Columbia	49.18983	10.7	23.6	13.4	30.0	19.4

Note: Blight severity was scored as a percentage of blighted leaf area on the most severely impacted current season leaves. Blight incidence was based on percent of the whole tree with leaves having the severity rating described above. Severity and incidence scores were multiplied together for an overall severity rating score ranging from 0 to 100, with 100 being highly susceptible.

higher blight severity while some southern sources such as PL, CA (Placerville & Calistoga, CA) had lower blight severity. These differences may be related to the climate at the site where the seed was collected being similar to that of a southern or northern site, respectively, and will be investigated further (Wilhelmi et al. 2017). The source by common garden site interaction (i.e., genotype X environment) was significant for all years ($p < 0.001$).

These results suggest that wetter, cooler conditions at a site seem to increase blight severity. Sources that moved the farthest north generally had more severe blight. These sources may have been less well adapted, thus more stressed and less able to resist leaf blight. In addition, the results suggest that resistance to leaf blight might exist. Madrone seed sources collected within the ecoregion of a common garden had both high and low blight severity. Blight severity also varied among half-sibling families within seed sources. Relative blight severity of some sources was not consistent across all common garden sites. This indicates that blight symptoms are likely caused by a complex of biotic and abiotic factors. Relatively few details of the blight dynamics were known and assessments of these trials increase it substantially. Detailed observations in 2018 on a small subset of seedlings indicate that blight severity continues to increase from November through May. In addition, some trees double or triple flush and the later flushes tended to have lower blight severity. This information will be useful in planning the timing of future assessments to examine genetic variation in blight. Further study should also include a more refined study of the individual causes of leaf blight in Pacific madrone.

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LITERATURE CITED

Elliott, M.; Chastagner, G.A.; Coats, K.P. [et al.]. 2014. First report of a new leaf blight caused by *Phacidiopycnis washingtonensis* on Pacific madrone (*Arbutus menziesii*) in Western Washington and Oregon. Plant Disease. 98: 1741.

Lintz, H.E.; Gray, A.N.; Yost, A. [et al.]. 2016. Quantifying density-independent mortality of temperate tree species. Ecological Indicators. 66: 1–9.

Wilhelmi, N.P.; Shaw, D.C.; Harrington, C.A. [et al.]. 2017. Climate of seed source affects susceptibility of coastal Douglas-fir to foliage diseases. Ecosphere. 8(12): e02011. 10.1002/ecs2.2011.

Zeller, S.M. 1934. Some new or noteworthy fungi on Ericaceous hosts in the Pacific Northwest. Mycologia. 26(4): 291–304.

BACK FROM THE BRINK: FOREST SERVICE EFFORTS TO CREATE DUTCH ELM DISEASE TOLERANT TREES FOR USE IN URBAN AND RURAL RESTORATION

Charles E. Flower[1], Cornelia C. Pinchot[1], Kathleen S. Knight[1], Keith Woeste[2], and James M. Slavicek[3]

Introduced into North America in the 1920's, the causal agent of Dutch elm disease (*Ophiostoma ulmi*) and the more aggressive variant of the pathogen (*O. novo-ulmi*, collectively referred to herein as DED) swept across North America resulting in a widespread decline of elms across urban and rural landscapes. Restoration of American elm (*Ulmus americana* L.) necessitates the development of new selections that are DED tolerant, in addition to enhancing the genetic variability of tolerant elms across the landscape. Ongoing research and breeding efforts have focused on (1) identifying survivor trees that have likely been exposed to DED yet have thrived, (2) crossing these large survivor elms with DED-tolerant American elms, (3) testing cloned survivor trees and progeny trees with DED inoculations, and (4) identifying genes which confer tolerance. This study examined the 2-year DED-induced canopy decline response of 27 American elm cultivars planted at the U.S. Department of Agriculture Forest Service Laboratory in Delaware, OH. Results suggest differences in DED-induced canopy decline among clones of large survivor trees collected across the Midwestern United States, indicating that unique tolerance mechanisms may be present in the natural elm population.

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FIELD PERFORMANCE OF PREVIOUSLY SELECTED ROOT ROT TOLERANT TROPICAL ACACIAS

Abdul Gafur[1,2], Ching Y. Wong[1], and Mukesh Sharma[1]

Reforestation programs have been established in some countries to anticipate the increase of the global demand for wood. The effort was initiated to sustain the supply of forest products while preserving the natural forests. In line with the policy, areas of industrial plantation forests of fast-growing species in Indonesia, especially acacias and eucalypts, have been increased to meet the ever-increasing demands. Disease infection has occurred since the establishment of the plantation forests. Root rots caused by red root rot (*Ganoderma philippii*) and *Phellinus noxi*us (brown root rot) are among the most economically important diseases on the two fiber trees. Research on field controls of the diseases has focused on certain components of integrated disease management such as inoculum reduction, silviculture practices, application of biological control agents, and tolerant genotypes. We have developed a method of screening for root rot tolerance in acacia seedlings. Using the method, identification of variations in root rot tolerance is possible. Field experiments and commercial plantations indicated that tolerant materials previously selected in the nursery screening had less root rot incidence, paving the way for plant tolerance to be incorporated as the core component of integrated management of the diseases in tropical acacia plantation forests.

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BUILDING A *CERATOCYSTIS* RESISTANCE PROGRAM FOR *METROSIDEROS* IN HAWAII

Christian Giardina[1], Lisa Keith[2], Phil Cannon[3], Richard Sniezko[4], Douglas Jacobs[5], and J.B. Friday[6]

The loss of ʻŌhiʻa to *Ceratocystis* wilt is an unprecedented threat to the ecological integrity of native Hawaiian forests and to native Hawaiian culture. The genetic basis for disease resistance by ʻŌhiʻa against rapid ʻŌhiʻa death (ROD) is poorly understood, yet represents a centrally important piece of any long-term strategy for perpetuating ʻŌhiʻa as a dominant component of Hawaii's native forests. Specifically, because ROD is infecting and killing trees across Hawaiʻi Island, with infection in some areas causing high rates of mortality, quantifying the presence and strength of resistance for various genotypes of Hawaiʻi Island ʻŌhiʻa is critical to anticipating long-term consequences of ROD for forest biodiversity and conservation in Hawaii, as well as informing restoration strategies including decisions about whether ʻŌhiʻa should be considered for restoration, and if so what genotypes. Questions about resistance need to be addressed for ʻŌhiʻa genotypes and species across the Hawaiian Islands, and so we have initiated an ʻŌhiʻa ROD resistance program that is relying a variety of nursery and in-field methods, focusing on Hawaiʻi Island genotypes and varieties initially, but if indicated, expanding to genotypes, varieties and other *Metrosideros* species on other Hawaiian Islands. Selections are being made based on morphological and geographic features. Molecular tools are being used to examine the genetic basis for and distribution of resistance. This work aligns with the State of Hawaii 2017–2019 ROD Strategic Response Plan (www.ctahr.hawaii.edu/dl/rod/strategicresponseplanfinal.pdf) which identifies ROD resistance research as a priority investment. This research will lead to improved understanding of ʻŌhiʻa disease resistance, identify the most resistant genotypes for propagation, and create a source of trees that will optimize the restoration of ROD impacted Hawaiian forests.

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THE IMMINENT INVASION OF THE EMERALD ASH BORER IN SOUTHERN EUROPE AND THE THREAT TO NATIVE OLEACEAE

Ignazio Graziosi[1] and Lynne Rieske-Kinney[2]

European countries are increasingly under invasion by non-native insects that are highly invasive in North America. For instance, the Japanese beetle *Popillia japonica*, the brown marmorated stink bug *Halyomorpha halys* and the Asian chestnut gall wasp *Dryocosmus kuriphilus*, notorious non-native pests of crops and forests in North America, have been recently introduced in Europe, with devastating consequence. The globally invasive emerald ash borer (EAB) *Agrilus planipennis* is currently expanding its range in both North America and European Russia, and its introduction in Southern Europe appears imminent. Furthermore, the spread of forest pathogens such as the ash dieback caused by *Hymenoscyphus fraxineus*, are adding concerns to the conservation of native forest habitats and *Fraxinus* species in particular. In addition, the recently reported utilization of non-*Fraxinus* hosts in the family Oleaceae is opening new scenarios for EAB invasion dynamics and future management challenges in Southern Europe. We seek to: (a) resume most recent knowledge on the pest's current distribution in Europe, (b) present natural distributions of native Oleaceae in the southern part of the continent, and (c) propose tools for predicting EAB invasion pathways and impact in Southern Europe.

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A COLLABORATIVE RESEARCH APPROACH FOR DIAGNOSING AND EVALUATING THOUSAND CANKERS DISEASE IN WALNUT: CURRENT PROGRESS AND FUTURE DIRECTIONS

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Thousand Cankers Disease (TCD) results from interactions between the canker-producing fungal pathogen *Geosmithia morbida*, an insect vector *Pityophthorus juglandis* (walnut twig beetle), and the susceptible plant hosts, *Juglans* spp. (walnuts) and *Pterocarya* spp (wingnuts). In the past two decades, TCD has expanded from the western to the native range of black walnut (*J. nigra*) in the Eastern United States. In 2013, TCD was discovered in northwestern Italy affecting both black and native English walnuts (*J. regia*). Although TCD has caused significant mortality among native and non-native walnut tree populations, our understanding regarding the genetic diversity of the insect vector and the fungal pathogen remains limited. One factor that may be contributing to the spread of the disease is the difficulty of its diagnosis due mainly to non-unique external symptoms. Based on the speed at which TCD has expanded its range and the potential global spread and invasion of areas where susceptible host plants are commercially grown, there is a critical need to understand the genetic diversity presented by the causal agent and its primary vector. Moreover, to limit the spread of the disease we need to improve our detection methods by utilizing specific and sensitive molecular tools that enable quick identification. Rapid and accurate detection of TCD will facilitate quarantine implementation in infested areas. Collaborative research presented here showcases our current understanding of the population structure of both pathogen and the vector of this disease, as well as a recently developed molecular protocol to rapidly detect *G. morbida* and *P. juglandis* directly from woody tissue samples. Our results indicate high genetic diversity, presence of population structure, and evidence of gene flow among subpopulations of *P. juglandis* and *G. morbida*. In particular, our work reveals that human mediated movement of infested plant material from multiple sources and on multiple occasions, has significantly contributed to TCD range expansion. Our results support an earlier hypothesis that the disease has been established in western TCD-affected areas for a long period of time and can't be considered a recent introduction. An important by-product of our population genetics research was the development of a set of specific microsatellite regions that have been extremely useful in the detection of *G. morbida* directly from infested wood tissue, even at low concentrations. Here, we address how we developed this novel molecular detection tool and explain how this approach can serve as a model for future research on disease outbreaks caused by similar disease complexes.

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COLLABORATIVE RESEARCH FOR SUSTAINABLE MANAGEMENT OF SOUTHWESTERN WHITE PINE

Kristen Waring[1], Sam Cushman[2], Andrew Eckert[3], Lluvia Flores[4], Richard Sniezko[5], Christopher Still[6], Christian Wehenkel[7], Amy Whipple[1], Michael Wing[6], Justin Bagley[3], Emily Boes[5], Ethan Bucholz[1], Jonathan Burnett[6], Antonio Castilla[1,5], Jessica DaBell[1], Chris Friedline[3], Cory Garms[6], Evan Heck[5], Jeremy Johnson[1,5], Erin Landguth[8], Alejandro Leal Sáenz[7], Briana McTeague[5], Mitra Menon[3], Ehren Moler[1], Gerald Page[6], and Andrew Shirk[9]

A collaborative team of researchers from the United States and Mexico have begun an exciting new research project funded by The National Science Foundation's Macrosystems Biology program. The project is to study ecological and evolutionary processes affecting the distribution of southwestern white pine, an important tree species of mixed conifer forests in the Southwest and Mexico. Southwestern white pine sustainability is threatened by changing climate, and a non-native tree disease, white pine blister rust. White pine blister rust causes extensive tree decline and mortality where it occurs in North America, including where it overlaps with southwestern white pine, an ever-expanding area. The climate may change too rapidly for southwestern white pine to adapt. The dual threats of climate change and invasive species make forecasting future tree distributions across continental scales an urgent challenge. The goal is to determine how gene movement among populations, adaptation to disease and drought, heritable changes beyond DNA mutations, and a changing environment interact to govern the success of southwestern white pine. This project will develop tools to help forecast and manage the future of the species, including genomics, common gardens, tree disease resistance testing, engineering and technology innovation to measure drought tolerance, and computer modeling in landscape ecology and genomics. The research team is using the Southwest Experimental Garden Array, set of common gardens, that allows scientists to quantify the ecological and evolutionary responses of species to changing climate conditions.

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BUTTERNUT CONSERVATION AND BREEDING TO MITIGATE BUTTERNUT CANKER DISEASE

James R. McKenna[1], Paul Berrang[2], Aziz Ebrahimi[3], Scott Rogers[4], Stuart Seaborne[5], and James Jacobs[6]

Butternut (*Juglans cinerea*), a valuable fine hardwood in North America, is no longer regenerating in the wild due its demise from an exotic fungal stem canker pathogen butternut canker disease (BCD) caused by the Ascomycete (*Ophiognomonia clavigignenti-juglandacearum* [*O-cj*]). The most effective way to mitigate BCD, will be to develop genetically resistant trees. Natural hybrids with the Japanese walnut (*J. ailantifolia*) have been found to have much wider resistance than pure *J. cinerea*; however, some *J. cinerea* families have shown a significant level of resistance but will require more breeding. Seed from our clone banks and orchards has become available in enough quantity to support conservation plantings. Our project aims to deploy and conserve regionally adapted and genetically diverse pure butternut seedlings. Conserving genetically pure butternut is important since natural selection over the last decades has left only hybrid trees alive in many places.

Dramatic differences in disease between upland and bottomland sites has been observed for nearly a decade. Artificial inoculation on bottomland sites has led to complete disease across the entire planting within 8–10 years. In contrast, two inoculated upland sites have shown as little as 3 to 10 percent natural disease spread. We will present new results from two new bottomland and one new upland site in Union County, Indiana established in 2013. Resistant stock combined with site selection and other cultural or biological controls can integrate several pest management strategies to minimize butternut canker disease.

From a large seed crop from our regional seed orchards and clone banks in 2013, we planted a new screening block in 2015 under naturally diseased trees. This extreme screening produced significant BCD in the first year after planting rapidly eliminating highly susceptible trees. New data will be presented on this and on our 10- and 9-year canker ratings from our 2nd screening blocks established in 2008–09. Our most productive seed orchard, located in the Pacific Northwest in Walla Walla, WA, comprised of 26 grafted trees of 15 genotypes, has yielded over 6,000 seeds the last few years. This past winter, we used DNA markers to determine each trees identity and hybridity and to thin it down to one individual *J. cinerea* tree per genotype.

Conserving pure and largely susceptible *J. cinerea* began last year and is continuing through 2019. We are providing seedlings from our Walla Walla, WA and Hoosier National Forest seed orchards to state and private foresters and interested woodland owners to plant in southern Indiana and Ohio, the geographic region of these orchard clones. 2018 marks the 10th consecutive year that our collaborators in the Indiana DNR have sold random hybrid butternut seedlings in Indiana. We harvested our first seed crop from a second generation resistant orchard in the fall of 2017 (300 nuts) which contain a mix of the most resistant hybrid and pure selections from our screening. Such seedlings, or supplemental pollen, can be a source of resistance to add into these new conservation plantings over the next decade. Our presentation will show seed production over the last 10 years, the new conservation sites being established, and discuss our various breeding and screening strategies.

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DIE BACK SYNDROME CAUSED BY *LASIODIPLODIA THEOBROMAE* AND *FUSARIUM PROLIFERATUM* IN ELITE GENOTYPES OF *TECTONA GRANDIS* LF., COSTA RICA

Dawa Méndez-Álvarez[1], Arantxa Rodríguez[1], Yorleny Badilla-Valverde[1], and Olman Murillo-Gamboa[1]

Tectona grandis L. (teak) is native to the tropical and subtropical regions of India, Burma, Thailand, and Laos in Asia and it is one of the most important timber species in the tropics because of its economic use. Costa Rica has been a pioneer in teak intensive cultivation, and a regional leader in tree improvement and conservation programs with GENFORES (international tree improvement cooperative). Forest plantation productivity and economical value has risen significantly; however, teak's dieback syndrome in commercial plantations become worst year by year. The main objective of this research is to contribute with the development of prevention options through putative tolerant genotypes utilization, as a transdisciplinary approach between pathology and tree breeding. This research used plant material from GENFORES. Syndromes causal agents were isolated, characterized and identified from five trees (four with symptoms and one healthy) of three different sites. Affected vascular tissue segments were cultivated in potato dextrose agar (PDA) to isolate pure cultures. According to the morphological characteristics, the fungus was identified as *Lasiodiplodia theobromae* and *Fusarium proliferatum*. To confirm the morphological identification, portions of the DNA were sequenced. Later, GENFORES elite genotypes teak were inoculated with *Lasiodiplodia theobromae* and *Fusarium proliferatum* (separated and together) to fulfill Koch's postulates and to screen out potential tolerant clones. Inoculation was carried out on 6 month old teak's clones, by placing 1.5 mm diameter mycelial plugs of the isolates grown on PDA in artificially made wounds (2 cm length) in the vascular cambium tissue. All elite clones displayed symptoms 120 days after inoculations. The fungus was re-isolated from all inoculated plants, fulfilling Koch's postulates. The research continues in order to obtain resistant or tolerant clones.

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SOMATIC EMBRYOGENESIS AND CRYOSTORAGE FOR RESTORATION OF ASH FORESTS DEVASTATED BY EMERALD ASH BORER

Scott A. Merkle[1], Paul M. Montello[1], A. Ryan Tull[1],
Daniel A. Herms[2], and Kamal J.K. Gandhi[1]

Emerald ash borer (EAB), *Agrilus planipennis* has devastated populations of ash trees in at least 20 U.S. States and Canada over the past decade. To date, control measures have had minimal impact on halting the infestation. However, there is some evidence of genetic resistance or tolerance to EAB in natural populations of white ash (*Fraxinus americana*) and green ash (*Fraxinus pennsylvanica*) trees, as demonstrated by the continued survival and growth of scattered individual trees for several years following infestations that killed over 99 percent of the ash trees in infested populations. These "lingering" or "surviving" ash individuals may form the basis for reforestation programs in EAB-impacted areas, if these genotypes or their progeny can be mass-propagated. We initiated cultures from seeds collected from several surviving white ash trees in southeastern Michigan. Cultures were initiated by dissecting immature seeds and culturing the developing zygotic embryos on a semisolid modified Woody Plant Medium (induction-maintenance medium; IMM) with 2 mg/l 2,4-D. Multiple highly productive embryogenic culture lines representing six different lingering ash parents were obtained. Embryogenic cultures were grown in suspension culture in liquid IMM, followed by size fractionation on stainless steel sieves and plating on nylon mesh overlaid on semisolid basal medium to produce populations of somatic embryos. Somatic embryo germination and conversion were improved by a combination of pre-germination cold treatment for eight weeks and addition of gibberellic acid to the germination medium. Ash somatic seedlings grew rapidly following transfer to potting mix and almost 100 trees representing 6 white ash clones have been acclimatized and grown in the greenhouse in preparation for clonal screening. Future research will focus on expanding the embryogenic work on other surviving ash populations, cryostoring copies of all ash embryogenic cultures and planting and testing of the clonal ash saplings for EAB resistance in the field.

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QTL MAPPING OF *SPHAERULINA MUSIVA* CANKER RESISTANCE/SUSCEPTIBILITY IN A POPLAR T x D HYBRID POPULATION

Kyle Mondron[1], Susanna Keriö[2], Sandra Simon[3], Stephen DiFazio[3], and Jared M. LeBoldus[1,2]

Poplar trees impact our ecological and economic well-being. Poplar hybrids benefit from heterosis, but the *P. trichocarpa* x *P. deltoides* (T x D) hybrid is very susceptible to infection by the fungal pathogen *Sphaerulina musiva*, which can cause premature mortality due to breakage or stem-girdling cankers. Breeding for resistance is currently the best technique for disease management. A prior Genome-Wide Association Study (GWAS) of *P. trichocarpa* found three genes that are thought to confer resistance (RLP1, RLP2, L-type lecRLK) and one gene that is thought to confer susceptibility (G-type lecRLK). A conidia-based greenhouse inoculation experiment was performed on T x D poplar hybrids using three isolates of *S. musiva*. Stem cankers were counted and disease severity scores (1–5) were assigned. The resulting phenotypes were analyzed using the R/qtl package with two models and a QTL map was created along with corresponding gene intervals. The four genes from the GWAS experiment were not found in the QTL gene intervals, nor were homologs found using BLAST analysis methods. Several candidate genes are listed and may be instrumental for further resistance breeding improvements.

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THE RESISTANCE OF MULBERRY TREE SPECIES TO TWO FOLIAR DISEASES IN THE RAINFOREST ECOLOGICAL ZONE OF NIGERIA

Oduntan, O. Akinlabi[1], Onilude Q. Ayansola[1], and Charles O. Ogunremi[2]

The capability of mulberry tree species to produce high quality leaf materials for silkworm growth and cocoon production has been dampened by incidence of insect and pest diseases. This study was carried out at the Sericulture plantation unit, Forestry Research Institute of Nigeria, located in the rainforest ecological zone to assess the resistance of six different mulberry tree accessions (S30, S14, S41, K2, N17, S54) to two foliar diseases (Leaf spot and powdery mildew). The study laid out in a Randomized Complete Block Design (RCBD) with five replications and Analysis of variance (ANOVA) used to test significance of the different means. The result revealed the percent incidence for leaf spot was highest in S14 (56.23 percent) and lowest in K2 (2.32 percent) where as in powdery mildew, it was highest in S40 (67.34 percent) and lowest in K2 (3.56 percent). It was also observed that the disease severity was ranging from a few individual spots to numerous lesions nearly covering the entire leaves. The results of different foliar anatomical characteristics showed that it plays a significant role in inhibiting the spread of the pathogen causing the diseases. Varietal rating to insect pest susceptibility showed that K2 accession was least affected or invested. It was concluded that K2 accession has high potentials that could be exploited for mass production of mulberry germplasm for silkworm, livestock, and human consumption. However, recommendations were also suggested for the study.

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NURSERY PEST RESISTANCE OF *MANSONIA ALTISSIMA* SEEDLINGS TO *GODASA SIDAE* ATTACK IN THE RAINFOREST ECOLOGICAL ZONE OF NIGERIA

Oduntan, O. Akinlabi[1], Omotade R. Ogunremi[2],
Onilude Q. Ayansola[1], and Awotedu B. Folake[1]

The establishment of plantations of *Mansonia altissim*a has been constrained by the insect pest *Godasa sidae* that causes extensive damage to the seedlings in the nursery. This study was carried out at Forestry Research Institute of Nigeria nursery site, using 120 seedlings with the aim to prevent *Godasa sidae* attack on *Mansonia altissima* seedlings in order to achieve an ecologically based pest resistance management strategy. Seedlings were placed in a screen house (SHS); in the open treated with a methanoic extract from *Gliricidia sepium* once a week (MTH); also, in the open treated with a low concentration (0.05 percent) of water-based insecticide (Lambda-Cyhalothrin) once a week (CHM), all replicated 30 times. Growth data recorded were subjected to ANOVA and DMRT (post mortem). The results revealed that the SHS, MTH, and CHM were all effective in the control of the studied insect pests except the experimental control. However, the results revealed a significant difference at 5 percent level of probability among the treatments in terms of seedling height, collar diameter, and leaf production with highest mean value of 15.41 ± 2.36 cm observed for seedling height for SHS, followed by CHM (14.11 ± 2.18 cm) and MTH (13.87 ± 2.16 cm). The values of collar diameter were 0.49 ± 0.05 mm, 0.43 ± 0.03 mm and 0.40 ± 0.03 mm for SHS, CHM, and MTH respectively. Also, the mean number of leaves in each treatment was 23 ± 3.23, 20 ± 3.15 and 19 ± 3.10 for SHS, CHM and MTH, respectively. It was concluded that the screen house (SHS) performed best. However, because of cost implications, an alternative is suggested. Due to the hazardous effects of the chemical on the environment, the methanoic extract which can be a potent pesticide should be adopted for the control of insect pest of *Mansonia altissima* at nursery stage.

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QTL MAPPING POPULATION DEVELOPMENT IN *CORNUS FLORIDA* USING OPEN POLLINATED SEEDLINGS AND SSR MARKERS

Erin Pfarr[1], Josh Honig[1], Jennifer Vaiciunas[1], Christine Kubik[1], John Capik[1], and Thomas J. Molnar[1]

Flowering dogwood (*Cornus florida*) is an important understory tree in deciduous mesic hardwood forests of the Eastern United States, from Massachusetts to Florida and as far west as Texas (Fulcher et al. 2012). In addition, flowering dogwood is a popular tree in managed landscapes thanks to its graceful form and spring blooms with showy bracts. It is honored as the State flower or tree of North Carolina, Virginia, and Missouri. However, since the early 1990s, powdery mildew (PM) (*Erysiphe pulchra*) has been one of the most problematic diseases for this species (Li et al. 2009). Infected leaves can be curled and stunted with unsightly white fungal growth or increased red pigmentation (Li et al. 2009). In the seedling stage, a heavy infection can be fatal (Parikh et al. 2016). For mature trees, the disease negatively affects bloom and repeated severe infections can stunt a tree's growth and reduce its appeal in the landscape (Parikh et al. 2016).

One selection from the Rutgers dogwood breeding program, *C. florida* H4AR15P25, shows excellent resistance to powdery mildew. The resistance holds up in clones across multiple locations and appears to be heritable. The quantitative trait loci (QTL) underlying this resistance will be investigated and mapped in a pseudo F_2 population using genotyping by sequencing (GBS)-derived single nucleotide polymorphism (SNP) markers. To expedite population development, open-pollinated (OP) seeds were harvested from H4AR15P25 grown in a crossing block of limited PM-susceptible male parents. The purpose of this study is to determine the pollen parents of the seedlings using simple sequence repeat (SSR) markers and assemble a full-sibling population of 150 seedlings from the same PM-susceptible pollen parent for the future QTL PM resistance study.

Mature fruit were harvested from *C. florida* H4AR15P25 at Rutgers University New Brunswick Agricultural Experiment Station in October 2017. Seeds were cleaned, stratified, and germinated in the greenhouse, resulting in ~650 healthy seedlings.

Newly expanding leaves were collected from H4AR15P25, 16 possible pollen parents, and a subset of 37 seedlings. DNA was extracted using the Qiagen DNeasy Plant Kit. The samples were genotyped with 11 SSR primer pairs developed by Wang et al. (2008) and Wadl et al. (2008) (table 1) (Wadl et al. 2008, Wang et al. 2008).

Briefly, the primer pairs were synthesized by Integrated DNA Technologies (Coralville, IA) with a 7 bp PIG-tailing sequence added to the reverse primers to aid in scoring true vs. plus A alleles,

Table 1—SSR marker summary statistics for *Cornus florida* H4AR15P25, 16 possible pollen parents, and 37 open pollinated progeny

Locus	k	N	HObs	HExp	PIC	F(Null)
CF020	8	54	0.630	0.620	0.582	-0.002
CF273	8	52	0.808	0.759	0.712	-0.039
CF125	4	54	0.889	0.639	0.566	-0.185
CF701	7	54	0.870	0.814	0.778	-0.043
CF048	5	54	0.667	0.589	0.550	-0.071
CF597	8	54	0.722	0.673	0.615	-0.041
CF1045	3	54	0.333	0.334	0.287	-0.010
CF055	4	54	0.630	0.485	0.413	-0.156
CF646	5	54	0.870	0.765	0.720	-0.067
CK040	3	54	0.222	0.283	0.246	0.114
CK015	4	54	0.556	0.553	0.448	-0.005
Average	5.4	53.8	0.654	0.592	0.538	-0.046

k = the number of alleles for each SSR marker.
N = the number of individuals genotyped at a locus.
HObs = the Observed Heterozygosity.
HExp = the Expected Heterozytosity.
PIC = Polymorphic Information Content.
F(Null) = the null allele frequency estimate.

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and an 18 bp M13 sequence added to the forward primers for economic PCR florescent labeling (Brownstein et al. 1996, Schuelke 2000).

The following were combined in a 96 well PCR plate with 13 µl reaction volumes: 0.5 pmol forward primer, 1 pmol reverse primer, 1 pmol florescent dye M13 primer (FAM, NED, PET, or VIC), 5 ng DNA, and 10xRamp-Taq PCR buffer (Denville Scientific, Metuchen, NJ), 2 mM MgCl2, and 0.25 mM of each dNTP (Denville Scientific). PCR was performed on GeneAmp 9700 thermalcyclers (Applied Biosystems, Foster City, CA) with the following parameters: initial denaturation of 94 °C for 5 min followed by 30 cycles of 94 °C for 30 s, 55 °C for 45 s, and 72 °C for 45 s, followed by 20 cycles of 94 °C for 30 s, 53 °C for 45 s, and 72 °C for 45 s, followed by a final extension of 72 °C for 10 min. The PCR products were run with LIZ 600 size standard on a capillary electrophoresis genetic analyzer (ABI 3500xl; Applied Biosystems). SSR alleles were binned and assigned with Genemapper 4.0 (Applied Biosystems). Allele frequency analysis and paternity parentage analysis was performed with CERVUS 3.0 (Kalinowski and others 2007), which uses a likelihood-based approach and parentage analysis simulation to determine confidence levels for parentage assignments.

The SSRs were highly polymorphic with 3 to 8 alleles per marker and polymporphic information content (PIC) values between .25 and .78 (table 1.). The SSR marker data confirmed the pedigrees of *C. florida* H4AR17P05 and H4AR17P48, two of the possible pollen parents, as progeny of *C. florida* forma rubra × *C. florida* "Red Beauty", which were also genotyped for this study.

H4AR15P28, the closest tree to H4AR15P25, was identified as the pollen parent of 10 of the seedlings, the most of any other possible parent (fig. 1 and table 2.). H4AR15P28 would be the best susceptible pollen parent for the mapping population because it is the most PM susceptible tree in the breeding block. With slightly over a fourth of genotyped seedlings in this subset assigned to H4AR15P28, we are optimistic that upon screening all 650 OP seedlings, we will find 150 full siblings from this pollen parent for the bi-parental QTL mapping population.

Six plants, H4AR17P05, H4AR15P49, H4AR15P58, forma rubra, H4AR17P48, "Red Beauty", and H4AR15P40, did not have any seedlings in this subset (fig. 1). This may be due to a variety of factors, such as increased distance from and asynchronous flowering with the mother plant, or limited flower set in the case of H4AR15P40 (Rhoades et al. 2011).

Interestingly, 7 seedlings could not be assigned with 80 percent confidence to any of the possible pollen parents. F-003, F-014, and F-106 had one or more alleles that were not present in any of the possible parents. It is unclear where this pollen flow is coming from as this breeding block is separated from the closest *C. florida* trees by 1,100 feet (including a 400 foot-wide river).

The set of 11 markers used here will be paired down to the 8 most informative for more efficient screening. They will be used to screen the rest of the OP seedlings from H4AR15P25 to assemble the 150-individual mapping population. This *C. florida* population will be genotyped using GBS and evaluated for PM response at 2 and 3 years of age. The final goals are to better understand PM resistance inheritance and to subsequently use QTLs for PM resistance to aid in marker assisted selection in the Rutgers dogwood breeding program. This would allow for early culling of PM susceptible trees, maximizing space and resources for a plant with a relatively slow generation time.

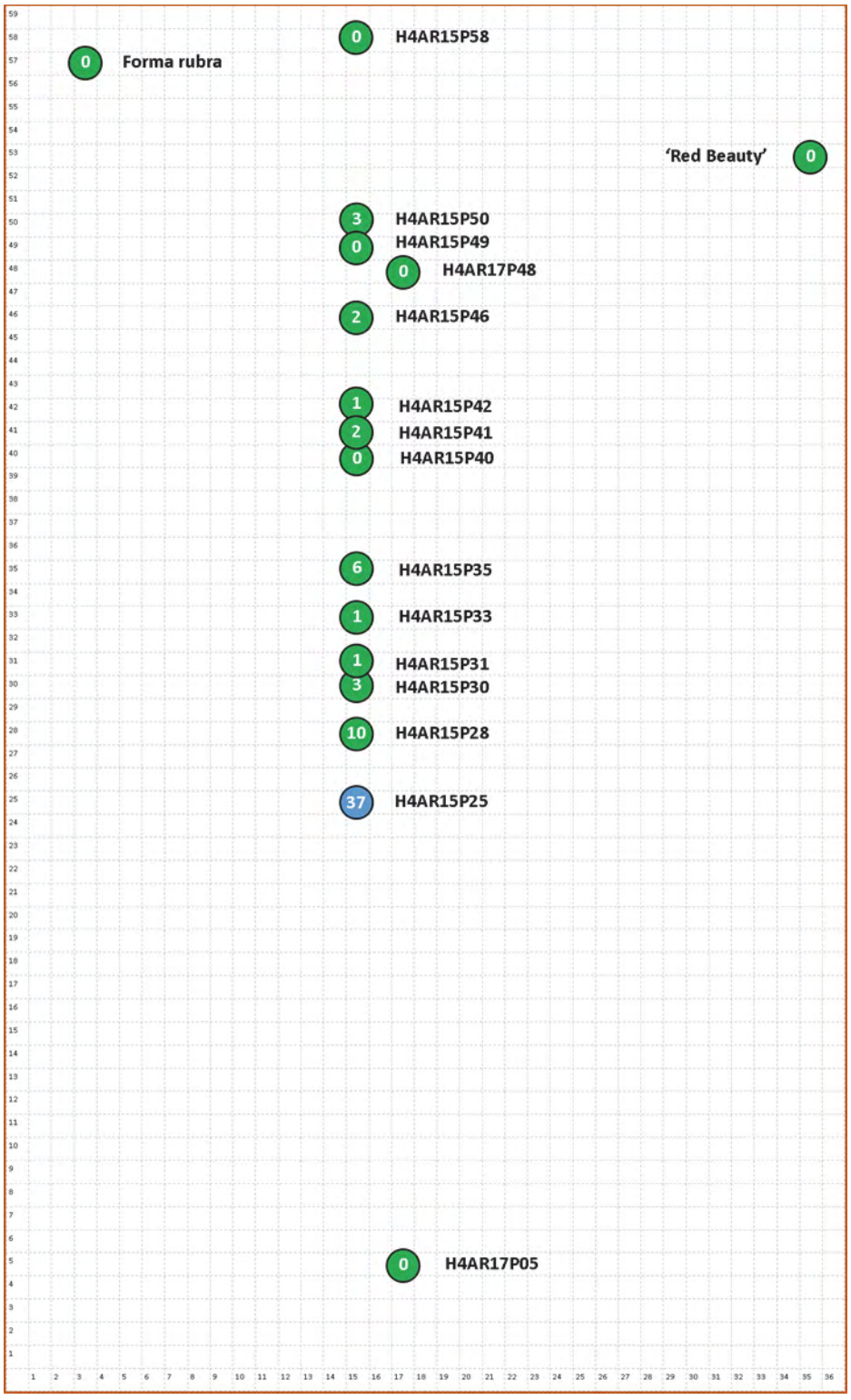

Figure 1—Approximate spatial relationships of *C. florida* H4AR15P25 (blue circle) and the possible pollen parents (green circles). Numbers inside of the circles represent number of progeny assigned to trees with 95% trio confidence or greater by CERVUS.

Table 2—CERVUS program parentage assignments for 37 open pollinated *Cornus florida* seedlings

Offspring ID	Loci typed	Candidate mother ID	Pair loci mismatch	Candidate father ID	Trio loci mismatch	Trio LOD score	Trio Delta	Trio confidence
F-006	11	H4AR15P25	0	H4AR15P28	1	4.23	4.23	*
F-011	11	H4AR15P25	0	H4AR15P28	1	5.12	5.12	*
F-015	11	H4AR15P25	0	H4AR15P28	1	4.78	4.78	*
F-038	11	H4AR15P25	0	H4AR15P28	0	9.72	6.51	*
F-047	11	H4AR15P25	0	H4AR15P28	1	4.52	4.52	*
F-073	11	H4AR15P25	0	H4AR15P28	1	4.74	4.74	*
F-075	11	H4AR15P25	0	H4AR15P28	0	10.5	10.5	*
F-096	11	H4AR15P25	0	H4AR15P28	0	9.73	6.51	*
F-104	11	H4AR15P25	0	H4AR15P28	1	4.52	4.52	*
F-129	11	H4AR15P25	0	H4AR15P28	1	4.51	4.51	*
F-020	11	H4AR15P25	0	H4AR15P30	0	12.4	12.4	*
F-024	11	H4AR15P25	0	H4AR15P30	1	5.33	5.33	*
F-029	11	H4AR15P25	0	H4AR15P30	0	10	10	*
F-018	11	H4AR15P25	0	H4AR15P31	0	14.1	14.1	*
F-004	11	H4AR15P25	0	H4AR15P33	0	9.69	9.69	*
F-016	11	H4AR15P25	0	H4AR15P35	0	8.15	8.15	*
F-021	11	H4AR15P25	0	H4AR15P35	1	3.29	3.29	*
F-026	11	H4AR15P25	0	H4AR15P35	1	5.25	5.25	*
F-042	11	H4AR15P25	0	H4AR15P35	0	2.54	2.54	*
F-101	11	H4AR15P25	0	H4AR15P35	0	8.47	8.47	*
F-109	10	H4AR15P25	0	H4AR15P35	0	1.16	1.16	*
F-098	11	H4AR15P25	0	H4AR15P41	0	4.26	1.51	*
F-110	11	H4AR15P25	0	H4AR15P41	0	6.39	6.39	*
F-007	11	H4AR15P25	0	H4AR15P42	0	8.24	8.24	*
F-113	11	H4AR15P25	0	H4AR15P46	1	0.79	0.79	*
F-128	11	H4AR15P25	0	H4AR15P46	0	2.48	2.48	*
F-009	11	H4AR15P25	0	H4AR15P50	0	7.37	4.89	*
F-017	11	H4AR15P25	0	H4AR15P50	0	7.08	7.08	*
F-095	11	H4AR15P25	0	H4AR15P50	0	7.88	7.88	*
F-022	11	H4AR15P25	0	H4AR15P28	1	5.37	0.16	+
F-071	11	H4AR15P25	0	H4AR15P28	2	#######	0	
F-106	10	H4AR15P25	0	H4AR15P41	2	-0.167	0	
F-003	11	H4AR15P25	0	H4AR15P46	5	#######	0	
F-013	11	H4AR15P25	0	H4AR15P46	1	-0.11	0	
F-005	11	H4AR15P25	0	H4AR15P50	3	#######	0	
F-014	11	H4AR15P25	0	H4AR15P50	5	#######	0	
F-136	11	H4AR15P25	0	H4AR15P50	2	#######	0	

Summary statistics for CERVUS program parentage assignments.
The most likely pollen parent ID is listed for each seedling.
For trio confidence levels * = 95 percent; + = 80 percent.

LITERATURE CITED

Brownstein, M.J.; Carpten, J.D.; Smith, J.R. 1996. Modulation of non-templated nucleotide addition by Taq DNA polymerase: primer modifications that facilitate genotyping. Biotechniques. 20(6): 1004–1006, 1008–1010.

Fulcher, A.F.; Hale, F.A.; Windham, A.S. 2012. Dogwood - *Cornus* spp. In: Fulcher, A.F.; White, S.A. IPM for select deciduous trees in southeastern U.S. nursery production. Knoxville, TN: Southern Nursery IPM Working Group: 137–158.

Kalinowski, S.T.; Taper, M.L.; Marshall,.T.C. 2007. Revising how the computer program cervus accommodates genotyping error increases success in paternity assignment. Molecular Ecology. 16(5): 1099–1106.

Li, Y.; Mmbaga, M.T.; Windham, A.S. [et al.]. 2009. Powdery mildew of dogwoods: Current status and future prospects. Plant Disease. 93(11): 1084–1092.

Parikh, L.; Mmbaga, M.; Kodati, S. [et al.]. 2016. Broad-sense heritability and genetic gain for powdery mildew resistance in multiple pseudo-F2 populations of flowering dogwoods (*Cornus florida* L.). Scientia Horticulturae. 213.

Rhoades, P.; Wadl, P.A.; Klingeman, W.E. [et al.]. 2011. Using simple sequence repeats (SSRs) to measure pollen flow in a flowering dogwood orchard. Journal of Environmental Horticulture. 29(4): 175–179.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology. 18: 233.

Wadl, P.A.; Wang, X.; Scheffler, B.E. [et al.]. 2008. Microsatellites from kousa dogwood (*Cornus kousa*). Molecular Ecology Resources. 8(4): 780–782.

Wang, X.; Trigiano, R.; Windham, M. 2008. Development and characterization of simple sequence repeats for flowering dogwood (*Cornus florida* L.). Tree Genetics & Genomes. 4(3): 461–468.

GENETIC BARCODES FOR IDENTIFICATION OF PURE AND HYBRID *FRAXINUS* SPECIES

William J. Plumb[1,2], Laura J. Kelly[1], Gerry Douglas[3], and Richard Buggs[1,2]

Native populations of species in the genus *Fraxinus* (ash) are being devastated in North America by the emerald ash borer, and in Europe by ash dieback. There is evidence that some Asiatic species of *Fraxinus* have low susceptibility to both of these threats. It is therefore possible that hybridisation between Asiatic species and congeners native to Europe and North America may be one way of retaining ash as a viable forestry species on those continents. We have developed a set of genetic barcodes for the confirmation and identification of ash hybrids. Using whole genome assemblies for 29 individuals from 28 *Fraxinus* taxa (www.ashgenome.org), we identified and aligned putative orthologs from low copy number genes. A subset of 1396 variable gene alignments were analyzed using the statistical programme CONTEXT to identify candidates with a pattern of variability suitable for barcoding the individual species of *Fraxinus*. Primers were designed for the selected barcoding regions, and tested using several example species from the living collection at the Royal Botanic Gardens, Kew, UK and hybrids developed at Ashtown Research Station (Teagasc, Ireland). Sequence data were generated for all regions using Polymerase Chain Reaction (PCR) amplification and Sanger sequencing. The results of this study show that these barcodes are capable of identifying various different *Fraxinus* species, as well as being suitable for both the confirmation and identification of hybrids.

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CONFOCAL MICROSCOPY CONFIRMS THE FEASIBILITY OF GENE SILENCING FOR EMERALD ASH BORER SUPPRESSION

Thais B. Rodrigues[1], Flavia Pampolini[1], Tomokazu Kawashima[2], and Lynne Rieske-Kinney[1]

Emerald ash borer (EAB) (*Agrilus planipennis*) is an aggressive, invasive forest and urban tree pest that feeds and develops beneath bark, killing trees rapidly. We are investigating the use of RNA interference (RNAi) as a tool for managing EAB. Previously, we showed that oral ingestion of double-strand RNA (dsRNA) can silence essential and specific genes, and subsequently found in droplet assays that silencing heat shock protein and shibire genes kills up to 90 percent of neonates and adults after 1–2 weeks of exposure. To evaluate our findings in vivo, we assessed dsRNA uptake through plant material and through the egg chorion. Twigs of greenhouse-grown tropical ash, *Fraxinus uhdei*, were treated with labeled dsRNA and infested with EAB eggs; twigs treated with unlabeled dsRNA were used as controls. Confocal microscope images were then taken and analyzed to detect fluorescence in both plant and insect material. Additionally, EAB eggs were soaked in labeled dsRNA, and eggs and hatched neonates analyzed by confocal microscopy. Gene silencing was performed to corroborate imaging results. After 8 days of exposure, we detected fluorescence in ash bark and xylem, and larvae fed on these plants showed strong fluorescence inside their alimentary canal. When eggs were soaked with labeled dsRNA, fluorescence was detected in eggs and the hatched larvae, confirmed by gene expression. Our twig assay demonstrated that dsRNA is taken up through plant material, suggesting that trunk injections or soil treatments with dsRNA may potentially be an efficient delivery method for this technology. Absorption of dsRNA by eggs demonstrated that spray-able dsRNA may be a potential delivery method. EAB is rapidly expanding its geographic range, and innovative means of management are essential to mitigate its impacts. We've shown that RNAi in EAB is feasible; here we demonstrate the potential of topical applications of this technology.

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VARIOUS PROPAGATION WAYS IN *ALNUS GLUTINOSA* WITH DIFFERENT SENSITIVITY TO *PHYTOPHTHORA × ALNI*

Jana Šedivá[1], Petra Štochlová[1], Kateřina Novotná[1], and Karel Černý[1]

The hardwood tree *Alnus glutinosa* L. (black alder) is a typical riparian plant and originally ranged throughout most of Europe and also into North Africa, Asia Minor, and Western Siberia. One of the most serious pathogens of *Alnus* is *Phytophthora × alni* causes a rapid necrosis of the inner bark of the collar, stems, and roots of infected trees. The identification of black alder genotypes resistant to the disease and successful propagation methods would be very beneficial in the rejuvenation of damaged riparian ecosystems and the prevention of possible losses in the future. In seeking to rapidly propagate plants possessing a rare but highly desirable character, it is more appropriate to use vegetative means. The feasibility of propagating mature *A. glutinosa* trees by vegetative means that could be used to propagate trees resistant to *P. × alni* was examined. Both softwood and hardwood cuttings were tested. The different treatments were employed, such as growth regulator concentration and method of application. The rooting success was highly dependent on the type of cuttings and treatments used. The softwood cuttings collected in the middle of July and then treated with 1 percent IBA rooted the best of all, with 42.5 percent of cuttings rooting successfully. Micropropagation is an effective and rapid propagation method for valuable genotypes of trees. An important role in the establishment of *in vitro* cultures of black alder plaid the physiological state of the donor trees, the time of explant collection and the composition of the nutrient medium, especially in terms of the content of the growth regulators.

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USING A POPLAR HYBRID TO EXPLORE HOST PLANT GENETIC CONTROL OF ASSOCIATING INSECT AND FUNGAL SPECIES

Sandra J. Simon[1], Stephen P. DiFazio[1], and David Macaya-Sanz[1]

Community genetics is the study of the influence of host genotype on associating arthropods, fungi, pathogens, and predators, such that related individuals support similar, heritable communities. Understanding how shifts in the genes of a population contribute to ecosystem function effectively links ecology and evolutionary biology. Observing these interactions allows genes associated with pest resistance and susceptibility to be identified within a host plant. This in turn could aid in the cross design of commercially valuable biofuel crops such as those in the Salicaceae family, which include poplar and willow species. To test the extent to which genes of *Populus* have extended phenotypes on arthropod communities, surveys were conducted in a *P. trichocarpa* x *P. deltoides* pseudo-backcross plantation. Trees were observed for the arthropods *Pemphigus populitransversus*, *Mordwilkoja vagabunda*, *Phyllocolpa bozemani,* and *Phyllocnistis populiella*. Individuals were also scored for Melampsora leaf rust, *Septoria musiva* leaf spot, and *Septoria musiva* stem canker fungal infections. Broad-sense heritability (H^2_c) revealed a significant genetic factor controlling both insect and fungal pests. Several quantitative trait loci (QTL) intervals were discovered to be associated with these biotic stressors that contain numerous candidate genes which mediate their interactions. There was also an elevated number of tandem duplications and chromosome rearrangements in the intervals for several biotic phenotypes. Future work will be aimed at understanding the contribution of structural variation in mediating biotic interactions.

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GENETIC RESISTANCE TO BLISTER RUST IN DECLINING LIMBER PINE (*PINUS FLEXILIS*) IN ALBERTA—THE PATH TO RESTORATION?

Richard A. Sniezko[1], Jodie Krakowski[2], Robert Sissons[3], Robert Danchok[1], Angelia Kegley[1], and Douglas P. Savin[1]

Limber pine (*Pinus flexilis*) is a long-lived conifer native to western North America. The northernmost populations of limber pine are in Canada, of which over 90 percent are in Alberta. These populations are heavily infected by white pine blister rust (WPBR), caused by the non-native pathogen *Cronartium ribicola*. Many natural populations are more than 70 percent infected, with some areas having > 95 percent infection. Limber pine is listed as endangered in Alberta and has been recommended as endangered by the Committee on the Status of Wildlife in Canada. Efforts to restore the species are underway, such as finding a diverse complement of genetically resistant parent trees and assembling seed collections for genetic conservation and restoration. Two small tests of Alberta parent tree progeny at the USDA Forest Service's Dorena Genetic Resource Center (DGRC) detected complete resistance to WPBR major gene resistance (MGR). Only low levels of partial resistance have been detected to date. Currently, Alberta Agriculture and Forestry and Waterton Lakes National Park are working with DGRC to greatly expand the number of seedling families being screened for resistance, using progeny of putatively resistant field selections. Sixty half-sib families were inoculated with WPBR spores in fall 2017, 50 additional families inoculated in 2018, and additional families are scheduled for inoculation in 2019 and 2020. Resistant seedlings from the first MGR tests at DGRC were included in restoration plantings in 2018. These seedlings and their parent trees will serve as sentinels to monitor changes in the rust fungus or environmental conditions that may affect the durability or stability of resistance. Within 5 years we anticipate having an increased knowledge of the frequency, type, and level of genetic resistance in northern Canadian populations, as well as establishment of more restoration plantings.

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GENETIC RESISTANCE AND RESTORING A THREATENED SPECIES: WHITEBARK PINE AT CRATER LAKE NATIONAL PARK

Richard A. Sniezko[1] and Jennifer S. Hooke[2]

Whitebark pine (WBP), *Pinus albicaulis* is a wide-ranging conifer species found at high elevations in western North American forests. It is a keystone species, but is facing serious challenges, including high mortality in many parts of its range due to the presence of white pine blister rust (WPBR), caused by the non-native pathogen *Cronartium ribicola*, as well as the impacts of mountain pine beetle infestation, climate change, and altered fire regimes. Due to impacts of these daunting challenges WBP has been listed as endangered in Canada, while in the United States it is being considered for listing under the Endangered Species Act. The consensus among scientists working with WBP is that for restoration efforts to be successful in most areas, they will have to include seedlings with genetic resistance to WPBR. Dorena Genetic Resource Center has been evaluating resistance of WBP from Crater Lake National Park (CRLA) since 2004. These trials show that at least some populations, including CRLA populations, have genetic resistance that will allow restoration to proceed successfully. From 2009 to 2016, six restoration plantings have been established using seedlings from resistant CRLA parent trees. Through fall 2013, survival in the four oldest restoration plantings ranged from 77.6–90.6 percent. Rust infection has been very low to this point. Each planting has documented family identification of each seedling, which allows for WBP restoration and conservation, along with facilitating further research on how survival, rust resistance, growth, and specific traits vary among and within family groups. These restoration plantings also serve as long-term source populations of resistance alleles to help the natural regeneration of resistant WBP throughout neighboring areas. In addition, the current living rust-resistant parent trees in CRLA are being protected from mountain pine beetle infestation using verbenone pouches, to further help spread genetic resistance. The CRLA plantings provide a successful example of the use of genetic resistance to begin the restoration of a non-commercial forest tree species. They also provide a conservation education tool to raise public awareness of the potential for restoration using genetic resistance.

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INVESTIGATING ANATAGONISTIC ACTIVITY OF FUNGAL ENDOPHYTES TOWARD PATHOGENS OF WESTERN WHITE PINE (*PINUS MONTICOLA*)

Emily Martin[1], Beau Larkin[1], Richard Sniezko[2], and Lorinda Bullington[1]

North American white pine populations have experienced increased mortality due to the invasive fungal pathogen *Cronartium ribicola*. Research has shown that white pines may defend against *C. ribicola* infection by hosting diverse fungal endophyte communities. Tree genetics appear to influence these endophyte communities and some fungal endophytes can be vertically transmitted, through seeds, from parent to offspring. Vertically transmitted fungi are often mutualists of plants and can facilitate host defenses against pathogens as seedlings mature. We plan to isolate and screen vertically transmitted fungi from resistant western white pines (*Pinus monticola*), for antagonistic activity that may inhibit infection, survival, and growth of *C. ribicola*. Western white pine seeds and needles will be collected from healthy trees throughout the Pacific Northwest and MPG North, Swan Valley, MT. We will culture tissue to isolate fungal endophytes and test for their ability to inhibit pathogen growth with microspectrophotometry, microscopy, and dual cultures. Fungi previously found to occur more often in resistant white pines will also be screened against pathogens. Microspectrophotometry will allow us to monitor pathogen growth and measure the inhibitory effects of toxic antimicrobial compounds produced by fungal endophytes. Dual cultures and microscopy will allow us to observe competition and mycoparasitism between pathogens and endophytes. With these methods we can screen many fungal endophytes at a time and quantify and compare their inhibitory effects. Fungal endophytes exhibiting the most effective antagonistic activity will be selected and used in western white pine seedling inoculation trials in 2019.

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SUDDEN OAK DEATH IN SOUTHERN OREGON: COMPARING THE EU1 AND NA1 LINEAGES OF *PHYTOPHTHORA RAMORUM*

Kelsey L. Søndreli[1], Alan Kanaskie[2], Richard Sniezko[3], Everett Hansen[1], and Jared M. LeBoldus[1]

Phytophthora ramorum is the cause of Sudden Oak Death and is an invasive pathogen in California and southwestern Oregon forests. Until recently, forest infestations in Oregon and California have all been the NA1 clonal lineage. However in 2015, the EU1 lineage of *P. ramorum* was isolated from a tanoak tree in the forest in Curry County, Oregon. In this region, tanoak (*Notholithocarpus densiflorus*) is the most susceptible species to *P. ramorum*, developing lethal stem cankers and sporulating on infected leaves and branches, spreading the disease. A variety of experiments were conducted, including field and greenhouse inoculations, in order to determine the relative threat of the new EU1 lineage compared to the NA1 lineage in Oregon forests. Overall, EU1 was shown to be more aggressive on Oregon trees than NA1. A sporulation assay demonstrated a 10-fold increase in sporulation of the EU1 isolates compared to NA1 isolates on tanoak. Results from a preliminary field experiment suggest that greater infection rates of a larger number of species occur under EU1 infested trees. An analysis of resistance in tanoak families inoculated with EU1 and NA1 isolates of *P. ramorum* indicated significantly larger cankers developing on EU1 inoculated trees compared to NA1 in 3/14 families. No trees were completely resistant to the pathogen. The accumulated evidence indicates that the EU1 lineage of *P. ramorum* is more aggressive and potentially poses a greater risk to Oregon forests than the NA1 lineage.

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SCREENING FOR WHITE PINE BLISTER RUST IN WHITEBARK PINE: BRITISH COLUMBIA, CANADA

Ward Strong[1]

Whitebark pine, *Pinus albicaulis*, is a high-altitude 5-needle pine native to Western North America. It is a keystone ecological species, essential to ecosystem functioning in many subalpine and treeline forests. In 2012, this species was identified as Endangered by the Canadian Species at Risk Act, due in large part to mortality by White Pine Blister Rust (*Cronartium ribicola*). This Old World disease was introduced into North America at the beginning of the 20th century; there is very little genetic resistance to it in endemic 5-needle pine populations. The British Columbia government has started a screening program to identify genetic resistance in Whitebark pine, with the objective of inter-situ gene conservation and in-situ species recovery. The process starts by identifying surviving trees in high-mortality areas. The progeny of these parent trees are artificially inoculated with blister rust basidiospores in a climate-controlled room, then scored over the next 2–4 years. We have now made our first rust-resistant parent selections, which have been grafted for deployment in clone banks or seed orchards. 2018 will be our 7th year of parent tree seed collections in this ongoing, long-term project.

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DIFFERENTIAL RESISTANCE TO *PHYTOPHTHORA CINNAMOMI* IN TROJAN FIR

Alexander J. Trouern-Trend[1], William H. Kohlway[2], Lilian Matallana[2], Ross W. Whetten[2], John Frampton[2], and Jill L. Wegrzyn[1]

RNA was sequenced from roots tissue samples of *Abies equi-trojani* that demonstrated differential susceptibility to dieback (root rot) when experimentally inoculated with the oomycete pathogen *Phytophthora cinnamomi*. Eight pooled RNA libraries of 10 inoculated individuals based on the progression of tree morbidity over the course of the inoculation experiment represented three conditions, uninoculated control (2 libraries), resistant (4 libraries), and susceptible (2 libraries). These data were used for differential expression (DE) analysis, which uncovered 12,680 instances of differential expression among the three groups; 326 upregulated and 1,219 downregulated in resistant relative to control, 5,955 upregulated and 343 downregulated in resistant relative to susceptible, and 4,722 upregulated and 115 downregulated in control relative to susceptible. Our analysis of DE genes leveraged a new functional annotation pipeline, Eukaryotic Non-Model Transcriptome Annotation Pipeline (EnTAP), which automates similarity searches across multiple databases and includes protein domain, gene ontology, and gene family assignment, Kyoto Encyclopedia of Genes and Genomes (KEGG) annotation and contaminant filtering. Functional annotation clustering of DE genes yielded patterns of increased expression of stress, metabolic and apoptosis related genes in resistant trees.

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PHENOLIC COMPONENT OF LOBLOLLY PINE DEFENSE RESPONSE TO BLUE-STAIN FUNGI ASSOCIATED WITH ROOT-FEEDING BEETLES

Zackary N. Parker[1], Brittany F. Barnes[1], and Caterina Villari[1]

Southern pine species are integral components to southeastern forests, comprising a significant percentage of the forested lands. Among these species, loblolly pine (*Pinus taeda* L.) is the dominant timber species grown commercially, thus being extremely important for both the economy and the ecology of southeastern regions. Extensive studies on pathogens and insect pests affecting this species have been conducted, but for obvious reasons, most of them have focused on major tree-killing species, such as the southern pine beetle (SPB) [*Dendroctonus frontalis* Zimmerman (SPB)] and its associated fungal complex. However, many other beetles and associated fungi colonize the species, and are still worth being investigated, as they too are integral components of the loblolly pine planation system. The root-feeding beetle system and associated fungi, for instance, have largely been overlooked in the past, and only recently become of interest (Coyle et al. 2015, Eckhardt et al. 2007). These beetles are generally secondary invaders, colonizing tree hosts only after their defenses have already been weakened (Matusick et al. 2013). Additionally, their associated ophiostomatoid fungi, which are carried externally on the exoskeleton, are usually considered weak pathogens (Jacobs and Wingfield 2001). However, there is still relatively too little known about the system, and in particular, nothing is known about how loblolly pine responds to colonization by the fungal species.

In this study, we focused on the phenolic metabolisms of loblolly pine, which, together with the terpenoid component, is one of the main defense mechanisms of the species. In fact, while terpenoids of loblolly pine have been widely explored, surprisingly, phenolics are currently totally unexplored. Objectives of the study were: (1) to characterize the constitutive phenolic profile of loblolly pine phloem, and (2) to investigate the qualitative and quantitative induced responses of the phenolic metabolism to inoculation with blue-stain fungi commonly associated with root feeding beetles in the region.

Using a nested randomized complete blocked design, 45 mature loblolly pines were selected from a planted stand in Whitehall Forest (Athens, GA). Trees were assigned to one of five different treatments: (1) inoculation with *Grosmannia alacris*, (2) *G. huntii*, (3) or *Leptographium profanum*, (4) sterile wounding and (5) non-wounded control. For inoculations, four plugs of phloem were removed from each tree with a cork borer, and then substituted with another bark plug that had been previously sterilized and then colonized by the fungi (fig. 1). All fungal isolates used in this study had been isolated from the surface of root-feeding beetles collected in the area. For sterile wounding, the plug was not replaced after removal, while control trees were left unwounded. Plugs collected at the time of treatment (September 2017) were used for the characterization of the constitutive phenolic profile. Four weeks after treatment, the outer bark surrounding inoculation sites was removed and vertical length of each induced lesion (fig. 2) was measured. Phloem tissues surrounding the inoculation sites and sterile wounding sites were collected and immediately stored in liquid nitrogen. Also at this time, phloem samples from non-wounded control trees were collected. All samples were then transported to the lab and stored at -80˚C until further processing.

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Figure 1—Loblolly pine bark plugs previously sterilized and then colonized by one of the fungal species used in this experiment. Inoculations consisted in removing bark plugs from the experimental trees and substituting them with colonized ones. (courtesy photo by Caterina Villari)

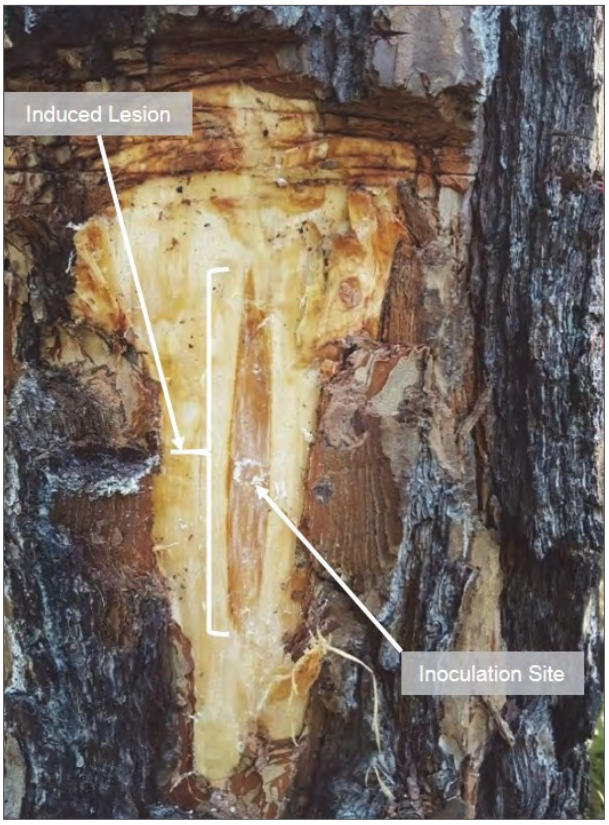

Figure 2—Fungal induced lesion in loblolly pine phloem. Lesions were measured in centimeters and used to estimate virulence of pathogens. (courtesy photo by Zack Parker)

Collected tissues were ground to powder in liquid nitrogen, and phenolics were extracted in methanol. Extracts are currently being analyzed using a combination of ultra-high pressure liquid chromatography—diode array detector (UHPLC-DAD) and high-pressure liquid chromatography—mass spectrometry (HPLC-MS) approaches.

Preliminary results show that all fungal inoculations produced significantly longer lesions than sterile wounding alone, and that inoculations with *G. huntii* produced the longest lesions. Additionally, preliminary observations of the phenolic profile indicate distinct quantitative changes in specific compounds after inoculation. Results from this study will provide valuable insights into the phenolic response of loblolly pine to fungal infections. Findings will also allow for further investigations regarding the effects of those phenolic compounds most responsive to the fungal induction on the survival and fitness of the same inoculated fungal species.

LITERATURE CITED

Coyle, D.R.; Klepzig, K.D.; Koch, F.H. [et al.]. 2015. A review of southern pine decline in North America. Forest Ecology and Management. 349: 134–148.

Eckhardt, L.G.; Weber, A.M.; Menard, R.D. [et al.]. 2007. Insect-fungal complex associated with loblolly pine decline in central Alabama. Forest Science. 53: 84–92.

Jacobs, K.; Wingfield, M.J. 2001. Leptographium Species: tree pathogens, insect associates, and agents of blue-stain. American Phytopathological Society Press. 207 p.

Matusick, G.; Menard, R.D.; Zeng, Y.; Eckhardt, L.G. 2013. Root-inhabiting bark beetles (Coleoptera: Curculionidae) and their fungal associates breeding in dying loblolly pine in Alabama. Florida Entomologist. 96: 238–241.

BREEDING FOR RESISTANCE TO PATHOGENIC PINE WOOD NEMATODES

Kwan-Soo Woo[1], Donghwan Shim[1], Sung-Joon Na[1],
Jinjoong Kim[1], and Il Hwan Lee[1]

We conducted an inoculation test using 231 open pollinated families (total 6,869 seedlings) of *Pinus densiflora* Sieb. et Zucc. and 301 open pollinated families (total 9,020 seedlings) of *Pinus thunbergii* Parl. to evaluate their susceptibility and mortality with pathogenic nematodes, *Bursaphelenchus xylophilus*. The 2-year-old seedlings were inoculated with the 3,000 nematodes/seedling in a nursery bed from July 5 to 20, 2017. The susceptibility of pine species to infection by *B. xylophilus* varied between pine species and among families. The average survival rate of *P. densiflora* at the time of the final sampling (about 60 days after inoculation) was 48.3 percent, and that of *P. thunbergii* was 12.5 percent. The average number of *B. xylophilus* recovered from 11 stems of *P. densiflora* and 12 stems of *P. thunbergii* was ranged from 40 to 398 and from 59 to 470 at 60 days after inoculation, respectively. Additional inoculation tests are suggested to identify tolerant families and individuals, particularly those that prevent nematode reproduction.

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VARIATIONS IN SUSCEPTIBILITY TO DOTHISTROMA NEEDLE BLIGHT AND PINE PITCH CANKER SCOTTISH POPULATIONS OF SCOTS PINE

Stuart Fraser[1,5], Steve Woodward[1], Anna Brown[2], Eugenia Iturritxa[3], Julio Casero Diez[4], and Jorge Martin-Garcia[4]

Pinus sylvestris is the national tree of Scotland, where it occurs naturally in a number of fragmented remnant populations. These populations show local adaptation in several traits, including susceptibility to pests and pathogens.

Experiments were undertaken to investigate among-population variation in susceptibility to two important diseases. The first, dothistroma needle blight (DNB), caused by *Dothistroma septosporum*, is already an important disease in British pine plantations. The second, pine pitch canker (PPC), caused by *Fusarium circinatum*, is an important future threat to UK forestry, being established in pine plantations and forest nurseries on the Iberian Peninsula.

Artificial inoculations with *D. septosporum* suggested that among-population variation in susceptibility exists within native Scottish *P. sylvestris*. Natural infection experiments at two field sites in Scotland, however, indicated that apparent relative susceptibility of *P. sylvestris* populations to DNB was not stable between sites or between years. When DNB severity was greatest in the field, the relative susceptibilities of populations correlated with those seen in artificial inoculation experiments. This variation was unrelated to the relative-continentality of populations.

Variations in susceptibility to PPC of 2–3 year old plants was examined in inoculations made in Phytotrons in Palencia and in glasshouses in Vitoria, Spain. Inoculation resulted in resin bleeding in most plants. Variations in susceptibility to *F. circinatum* between populations was unclear: most plants died within 80 days of inoculation. Numbers of survivors were greatest in the North Central population. Small numbers from 4 populations remained alive at 132 days after inoculation. Glasshouse inoculations largely corroborated the results of the Phytotron experiments: a few plants survived inoculations, but most died within the first 90 days after inoculation. Field inoculations, carried out in an area of Cantabria with plantations of radiata pine badly affected by PPC, gave inconclusive results, with inoculated plants often re-growing from points below the infection.

Very low numbers of *F. circinatum* conidiospores (50 in total) were required to kill most germinating *P. sylvestris* seed; greater losses occurred with increasing spore numbers. Highest rates of survival occurred in the Ballochbuie population.

Further work is required to determine the reasons for between-site variation seen in Scots pine responses to DNB and the roles of potential resistance mechanisms in this host-pathogen system. The findings presented are guiding improved DNB management in Britain. For the potential impacts of PPC, future research should focus on whether the lower susceptibility of some plants to the pathogen was due to genetic diversity in the Scottish provenance of *P. sylvestris*. Moreover, the susceptibility of Scots pine to multiple pathogens must be considered in future work.

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BREEDING AND DEPLOYMENT OF INSECT AND DISEASE RESISTANCE TREES IN BRITISH COLUMBIA: A 2018 UPDATE

Alvin Yanchuk[1]

At the 2011 meeting in Eugene, OR, I provided a "thought piece" where I assumed we won't have much success if we continue to breed trees for any individual pests or diseases, one at time. But most of us were somewhat overwhelmed of what that could mean, in terms of our research program, building new partnerships, obtaining funding for something that may not exist (i.e., the pest or disease) at this time, and the complexity in what to screen or test for. We are making small steps in the respect, and our program is expanding to include more pest and disease traits. For instance, (1) Swiss Needle Cast in Douglas-fir, (2) lodgepole pine rusts (western gall rust, commandra), and needle disease (*Dothistroma*), (3) a new cedar leaf rust, (4) western tent caterpillar in alder, and (5) western spruce budworm in interior Douglas-fir (see Strong presentation), to mention a few. We are still actively trying to improve resistance in our long-term programs (i.e., blister rust, spruce weevil, etc.), and hopefully over time, with more research, we might uncover more traits that could provide more durable trees (i.e., trees with "cross resistance"). Without a doubt, climate change, new exotic pests, the outbreak of our current pests, and major disturbances by fires, haven't gone away and forest tree improvement programs will continue to show they have a large part to play in managing our forests.

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RESTORATION AFTER THE LAUREL WILT DISEASE EPIDEMIC

Katherine Smith[1], Junli Zhang[2], Marc Hughes[3], Craig Echt[1],
Sedley Josserand[1], C. Dana Nelson[4], and Jason Smith[2]

The causative fungus of laurel wilt disease, *Raffaelea lauricola*, is a symbiont of the redbay ambrosia beetle, *Xyleborus glabratus*. The beetle was likely introduced to the United States through the port of Savannah in 2002. Since then the disease has spread across the southeast causing massive losses to redbay (*Persea borbonia*) and swamp bay (*Persea palustris*) populations. Restoration efforts will involve replanting of resistant genotypes and preservation of germplasm at botanical gardens and on public lands. Beginning in 2007, redbay survivor trees were collected from epidemic areas and propagated with rooted cuttings. This material was then screened for resistance using fungal inoculation. In this work, 22 genotypes with 3 to 21 replicates were tested. Least squared means rankings for 12 of the genotypes were compared to previous inoculation experimental rankings. This showed that most genotypes are highly susceptible to fungal inoculation, while only one genotype showed high tolerance in multiple experiments. In addition, many other *Lauraceae* species, including silkbay, sassafras, and avocado have been affected by laurel wilt disease. In further work, the population genetic structure of three ecologically important *Persea* species redbay, swamp bay, and silk bay (*Persea humilis*), from five different Florida sandhill habitat locations were studied. Twenty trees were sampled per species at each location and data for 16 microsatellite loci were determined for each sample. Results show genetic differences between species and the strongest location differences among redbay. Taken together these data can be used to aid restoration efforts of both the *Persea* species in Florida and redbay across the southeast.

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THE EFFECTS OF LEAF WAX COMPOSITION AND RATIO ON RUST RESISTANCE OF POPLARS

Zhongdong Yu[1], Wei Zheng[1], Kuocheng Shen[1], Dan Yu[1], and Zhimin Cao[1]

Populus tomentosa, *Populus deltoides* Marsh and *Populus purdomii* Rehd are three typical poplar which show different resistant type to *Melampsora larici-populina*. Leaf surface waxes were extracted by chloroform, and detected by gas chromatography-mass spectrometry (GC-MC), scanning electronic microscope (SEM). Leaves of *Populus tomentosa* fixed the highest wax content of 5.6628μg · cm^{-2}, followed by *Populus deltoides* 4.1371μg · cm^{-2}, and then *Populus purdomii* Rehd with 1.6667μg .cm^{-2}, which conformed to the thickness under the SEM and the phenotype of rust resistant poplars. Alkanes, alcohols, aldehydes, fatty acids, esters and a small amount of some phenols, ethers, ketones, amides substances are the main composition of leave surface wax. Among them, C12 and C15 phenols, C8 and C18 amides, are both directly correlated with poplar tree rust resistance. Additionally, amides show higher rights than phenol substances for evaluation of poplar rust resistance. Wax content ratio is an important index for evaluating poplar rust resistance, especially the C18 amides ratio can directly be used for Melampsora resistance in poplar breeding.

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The Sixth International Workshop on the Genetics of Host-Parasite Interactions in Forestry—Tree Resistance: Putting Promise into Practice was held the first week of August 2018 at Deer Creek State Park in Mt. Sterling, Ohio. The workshop provided a continuing forum for researchers, tree breeders, and forest managers to focus on perhaps the most salient action society can take to restore tree species imperiled by invasive pests and pathogens—developing and deploying resistant tree populations. The 95 presentations (oral and poster) covered a wide range of tree species and associated pathogens and pests from throughout the world. Ash species (genus *Fraxinus*) throughout Europe and North America face relatively new perils (ash dieback disease and emerald ash borer) and a special session was devoted to this genus. Screening for resistance is a fundamental component of resistance development and the topic of another full session. Additional presentations detailed efforts to develop genomics and biotechnology tools and resources that have the potential to increase the efficiencies of applied resistance programs. Other presentations provided inputs on collaborative breeding approaches, citizen science in forest health, the potential role of endophytes in managed forests, threats to agroforestry species, prioritization of species in need of resistance programs, considerations necessary for the use of new biotechnologies, and concepts for integrating genetic, phenotypic and environmental data across host-parasite systems. Several presentations provided updates on "the ultimate goal"—progress of applied resistance programs and actual restoration efforts—leading into discussions on key topics such as the durability, stability, and usability of resistance in forest tree species. Taken together the presentations and discussions provided ample evidence that developing resistant populations is a viable (and in some cases necessary) approach for society to use to ensure healthy forests for future generations. These proceedings document the presentations given as lightly reviewed full papers, extended abstracts, and standard abstracts. In concluding the workshop, the attendees approved Spain by acclamation as the host of the Seventh Tree Resistance Workshop to be held in 2020.

Keywords: Durable resistance, ecosystem restoration, forest health, genetics of resistance, insect and disease mitigation, invasive pests and pathogens, resistance breeding.

A copy of this general technical report is available for download at www.srs.fs.usda.gov/pubs/.