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Proceedings of Workshop on Gene Conservation of Tree Species–Banking on the Future

May 16–19, 2016,
Holiday Inn Mart Plaza, Chicago, Illinois, USA

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Proceedings of Workshop on Gene Conservation of Tree Species—Banking on the Future

May 16–19, 2016
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Chicago, Illinois, USA

Richard A. Sniezko, Gary Man, Valerie Hipkins, Keith Woeste, David Gwaze, John T. Kliejunas, and Brianna A. McTeague

Technical Coordinators

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Preface

Trees play an important and critical role in our forests and woodlands. People depend on trees for food, fuel, paper and wood products, recreation, and livelihoods. Trees are the foundation of biodiversity, creating functioning ecosystems that provide clean air, water, and other benefits. The “Gene Conservation of Trees – Banking on the Future” workshop brought together people with a broad array of perspectives and from varying organizations to discuss and explore collaborative solutions to conserve the genetic tree material that could one day provide the product that cures cancer, or advances the social and economic well-being of our society. This unique meeting provided an opportunity for genetic conservationists from botanic gardens and arboreta, universities, the Forest Service, and other state and federal agencies to meet, often for the first time. To strengthen and formalize this growing relationship, the American Public Gardens Association, the Botanic Gardens Conservation International, the Center for Plant Conservation, and the Plant Conservation Alliance signed a memorandum of understanding (MOU) with the Forest Service at the meeting. The MOU sets up a framework for collaboratively sharing information and genetic material, conducting research of mutual interest, and developing activities that enhance in situ and ex situ genetic conservation of at-risk tree species.

Much of the meeting focused on the current work of conservationists with an eye to the future, but we must not forget, and in fact build upon, the work of our predecessors. The efforts of Thomas Ledig, Bruce Zobel, Gene Namkoong and many others set the ground-work for much of what we do today in tree genetic conservation in the U.S. and internationally. With an understanding and appreciation for past accomplishments, present work, and future opportunity, we will be able to achieve much more through effective collaboration than we can realize through individual efforts. We look forward to engaging with genetic conservationists as we work together for our future

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Participants in the Gene Conservation of Tree Species–Banking on the Future workshop - May 16-19, 2016, Holiday Inn Mart Plaza, Chicago, IL, USA (Photo: Murphy Westwood)

Abstract

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The 'Gene Conservation of Tree Species—Banking on the Future Workshop' provided a forum for presenting and discussing issues and accomplishments in genetic conservation of trees, and notably those of North America. The meeting gathered scientists, specialists, administrators and conservation practitioners from federal, university, non-governmental and public garden institutions worldwide. The 81 submissions included in this Proceedings are from oral and poster presentations at the 2016 workshop held in Chicago, Illinois. They update the science and policy of genetic conservation of trees, showcase current successes, and provide guidance for future efforts. This Proceedings is complemented by 11 related papers gathered in a special issue of the journal New Forests (Vol 48, No. 2, 2017). In addition to plenary talks that provided overviews of some national and international efforts, there were concurrent sessions with themes of Conservation Strategies, Pest and Pathogen Resistance, Genetic Conservation, Tools for Tree Genetic Conservation, Conservation Program Case Studies, Designing Seed Collections, Ex Situ Conservation, and Science in Support of Conservation. The meeting was also the venue for special sessions on Coordinating the Red List of North American Tree Species, Innovative Approaches for Assessing and Prioritizing Tree Species and Populations for Gene Conservation, Community Standards for Genomic Resources, Genetic Conservation and Data Integration, and Development of Seed Zones for the Eastern U.S., and a group discussion on Improving Genetic Conservation Efforts.

Key words: Genetic conservation, threatened & endangered species, climate change, in situ, ex situ

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Plenary Talks

The Importance of Gene Conservation in the USDA Forest Service[1]

Robert D. Mangold[2]

Abstract

Aldo Leopold once said "to keep every cog and wheel is the first precaution of intelligent tinkering." The USDA Forest Service has embarked on a long-term effort to do just that. Our gene conservation efforts in forest trees are a modest beginning to this urgent need. In the early 2000s, the Forest Health Protection Program and its partners in the National Forest System and Research and Development Deputy Areas of the Forest Service initiated seed collections in several five-needle pine species that were succumbing to white pine blister rust and bark beetle attacks at accelerated rates. We began with a simple plan to start small and try to build momentum over time. We held a number of important meetings, one in particular at the Dorena Genetic Resource Center, where we laid out principles for a potential gene conservation effort. The efforts of the Conservation Assessment and Prioritization of Forest Trees under Risk of Extirpation (CAPTURE) group who have led this endeavor, are also presenting at this workshop.

This paper will discuss the value and importance of gene conservation work. Given the rapid changes we are witnessing in our environment, this work takes on a new urgency. Climate change, invasive species and other vectors of change will catapult us into unchartered territory. I will talk about the challenges we face as an agency and in the forest community at-large to ensure ample and resilient forests in the 21st century. Hopefully, this discussion will help set the stage for adapting to and mitigating the challenges that await us in the very near future.

[1] A version of this paper was presented at the Gene Conservation of Tree Species – Banking on the Future Workshop, May 16-19, 2016, Chicago, IL.

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Plant Conservation Progress in the United States[1]

Kayri Havens,[2] Andrea Kramer,[2] and Ed Guerrant[3]

Abstract

Effective national plant conservation has several basic needs, including: 1) accessible, up-to-date information on species distribution and rarity; 2) research and management capacity to mitigate the impact of threats that make plants rare; 3) effective networks for conserving species *in situ* and *ex situ*; 4) education and training to make sure the right people are addressing the issues; 5) policy that supports conservation; 6) funding to maintain the infrastructure for plant conservation; and 7) effective communications so that plants are valued and supported by the public. The more coordinated these efforts are, the more strategic, efficient and effective they can be. We argue that plant conservation still has a long way to go because plant species are becoming increasingly rare, threatened by habitat loss, fragmentation, climate change, and the continued introduction of new invasive species (pests, pathogens and plants). Here we outline areas where the United States is strong, and areas where it needs to improve in order to meet its plant conservation needs. We will discuss the role of the updated Global Strategy for Plant Conservation, the National Seed Strategy, and other recent policy documents in providing a road map for successful gene conservation.

[1] A version of this paper was presented at the Gene Conservation of Tree Species – Banking on the Future Workshop, May 16-19, 2016, Chicago, IL.

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Forest Gene Conservation From the Perspective of the International Community[1]

M. Hosny El-Lakany[2]

Abstract

As a background, this presentation begins by briefly highlighting the interests of the international community in the conservation of forest genetic resources (FGR). After presenting internationally adopted definitions of some terms related to FGR, the characteristics of the current state of FGR conservation from a global perspective are summarized.

Many international and regional organizations and institutions are engaged in the conservation of FGR at degrees ranging from core mandate to indirect interest. Relevant actors are listed under each of the following categories: United Nations agencies and programs; the Rio Conventions; Consortium of International Agricultural Research Centres (CGIAR); advocacy and special interest groups; discussion and policy making fora; panels of experts and data bases. This presentation outlines their mandates, *modus operandi* and activities as far as conservation of FGR is concerned.

Overlaps in the mandates and strategic objectives of international organization are identified in general, then illustrated using Food and Agriculture Organization (FAO) and Convention on Biological Diversity (CBD) as examples. The need to enhance coordination and collaboration among international organizations is highlighted followed by proposals to help manage, with a view to minimize, overlaps. Potential roles for FGR conservation in the operationalization of some recent inter-institutional programs as well as inter-governmental policies and agreements such as reducing emissions from deforestation and forest degradation in developing countries (REDD+); and the role of conservation, sustainable management of forests and enhancement of forest carbon stocks in developing countries), sustainable development goals (SDG's); and the Paris Agreement, are described briefly.

Finally, the presentation concludes with recommendations for the way forward towards achieving a meaningful global conservation of FGR. A list of the main literature consulted is given at the end.

[1] A version of this paper was presented at the Gene Conservation of Tree Species – Banking on the Future Workshop, May 16-19, 2016, Chicago, IL.

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BGCI's Role in Co-ordinating a Botanic Garden-Centred Global System for the Conservation of All Tree Diversity[1]

Paul Smith[2]

Abstract

It is estimated that there are at least 60,000 tree species globally and that more than 20 percent of them are threatened with extinction. This threatened tree diversity will have a critical impact for solving some of this century's major challenges in the areas of energy, water scarcity, human health, food security, climate change and habitat degradation.

Botanic gardens and arboreta offer the opportunity to conserve and manage a wide range of plant diversity *ex situ*, and *in situ* in the broader landscape. The rationale that botanic gardens have a major role to play in preventing plant species extinctions is based on the following two assumptions:

- There is no technical reason why any plant species should become extinct. Given the array of *ex situ* and *in situ* conservation techniques employed by the botanic garden community and other partners, we can avoid species extinctions.
- The professional community associated with botanic gardens possesses a unique set of skills that encompass finding, identifying, collecting, conserving and growing plant diversity across the entire taxonomic spectrum.

Botanic Gardens Conservation International (BGCI) sits at the centre of a global network of about 2,600 botanic gardens and arboreta, that includes: living collections representing at least one third of known plant diversity; world class seed banks, glass houses and tissue culture infrastructures, and; technical knowledge networks covering all aspects of plant conservation. However, current estimates suggest that only 25 percent of threatened tree diversity is currently held in the living collections and seed banks of botanic gardens and arboreta.

Following the example of the crop conservation community, BGCI is promoting the concept of a cost-effective, rational, botanic garden-centred Global System for the conservation and management of tree diversity. This system will aim to collect, conserve, characterise and cultivate samples from all of the world's rare and threatened trees as an insurance policy against their extinction in the wild and as a source of plant material for human innovation, adaptation and resilience.

BGCI leads or helps to co-ordinate the following components of the Global System:

- A comprehensive, geo-referenced list of all known tree species – GlobalTreeSearch;
- The Global Tree Assessment which aims to assess the conservation status of all tree species by 2020;
- The Global Trees Campaign which provides technical and financial resources for integrated tree conservation on the ground, and;
- Co-ordinating mechanisms for deploying targeted expertise and resources, including IUCN's Global Tree Specialist Group; BGCI's Global Seed Conservation Challenge; technical consortia such as the Global Oak Initiative, and; BGCI's Twinning programme matching gardens with similar interests.

The speaker will set out BGCI's rationale, vision and mechanisms for mobilizing the global community of botanic gardens and arboreta for tree conservation using this cost-effective, rational approach.

[1] A version of this paper was presented at the Gene Conservation of Tree Species – Banking on the Future Workshop, May 16-19, 2016, Chicago, IL.

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Special Sessions

Improving Genetic Conservation of Tree Species[1]

Pam Allenstein,[2] Jennifer DeWoody,[3] David Gwaze,[4] Valerie Hipkins,[5] Gary Man,[6] Anna Schoettle,[7] Kirsty Shaw,[8] and Murphy Westwood[9]

Background

The aim of this workshop breakout group session was to review significant gaps within each of three major themes (*In-situ* Conservation, *Ex-situ* Conservation, and Restoration of Species and Ecosystems) and to identify actionable solutions to move genetic conservation efforts forward. In order to identify solutions and action items for the tree conservation community, participants were asked to consider the session goals throughout the proceedings, provide examples of gaps in the field, and suggest actions to overcome roadblocks. To maximize participant feedback, a number of easel pads were made available throughout the workshop so that people could write comments at will. In addition, a discussion session was held at the end of the workshop to contemplate and discuss issues. Comments from the session were recorded separately and added to those captured on the easel pads.

Following the workshop, all written comments were transcribed into a spreadsheet and logged by theme, participation method (easel pad or discussion session), and (if provided) whether the comment identified a gap or suggested an action. The comments were then assessed for similarities in order to define a set of categories uniting remarks. The categories were developed post hoc by a single reviewer who did not attend the Workshop, and therefore had no prior knowledge of the discussion of each theme, but instead grouped comments based solely on the transcribed notes. The use of an outside reviewer was made to eliminate bias in capturing and synthesizing comments.

During the synthesis of the comments, it became clear that the distinction between "gap" and "action" was purely grammatical, and was not a meaningful way to group responses. For instance, two comments, "Need a centralized database" and "Develop plant search engine for gardens, etc.", identify the same issue (a need for better information technology infrastructure that captures the facilities and people working to conserve specific plant species), so the distinction between gap and action was solely due to sentence construction. In addition, we made no effort to collapse multiple comments on a single topic into one item. We counted every comment recorded, which may have inflated the input of vocal participants, but should reflect the proportion of time spent discussing each issue, a proxy for the complexity of or need for the action.

Responses were then synthesized as the number of comments in each category within each theme, with the distribution of comments across themes qualitatively and quantitatively assessed. Individual topics that crossed themes or appeared critical to one theme were identified for discussion here, as were those items that appeared the most actionable or offered the greatest return on investment. The latter items are proposed as Actionable Items.

Outcomes

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[1] A version of this paper was presented at the Gene Conservation of Tree Species – Banking on the Future Workshop, May 16-19, 2016, Chicago, IL.

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A total of 193 comments were recorded throughout the workshop. Eleven of the comments were classified into two categories, producing a final tally of 204 theme-comment-category items synthesized here. The *Ex-situ* theme received the greatest number of comments (n = 86), followed by the Restoration theme (n = 67) and then the *In-situ* theme (n = 51).

Nine categories were found to be sufficient to group all comments during the post hoc synthesis (table 1). Comments were assigned to one category, with the exception of 11 comments that were placed into two categories.

Table 1—Nine categories identified post hoc were sufficient to group all comments on *In-situ*, *Ex-situ*, and Restoration themes during the workshop

Category	Description
Authority/Jurisdiction	Federal or local code, or agency direction, providing the ability to conduct conservation or restoration activities.
Collections	Either existing or ongoing assemblies of living material or germplasm, with particular interest in tree species. May be publicly or privately held, with various management and funding structures. Includes how to coordinate, design, and maintain collections; financing issues were classified as "Funding".
Communication – Public	Dissemination to or coordination with public, either private individuals or non-conservation organizations. Does not imply collaboration or cooperative agreements but rather the transfer of information.
Communication/Collaboration	Coordination between conservation organizations, including governmental agencies, universities, and non-governmental organizations, as part of conservation or restoration activities.
Definitions/Goals	Descriptions of aims and objectives of individual projects or larger subtopics within conservation genetics.
Funding	Programmatic or project funding from any source.
Information Technology	Database and web infrastructure for data maintenance and collaboration. Specific platforms, owners, and support vary.
Protocols	Detailed methods or guidelines for specific conservation actions or goals. "Technology transfer", for example.
Research	Basic or applied research to develop protocols or guidelines for conservation programs or activities.

Categories

Comments were not distributed evenly among categories (fig. 1). Comments related to Definitions/Goals and Communication/Collaboration were the most numerous, followed by comments related to Research. The remaining six categories contained a more even number of comments, with Authority/Jurisdiction having the fewest.

Comments for each theme were not evenly assigned to categories (fig. 2). The distribution of comments among themes (*Ex-situ*, *In-situ*, and Restoration) within each category was tested using Chi-Squared analyses with a simple Bonferroni correction for multiple comparisons. Five categories displayed an uneven distribution of comments among the three themes at an uncorrected $P < 0.05$, and two, Funding and Information Technology, were significantly different even after the multiple test correction ($P < 0.005$).

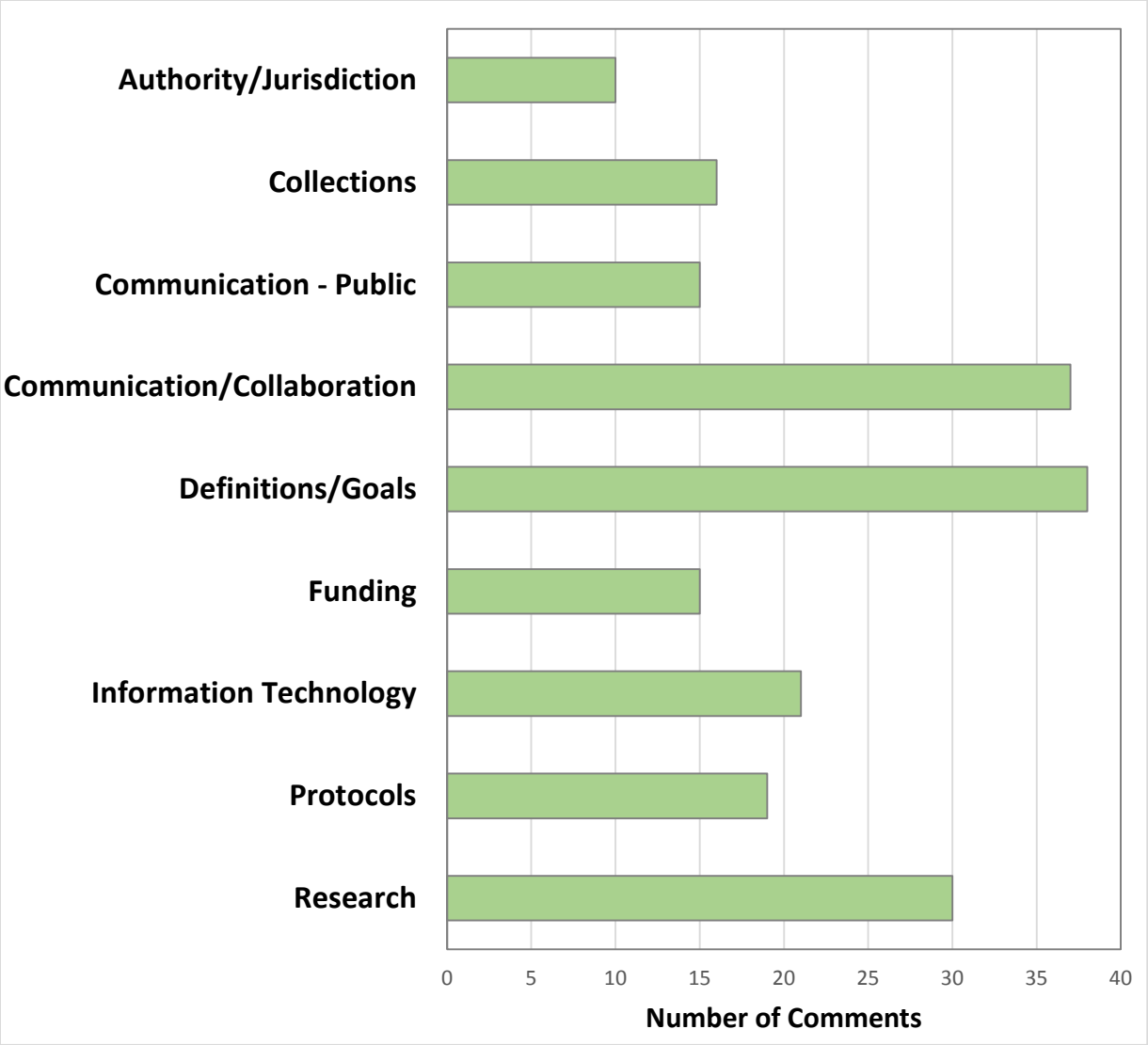

Figure 1—Distribution of 193 comments assigned to nine categories. Categories were defined post hoc during synthesis of the workshop responses. Eleven comments were assigned to two categories each (total count displayed = 204).

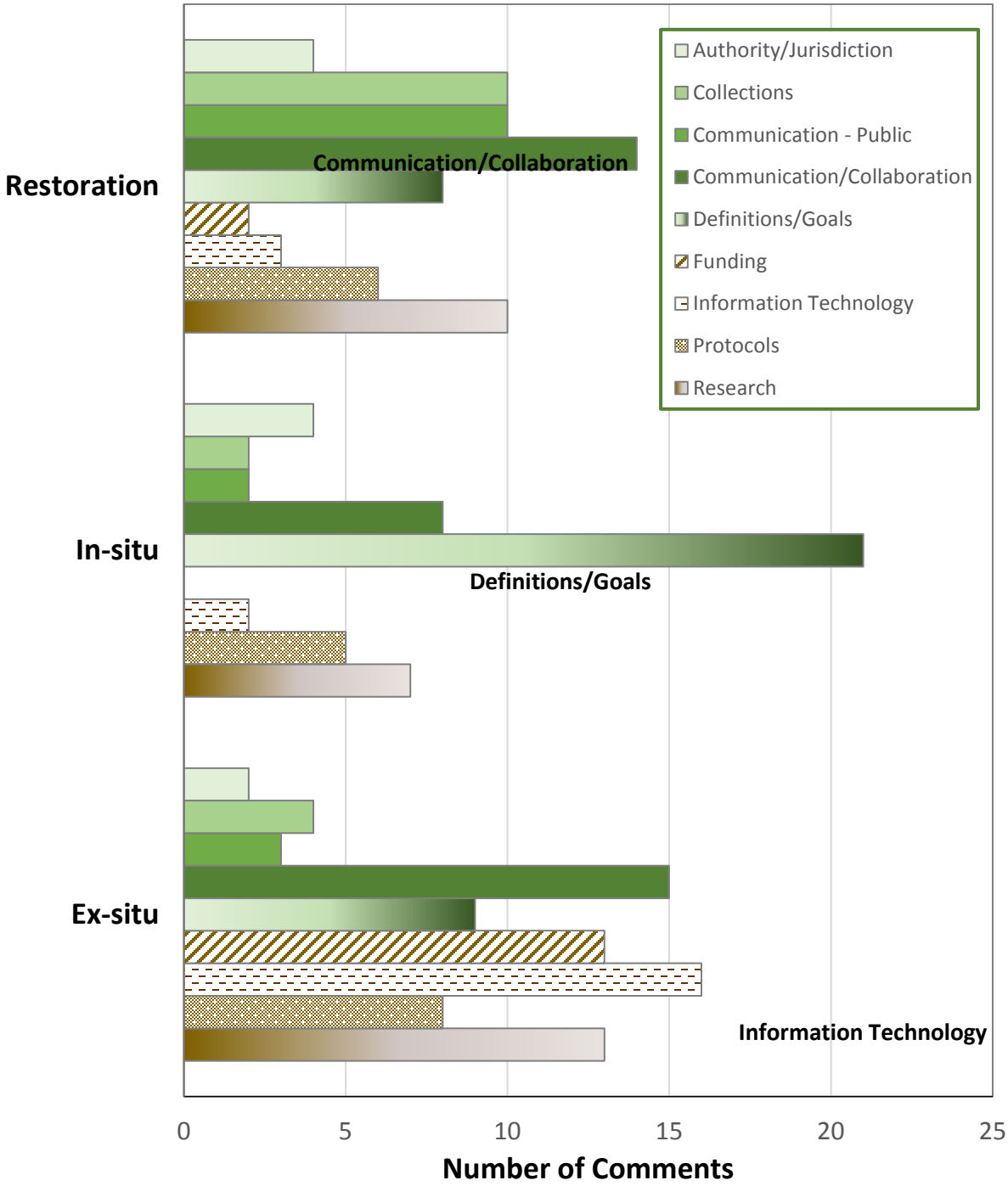

Figure 2—Distribution of comments among categories varied across the three themes of the workshop. The category containing the greatest number of comments is labelled for each theme.

Synthesis of Comments

Needs related to Communication/Collaboration were evenly distributed across themes, but represented the greatest proportion of comments within the Restoration theme. Different needs were identified within each theme. Comments pertaining to the *Ex-situ* theme focused on a need for networks of botanic gardens/arboreta/plant or seed collections to better coordinate conservation efforts, with particular

mention of tropical species. Coordination between *ex-situ* and *in-situ* efforts to improve efficiency and effectiveness of programs was also discussed. For *In-situ* conservation, comments identified a need to improve coordination across all levels of land ownership: private land owners, local municipalities, state agencies, federal departments, and even international organizations. Comments related to Restoration focused on improved dissemination of management plans and germplasm resources, especially between public and private owners and seed sources. Several examples of local or regional success stories or examples of collaborations were provided.

Comments identifying needs or opportunities for research were also evenly distributed across themes. Within each theme, many responses indicated the need for better models of future conditions to help plan collections or reserves. In the *Ex-situ* theme, comments indicated a need for predictive models and guidelines for future needs, including climate models and recommendations for proactive collections to use in future restoration efforts. In addition, research into methods to preserve recalcitrant and disease-prone seed was identified in more than one session. One comment identified the need for economic estimates to put values on collections, demonstrating the interdisciplinary nature of conservation efforts. *In-situ* conservation would benefit from better definitions for and measures of local provenance, and methods to assess the adaptability of collections as environments change. In context of declining populations and changing climate, it would be helpful if researchers' could determine if existing reserves will protect species and if reserves are sufficient for future needs. Comments in the Restoration theme identified a lack of pest and disease resistant planting stock for restoration activities. In addition, better guidance on "locally appropriate" and seed transfer zones was requested. Commenters also requested a method to identify species of greatest conservation need (deficit-based models), and estimate the economic value of restoration activities.

Comments related to Authority/Jurisdiction and Protocols were evenly distributed among themes, indicating that all three conservation approaches (*Ex-situ*, *In-situ*, and Restoration) face issues related to legal standing or organizational priorities, and all three would benefit from additional protocol development and distribution. Issues related to Authority/Jurisdiction included memoranda of understanding among multiple agencies and challenges related to conservation when species occur on private lands. Specific protocols identified in comments included needs for best management practices (BMPs) and methods to conserve species with recalcitrant seed.

Restoration

The majority of all comments classified as "Collections" were related to the Restoration theme (10 of 16 total comments). Most of these comments concerned the availability of plant material of known provenance appropriate for restoration projects. Specific comments identified a general lack of appropriate seed from commercial sources (especially native and recalcitrant species), a lack of identified seed sources, a lack of commercialization of native plant genetic resources, and a dwindling number of seed collectors and nurseries. One comment identified confusion about whether the quantity of existing seed collections is sufficient for restoration activities or only for *ex-situ* conservation of variation. While all of these concerns may be common to both *ex-situ* and *in-situ* projects, the vast number of comments provided to the Restoration theme indicated that obtaining appropriate seeds/propagules in sufficient quantity for restoration of trees may be a key barrier to implementing management plans.

The Restoration theme also received the majority of comments related to communicating with the public (10 of 15 comments), demonstrating the importance and difficulty of describing restoration plans to interested and affected parties. Comments focused on the importance of informing and educating the public on restoration activities, because public backlash may halt projects, and public support can help stabilize funding. Commenters suggested building a message ("Restoration Revolution") and applying a public relations approach to projects, including building demonstration gardens, educating private landowners on the value of appropriately-sourced seed, and even gaining funding through a Super-PAC.

Together, comments identified as barriers to restoration activities under the Restoration theme were limitations of source material, seed transfer guides in the context of a changing environment, and public support.

In-situ Conservation

The *In-situ* Conservation theme received the majority of total comments related to Definitions/Goals (21 of 38 comments). Comments in this category, and for the *In-situ* Conservation theme in particular, depicted a lack of investment in and attention by conservation experts to the exploration and development of *in-situ* conservation approaches, especially those that include managed conservation areas. Many comments contained phrases such as, "decide on goals", "need…clear picture of what we are trying to do and why", "what are we conserving?", and "differences of scale in definitions." In particular, several comments illustrated the vague differences between *in-situ* preserves and wilderness areas, and the potential conflict between a laissez-faire approach (e.g., wilderness areas) and active management efforts (e.g., conservation activities), typified by the differences between the U.S. Department of the Interior National Park Service and the U.S. Department of Agriculture (USDA) Forest Service. Multiple comments depicted the difficulty of defining *in-situ* areas when faced with potential habitat shifts due to climate change, and the need to manage for ecosystem function not solely for species occurrence. In the past, "setting aside" land for conservation (e.g., wilderness) was considered sufficient, but with climate change, land use change, changing disturbance regimes, and invasion by non-native organisms, we now see that these areas may be inadequate to conserve ecosystems, their functions, or target species. Hence, the concept of managed, *in-situ* conservation areas or proactive conservation (to sustain threatened but not yet degraded ecosystems or species, facilitate adaptation or migration, etc.), is relatively new and more research and development is needed to fully realize its application and potential.

No comments assigned to the Funding category were made under the *In-situ* Conservation theme. This may indicate that defined goals and conceptual frameworks, not funding, most limit in-situ activities.

Also of note for the *In-situ* Conservation theme was one comment assigned to the Collections category that identified a need to maintain detailed histories of seedling and provenance trials. This suggestion is applicable to all three themes, and was identified as a need for collections used in restoration activities.

Ex-situ Conservation

The majority of total comments related to funding (13 of 15) were provided for the *Ex-situ* Conservation theme. Funding was broadly defined to include potential funding opportunities (e.g., USAID grants for overseas collaboration), direct funding (e.g., budgets and accounting), and non-Information Technology infrastructure (e.g., building capacity). Several comments questioned the current capacity of the national seed banks and collections to maintain the quantity of seed required for forest species. Another theme described the need for long-term, stable funding to maintain living collections of tropical species. Additional investment in the management and measurement of long term provenance tests was also identified as a need, along with a succession plan to maintain institutional knowledge of ongoing projects. Lack of funds to grow seed of sufficient quality for restoration efforts was also identified as a limiting factor. One comment suggested offering a prize for developing techniques to meet specific needs (e.g., recalcitrant seed).

The majority of total comments classified as Information Technology (16 of 21) were provided under the *Ex-situ* Conservation theme. Most comments identified a need for easy-to-use, high-functioning, international databases accessible via web interfaces to maintain and distribute information relating to seed collections, living collections, provenance tests, protocols, and genetic data (essentially all aspects of genetic conservation). Comments also identified barriers to sharing information, including incompatible existing databases, lack of national networks, and the lack of participation in existing databases by some collectors (i.e., some desire not to have data fully open access). Additional suggestions included a list serve to disseminate opportunities for collections, collaborations, jobs, and funding sources.

The *Ex-situ* Conservation theme also received comments related to public communication. Some comments provided suggestions to improve science outreach, such as refocusing or repurposing established behaviors such as scientific meetings toward effective communication with the public, and using citizen scientists by developing a "master conservationist" program (similar to the "master gardener" programs). As with the Information Technology suggestions, these actions may benefit any conservation activity.

Together, the comments listed under the *Ex-situ* Conservation theme identified specific needs to increase capacity and collaboration in order to improve collections.

Actionable Items

The following tasks were identified from participant comments during the breakout discussion and are based on their potential for timely implementation in the near-term, ideally across more than one theme. These are listed in no particular order, and they may be moot after additional research into specific needs or input from workshop attendees.

1. Develop a listserv or web page to disseminate information related to:
    a. Collections, protocols, jobs, funding, institutional knowledge of plantings, and federal policies and authorities.
    b. Public-focused websites to communicate issues and techniques related to restoration and conservation.
2. Conduct feasibility studies into the potential to integrate existing databases of collections.
3. Convene an expert panel or working group to develop or disseminate protocols to:
    a. Identify disease resistant material of forest species currently underrepresented on the landscape.
    b. Develop a research strategic plan, and disseminate it to major funding agencies (USDA, National Science Foundation).
4. Convene an expert panel or working group with a focus on living collections, including:
    a. Developing a consortium for tropical species conservation.
    b. Identifying research and protocol needs for conservation of species with recalcitrant seed.
5. Develop a Master Conservationist program to engage citizens as collectors and caretakers on private lands.
6. Within agencies and programs, expand the concept of in-situ conservation to include managed ecosystems, while maintaining the use of Research Natural Areas, National Parks, wilderness, and other preserves.

The future success of tree genetic conservation depends in part on the personal commitment of individuals to be leaders in this effort. At the conclusion of the breakout session, participants were asked to write down their own personal commitments to further gene conservation of tree species within their home organization upon their return following the workshop. A total of 36 commitments were made by the workshop participants. Half of those commitments were made for actions within the Restoration theme, 44 percent were actions in the *Ex-situ* Conservation theme, and 6 percent fell under *In-situ* Conservation. For the *Ex-situ* Conservation theme, the commitments were evenly distributed among the Communication/Collaboration, Information Technology, Protocols, and Collection categories. Commitments were similarly distributed among the same four categories within the Restoration theme, with the addition of Research actions identified. The two commitments made for *In-situ* Conservation fell into the Communication/Collaboration and the Research categories, reflecting the overall impression from the breakout session that *In-situ* Conservation is an under-developed approach, and awareness of its utility is under-explored compared to *Ex-situ* Conservation and Restoration efforts. There were strong personal commitments by participants to share specific information, protocols, and data; refine or develop seed zones; advance collaborations; and develop new partnerships.

Coordinating the IUCN Red List of North American Tree Species: a Special Session at the USFS Gene Conservation of Tree Species Workshop[1]

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Abstract

Conservation status assessments are a valuable tool for the management and protection of rare and endangered species. Categorizing and defining rarity, threats, and population trends is often the first step toward understanding and documenting the health of the world's plant diversity. Having up to date conservation status assessments for all of North America's native tree species, based on a globally standardized system, would enable an objective and systematic prioritization of species for future conservation action and enable stakeholders from a wide range of sectors to engage in informed conservation efforts. For over 50 years, the IUCN Red List of Threatened Species has been the international standard for evaluating the extinction risk of plant and animal species on a global scale. Currently, the tree flora of North America is poorly represented in the IUCN Red List. However, there are other more regionally focused threat assessment platforms being used in North America, creating an opportunity to streamline assessment efforts, share information, and ensure that all of the tree species in the region are evaluated for their level of imperilment. This initiative will require coordination and collaboration among multiple sectors and organizations to ensure that limited resources are maximized to cover all tree species and prevent any threatened taxa from slipping through the cracks. To initiate this collaborative effort, a special session was convened at the Gene Conservation of Tree Species workshop during which experts from each of the four complementary threat assessment platforms (IUCN Red List, NatureServe, United States Endangered Species Act, and U.S. Department of Agriculture Forest Service CAPTURE Program) presented their methods, applications, and progress for evaluating imperilment of North American tree species. A productive discussion session followed that sparked the development of a two phase collaborative project: 1) create a comprehensive, unified checklist of the tree species of North America that can be used to identify gaps and missing taxa from the various assessment platforms, and 2) fill those gaps by systematically and strategically evaluating species so that the threat level of all native trees of North America is known by 2020.

Introduction to the Special Session

For over 50 years, the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species has been the international standard for evaluating the extinction risk of plant and animal species. It is important to ensure that the trees of North America are assessed for the IUCN Red List so that the rich tree flora of that continent can be included in globally standardized biodiversity metrics such as the Barometer of Life (Stuart et al. 2010) and the Red List Index, and to contribute to international conservation policy objectives like the Global Strategy for Plant Conservation (Sharrock 2012). Regionally, there are several different mechanisms for assessing the imperilment of a species in North America (for this initiative we are following the geographic delimitation from the Flora of North America

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– "all trees native and naturalized found in North America north of Mexico." See Flora of North America Editorial Committee, 1993), including: 1) the IUCN Red List, 2) NatureServe's Conservation Status Assessments, 3) the U.S. Endangered Species Act, and 4) U.S. Department of Agriculture Forest Service's (USDA FS) Project CAPTURE (Conservation Assessment and Prioritization of Forest Trees Under Risk of Extirpation). All of these processes evaluate demographic, distribution, population trend, and threat data for a given species to quantify its risk of extinction, but how are they related to each other? What are the requirements and data inputs for each process? What are the pros and cons of each application? Do they build off of each other? And most importantly, can they be streamlined and coordinated to achieve a comprehensive Red List of North American Tree Species, ensuring that extinction risk data from this region is included in global analyses and policy frameworks? During this special session, attendees heard from four experts who explained the fundamentals of each of these conservation status assessment platforms and outlined how each could contribute to a Red List of North American Tree Species. A group discussion followed the presentations, during which much progress was made on establishing a North American tree working group for the purpose of undertaking an initiative to complete Red List assessments for all North American tree species.

Why Conduct Conservation Status Assessments?

Conservation status assessments are a valuable tool for the management and protection of rare and endangered species. Categorizing and defining rarity, threats, and trends is often the first step toward prioritizing which plants are in most urgent need of conservation action. Having up to date conservation status assessments for all of North America's native tree species, based on a globally standardized system, would enable an objective and systematic prioritization of species for future conservation action and enable stakeholders from a wide range of sectors to engage in informed conservation efforts. Conservation status assessments also provide policy makers with clear evidence to support protective regulations for threatened species. Furthermore, many funding agencies and conservation nonprofits require such an assessment as a component of funding proposals targeting threatened plant or animal species.

Target 2 of the Global Strategy for Plant Conservation calls for "an assessment of the conservation status of all known plant species […] to guide conservation action" (CBD 2012). At a more regional level, Target A2 of the North American Botanic Garden Strategy for Plant Conservation mandates that "all botanic gardens with the capacity will review and contribute to assessments of the conservation status of plant species, using criteria and standards developed by NatureServe and the IUCN" (BGCI 2016). These two international policy documents provide the framework and foundation for meeting ambitious plant conservation goals that have been identified as crucial to sustaining healthy ecosystems and global biodiversity.

Comparison of Conservation Status Assessment Platforms in North America

The IUCN Red List of Threatened Species

Established in 1964, the IUCN Red List of Threatened Species is the world's most widely adopted system for evaluating the threat level of plant and animal species (IUCN 2001). The Red List aims to provide an objective baseline from which to measure and monitor the state of the world's biodiversity and puts species into a global context for setting conservation priorities. IUCN Red List assessments are based on applying a well-defined and rigorous set of Categories and Criteria, which are tiered thresholds for various population and demographic metrics, such as population growth trends, geographic range size, number of mature individuals, and habitat quality. Based on meeting the predetermined thresholds, species may qualify for one of three threatened categories: Critically Endangered (CR; extremely high risk of extinction in the wild), Endangered (EN; very high risk of extinction), or Vulnerable (VU; high

risk of extinction). Other categories include Extinct (EX; no longer extant anywhere in the world) and Extinct in the Wild (EW; existing only in captivity or ex situ collections), as well as Near Threatened (NT; does not currently reach a threatened threshold, but is likely to qualify in the near future if no intervention is taken), Least Concern (LC; widespread and abundant) or Data Deficient (DD; inadequate information to confidently determine the category, or ambiguous/conflicting information that places the species in many different categories). Species that have never been processed through the IUCN Red List Categories and Criteria are considered Not Evaluated (NE), the category to which the vast majority of plants are currently assigned. The IUCN Red List is a dynamic system, designed to provide a baseline or snapshot in time for each species based on the best available information *at that time*. Assessments officially expire after 10 years, so assessors are encouraged to reassess species at least that often, prioritizing those taxa that are threatened (CR, EN, VU) or Near Threatened. The dynamic nature of the Red List allows for the IUCN to generate the Red List Index and Barometer of Life—indicators of global biodiversity trends over time. An illustration of the IUCN Red List threat assessment categories can be seen in fig. 1.

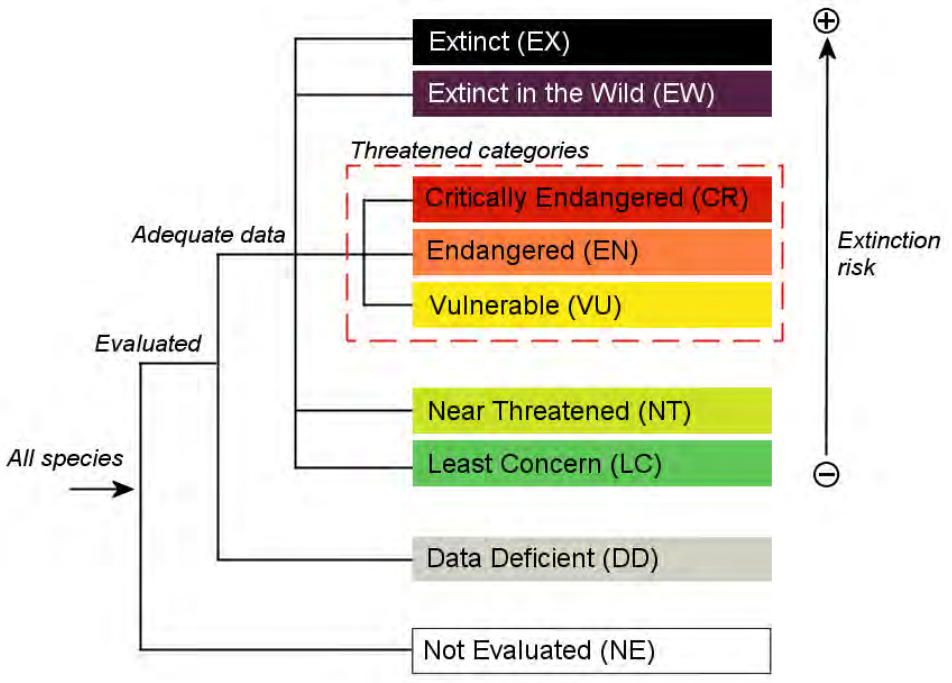

Figure 1—The IUCN Red List of Threatened Species categories.

Anyone can become certified by the IUCN through an online training system to participate in compiling or reviewing an IUCN Red List threat assessment, although the vast majority of assessments are completed by members of IUCN Species Survival Commission Specialist Groups. The specialist groups are made up of taxonomists, researchers, and field biologists who are experts of particular taxonomic groups, geographic regions, or specific habitat types. For example, there are specialist groups that focus on cacti, amphibians, plants of the Hawaiian Islands, arctic plants and crop wild relatives, to name a few. There is also a Global Tree Specialist Group (GTSG) that coordinates and leads Red List efforts for all of the world's tree species. The GTSG has recently launched an ambitious project, the Global Tree Assessment, which aims to have threat assessments completed for all of the world's estimated 60,000 to 80,000 tree species by 2020. Botanic Gardens Conservation International (BGCI), which provides the secretariat for the GTSG, is also in the process of developing the most comprehensive database of tree species, GlobalTreeSearch, the only full list of the world's tree species geo-referenced to

country level. GlobalTreeSearch provides the backbone for tracking progress of the Global Tree Assessment.

The IUCN does not have its own strict definition of a tree, and leaves the growth habitat designation for a plant species up to the discretion of the individual assessor. As of April 2017, there were 245 tree species published on the IUCN Red List from continental North America (Canada and the United States, excluding Hawaii; the IUCN SSC Hawaiian Plant Specialist Group is currently systematically assessing all Hawaiian trees, so those taxa are excluded from this analysis), of which 9 percent were last assessed at least 10 years ago and are out of date. Estimates from BGCI, NatureServe, and the USDA indicate there are around 1000 tree species in continental North America, so there is clearly work to be done to evaluate threats to trees in this region and to ensure the threat assessments are globally standardized and accessible. Of the four threat assessment platforms, the IUCN Red List is the least complete for tree species of North America.

NatureServe Conservation Status Assessments

The NatureServe Network represents a public-private consortium of independent organizations operating across the Western Hemisphere that gathers, analyzes, and distributes biodiversity data on species and ecosystems to advance scientifically informed conservation actions. This network of United States Natural Heritage Programs and Canadian Data Centres has been assessing the conservation status of North American species for over 30 years. It has compiled over 70,000 plant and animal conservation status assessments based on its own system of evaluation of potential extinction or extirpation risk considering rarity, threats and population trends (Faber-Langendoen et al. 2012, Master et al. 2012). The NatureServe conservation Ranks are completed at three nested, geographic scales: Global (G), National (N), or Subnational (S). Species and infraspecific taxa (varieties and subspecies) are ranked from most endangered to least endangered on a scale of 1 to 5 (table 1). NatureServe ranks also include GX (Presumed Extinct) and GH (Possibly Extinct), as well as variant ranks and rank qualifiers (table 1). Uncertainty in a Global Rank is expressed through a Range Rank or a rank qualifier of ? or Q.

Table 1—The NatureServe conservation status assessment global ranks, variant global ranks, and rank qualifiers

Global (G) Rank	Definition
GX	Presumed Extinct—Species not located despite intensive searches and virtually no likelihood of rediscovery.
GH	Possibly Extinct—Known from only historical occurrences but still some hope of rediscovery.
G1	Critically Imperiled—At very high risk of extinction due to extreme rarity, very steep declines, or other factors.
G2	Imperiled—At high risk of extinction or elimination due to very restricted range, very few populations, steep declines, or other factors.
G3	Vulnerable—At moderate risk of extinction or elimination due to a restricted range, relatively few populations, recent and widespread declines, or other factors.
G4	Apparently Secure—Uncommon but not rare; some cause for long-term concern due to declines or other factors.
G5	Secure—Common; widespread and abundant.
Variant Global Ranks	
G#G#	Range Rank—A numeric range rank (e.g., G2G3, G1G3) used to indicate uncertainty about the exact status of a taxon.
GU	Unrankable—Currently unrankable due to lack of information or due to substantially conflicting information about status or trends.
GNR	Unranked—Global rank not yet assessed.
GNA	Not Applicable—A conservation status rank is not applicable because the species is not a suitable target for conservation activities.
Rank Qualifiers	
?	Inexact Numeric Rank—Denotes inexact numeric rank; this should not be used with any of the Variant Global Conservation Status Ranks or GX or GH.
Q	Questionable taxonomy that may reduce conservation priority— Distinctiveness of this entity as a taxon at the current level is questionable; resolution of this uncertainty may result in change from a species to a subspecies or hybrid, or inclusion of this taxon or type in another taxon or type, with the resulting taxon having a lower-priority (numerically higher) conservation.
C	Captive or Cultivated Only—At present presumed or possibly extinct in the wild across entire native range but extant in cultivation, in captivity, as a naturalized population outside their native range, or as a reintroduced population, not yet established. Possible ranks are GXC or GHC.

The thresholds and criteria of the NatureServe assessment process consider much of the same information and metrics that would be used to conduct an IUCN Red List assessment. Like the Red List, a standardized, scientific, empirical and objective methodology has been established and improved over several decades. Many of the concepts and terms are interchangeable between the two platforms, such as the Area of Occupancy, Extent of Occurrence, Population Size, and the way in which threats are classified and coded (Salafsky et al. 2008). Furthermore, many of the thresholds between the different categories are set at the same level, so in the vast majority of cases the NatureServe rankings and the Red List categories are largely in alignment (table 2). However, NatureServe rankings cannot automatically be transferred over to a Red List category—the assessment process must be done independently for each platform. Like the Red List, NatureServe rankings are also dynamic and are regularly monitored and prioritized for updating, based on known new threats or changing population trends.

Table 2—Comparison of NatureServe and IUCN Red List Global Statuses (adopted from Master et al. 2012)

NatureServe Global Status	IUCN Red List Status
Presumed Extinct (GX)	Extinct (EX)
Presumed Extinct in the Wild[a] (GXC)	Extinct in the Wild (EW)
Possibly Extinct (GH)	Critically Endangered (CR) (possibly extinct)
Possibly Extinct in the Wild[a] (GHC)	Critically Endangered (CR) (possibly extinct)
Critically Imperiled (G1)	Critically Endangered (CR)
Critically Imperiled (G1)	Endangered (EN)
Imperiled (G2)	Vulnerable (VU)
Vulnerable (G3)	Near Threatened (NT)
Apparently Secure (G4)	Least Concern (LC)
Secure (G5)	Least Concern (LC)
Unrankable (GU)	Data Deficient (DD)

[a] Species ranked GXC and GHC are presumed or possibly extinct in the wild across their entire native range, but are extant in cultivation, in captivity, as a naturalized population (or populations) outside its historical native range, or as a reintroduced population not yet established. The C modifier is only used with status ranks at a global level, and not a national or subnational level. Similarly, IUCN's EW status is only used at a global level.

Where the IUCN Red List and NatureServe begin to diverge is in the process of evaluating the available population trend and rarity data. NatureServe ranks follow a weight-of-evidence approach with minimum criteria, whereas the Red List is based on criteria (rules) with greater emphasis on trends rather than rarity. NatureServe Ranks have been used extensively by United States and Canadian state and federal agencies, including state natural heritage programs, and as such is much more complete than the Red List for the United States and Canada. Because the Ranks are nested within three geographic scales, data from Subnational and National Ranks are used to inform Global Ranks. Nearly every vascular plant in the United States and Canada has been assessed at least once on the NatureServe platform. Of the estimated 1000 tree species, over 97 percent have been assigned a Global Rank by NatureServe. The NatureServe Ranks indicate that while most North American tree taxa are Apparently Secure (GT4; the "T" in the rank indicates that both species level and infrataxa—trinomials—are included in the analysis) or Secure (GT5), about 14 percent are Critically Imperiled (GT1), Imperiled (GT2), or Vulnerable (GT3) (Figure 2). However, about 75 percent of the NatureServe assessments have not been reviewed in over 10 years. These assessments need to be reviewed to incorporate current threats and trends. For a thorough (although now outdated) review of the NatureServe platform compared to the Endangered Species Act and the IUCN Red List, see Master et al. 2000.

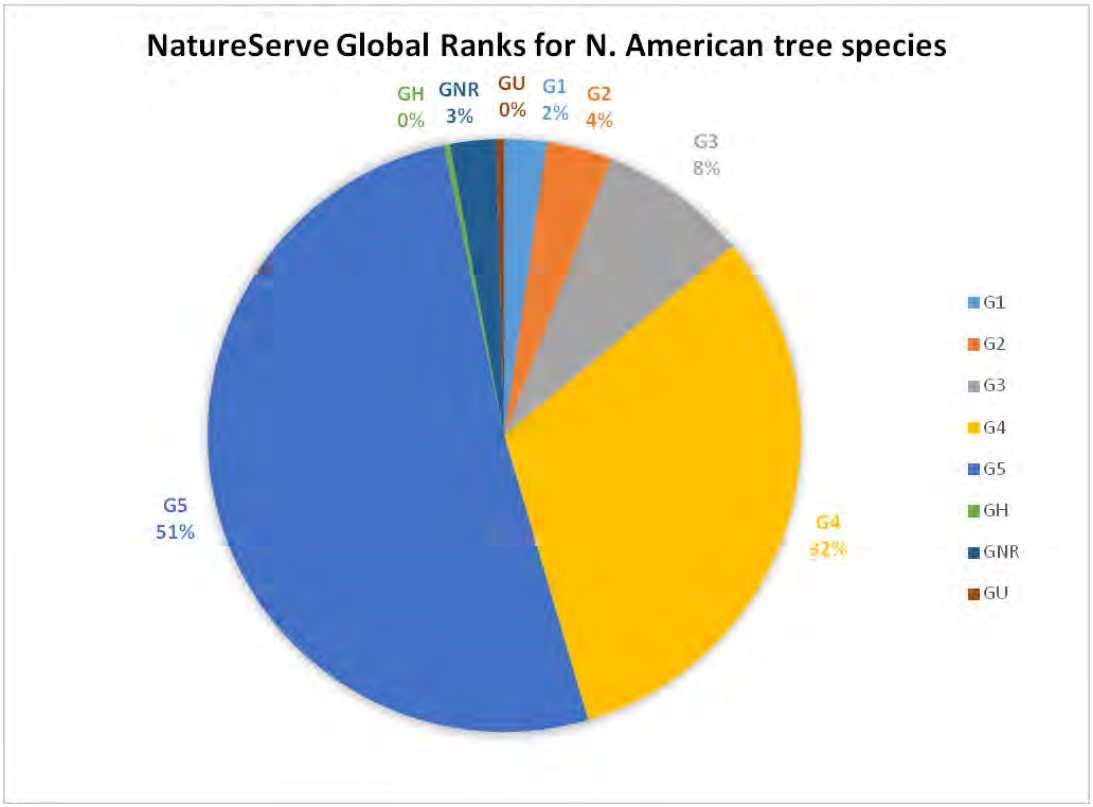

Figure 2—Conservation status of continental North American tree taxa based on NatureServe global rounded ranks. The "T" in the rank (e.g., GT1) indicates that both species level and infrataxa (Trinomials) are included in the analysis. Data from NatureServe's Biotics database accessed April 21, 2017.

Conservation Assessment and Prioritization of Forest Trees at Risk of Extirpation – Project CAPTURE

In 2010, the USDA FS started a focused effort in conservation of at-risk forest tree species for the purpose of categorizing and prioritizing species and developing a scientifically informed strategy for seed collection, storage, and propagation of threatened forest trees. Project CAPTURE is a data-driven and expert-guided assessment framework and a scalable tool to help decision makers address and prioritize forest resilience and restoration planning, genetic conservation efforts, and threat mitigation efforts based on species' threats and life history traits (Potter and Hargrove 2013, Potter et al. 2017). The assessment framework integrates threat vulnerability projections with species trait data metrics to categorize each species based on their scores within three vulnerability dimensions: 1) sensitivity to a threat, 2) severity of the threat, and 3) adaptive capacity (fig. 3). A species with high scores in all three dimensions, for example, would have the highest vulnerability and need for conservation action.

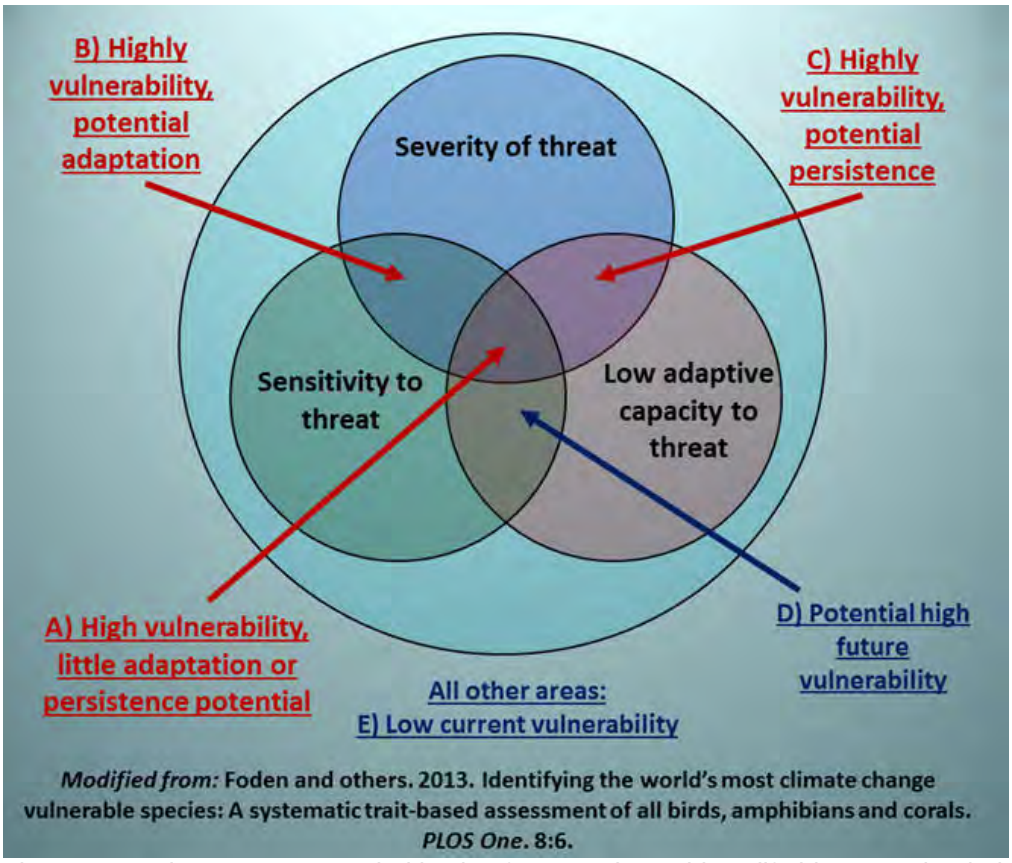

Figure 3—Project CAPTURE prioritization framework combines life history and trait data with climate change modeling to rank species' inherent risk of extirpation.

Collaborators on Project CAPTURE have evaluated around 420 United States native tree species for vulnerability to projected climate change using this detailed and sophisticated methodology (Potter et al. 2017), providing prioritized lists of species for recommended conservation action. The number of tree species evaluated for that study was fewer than the 1,000 estimated for North America, because the assessment was limited only to species of the United States (excluding the high plant diversity of Mexico) and that met the USDA FS's Forest Inventory and Analysis Program's definition of a tree: woody perennial plants that usually have a single well-defined erect stem having a more or less definitely formed crown of foliage, a stem diameter at maturity of at least 7.62 cm, and a height at maturity of at least 4.75 m. While the Project CAPTURE framework has been applied so far in the context of species vulnerability to climate change, insect and disease infestations are arguably a more serious and immediate threat to the genetic integrity of tree species. An effort applying the Project CAPTURE framework to pest and pathogen threats for North American tree species is currently underway. As with the climate change assessment, it will incorporate expert opinion regarding which species attributes should be included, and to which of the three vulnerability dimensions (threat severity, threat sensitivity, and adaptive capacity) they will be designed. When that assessment is complete, species' vulnerability to both climate change and pests and pathogens will be combined to prioritize those at greatest risk; this step will incorporate expert opinion on the final species ratings.

Similar to the IUCN Red List and NatureServe, CAPTURE uses demographic data (e.g., population size, density), range size and threats, but also includes other life history characteristics such as attributes associated with genetic diversity, ecological limitations, and propagule dispersal ability. These attributes are organized into the three vulnerability dimensions based on expert opinion. The framework also can include additional weighting factors such as ecological and economic importance, evolutionary distinctiveness, and regional conservation responsibility when giving species final scores within

vulnerability classes (A through E in fig. 3). As such, the amount, type, and depth of data gathered for these assessments may be much different than for an IUCN Red List or NatureServe assessment. The primary emphasis of the Project CAPTURE framework is different than that of the other assessments in that it focuses specifically on categorizing species based on the degree to which they may be vulnerable to genetic degradation, defined as a significant reduction in the ability of species to persist for the next century while maintaining sufficient genetic variation to adapt to changing environmental conditions (Potter et al. 2017). The application of the framework, then, is to identify groups of species requiring similar sets of strategies to maintain adaptive genetic variation, whether or not the species are currently at risk of extirpation in all or parts of their distributions. The strategies needed for groups of species may include conservation activities, but may also include routine monitoring and management. Given its reliance on detailed species-level data, the CAPTURE framework may be more difficult to apply to very rare and poorly known species and could exclude species from high-vulnerability categories if it were applied in regions of the world where botanical data are lacking, such as in biodiversity hotspots like tropical forests. The framework is flexible to regional differences in data availability, however. Data are currently being collected for separate vulnerability assessments of 562 tree species in Puerto Rico and the United States Virgin Islands, and of 304 tree species in Hawaii. These assessments will, by necessity, incorporate less information than is available for species native to the continental United States.

U.S. Endangered Species Act

Within the United States, the Endangered Species Act (ESA) is a law designed to conserve imperiled species and the ecosystems upon which they depend. Plant and animal species in the United States are listed under the ESA as either Endangered (in danger of extinction throughout all or a significant portion of its range) or Threatened (likely to become endangered within the foreseeable future). Before a plant or animal species can receive the protection provided by the ESA, it must first be added to the federal lists of endangered and threatened wildlife and plants. The List of Endangered and Threatened Wildlife (50 CFR 17.11) and the List of Endangered and Threatened Plants (50 CFR 17.12) contain the names of all plant and animal species that have been determined by the United States Fish and Wildlife Service (USFWS) or the National Marine Fisheries Service (for most marine life) to be in the greatest need of federal protection. A species is added to the list when it is determined to be endangered or threatened because of any of the following factors: 1) the present or threatened destruction, modification, or curtailment of its habitat or range; 2) over utilization for commercial, recreational, scientific, or educational purposes; 3) disease or predation; 4) the inadequacy of existing regulatory mechanisms; and 5) other natural or manmade factors affecting its survival.

There is no program under the ESA for systematically evaluating all plant and animal species in the United States. Species are evaluated for listing under the ESA either through a petition process or through the candidate assessment process. The ESA provides that any interested person may petition the Secretary of the Interior, or the Secretary of Commerce for most marine life, to add a species to, or to remove a species from, the lists of endangered and threatened species. Through the candidate assessment process, USFWS biologists identify species as listing candidates. Once a species is listed as threatened or endangered under the ESA, it is eligible for federal protection, recovery planning, and funding (if available) for conservation actions. Furthermore, federal agencies must ensure their actions (e.g., building a highway) do not jeopardize the continued existence of the species..

Summary

Of all of the threat platforms described, the ESA is the only legally binding, policy-driven platform that requires stakeholders to modify actions to protect the species. The other three platforms provide information and recommendations and enable species to be prioritized for action, but do not result in any legally mandated conservation activities. However, it is worth noting that the USDA FS uses NatureServe rankings as a tool to identify Species of Conservation Concern as required by land management planning regulations. A comparison of the four different threat assessment platforms is presented in table 3.

Table 3—Comparison of the four different threat assessment mechanisms presented in the special symposium

	IUCN Red List	NatureServe	USFS Project Capture	USFWS Endangered Species Act
Geographic focus of platform	Global	Regional	National	National
Geographic scope of assessments	Global, Regional, National	Global, National, Subnational	National	National
Biological scale	Plants, Animals, Fungi	Plants, Animals, Fungi	Trees	Plants, Animals
Amount of data needed	Moderate	Moderate	High	High
Accessibility of assessments	High	High	Moderate	High
Accessibility of underlying data	Moderate	High	Low	Moderate
Degree of completion (relative to scope)	Low	Medium/High	High	Low
Assessors	Gardens, academia, taxonomic experts, gov't agencies (anyone certified)	Natural heritage and partner organizations, taxonomic experts, academia	USFS staff, academia, taxonomic experts, (CAPTURE) collaborators	USFWS staff
Legally binding protections for threatened species	No	No	No	Yes

While the four different conservation status assessment mechanisms highlighted in this special symposium all rely on similar underlying data, they each have unique applications, purposes, scopes, benefits, and drawbacks. The IUCN Red List and NatureServe are focused on assessing all flora and fauna across broad geographic scales to prioritize species in need of conservation, whereas Project CAPTURE and the ESA have much more specific and targeted purposes. The IUCN Red List and NatureServe require less underlying data, are higher throughput platforms, and are used by and accessible to a wider audience than the other two. They also often rely on expert opinion and unpublished data from those with firsthand knowledge of each species' threats and trends. Because CAPTURE and the ESA have more targeted purposes, these assessments typically are more involved and detailed than the IUCN Red List or NatureServe, but the emphases and objectives of each are different as well. For example, NatureServe's Ranks and IUCN Red List assessments are often used as supporting information to petition the listing of a species as Threatened or Endangered under the Endangered Species Act. Once a species is designated as Threatened or Endangered, and therefore a priority for conservation action, the ESA supports and undertakes detailed research that will inform regulations and recovery planning. Likewise, Project CAPTURE involves more quantitative data, sophisticated evaluation, and analysis than the IUCN Red List or NatureServe. There are inherent tradeoffs in balancing the amount of data required for an assessment and the rigor of the evaluation and review process with the time and money needed to complete a single assessment.

Panel Discussion During the Special Session

Following the presentations on the four threat assessment mechanisms at play in North America, an open discussion with the audience commenced. It was agreed that there was a pressing need to complete and update threat assessments for all of the North American tree species, and that coordination and collaboration between stakeholders operating under the various assessment platforms was of the upmost importance. Moving forward, the opportunities for assessment coordination and streamlining are highest for the IUCN Red List and NatureServe processes. Of the four assessment processes reviewed here, these two are the most similar, rely on the same underlying data inputs, and follow a well-aligned ranking system. Both systems could be adapted to efficiently and systematically incorporate data and results from each other. In fact, initiatives are already underway between the Red List, NatureServe, and USDA FS to streamline and coordinate threat assessment efforts. An ongoing project to increase efficiency is focusing on a tool to import existing conservation status data into the Red List database assessment system. NatureServe is working with the IUCN to determine a process by which each systems' data can be exported and imported into the other database.

Interestingly, despite having a thoroughly well studied native flora, the United States and Canada do not have a single, centralized database of native tree species. Several floras and checklists exist for the region, including the Flora of North America, the USDA PLANTS database, and Biotics (the backbone of the NatureServe database), which all have varying degrees of agreement over taxonomic concepts and which taxa are considered trees. One of the challenges to achieving a comprehensive list of North American trees lies in establishing a widely agreed upon definition for what makes a tree a tree. GlobalTreeSearch, the global database of trees developed by BGCI, relies on information provided by individual countries, so by developing a definitive list of trees of North America, the global list of trees would also be strengthened.

Progress Since the Special Session: Creating a Red List of North American Trees

As a result of this Special Session, a collaborative effort is now underway between NatureServe, The Morton Arboretum, BGCI, and the USDA FS to create a definitive list of tree species of continental North America and complete threat assessments for all of the tree species under both the IUCN Red List and NatureServe platforms simultaneously (a complementary initiative is underway to assess all of the Hawaiian native tree species by the IUCN Hawaiian Plants Specialist Group). The checklist of trees will be used to update BGCI's GlobalTreeSearch database, and will provide the foundation for the longer term initiative to update and/or complete NatureServe Global Ranks and IUCN Red List assessments for all North American trees.

Several steps are needed to produce the North American tree checklist. First, the designation of a species as a tree must be articulated and agreed upon through a review process with a variety of botanical experts. Second, existing checklists and authorities must be consulted and cross-referenced to ensure taxonomic accuracy and legitimacy. This process will be conducted in coordination with related taxonomic and checklist efforts underway by the database coordinators of NatureServe, the Flora of North America, USDA PLANTS, Tropicos (Missouri Botanical Garden), BGCI's PlantSearch, the IUCN Red List, and other taxonomic experts. Once tree designations and preliminary checklists are created, the list must be quality checked and put through a review process by taxonomic experts and other relevant stakeholders. After review and general approval, the list will be cross-referenced to the IUCN Red List, USDA FS's Project CAPTURE database, and NatureServe Global Ranks, which will provide a preliminary threat category assessment for every species, including data deficient (DD) and not evaluated (NE) taxa. Upon reaching this milestone, we will get a sense of the scope of the task at hand to complete or update a NatureServe Global rank and IUCN Red List threat assessment for every tree species—phase two of this initiative. In phase two, funds will be raised to support the coordination and streamlining of NatureServe and IUCN Red List assessments for priority species, so that all North American tree species

have been evaluated on at least one platform in the past 10 years. Being dynamic systems, the assessments on these platforms can be updated as new threats emerge, additional information becomes available, or the positive impact of conservation efforts on threatened species become realized.

The results of these two efforts (the checklist of tree species and the completed threat assessments) will provide the critical knowledge needed to inform and coordinate tree conservation actions across an entire continent. The impact of this initiative will be wide reaching and provide the opportunity to launch a public awareness campaign for the need for tree conservation in North America. In turn, the public awareness campaign will leverage funding opportunities and inspire community engagement. The Red List of North American Tree Species will also significantly contribute to the Global Tree Assessment and to achieving the targets of the Global Strategy for Plant Conservation and the North American Botanic Garden Strategy for Plant Conservation. Ultimately, it will provide a comprehensive picture of the extinction risk of North American trees based on a globally standardized and recognized system of threat assessment.

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Institute of Forest Tree Breeding: Improvement and Gene Conservation of Iconic Tree Species in the 21st Century[1]

C. Dana Nelson[2] and Jennifer L. Koch[3]

Background

Our nation's forests and forest trees are undergoing unprecedented stress from invasive pathogens and pests, climate change, land fragmentation, and urbanization. Some of these stresses are acute, either regionally or locally, and are having significant negative impacts on regional and local economies and ecosystems. Managing and improving the genetic resources of impacted iconic[4] forest tree species is key to ensuring their existence into the future. However, our national capacity in forest genetics and tree improvement has been declining for decades (Wheeler et al. 2015). Many of the current programs addressing genetic improvement of our hardwood and non-commercial softwood species are isolated, under-staffed and under-funded, resulting in limited success in achieving and deploying improved trees. In addition, they lack a committed source of long-term funding to make them sustainable across the time periods that are needed for genetic improvement in long-lived organisms such as forest trees. Experience has shown that most hardwood tree improvement programs have failed to outlive their initial phase and usually are suspended indefinitely or terminated upon retirement of the founding forest geneticist/tree breeder.

Despite these obstacles, there are exceptions; programs that have had success including some that have achieved success in relatively short periods. The American Chestnut Foundation (TACF) has been breeding hybrid chestnuts (*Castanea* spp.) to develop improved resistance to chestnut blight (caused by the fungus *Cryphonectria parasitica*) for over 25 years (spanning nearly the complete career of the lead tree breeder). This program has achieved longevity through concerted efforts that included following a well-reviewed breeding plan, engaging in a participatory breeding model, and maintaining stable funding (through an active membership program). In addition, TACF has hired a new breeder to continue to work toward the production of ample resistant planting stock with r regional adaptation for use in species restoration efforts. Other notable longer-term programs include the University of Tennessee Tree Improvement Program and a program run by the University of Missouri. These later programs rely on state-funding to distribute work on a number of important species.

Within the U.S. Department of Agriculture Forest Service (USDA FS), the Dorena Genetic Resource Center (DGRC) in Oregon, celebrating their 50 year anniversary this year, is a successful long-term program that began in response to white pine blister rust (*Cronartium ribicola*). Originally focused on western white (*Pinus monticola*) and sugar (*P. lambertiana*) pines, it now has blister rust resistance programs in all impacted western five-needle pine species, including the threatened whitebark pine (*P. albicaulis*). The DGRC has also expanded to include resistance programs to newer invasive pathogens including *Phytophthora lateralis*, threatening Port-Orford-cedar (*Chamaecyparis lawsoniana*). In the case of Port-Orford-cedar and whitebark pine, sufficient resistance was developed to begin restoration within a relatively short period of time (~10 years). Such rapid success is due to several factors, including the availability and types of resistance, having a tree improvement program in place that included stable

[1] A version of this paper was presented at the Gene Conservation of Tree Species – Banking on the Future Workshop, May 16-19, 2016, Chicago, IL.

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[4] We define iconic species as foundational or keystone species in the ecological sense indicating that they disproportionately affect the forest's flora and fauna and the succession of the forest itself.

funding, experienced staff—including breeders and geneticists— and physical infrastructure (e.g., nursery, screening facilities, land for research plantings). The USDA FS resistance breeding programs in the West include not only those at DGRC, but also the Institute of Forest Genetics (Pacific Southwest Research Center and Pacific Southwestern Region) and the Coer d'Alene nursery (Intermountain Region). The value of putting tree breeding principles to practice to save tree species from potential extirpation is illustrated by the western United States resistance programs in Port-Orford-cedar and whitebark pine (Sniezko and Koch[5]).

Although the eastern United States lacks the longevity and infrastructure of programs in the West, there are USDA FS programs that have been established more recently including the Hardwood Tree Improvement and Regeneration Center at Purdue University, a collaborative effort between the university and the USDA FS's Northern Research Station (NRS), that is focusing on a few high-value hardwood species largely within the state of Indiana but also species threatened by invasive diseases including butternut and black walnut. Other programs in the NRS include, for example, a beech bark disease (caused by an interaction between the beech scale insect, *Cryptococcus fagisuga* and the fungal pathogen *Nectria coccinea* var. *faginata*) resistance program and a research-oriented program that is developing materials in ash (*Fraxinus*) species to study the genetics of resistance to the emerald ash borer (*Agrilus planipennis*) and provide resistant materials for further breeding work. These eastern USDA FS programs, like many modern tree improvement efforts, rely in part on piecing together funding in the 1- to 3-year cycles dictated by the few granting programs that will fund this type of applied research, putting them and the species they aim to save, at high risk for failure over the long-term.

A potential approach to solving this problem, in general, is the formation of an institute of tree breeding that provides an avenue for stable, long-term funding to support the breeding work that is critically important to saving iconic forest tree species in the eastern United States. Such an institute would support development and retention of necessary infrastructure and provide the continuity, skill and longevity needed for success by employing a tree breeder and an assistant (apprentice) for each high priority major tree species or group of related species (species/group). The institute's breeders would work in conjunction with experienced USDA FS and university geneticists to develop peer-reviewed, range-wide breeding plans. Programs that are part of the institute will collaborate with each other and other partners to develop region-wide testing programs for each species, web- and mobile-enabled data management software, curriculum for training new tree breeders and their assistants, and production and distribution systems for the improved materials. We propose to form the Institute of Forest Tree Breeding (IFTB) with funding from the USDA FS and matching funds from associated universities, state forestry agencies, non-government organizations, non-profit foundations and private individuals. Some USDA FS funding is already committed to tree breeding efforts with various levels of matching assets and we suggest that these efforts be consolidated within the IFTB. Merging these efforts into the IFTB would provide for an initial critical mass for the institute and a solid base to build and grow the institute to meet current critical needs and to be positioned to address future needs as they arise (e.g., new invasive insects and pathogens, climate change) in a timely, efficient and cost-effective manner.

Mission and Goal of the IFTB

To cost effectively provide for long-term, region-wide breeding programs for iconic forest tree species that are critically important to national forests, state agencies and private landowners in the eastern and southern regions.

[5] **Sniezko, R.A.; Koch, J.L.** Breeding trees resistant to insects and diseases-putting theory into application. Manuscript submitted to Journal.

General features of the IFTB

- Networked consortium of tree breeders and their breeding programs or components of their programs.
- A lead tree breeder and an assistant (apprentice) for each species/group, with an operating budget and in kind institutional support.
- A set of quality assurance standards for entering a species/group into the IFTB, including economic and ecologic justification, a well-documented user's group, and a sustainable funding plan.
- Peer reviewed regional breeding plans that cover appropriate portions of the species' native ranges and annual progress reports for each species.
- Breeders collaborate with each other to facilitate progress and success in all programs while being responsible for their own program. Collaboration among IFTB breeders will include sharing test sites, facilitating test establishment and germplasm collections, developing and sharing data management and analysis software, sharing best practices for recruiting and utilizing volunteers, developing curriculum for training new tree breeders and volunteers.
- Breeders individually and as an institute report to a board of directors, where the board consists of representatives (one person each) of the following: USDA FS Southern Research Station and NRS Station Directors or Assistant Directors, USDA FS Region 8 (Southern) and Region 9 (Eastern) Regional Foresters or Deputy Regional Foresters, and a northern and southern representative state forester, as well as representative as appropriate from associated universities and other nongovernmental organizations.
- USDA FS funding committed in 5 year intervals, not to exceed four renewals or 25 years total. Programs must stay on track in order to be renewed for subsequent 5 year periods, with the goal of developing a path to full funding. A sliding scale of matching requirements will be developed, for example, with USDA FS funding covering 50 percent in first two periods (years 1 to10), 25 percent in the next two periods (years 11 to 20) and 10 percent in final period (years 21 to 25).
- USDA FS funding permitting, new species/groups may apply for funding and entry into the IFTB. Applications will be reviewed by expert panel with recommendations made to IFTB's board of directors.
- Appropriate genetic materials produced by the IFTB will be publicly available through material transfer agreements (MTAs) for research purposes, seed orchard establishment for forest management and restoration activities and continued breeding (i.e., cultivar development). Cultivars developed through such MTAs will be jointly owned by IFTB and the collaborator developing the cultivar.
- Affiliated breeders would agree to provide certain materials or services to IFTB in exchange for funding or other services or materials. All expectations for both parties will be formally documented in a MTA. Materials provided by affiliated breeders would be part of IFTB with respect to being subject to peer-review and being available through MTAs.

Specific Objectives of the IFTB

1. Provide sustainable (including funding plan), long-term, region-wide breeding programs for iconic forest tree species with critical ecologic or economic need. Initially we propose these to be:
    a. Black walnut (*Juglans nigra*) and butternut (*J. cinerea*) (walnut group)—black walnut is the most economically valued hardwood species and butternut is a highly endangered (from butternut canker, caused by *Sirococcus clavigignenti-juglandacearum*) and valued hardwood species.

   b. Green ash (*F. pennsylvanica*) and white ash (*F. americana*) (ash group)—two of the most important forest and urban landscape trees and economically valued hardwoods; both under extirpation pressure from emerald ash borer.
   c. Redbay (*Persea borbonia*), sweet bay (*Magnolia virginiana*), sassafras (*Sassafras albidum*) (laurel group)—three important coastal and inland forest trees with very high ecological value; all three under intense extirpation pressure from laurel wilt (caused by *Raffaelea lauricola*).
   d. White oak (*Quercus alba*), northern red oak (*Q. rubra*) and American beech (*Fagus grandifolia*) (oak group)—the oak species are among the most economically and ecologically important hardwood species across the eastern United States, and the closely related American beech is an important wildlife species.
2. Work with university partners to develop and deliver curriculum for a Master Tree Breeder certificate to train professionals, landowners and others in forest tree breeding.
3. Develop participatory tree breeding methods and implement these methods in each species' program and new programs as they enter the institute.

Path Forward

We suggest the following steps to move this proposal abstract to a full proposal and ultimately a functional institute:

1. Convene a writing team to complete the proposal/business plan.
2. Enlist letters of interest from potential participants and funding sponsors.
3. Submit the proposal to peer review by forest geneticists.
4. Present revised proposal to the USDA FS and others for consideration of funding.

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Community Standards for Genomic Resources, Genetic Conservation, and Data Integration[1]

Jill Wegrzyn,[2] Meg Staton,[3] Emily Grau,[2] Richard Cronn,[4] and C. Dana Nelson[5]

Genetics and genomics are increasingly important in forestry management and conservation. Next generation sequencing can increase analytical power, but still relies on building on the structure of previously acquired data. Data standards and data sharing allow the community to maximize the analytical power of high throughput genomics data. The landscape of incomplete submissions, minimal metadata, and distributed raw data inhibits aspects of data re-use, validation, and discovery. Community databases can address these challenges for their research communities by collecting and integrating data, as well as providing analytical tools.

The Hardwood Genomics Web (HWG, http://www.hardwoodgenomics.org) and TreeGenes Database (http://treegenesdb.org) are community resources for forest tree genetics research. These web-based repositories host a variety of data types, including genome sequences, transcriptomes, genetic maps, and supporting marker information for 23 tree species in HWG and another 1780 species in TreeGenes. TreeGenes and the HWG host data visualization and analytical tools to further integrate and interrogate these datasets. Specific analytical functions include gene expression, functional annotation, and association genetics.

Recent developments in TreeGenes and HWG have focused on the organization and integration of phenotypic and environmental data for georeferenced tree accessions originating from studies around the world. The majority of phenotypic data is not collected in a form that can easily be integrated and generally resides in flat files or in disparate databases. Genomics is more centralized but the critical metadata components are often not required by primary repositories such as Genbank and therefore, never documented. Environmental data, in the form of layers, is distributed by numerous independent sources and there are few standards imposed to facilitate cross-query and integration. The tree databases have implemented web-based modules designed to capture data, metadata, and relevant accessions on all aspects of association genetics and population genomics studies. The acquisition of this data has made it possible to develop interfaces, such as CartograTree (http://cartogratree.org), which allows users to interact with map-based utilities to query georeferenced forest tree accessions, integrate complex environmental and phenotypic data, and perform association genetic studies through high-performance computing resources in the Cloud. HWG and TreeGenes are collaborating to offer increased connectivity and analytical capabilities through involvement with the Tripal project (http://tripal.info). Tripal is an open source software project aimed at streamlining the creation and maintenance of web-based community databases.

Efforts to expand offerings through HWG and TreeGenes are coordinated with the Forest Health Research and Education Center (FHREC, http://www2.ca.uky.edu/Forestry/fhrec/index.html). This center represents the intersection of participating U.S. Department of Agriculture Forest Service research stations, academic institutions, and related organizations. The FHREC aims to understand the genetics of pests and pathogens, contribute to technology development for tree improvement, support education and outreach activities, and coordinate collaboration between different players in forest tree conservation. The

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goal to improve sustainability and address forest health concerns is met through coordinating outreach activities, information resources, and research objectives. Community databases collaborate with the FHREC by facilitating data and resource sharing with the scientific community. Collaboration among community databases and these organizations increases data availability, data integration, and data analysis capabilities.

The HWG and TreeGenes databases conducted a community survey to determine user priorities for data standards and sharing. The results formed the basis for a focused discussion at the 'Community Standards for Genomic Resources, Genetic Conservation, and Data Integration' session. The survey gathered 42 responses from academic, government, and private organizations. Users identified the integration of phenotypic, environmental, and genetic marker data as the highest priority. Only 30 percent of respondents directly interact with high performance computing (HPC) resources that might enable the analysis and integration of this data. The discussion supported the idea that community databases can better serve the forest tree research community by providing access to this integrated data as well as analysis tools directly linked to HPC resources. Discussion also focused on the implementation of data standards in terms of genetic marker types. This discussion also spoke to limitations of frequently used genetic and genomic resources for assessing forest populations. This was not limited to the data itself but rather encouraged discussion on what markers were most useful in a comparative genomics context. Experimental implementation of non-standardized markers produces results that are less informative and not cumulative. Parallels can be made to the human genetics community where standardized marker types have vastly improved the ability to build on studies on the biomedical field. Proper marker development requires a list of high quality gene sequences from the target species or a close relative (with less than 5 percent sequence divergence). Continued focus on developing high quality genomic resources (full transcriptome or genome) can achieve this.

Components of the workshop, survey, and discussion revealed that the community is concerned about data access, distribution, and integration. Model and clade organism databases remain critical to achieve this goal. The challenges lie in not just generating the appropriate genomic resources, but also in how to store this information so that it can promote access, validation, and discovery.

Development of Seed Zones for the Eastern United States: Request for Input and Collaboration![1]

Carrie C. Pike,[2] George Hernandez,[3] Barbara Crane,[4] and Paul Berrang[5]

Artificial regeneration is necessary for meeting a variety of management objectives following timber harvests and other disturbances. While foresters use ecological classification to identify the most appropriate species to plant on a particular site, they generally use seed zones to identify the most suitable seed source of that species to plant. Seed zones have been utilized by public and private sector nurseries for many years in the western United States, but have not been accepted by nurseries for the eastern United States, corresponding to 33 states defined by the National Forest System as Regions 8 (Southeast United States) and 9 (Northeast United States). The national forests (NFs) in the Northeast and Southeast have historically used their own set of seed zones to define where the seed was collected to ensure that seed will be collected from appropriate areas to plant on NF lands. Similarly, state nurseries define their own seed zones to meet their needs. A common set of seed zones would facilitate the exchange of seed and seedlings among government agencies, seed brokers, nurseries and private landowners.

Seed zones have been developed for the Southeast United States, notably Ron Schmidtling's Southern Pine Seed Sources Guide (Schmidtling, R.C. 2001. Southern pine seed sources. Gen. Tech. Rep. SRS-44. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station. 25 p). Provenance trials for numerous species lacked robust sampling to generate genetically-based seed zones in the Northeast. Efforts to regionalize seed zones have failed to gain traction in the eastern United States for two main reasons. Firstly, the majority of land is privately-held and there is a fear that common seed zones would create a burden for growers and landowners. Secondly, the need for seed zones has been perceived, by some, as unnecessary because of the low levels of topographical relief in the eastern United States.

Climate change will create new challenges for land managers, both in terms of novel disturbance regimes, and the potential for failures of tree plantings. One recommendation is to plant at least a portion of seed or seedlings from non-local sources. This has created a conundrum for managers of NFs who are required to use appropriate seed sources of known origin and will need to rely more on seed collected outside the NFs by other landowners who do not track seed origin. Exchange of seed across land ownerships will be most effective if all land owners and managers use a common language to describe the origin of the seed that is needed or is for sale.

This special session of the Gene Conservation Workshop was organized by the U.S. Department of Agriculture Forest Service's National Forest System and State and Private Forestry divisions to explore the development of seed zones for the eastern United States that would be useful to both public and private land managers. At this session, geneticists from the National Forest System will present an overview of the need for seed zones; regeneration specialists will discuss the challenges and utility of seed zones for state nurseries and private landowners. We will lead an open discussion for participants to address four primary objectives: 1) develop a list of collaborators from across agencies and organizations who will partner on this work, 2) begin discussions on the tree and/or shrub species for which seed zones would most likely apply, 3) discuss possible schemes for delineating common seed zones, and 4) begin discussions on how best to communicate seed zones to the public. Lastly, we are seeking participants and input for an upcoming seed zone summit that we are organizing, slated for 2018, to finalize the development, communication, and application of seed zones for the eastern United States.

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Innovative Approaches for Assessing and Prioritizing Tree Species and Populations for Gene Conservation

Conservation Priorities for Tree Crop Wild Relatives in the United States[1]

Colin K. Khoury,[2,3] Stephanie L. Greene,[2] Karen A. Williams,[4] Chrystian C. Sosa,[3] and Chris Richards[2]

Abstract

Crop wild relatives native to the United States have proved useful as genetic resources in breeding more productive, nutritious, and resilient crops. Their utilization is expected to increase with better information about the species and improving breeding tools. But this utilization may be constrained by their limited representation in genebanks and the ongoing loss of wild populations to habitat modification, invasive species, pollution, over-collecting, and climate change. We report on a series of related initiatives contributing to conservation of crop wild relatives in the United States. An inventory of wild relatives has documented taxa related to a broad range of food, forage and feed, medicinal, ornamental, and industrial crops. Valuable species are threatened in the wild, and few accessions of these taxa are currently conserved *ex situ*. Potential distribution models based on historical occurrence information are clarifying where the species diversity of wild relatives is likely to be concentrated, and a gap analysis methodology is facilitating efforts to identify those taxa and geographic areas of particular conservation concern. A novel collaboration between the U.S. Department of Agriculture (USDA) Forest Service and USDA Agricultural Research Service (ARS) is making progress studying, collecting for genebank conservation, and protecting *in situ* a number of crop wild relative species. We discuss the value of broadening partnerships between agencies and aligning with ongoing regional and international initiatives to conserve, research, and utilize crop wild relative diversity.

Crop Wild Relatives- Useful but Threatened

Crop wild relatives—wild plants closely related to domesticated species—have proved useful as genetic resources in breeding more productive, nutritious, and resilient cultivars. Wild relative species native to the United States are no exception, with important contributions— especially to pest and disease resistance and stress tolerance—for corn (*Zea mays* subsp. *mays*), wine grape (*Vitis vinifera*), sunflower (*Helianthus annuus*), and English walnut (*Juglans regia*), among many others (Khoury et al. 2013).

The use of crop wild relatives is expected to increase with better information about the species (Castañeda-Álvarez et al. 2016, Wiersema et al. 2012) and improving breeding tools (Ford-Lloyd et al. 2011). But their use may be constrained by their limited representation in genebanks and the ongoing loss of wild populations to habitat modification, invasive species, pollution, over-collecting, climate change and other impacts (Castañeda-Álvarez et al. 2016). This is why the most important global agreements on agriculture, development, and conservation, such as the Sustainable Development Goals (Target 2.5) (United Nations Sustainable Development Platform 2016) and the Aichi Biodiversity Targets (Target 13) (Convention on Biological Diversity 2016), have explicitly highlighted the need to fully conserve crop wild relative diversity within the next few years. The United States has been recognized as one of the most important hotspots of crop wild relative diversity worldwide because it is home to many important native species that are inadequately represented in genebanks and are facing considerable threats *in situ* (Castañeda-Álvarez et al. 2016, Volk et al. 2015).

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One major challenge to overcome is that these unique sources of indirect ecosystem services have historically been given relatively low conservation priority in agricultural research, biodiversity, and natural resources management agencies' and organizations' mandates. Crop wild relatives have therefore been slipping between the cracks in conservation systems even while their characterization and use in crop breeding has been increasing. Information sharing and cooperation across different conservation approaches is needed to comprehensively resolve these deficiencies. The good news is that numerous recent initiatives are beginning to address conservation gaps for crop wild relatives through innovative collaborations among research and management organizations.

Crop Wild Relatives of the United States

The relatively short list of important crops originating in the United States includes squash (*Cucurbita pepo*) and sunflower (*Helianthus annuus*), which were domesticated before European contact, as well as blueberry (*Vaccinium* section *Cyanococcus*), cranberry (*Vaccinium* section *Oxycoccus*), blackberry (*Rubus fruticosus*, sensu lato and hybrids), and pecan (*Carya illinoinensis*), which were first cultivated more recently. The number of crop wild relatives native to the country would thus at first glance appear likely to be fairly small (Vavilov 1926).

Three additional factors lead to a considerably larger number of potentially valuable wild relatives documented in the region. The relatives of Mesoamerican crops, including species related to corn (*Tripsacum*), bean (*Phaseolus*), chili pepper (*Capsicum*), and cotton (*Gossypium*) are distributed in the southern regions of the United States. A number of crops domesticated in other temperate regions of the world are congeneric with wild species occurring in the country, for example onion (*Allium*), grape (*Vitis*), apple (*Malus*), strawberry (*Fragaria*), hops (*Humulus*), and walnut (*Juglans*). In addition, populations of introduced relatives of important staples such as wheat (*Aegilops*), oat (*Avena*), and sugar beet (*Beta*) have become quite successfully established (Khoury et al. 2013).

Taking a broad perspective both on what species can be considered crop wild relatives and what types of crops may be of interest (e.g., food, forage and feed, medicinal, ornamental, and industrial), a recent inventory of wild relatives occurring in the United States recognized 2500 taxa from 160 genera and 56 plant families (Khoury et al. 2013). Of these, ca. 250 native wild relative species were prioritized as close relatives of important food crops, including tree crop wild relatives of apricot, cherry, peach, and plum (*Prunus*), chestnut (*Castanea*), fig (*Ficus*), guava (*Psidium*), hazelnut (*Corylus*), mate (*Ilex*), pecan (*Carya*), persimmon (*Diospyros*), pistachio (*Pistacia*), sugar maple (*Acer saccharum*), and walnut.

Several well-documented examples of use of native wild relatives in crop breeding exist. North American wild grape germplasm proved critical in providing resistance to phylloxera (*Phylloxera vitifoliae*) as a rootstock in European grape production in the late 1800s, and these stocks continue to provide the basis for protection worldwide. Genes for resistance to rust, downy mildew, powdery mildew, broomrape, sclerotinia head and stalk rot, and sunflower moth have been successfully transferred from wild sunflowers into cultivars (Khoury et al. 2013). The foundation English and European hops cultivar "Brewer's Gold" is a hybrid carrying important introgressions from a North American species (*Humulus lupulus* var. *lupuloides*) (Townsend and Henning 2009). Tree crop wild relatives have primarily been utilized as pest and disease resistant and stress tolerant rootstocks, notably including American filbert (*Corylus americana*), Oregon crab apple (*Malus fusca*), northern California walnut (*Juglans hindsii*), and desert peach (*Prunus andersonii*) (Khoury et al. 2013).

Conservation Gaps

Sixty-two crop wild relative taxa native to the United States are listed endangered under the Endangered Species Act; 10 taxa as threatened; and 11 as candidates for listing. NatureServe, a non-profit organizational source of threat status information, has assessed eight native wild relative taxa as known or presumed extinct in the wild, 115 as globally critically imperiled, 111 as imperiled, and 337 as vulnerable. Threatened species with known or high potential value for crop breeding include northern California

walnut, which is used as a primary rootstock for English walnut and is critically imperiled in its native habitat, and Pecos sunflower (*H. paradoxus*) (fig. 1), a source of salt tolerance, as well as close relatives of squash, cotton, currant (*Ribes*), raspberry (*Rubus*), onion, wild rice (*Zizania* spp.), and plum (table 1) (Khoury et al. 2013).

Figure 1—Pecos sunflower (*Helianthus paradoxus*) at Blue Hole Cienega, Santa Rosa, New Mexico. Pecos sunflower is a source of salt tolerance for cultivated sunflower, and is listed threatened under the Endangered Species Act and globally imperiled in NatureServe (Khoury et al. 2013). (Photo by Laura Marek)

Table 1—Threatened United States wild relatives of major crops

Taxon	Endangered Species Act[a]	NatureServe	Number of accessions[b]
Allium munzii	LE	G1	4
Allium obtusum var. *conspicuum*		T2-3	0
Allium scilloides		G2-3	0
Cucurbita okeechobeensis	LE	G1	1 (+ 43 of subspecies)
Fragaria chiloensis (L.) subsp. *sandwicensis*		T2	2
Gossypium tomentosum		G2	41
Helianthus carnosus		G1-2	6
Helianthus niveus subsp. *tephrodes*		T2	15
Helianthus nuttallii subsp. *parishii*		TH	0
Helianthus paradoxus	LT	G2	22
Helianthus smithii		G2	10
Helianthus verticillatus	LE	G1	2
Hordeum arizonicum		G2-4	1
Ipomoea microdactyla		G2	2
Juglans hindsii (Jeps.)		G1	19
Lathyrus grimesii		G2	3
Lathyrus holochlorus		G2	1
Leymus pacificus		G2-3	0
Manihot walkerae	LE	G2	1
Phaseolus texensis		G2	0
Prunus eremophila		G1	0
Prunus geniculata	LE	G3	3
Prunus murrayana		GH	0
Ribes binominatum		G2-3	3
Ribes echinellum	LT	G1	3
Ribes erythrocarpum		G2	18
Rubus aliceae		GH	0
Rubus hawaiensis		G2-3	14
Rubus macraei		G2	0
Solanum incompletum	LE	G1	0
Solanum nelsonii	PE	G2	0
Solanum sandwicense	LE	G1	0
Solanum wallacei		G2	0
Tripsacum floridanum		G2	1
Vanilla mexicana		G2-4	0
Vicia menziesii	LE	G1	0
Vicia ocalensis		G1	1
Zizania texana	LE	G1	0

Data from Khoury et al. (2013), NatureServe (2016), and USDA NPGS Genetic Resources Information Network (2016).

[a] Taxa listed as endangered (LE), threatened (LT), or proposed endangered (PE) under the United States Endangered Species Act, and/or listed as known or presumed extinct in the wild (GH), globally critically imperiled (G1), imperiled (G2), vulnerable (G3), or apparently secure (G4) in NatureServe. T denotes global listing at the infraspecific level.

[b] Number of accessions denotes active USDA National Plant Germplasm System accessions.

Species distribution models of the ca. 250 prioritized crop wild relatives based upon historical occurrence records have indicated a surprisingly high concentration of taxa in the northeastern and eastern United States, upper Midwest, and Gulf coast regions (fig. 2). As these are areas of particularly high human population density, further analyses are urgently needed to verify extant populations and to prioritize zones of important crop wild relative genetic diversity for conservation. Preliminary assessments of the state of representation of these species in the U.S. Department of Agriculture (USDA) National Plant Germplasm System and other *ex situ* conservation programs indicate that considerable further collecting is needed to comprehensively represent native crop wild relatives in genebanks. Some 209 taxa related to 36 food crops were assessed through a gap analysis as high priority for further collecting due to very limited or no representation *ex situ*. States with the greatest numbers of under-represented taxa include New York, Virginia, Tennessee, Texas, North Carolina, West Virginia, Pennsylvania, Ohio, Illinois, Georgia, New Jersey, and Indiana, although collecting gaps were identified in all 50 states as well as Washington DC.

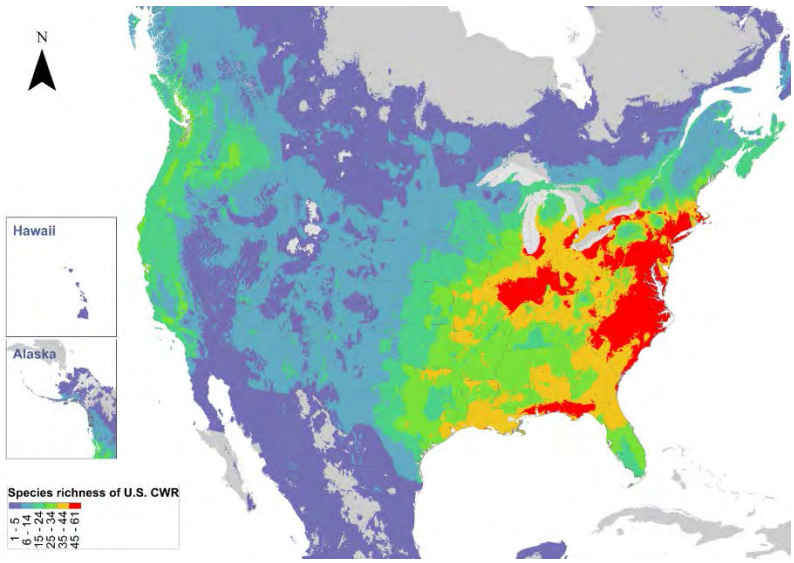

Figure 2—Priority crop wild relative taxon richness map. Areas colored light green, yellow, and red potentially possess the highest concentrations of species.

Progress Through Innovative Collaborations

Current efforts to collect crop wild relatives in the United States include the USDA ARS Plant Exploration Program, and the Bureau of Land Management Seeds of Success Program. A handful of conservation areas explicitly created to manage wild relatives *in situ* have been established, including for wild chilies (*Capsicum annuum* var. *glabriusculum*) in southern Arizona (USDA Forest Service 2016a). But given the considerable threats to wild relative populations in the United States and their relatively low level of representation in genebanks, both the urgent collection for *ex situ* conservation and the active management of taxa in conservation areas need to be enhanced considerably. Only a major increase in conservation action will enable the country to reach the global targets prioritized for completion by 2020.

In order to achieve these goals for the diversity of prioritized taxa, broad partnerships and networks between the federal, state, tribal and non-governmental organizations pursuing conservation activities are needed. An innovative example of such collaborations is current work on the conservation of wild cranberry by the USDA Forest Service (FS) and the ARS. Under the Strategic Framework on the Conservation and Use of Native Crop Wild Relatives in the United States established by the agencies (USDA Forest Service and USDA Agricultural Research Service 2014), populations of the two close

relatives of the crop are being collected for *ex situ* conservation in the National Plant Germplasm System, and assessed for unique genetic diversity in order to guide *in situ* conservation priorities (USDA Forest Service 2016b).

Such collaborations need to be extended to achieve comprehensive conservation. Moreover, because many of the taxa are distributed across national borders and the genetic resources of such species are potentially valuable globally, these efforts should be aligned with neighboring national strategies and with regional and global initiatives to conserve and provide access to crop wild relative diversity.

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Prioritizing Trees for Global Conservation Action: BGCI's Tree Conservation Agenda[1]

K. Shaw,[2] M. Rivers,[2] and E. Beech[2]

Botanic Gardens Conservation International (BGCI) is a global voice for plant conservation via its worldwide network of botanic gardens and partners. Tree conservation has been a strong focus of BGCI's program for many years. In collaboration with Fauna & Flora International, BGCI runs the Global Trees Campaign (GTC, www.globaltrees.org), the only international program dedicated to saving the world's threatened tree species. Through the GTC, we prioritise and protect the trees of greatest conservation concern, and improve and promote tree conservation action globally.

As a foundation of these efforts, BGCI is developing a world list of trees, GlobalTreeSearch, which currently contains over 55,000 accepted species. GlobalTreeSearch will be complete to species level and geo-referenced to country level by the end of 2016. GlobalTreeSearch will underpin GTC activities and provide critical support for conservation, restoration, forestry and agroforestry worldwide.

BGCI hosts the secretariat of the IUCN/SSC Global Tree Specialist Group (GTSG), a network of over 80 specialists who, working in their own institutions, carry out red listing of the world's trees. By 2020, the GTSG will leverage its global membership and expertise to ensure that we have conservation assessments for all of the world's tree species in the Global Tree Assessment. Red list assessments will be made available through published reports focusing on particular families or regions, and will be published onto the IUCN Red List of Threatened Species (www.iucnredlist.org). The Global Tree Assessment will enable identification of tree species at greatest risk of extinction. These species are priorities for conservation action.

BGCI's PlantSearch database is a global database of living plant collections with records from over 1,100 participating institutions (www.bgci.org/plant_search.php). PlantSearch enables BGCI to further prioritise where tree conservation action is most needed by identifying gaps in existing collections and mobilizing our network of botanic gardens, arboreta and seed banks, to bring missing and under-represented taxa into *ex situ* collections. In 2015, BGCI published (Rivers, M.; Shaw, K.; Beech, E.; Jones, M. 2015. Conserving the world's most threatened trees: a global survey of *ex situ* collections. Richmond, UK: Botanic Gardens Conservation International. http://globaltrees.org/wp-content/uploads/2015/10/webLR.pdf) a study that evaluated current collection records for 9,641 tree species identified as Critically Endangered and Endangered and determined that only one in four of the world's most threatened trees are represented in *ex situ* collections. While this result demonstrates that our network of partners has the technical ability to provide protection for some of the world's most at risk tree species, many of which are recalcitrant, there is clearly a lot of work to be done to provide *ex situ* protection for the remaining priority species. The report and accompanying annex enables conservation institutions to identify which trees require increased protection *ex situ* and to target their collecting programmes to address these gaps.

In addition to guiding conservation efforts, GTC works with partners on the ground to protect tree species *in situ*, carrying out reintroduction, restoration and sustainable use projects that trial new techniques and provide models of best practice. By sharing news and results from our projects, this direct action aims to provide inspiration so that our methods can be replicated for other threatened trees.

GTC also works to improve tree conservation practice by producing resources and delivering training. We draw upon the skills of specialists in our network and focus our efforts in areas where capacity is particularly limited. We provide recommendations including guidelines for the consideration of genetic diversity in both *ex* and *in situ* projects. All our resources are open access on our website and therefore available to a wide audience of conservation practitioners.

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By identifying which species are of greatest conservation concern, establishing replicable tree conservation projects, and providing technical and practical guidance, GTC guides, encourages, and enables action to be undertaken for a wider number of threatened trees than can be covered through GTC's direct actions. BGCI's network of botanic gardens, with sites for *ex situ* conservation, as well as research and horticultural expertise, provides a particularly valuable resource for tree conservation, as well as a platform for scaling up action.

To find out more about the Global Trees Campaign and BGCI's tree conservation agenda, please visit our websites: www.globaltrees.org and www.bgci.org/plant-conservation/globaltrees/.

Climate Change and Forest Trees in the Pacific Northwest: a Vulnerability Assessment and Recommended Actions for National Forests[1]

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Abstract

Climate change presents new challenges to land managers. At stake is our ability to make thoughtful, science-based decisions and to add climate change considerations to our project and management plans. We also must prioritize among the opportunities that can be included in adaptation strategies because funding and time are limited, now more than ever. In 2012, we conducted a vulnerability assessment of common overstory forest tree species for the Pacific Northwest and provided recommended actions based on the results of this assessment. These recommendations will sharpen the focus of activities on the most vulnerable species while simultaneously any recommended actions taken will help in conserving biodiversity and building resiliency. Our analytical approach did not include spatially explicit predictions of future tree species habitats. Rather, it uses life history traits, distribution, and pest and pathogen data for individual tree species, combined with consensus regional climate projections to rate each species' relative vulnerability to a changing climate. The analytic method we employed here with forest trees is transparent, flexible, and simple to apply and could be adapted to other native plants including forbs and grasses.

Vulnerability scores varied by species and geographic area, but there was a consistent positive relationship between vulnerability to climate change and mean elevation with many of the most vulnerable tree species occurring at the highest elevations. There were three overall recommendations for land managers that came out of this assessment: 1) learn about and track changes in plant communities as the climate changes, 2) maintain and increase biodiversity and increase resiliency, and 3) prepare for an uncertain future. Specific action items were proposed to address these recommendations based on the results of the vulnerability assessment.

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Genetic Conservation Planning for Forest Tree Species in Western North America Under Future Climate Change: Employing a Novel Approach to Identify Conservation Gaps[1]

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Among the 17 provinces, territories, and states that comprise western North America, approximately 18 percent of the 8.4 million km^2 of forested land base is designated as protected areas to ensure the *in situ* conservation of forest biodiversity. Jurisdictions vary substantially however, in their responsibilities, protected area coverage, and conservation policies. Here we demonstrate a novel approach to identify current and potential future conservation gaps for genetic populations across a tree species' range based on (1) statistical species distribution maps generated from 250 m remote sensing data and species frequency estimates from over 50,000 forest inventory plots, and (2) a multivariate velocity of climate change metric. We ask which tree species will be most vulnerable due to insufficient population protection in the future. Our goal is to determine sensible global conservation priorities that can be implemented in individual jurisdictions. The analysis of the putative genetic populations within major ecological zones revealed that forests are generally well represented with only four jurisdictions (Oregon, Saskatchewan, as well as the Yukon and Northwest Territories) protecting less than 10 percent of their forested land base. Within the 54 tree species analyzed, populations of western white pine (*Pinus monticola*), whitebark pine (*P. albicaulis*), and limber pine (*P. flexilis*) were found to be the least protected *in situ*. Under projected climate change, interior and boreal tree species are expected to be the most vulnerable, although they are currently among the most frequent and best protected species. To facilitate policy development, we present an example of how conservation efforts can be prioritized across multiple jurisdictions. We also provide data for resource managers that pinpoint the least protected tree populations as well as their relative vulnerability to climate change.

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Using Climate and Genetic Diversity Data to Prioritize Conservation Seed Banking for Imperiled Hemlock Species[1]

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Hemlock woolly adelgid (HWA, *Adelges tsugae*) is an invasive forest insect that has caused mortality of eastern (*Tsuga canadensis*) and Carolina hemlock (*T. caroliniana*) at an alarming rate. Now infesting 19 states and over 400 counties of the eastern United States, HWA poses a significant threat to native host species. The current biological and chemical methods for protecting these keystone species are expensive, time consuming, and short-lived. For the long-term preservation of both hemlock species, *ex situ* genetic conservation efforts such as seed collection and storage may be the best solution. With this in mind, it is urgent to prioritize populations within the native range of eastern and Carolina hemlock for *ex situ* conservation. Using a geographic information systems technique called gap analysis in congruence with eight genetic diversity estimates, areas of significant eastern and Carolina hemlock genetic diversity were located and threats to those areas were identified. Using the Multivariate Adaptive Constructed Analogs statistical downscaling method, climate projections averaged over 20 regional climate models were analyzed to display a minimum temperature threshold below which significant HWA mortality occurs. Models also show the temporal northward movement of that threshold to areas not yet exposed to HWA. The result is a spatially weighted index of hemlock populations prioritized by genetic significance and climatic risk. Through 12 years of seed collection and seed banking of eastern and Carolina hemlock, we have also collected the genetic diversity data needed to refine ongoing efforts to prioritize populations most at risk and those that encompass the highest levels of genetic diversity.

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Project CAPTURE: a U.S. National Prioritization Assessment of Tree Species for Conservation, Management, and Restoration[1]

Kevin M. Potter,[2] Barbara S. Crane,[3] and Valerie D. Hipkins[4]

A variety of threats, most importantly climate change and insect and disease infestation, will increase the likelihood that forest tree species will undergo population-level extirpation or species-level extinction during the next century. Project CAPTURE (Conservation Assessment and Prioritization of Forest Trees Under Risk of Extirpation) is a cooperative effort across the three U.S. Department of Agriculture Forest Service (USDA FS) deputy areas to establish a framework for conservation priority-setting assessments of forest tree species across the entire United States. Project CAPTURE uses extensive lists of life history trait data, as well as climate change and pest and pathogen threat information, to categorize and prioritize 368 native tree species for conservation, monitoring, management, and restoration across all forested lands in the contiguous United States and Alaska. The project has recently expanded to include 561 native tree species of Puerto Rico and the Virgin Islands, and will eventually include the native trees of Hawaii.

The foundation of this overall effort is a flexible framework that rates species based on risk factors relating to (1) intrinsic attributes, such as population structure, fecundity and seed dispersal ability; (2*)* external threats to genetic integrity; and (3) conservation factors, including evolutionary distinctiveness and regional responsibility. The Project CAPTURE framework allows for the quantitative grouping of species into vulnerability classes that may require different management and conservation strategies for maintaining the adaptive genetic variation of the species contained within each class. The framework was developed with input from a 2014 workshop that included USDA FS resource managers and scientists across the country and from the three deputy areas. An assessment of climate change vulnerability for species of the continental United States has been completed (Potter, K.M.; Crane, B.S.; Hargrove, W.W. 2017. A United States national prioritization framework of tree species threatened by climate change. New Forest. doi: 10.1007/s11056-017-9569-5.), while an assessment of pest and pathogen vulnerability is under way. The Project CAPTURE assessment tool should be valuable for scientists and managers attempting to determine which species and populations to target for monitoring efforts and for pro-active gene conservation and management activities.

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Prioritization of Gene Conservation Activities in the Face of Changing Climates[1]

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Several analyses have been done or are underway to evaluate the vulnerability of individual forest tree species to climate change. Species vulnerability assessments allow managers to allocate limited resources to the management of those species that are most threatened. But we also know that threats to individual populations of species may vary across the landscape, and that populations may contain unique and potentially valuable genetic variants that should be conserved. We propose a framework for prioritizing populations within species for gene conservation activities in which maps of risk of extirpation are overlaid with maps representing genetic variation and the species range. The average risk of extirpation is determined for each genetic classification representative of genetic variation—for example, seed zones as representative of adaptively significant variation, or, if available, maps of geographic variation in adaptive traits as determined from common garden studies. The highest priority is given to those zones with the highest risk of extirpation due to immediate factors other than climate change—fire, disease, insects, invasive species, and human development (although those factors may be indirectly related to recent climate change). The risk associated with each factor is overlaid with the genetic classification, and an average risk for each zone determined. Zones with the highest average risk are given the highest priority for *ex situ* collections. Meanwhile, zones with lower average risk may be the best candidates for *in situ* reserves since they may be expected to be most stable. The second highest priority is given to those populations that are at risk because they are expected to be outside of the future climatic niche of the species based on the best available models. Those populations may, however, have some potential for migration to new locations over time. The potential for migration within each zone may be evaluated using the average climate velocity for each zone. The third highest priority is given to those populations which lay within the future climatic niche of the species, but are at highest risk of maladaptation. Measuring risk of maladaptation involves knowledge of those climates that are most strongly associated with adaptive genetic variation. Such knowledge may come from genecology studies in which differences in adaptive traits are directly associated with differences in climates of seed sources. Average differences may be determined for each zone between current and future climates or associated traits, and higher priority for gene conservation given to those zones with the greatest differences. Consideration may also be given to the potential for migration for those higher priority zones of high risk of maladaptation. In all cases, special considerations may be given to disjunct populations and populations at the trailing edge of climate change. These populations are likely to be small with a greater risk of extirpation and higher likelihood of harboring unique variation. Trailing edge populations, in particular, may contain genetic variation associated with adaptation to warmer climates.

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Conservation Strategies (Overviews)

Benefits and Challenges For Gene Conservation: a View From The UK National Tree Seed Project[1]

Clare Trivedi[2] and Simon Kallow[2]

Abstract

Trees and woodlands in the United Kingdom are currently subject to a range of threats including loss and fragmentation of native woodland and escalating pest and disease outbreaks. The largely unknown impacts of climate change pose a number of questions when considering afforestation and reforestation. There are frequent calls to develop resilient woodlands, robust enough to deal with these challenges but there is uncertainty over what this means in terms of species mixes and use of local provenance versus non-local planting material. Conserving and making appropriate use of the current genetic diversity of native trees and shrubs should be an important element for meeting these challenges. For this reason, the United Kingdom National Tree Seed Project was launched by the Royal Botanic Gardens, Kew in 2013. Multi-provenance seed collections are being made, and conserved in Kew's Millennium Seed Bank. These genetic resources will be made available for research and conservation activities, ultimately facilitating access to appropriate planting materials for afforestation and reforestation.

Introduction

With one of the lowest rates of woodland cover in Europe, the United Kingdom's woodland resource is significantly impoverished. Woodland covers 12 percent of land area, of which only about one third can be considered native woodland (Atkinson and Townsend 2011). United Kingdom woodlands are also highly fragmented, and some are poorly managed. These issues are complicated by the significant rise in pest and disease outbreaks over the past decade and uncertain future climate change scenarios. For these reasons, protection, restoration and creation of woodlands are widely proposed (DEFRA 2012, Forestry Commission Scotland 2009, Welsh Assembly Government 2009, Woodland Trust 2016). For example, in England it is proposed that woodland habitat be enhanced through sustainable management, restoring ancient woodlands, and expanding tree cover with appropriate species (DEFRA 2012).

Reports frequently refer to the need to develop 'resilient woodlands' able to withstand varied challenges, but there is debate about what this means in terms of the benefits of native versus non-native species, and the use of local versus non-local planting material. One specific issue, highlighted by the 2012 ash dieback (caused by the pathogen *Hymenoscyphus fraxineus*) breakout, is that for many years much planting material was brought into the United Kingdom from continental Europe (Russell and Evans 2004). While there is now an increasing demand for homegrown planting material of native species, the supply can be difficult to maintain (Forestart 2014).

At the heart of these complex and intertwined issues is the need to maintain and utilize the full genetic diversity of native woodland resource. Coordinated gene conservation programs seek to address such challenges by conserving a genepool of genetically diverse, locally-adapted material which can be used for breeding for required traits, such as disease resistance, and facilitating the supply of appropriate native planting material.

A national gene conservation program to conserve forest genetic resources in the United Kingdom does not yet exist. Given that United Kingdom shares most of its woody flora with European neighbors, taking action to conserve its' own genetic resources will contribute to the wider conservation of European genetic resources. The islands of the United Kingdom are at the limit of the natural distribution range for a number of species and so may contain unique elements of natural variation worthy of conservation.

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It is in this context that in 2013, the Royal Botanic Gardens, Kew launched its United Kingdom National Tree Seed Project (UKNTSP), in order to establish an *ex situ* gene conservation program for the United Kingdom forest genetic resource. The aim of UKNTSP is to establish multi-provenance seed collections which in total will represent the majority of adaptive genetic diversity present. These collections are intended to provide a resource for science and practical efforts to meet the challenges outlined above.

The UK National Tree Seed Project

Based at Kew's Millennium Seed Bank (MSB), the project has a 5 year initial workplan with the following purpose:
'To provide a national repository of plant material and associated knowledge, for the purposes of long term conservation, and to make these resources available to users, in order to better understand and manage tree and shrub species in the United Kingdom landscape'.

The project will deliver three outputs by March 2018:

1. Establishment of an accessible, genetically representative, national seed collection of United Kingdom trees and shrubs.
2. Research to understand and overcome constraints to the *ex situ* conservation and use of United Kingdom tree species.
3. To raise public awareness of the project, and the role of *ex situ* conservation in general, to meet the challenges facing the conservation and management of United Kingdom trees, woods and forests. This output will not be discussed further in this paper.

Output 1

A detailed account of the process for developing the project's target species list and sampling strategy is provided in Kallow and Trivedi (Collecting genetic material on a small island, these proceedings). In summary, a list of 70 native taxa were chosen as target species, based on a scoring system that took into account tree health risks, conservation status, and prevalence in the landscape. Species which are prevalent in the landscape were scored highly to reflect the likely greater impacts of their loss.

Tree seed zones had already been developed for the United Kingdom (Herbert et al. 1999). In the absence of species-specific genetic knowledge, these biogeographic zones were adopted by the project to provide a framework for the establishment of seed collections that represented the majority of adaptive genetic variation in Britain for each target taxon. It was decided to ensure at least one collection was made within each seed zone in the native distribution of each targeted taxon, with collections at both high and low elevation where populations are present above 300 m above sea level. Botanical records, provided by the Botanical Society of Britain and Ireland, were mapped against the seed zones, leading to an initial list of 946 target collections. Over time, desk studies and ground-truthing reduced this target list to 663, illustrating the importance of both local and species-specific knowledge when planning seed collecting.

In order to meet the ambitious targets with limited resources, seed collecting is being carried out by more than 30 governmental and non-governmental agencies, and many trained volunteers. Guidance for tree seed collecting typically advocates the collection of 30 to 60 genetically distinct individuals (e.g., OECD 2013, Thomas et al. 2014) from a population. The highly fragmented nature of United Kingdom woodlands means it is rarely possible to meet such expectations. Collectors must then consider making collections from dispersed sites across a seed zone. Furthermore, the project is seeking several thousand seeds per collection, collected from across the canopy of each mother tree, in order to capture the genetic diversity associated with a range of fathers. Such seed collecting is extremely time consuming. Our experience shows a team of four can generally collect from a maximum of 15 trees in one day.

Seed from individual mother trees is stored separately as this will allow heritability estimates to be calculated for important adaptive traits. Each mother tree is geo-referenced and tagged. As of July 2016, 465 collections have been made, achieving 278 collecting targets.

Output 2

Output 2 seeks to identify and overcome constraints to seed banking the United Kingdom native woody flora. It is split into 2 parts:

2.1: Studies to better understand the population genetics of United Kingdom species and how to sample and use collections.

The project adapted a desk-based decision tree approach to identify what is known about the genetic structure and diversity of the target species (Neaves and Hollingsworth, personal communication). Overall, this approach confirmed there is not enough fine detail known at the United Kingdom level to inform species-specific sampling strategies. However, it did identify priority species for future studies such as *Juniperus communis* and *Taxus baccata.*

2.2: To identify and overcome constraints to the storage and germination of the United Kingdom woody flora.

Literature reviews and viability data from MSB collections were used to identify potential constraints and solutions. This assessment indicated there are few significant constraints to seed-banking, with the exception of *Quercus* species, which are recognised as recalcitrant.

To date, germination protocols are established for 64 target species, and these will be made publicly available. Studies are identifying species likely to be short-lived in conventional seed bank conditions, and these are prioritized for cryopreservation in order to ensure their long term availability. Particular attention is being paid to developing collecting and processing protocols for the Salicaceae to enable optimal storage of this difficult family. Studies to optimize seed maturity at the time of collection are planned for *Fagus sylvatica.*

Benefits and Challenges for the UKNTSP

The UKNTSP collections will conserve a representation of the genetic diversity present in the United Kingdom woody flora at this point in time. The collections will be available for a wide range of research studies, many of which we cannot anticipate at present. They will allow future researchers to understand changes that occurred due to climate change and disease and pest events which may change the composition of the United Kingdom genetic resource. Through its gene conservation approach, the UKNTSP can also make a significant contribution to meeting the contemporary challenges outlined previously.

Some of the seed collecting sites found by the UKNTSP could be suitable for exploitation as registered seed sources. This could facilitate an improved commercial supply of planting material of known provenance for afforestation and reforestation.

Furthermore, the project is enhancing skills and knowledge in tree seed collecting and storage. It is also raising awareness of issues of genetic diversity and seed quality. So, alongside commercial supply chains, a culture of high quality seed collecting and use of appropriate genetic material is developing among many non-government organizations who lead United Kingdom habitat management.

For several species, collecting directly from seed sources is unlikely to be efficient, either because sufficiently large native stands simply do not exist or they rarely produce seed (Karen Russell, personal communication). The UKNTSP collections could provide the founder stock for establishing seed orchards. For some species, an option would be to screen collections for traits such as disease resistance, ultimately aiming for the development of disease resistant seed orchards to supply material for reforestation of areas decimated by pests or disease.

The UKNTSP collections also provide useful material to better understand how to adapt United Kingdom woodland conservation and management to climate change. They are an ideal source of experimental material for provenance trials to measure the performance of materials from different seed sources across different parts of the United Kingdom. Of particular importance are adaptive traits such as drought resistance. Collections of known provenance from across the United Kingdom will also be useful for studies of seed physiology in support of natural regeneration of populations. Examples include

understanding gradients in seed viability, and varying requirements for cold stratification before germination.

Finally, the collections will be available for wider studies to better understand the interactions of trees with pests and diseases, which in turn will provide management solutions. For example, the seeds could be used to assess the efficacy of new treatments or preventive measures and the impact of these on related and associated species.

These likely benefits of the collections mostly fall outside the scope of the project itself. It is incumbent on the project staff to engage constructively with a wide range of researchers, foresters and conservation agencies to ensure the resource is used to its fullest advantage and sits within a coherent wider gene conservation framework. The key challenge remaining for the project team is to ensure the collections are sufficiently large and genetically representative to meet the needs of users. We need to make the best use of available resources to collect enough seed from sufficient trees to ensure the collection is representative of the adaptive genetic diversity contained by the United Kingdom native forest resource, and that it meets the needs of those who will use it in the future.

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Contributions of Public Gardens to Tree Gene Conservation[1]

P.A. Allenstein[2]

American Public Gardens Association, founded in 1940, represents over 600 member gardens spanning North America and 24 countries. Its diverse membership includes botanic gardens, arboreta, and other public gardens which contribute to tree gene conservation. Some maintain *ex situ* collections nationally accredited through the Association's Plant Collections Network, a 21-year collaboration with the U.S. Department of Agriculture (USDA) Agricultural Research Service. This program promotes excellence in curatorial practices while facilitating a continent-wide approach to germplasm preservation. Some collections have a global scope while others focus on a particular plant type or even historic cultivars. An increasing number of gardens are developing and strengthening collections for conservation purposes. A recent partnership with the USDA Forest Service focuses on tree gene conservation emphasizing those species which cannot be preserved through traditional seedbanking. Horticultural expertise and facilities at public gardens assist restoration efforts. Gardens manage herbaria of both wild-collected and cultivated plants. A number of gardens steward natural lands on their properties. Some public gardens have active research programs which include a focus on tree conservation, plant evaluation, and breeding. Individuals are trained as first detectors of high consequence pests and diseases through the Association's Sentinel Plant Network, while the Plant Heroes youth program provides educational materials. Public gardens provide a valuable role in raising conservation awareness among their 70 million annual visitors through displays, interpretive materials, and educational programs.

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Overview of the Camcore (NC State University) and USDA Forest Service Cooperative Gene Conservation Program for Threatened and Endangered Tree Species Native to the Southern United States[1]

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The southern United States is home to some of the world's most biologically diverse temperate forests. These forests range from the Atlantic and Gulf coastal plains to the Southern Appalachian Mountains and are home to more than 140 tree species which provide a number of ecosystem services, including clean air and water, carbon storage, recreational opportunities, wood, and fiber to feed a growing population's need for solid wood and paper products. Many of these species are threatened by natural and human-caused disturbances, including native and exotic insects, diseases, invasive plants, tropical weather systems, wildland fire, development, fragmentation, and climate change. Species with declining population sizes need dynamic approaches to *ex situ* gene conservation to secure seed resources for long-term preservation and the eventual restoration of the species and ecosystems. Since 2003, Camcore (Central American and Mexico Coniferous Resources Cooperative), International Tree Breeding and Conservation, North Carolina State University) and the U.S. Department of Agriculture Forest Service have collaborated on a cooperative gene conservation program to secure seed resources for tree species native to the southern United States recognized as threatened and endangered. Species targeted for seed collection to date include eastern hemlock (*Tsuga canadensis*), Carolina hemlock (*T. caroliniana*), Table Mountain pine (*Pinus pungens*), Atlantic white cedar (*Chamaecyparis thyoides*), red spruce (*Picea rubens*), Fraser fir (*Abies fraseri*), and four rare species of ash (*Fraxinus* spp.). Camcore employs studies of genetic and adaptive variation using microsatellite molecular markers, seed zone models, plant hardiness zones, and ecological sub-regions to design seed conservation strategies and to target seed collections to areas of high or unique variation. Substantial progress has been made with the hemlocks and Table Mountain pine, and project overviews have been published (Jetton et al. 2008. Forest Ecology and Management. 255: 3212–3221; Jetton et al. 2013. Tree Planters Notes. 56: 59–71; Jetton et al. 2015. Tree Planters Notes. 58: 42–52). To date, more than 2.5 million hemlock seeds representing 728 mother trees in 72 populations of eastern hemlock and 134 mother trees and 19 populations of Carolina hemlock have been placed into conservation. Seed collections from Table Mountain pine acquired nearly 400,000 seeds representing 262 mother trees in 38 populations. Projects with Atlantic white cedar, red spruce, and Fraser fir are ongoing, but have already acquired seed representing 205 mother trees in 28 populations of Atlantic white cedar, 83 mother trees in 12 populations of red spruce, and 129 mother trees in 10 populations of Fraser fir. The seed conservation project for ash is focused on four rare species that occur in the southern United States: Carolina ash (*F. caroliniana*), pumpkin ash (*F. profunda*), blue ash (*F. quadrangulata*), and Texas ash (*F. texensis*). Collections for these species are in the planning phases and will begin during the summer of 2016.

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State of the United States Forest Genetic Resources – Summary of a Report to FAO International Technical Working Group on Forest Genetic Resources[1]

Randy Johnson[2]

Abstract

Among forest-associated plant species in the United States, less than one percent has been determined to be extinct. However, 57 trees or trees/shrubs are officially listed as threatened or endangered by the U.S. Department of Interior Fish and Wildlife Service. Most of these listed species are tropical island endemics; 35 are from Hawaii and 13 from Puerto Rico and/or the United States Virgin Islands. As required by law, these listed species have restoration plans that are in some state of implementation. Federal land management agencies also strive to conserve species that are considered "at risk". In addition, federal agencies manage for native ecosystems; thereby providing aspects of *in situ* conservation on their land base; which represents one third of all forest land in the United States.

Ex situ conservation efforts within the United States are extensive. Specific conservation collections are made by a number of organizations, including: the Center for Plant Conservation (http://saveplants.org/), the U.S. Department of Agriculture (USDA) Agricultural Research Service National Plant Germplasm System, the U.S. Department of Interior Bureau of Land Management 'Seeds of Success' program, and the USDA Forest Service. Breeding and restoration programs, predominantly housed in federal agencies and universities, represent over 150 different *ex situ* collections which include over 100 species of trees and tree/shrubs.

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Camcore: Thirty-five Years of Mesoamerican Pine Gene Conservation[1]

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Abstract

Camcore is an international tree breeding and conservation program with headquarters at North Carolina State University. Camcore was founded in 1980 as a cooperative, non-profit organization to identify and save the dwindling natural populations of pines in the highland regions of Guatemala in Central America. Funded by the private sector, the program has played an important role as an international gene conservation cooperative. The program emphasizes *ex situ* conservation as a complement to *in situ* conservation to ensure that a wide range of genetic variation in a species is protected. Camcore has collected seed from almost 10,000 trees from 349 populations of 25 species of pines in natural stands in Mexico, Central America, and the Unites States. A goal of 20 trees per population ensures that collections include most genes that have a frequency of over 5 percent. Working with local people, Camcore staff determines the conservation status of some of the natural populations using the criteria established by the International Union for Conservation of Nature (IUCN). Out of the 105 populations assessed, 5 percent are critically endangered, 32 percent endangered, 61 percent vulnerable, and only 2 percent low risk. Camcore has established 1,250 pine genetic trials in 17 countries. Eighty-six percent of the provenances and 70 percent of the families of collected pine species have been planted in Camcore trials and conservation banks. Camcore has sent seeds to government organizations in Mexico and Guatemala for the establishment of reintroduction studies, including two studies of *P. patula* Schldl. et Cham. and one of *P. greggii* Engelm. ex Parl. planted in Mexico, and four studies of *P. maximinoi* H.E. Moore and one of *P. tecunumanii* Eguiluz and Perry planted in Guatemala. One of Camcore's latest efforts is the establishment of six large multi-species pine conservation parks in South Africa that will eventually be 20 hectares each.

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Genetic Diversity and Conservation of Mexican Forest Trees[1]

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Over the last 200 years, humans have impacted the genetic diversity of forest trees. Because of widespread deforestation and over-exploitation, about 9,000 tree species are listed worldwide as threatened with extinction, including more than half of the ~600 known conifer taxa. A comprehensive review of the floristic-taxonomic literature compiled a list of 4,331 recorded tree species in Mexico. The highest diversity of pine and oak worldwide is located in the Mexican temperate forests. Because species diversity and genetic diversity are often positively associated, there is expectation of very high trans-specific genetic diversity exists in Mexican tree species. Contrasting with its high species and genus richness, studies of genetic diversity within Mexican forest trees are rather scarce, and often biased to particular families, like the Pinaceae. Moreover, even within those particular families the available surveys have a penchant for a specific genus. The markers used in most of these studies include the traditional and "universal" isozymes and chloroplast microsatellites and, to a lower extent, the anonymous ISSRs, AFLPs, and RADPs. Additional studies on more varied taxa, using more advanced technologies and markers are needed. Because of the poor comparability of the genetic diversity estimates among the studied Mexican tree species, it is extremely difficult to discern general trends across species or regions. We therefore recommend that genetic diversity should be measured across species with an identical type of genetic marker, surveying similar numbers of loci, individuals, and populations, and while using identical indices of genetic diversity.

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Pest and Pathogen Resistance

Development of New Dutch Elm Disease-Tolerant Selections for Restoration of the American Elm in Urban and Forested Landscapes[1]

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Abstract

The goal of our research and development efforts is to restore American elm (*Ulmus americana*) as a species in both natural and urban landscapes. Accomplishing this goal requires identification/generation of additional American elm cultivars that are tolerant to Dutch elm disease (DED) caused by *Ophiostoma ulmi* and *O. novo-ulmi*, and development of methods to reintroduce American elm along the rural to urban gradient. To accomplish our goal we are screening large survivor trees for DED tolerance, generating DED-tolerant/site-adapted cultivars, generating three regional seed orchards, establishing experimental American elm restoration sites, using elm in the Appalachian Regional Reforestation Initiative, performing operational trials using elm seed along the Mississippi River, comparing local vs. DED-tolerant enriched seedlings, investigating fungal transmission in DED-tolerant selections, and investigating the basis for cold-hardiness in the American elm and the genetic as well as metabolic basis of DED tolerance. In the spring of 2016, branches containing flower buds were collected from large surviving American elm trees in New England. Pollen was collected from the flowers and was used in controlled pollinations with the DED-tolerant American elm cultivars 'Delaware 2', 'Princeton', R18-2, and 'Valley Forge'. The mean numbers of seeds produced per cross for each DED-tolerant mother tree ranged from 0.1 to 23 per flower. Scion wood was collected from the branches and used to graft buds and scion wood to potted American elm root stock using several types of grafting techniques. Veneer and top grafting had the highest success rates (30 percent and 22 percent, respectively), followed by bottle grafts (12 percent) and bud grafts (6 percent). Top grafts, veneer grafts, and bud grafts produced the greatest growth and number of cuttings. Plants growing from successful grafts were used to generate clones by vegetative cuttings using the rooting hormone indol-3-butyric acid at varying concentrations. Sixty-five percent of the cuttings had rooted by early August, and 42 percent of the trees that rooted had also produced shoots. Clonally propagated American elm selections generated from crosses among DED-tolerant cultivars, clones of large survivor trees found in Michigan, Ohio, Illinois, and Indiana, and susceptible and tolerant controls were inoculated with a mixture of *O. novo-ulmi* and *O. ulmi* spores. For each tree, the combined percentage of the crown exhibiting wilting, chlorosis, or necrosis was visually estimated to the nearest 5 percent. The percentage of the crown exhibiting DED symptoms in trees from the Midwest and New England inoculated trees ranged from 0 to 35 percent at 4 weeks post-inoculation. Control trees (inoculated with water) in both studies exhibited from 0 to 5 percent canopy decline. Trees with high levels of DED tolerance will be released to the tree nursery industry and retained in test plots to constitute a seed orchard. Over the next 6 years approximately 16,000 elm trees—clones of survivor trees and site-adapted progeny from crosses between DED-tolerant elms and elms from the upper Midwest and New England states—will be inoculated.

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Introduction

The American elm (*Ulmus americana*) was once widely distributed throughout the eastern United States before the arrival of Dutch elm disease (DED) caused by the fungal pathogens *Ophiostoma ulmi* and *O. novo-ulmi*. American elm's tall height coupled with its vase-like shape provides for a uniquely graceful tree that was commonly planted along city streets and boulevards. The crowns of mature elms spanned countless roadways, houses, and recreation areas, where they provided the benefits of cleaner air and cooler temperatures. American elm is one of the few native tree species capable of thriving within the harsh urban environment, where extreme summer temperatures, air pollution, and road salt are common. Before the invasion of DED, elm was an ecologically important tree species in riparian areas, bottomlands, and the urban environment, serving to enrich soils through the rapid decomposition of its nutrient-rich leaf litter. Its seeds were an important source of food for song birds, as elm seeds matured in the spring before most other seeds are available.

The DED fungal pathogen *O. ulmi* was introduced into the United States in 1930 and its spread has devastated North American species of elm, nearly eliminating the use of American elm as an urban shade tree. In Illinois in the 1940s the Eurasian race of *O. novo-ulmi* appeared, causing a second wave of elm mortality. Research on American elm from the 1970s to the present focused on the identification of American elm cultivars that could withstand the DED pathogen. Of the over 100,000 American elm trees tested for resistance to DED, only nine cultivars exhibited adequate levels of DED tolerance. While a few cultivars are commercially available, about 90 percent of the elms purchased in the United States are the 'Princeton' cultivar. The widespread use of one DED-tolerant cultivar presents the risk of another wave of elm mortality due to attacks by other pests/pathogens or the mutation of DED. Additional DED-tolerant cultivars representative of the genetic diversity of native American elm populations and suitable for both urban and forested settings are needed to ensure the long-term stability of DED-tolerance among American elm populations. Toward this goal, several research programs have carried out work on the selection and breeding of American elms (Schreiber and Domir 1994, Sherald 1993; Smalley and Guries 1993, Smalley et al. 1993, Townsend et al. 1995, Townsend 2000, Townsend et al. 2005), though all have largely ended due to limited funding.

We are engaged in an ongoing study to identify and generate additional American elm selections that can tolerate DED pathogens. Our approach is to clonally propagate survivor elm trees, cross them with known DED-tolerant selections and to test the clones and crosses for tolerance to DED. This paper focuses on methods used for collection of pollen, controlled pollinations in the field, clonal propagation through grafting and vegetative cuttings, inoculation of American elm selections—both orchard trees (5 to 7 years old) and small potted trees (1-year-old)—with *O. ulmi* and *O. novo-ulmi*, and evaluation of foliar stress metabolite concentrations as an early screening marker for DED tolerance. The results of the controlled pollinations, preliminary results of vegetative propagation, foliar symptoms observed at 4 weeks post-inoculation with DED, and pre-inoculation concentrations of foliar stress metabolites are described.

Polyamines (PAs) are a group of metabolites including a diamine putrescine (Put) whose cellular concentrations fluctuate significantly over and above baseline background variations when plants are under stress - making it an ideal metabolite for early detection monitoring of stress (Minocha et al. 2014). Two other common polyamines are spermidine, and spermine. These are small, positively charged, organic molecules that are found in all living organisms. Their simple structure, universal distribution in all cellular compartments, and involvement in various physiological processes is why fluctuations in their cellular concentrations are often related to varied responses of plants to different forms of stress. A strong positive relationship between abiotic stress and foliar putrescine has been proposed as a potential biochemical marker of persistent environmental stress in several species of trees where phenotypic symptoms of stress were not yet visible. Various types of stress lead to a modulation of putrescine levels, suggesting Put is a reliable indicator of cellular functional adjustments. An increase in putrescine indicates resistance to a stress and a decrease upon reversal of stress means amelioration from stress (Minocha et al. 2010, Wargo et al. 2002). Despite continued interest in polyamine metabolism in plants

subjected to abiotic stresses, work on polyamine metabolism relative to plant-pathogen interactions lags behind (Walters 2000, 2003). In several preliminary, yet unpublished, studies conducted in the Minocha laboratory using different tree species, higher concentrations of putrescine were found to be associated with tolerance to biotic stress at different forested sites (fig. 1). Metabolic markers respond to environmental and microsite soil chemistry differences related to infestation and tolerance.

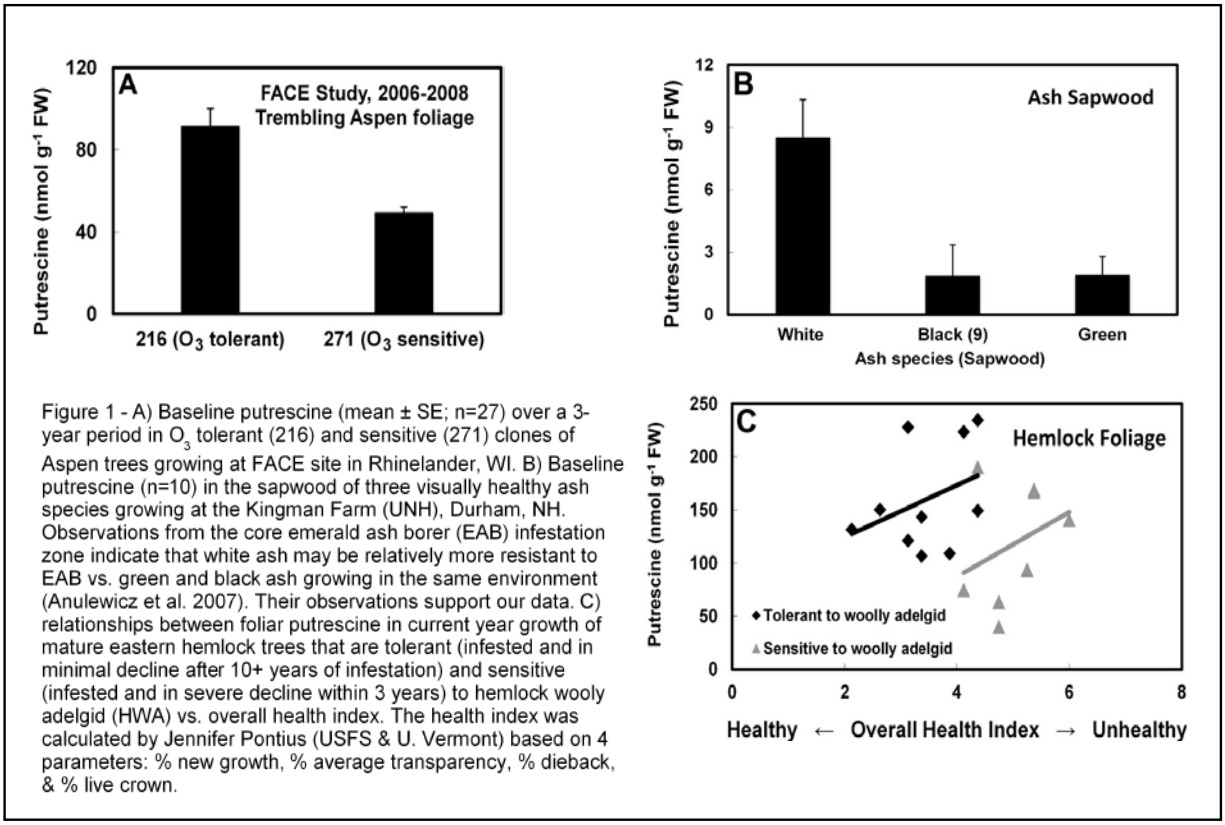

Figure 1 - A) Baseline putrescine (mean ± SE; n=27) over a 3-year period in O_3 tolerant (216) and sensitive (271) clones of Aspen trees growing at FACE site in Rhinelander, WI. B) Baseline putrescine (n=10) in the sapwood of three visually healthy ash species growing at the Kingman Farm (UNH), Durham, NH. Observations from the core emerald ash borer (EAB) infestation zone indicate that white ash may be relatively more resistant to EAB vs. green and black ash growing in the same environment (Anulewicz et al. 2007). Their observations support our data. C) relationships between foliar putrescine in current year growth of mature eastern hemlock trees that are tolerant (infested and in minimal decline after 10+ years of infestation) and sensitive (infested and in severe decline within 3 years) to hemlock wooly adelgid (HWA) vs. overall health index. The health index was calculated by Jennifer Pontius (USFS & U. Vermont) based on 4 parameters: % new growth, % average transparency, % dieback, & % live crown.

Thus, such markers will be very useful in following the physiological response of selected genotypes to disease tolerance (based on phenotype, e.g., historic survival – if available) as well as the development of management practices to ensure the survival of plantations. Changes in a biochemical marker due to disease infection appear much sooner than the appearance of visual symptoms, making its detection a faster way to assay tolerance than rating foliar symptoms. Additionally, markers can be used to detect differences in tolerance levels in the absence of infection, eliminating the need to inoculate the trees. Microsite factors, such as soil moisture and light availability will also affect levels of biochemical markers, however an effective marker will distinguish susceptible vs. tolerant elms even with variation caused by site differences. In situations where an identified genetic marker of resistance turns out to be a functional gene, epigenetic changes could affect its expression, and in that case the phenotype may no longer match with the presence of this genetic marker. Under such conditions, and in situations where no genetic markers of resistance have yet been identified, we may have to rely on additional metabolic markers to differentiate between ranges of tolerance to a disease among individuals within a clone or even within a species; the clonal material provides unique opportunities in this regard.

Materials and Methods

Pollen Collection, Controlled Pollinations, and Seed Production

During the spring of 2016, branches with flower buds from exceptionally large surviving American elms in New England states were collected and shipped to Ohio (table 1). We attempted to have elms

represented from a large geographic area, where we defined populations in terms of watersheds because American elm is primarily a riparian species in New England. In selecting trees, we used size as a proxy for age, so by considering size and isolation from other elms we hoped to select trees that were likely exposed to Dutch elm disease multiple times over their lives. We collected from elms only where we had permission from the landowner and only if the tree had sufficient flower buds at the time of collection (in early March just before flower buds open). Elms growing in the milder climate of the Lower Connecticut River region were taller and younger at the same diameter than elms from the Upper Connecticut River region. The 21 elms selected varied considerably in easily observable traits like branch architecture and bark, probably reflecting genetic diversity.

Table 1—Summary of American elms from which scions were collected (DBH = diameter at breast height (i.e.1.4 m (4.5 ft) above ground level (Isolation codes are: 0 for trees <30 m (100 ft), 1 for trees <91 m (300 ft), and 2 for trees >91 m (300 ft) from adjacent elms (live or dead), if present. Lower Connecticut River is defined as downstream of Turner's Falls Dam in MA. Upper Connecticut River is defined as upstream of Wilder dam in VT)

Number	Population	State	DBH cm (in)	Height m (ft)	Isolation
30	Housatonic River Valley	MA	94 (37)	22 (72)	0
29	Housatonic River Valley	CT	74 (29)	17 (56)	0
42	Lake Champlain Valley	VT	131 (52)	22 (72)	0
43	Lake Champlain Valley	VT	107 (42)		1
44	Lower Connecticut River	MA	128 (51)		1
45	Lower Connecticut River	MA	114 (45)	25 (82)	1
36	Lower Connecticut River	CT	107 (42)	26.5 (87)	1
32	Lower Connecticut River	MA	93 (36)	24 (79)	1
38	Lower Connecticut River	MA	75 (30)	26.5 (87)	0
31	Lower Connecticut River	MA	73 (29)		1
37	Lower Connecticut River	MA	61 (24)	30.7 (100)	0
33	Middle Connecticut River	VT	186 (73)	24 (79)	1
27	Middle Connecticut River	MA	111 (44)		1
25	Middle Connecticut River	MA	107 (42)	23 (76)	0
28	Middle Connecticut River	MA	80 (31)	17.5 (57)	0
34	Middle Connecticut River	VT	77 (30)	22.5 (74)	0
35	Middle Connecticut River	VT	75 (30)	20.5 (67)	1
26	Middle Connecticut River	MA	40 (16)		0
41	Upper Connecticut River	NH	91 (36)	23.5 (77)	2
39	Upper Connecticut River	VT	90 (35)	17 (56)	2
40	Upper Connecticut River	VT	81 (32)	16.6 (55)	0

To prevent seed predation by insects, a mixture of dormant oil and carbaryl was prepared using the manufacturer's specifications, were sprayed on the elm branches and allowed to dry. Prior to spraying, scion wood was collected from branch tips for use in grafting. The cut end of the branches were recut while submerged in water to prevent embolism, then disinfected by dipping in 10,000 ppm activated chlorine dioxide (Bio-Cide International), and placed in flasks containing water (fig. 2). Flasks were placed on tables covered with dry wax paper sheets and enclosed with a wood frame covered with plastic sheeting (to reduce airborne pollen transmission). The released pollen was collected by scraping the wax paper sheets with razor blades, placing the pollen in plastic vials that were then placed in 50 ml plastic tubes containing a desiccant. Individual flowers were counted on select branches of mature DED-tolerant elms cultivars ('Delaware 2', 'Princeton', R18-2 and 'Valley Forge'). Pollination bags were placed over these branches on March 2nd and 3rd with the assistance of a canopy lift. Pollen was added to the bags on March 9 through March15 (fig. 2), depending of the stage of flower development. A small hole was made at the top of the pollination bag and an inflator needle was inserted into the bottom of the pollination bag.

The nozzle was attached to the controller of an air compressor which was set to 138 kPa (20 PSI). Upon addition of pollen through the top hole, bursts of air were used to distribute the pollen to the flower heads within the pollination bags, and duct tape was applied to the bags to cover the holes. Pollination bags were collected on April 19th and 20th using a pole pruner. Seeds were removed from the bags, inspected for damage, and counted.

Figure 2 - American Elm pollen collection station (left) and pipetting pollen collected from survivor American elm into pollination bag (right). Photo credit: K. Lehtoma

Clonal Propagation of Trees Using Grafting and Cuttings

Branches with at least three vegetative buds were dipped in wax, placed in plastic bags, and refrigerated for 2 to 4 weeks. One-year-old containerized dormant elms from multiple selections of unrelated trees were used as root stock. Five root stocks were used for each of the 21 survivor tree selections, with multiple grafts attempted on each root stock. Multiple grafting techniques, including top (cleft) grafting, veneer grafting, bottle grafting, and bud grafts (chip budding) were used to propagate the elm selections in March 2016 (Garner 2013, Winieski 1959). A top grafting tool was used to make identical "V" cuts in the root stock and scion; care was taken to match the diameter of the stems (fig. 3). Veneer grafts and bud grafts were performed using a grafting knife, with the cambium layer aligned on at least one side of the graft junction. Bottle grafts were performed using a grafting knife using an "approach grafting" technique and tubes of water to support the grafted scion. Bud grafts were affixed with budding tape. The other grafts were tied with grafting bands and painted with wax. In mid-April, the budding tape was removed. When the grafts were well calloused, grafting bands were removed to prevent girdling on rapidly expanding branches. Growing grafts were supported with stretch fabric bands to prevent them from breaking. Pots were kept in the greenhouse, watered regularly, and supplied with 20-20-20 fertilizer (diluted to 350 ppm) once the scion was growing. Greenhouse pests and diseases were controlled using insecticide sprays and antifungal drenches as needed.

Figure 3 – Photographs depicting a top graft (left), tan buds on a branch indicating its suitability for taking cuttings (center), and rooted American elm cuttings (right).

Most of the grafts that calloused grew rapidly. When the buds on the new growth began to turn tan (fig. 3), we took cuttings from the new growth (leaving some buds to grow). Cut branches were submerged in water and cut into sections with three to four buds per section of stem. At least half of each leaf was cut off to reduce transpiration. The bottom of each section was stripped of bark in at least two areas, dipped in rooting hormone, and placed in Oasis Growing Medium Wedge Strips® (#5656) in a mist bed. For each selection, we treated some cuttings with each of three different concentrations of powdered indol-3-butyric acid hormone: 0.3 percent, 0.8 percent, and 1.6 percent (Hormex®). After a few weeks in the mist beds, cuttings were supplied with 10-30-20 fertilizer (diluted to 350 ppm). After 3 weeks, cuttings were checked weekly for roots (fig. 3). Rooted cuttings were potted into 7 cm x 25 cm pots containing a mixture of potting soil and fertilizer (1 bag Fafard 3B® potting mix, 300 g Osmocote®, 61 g Micromax® nutrients) and supplied weekly with 10-30-20 fertilizer (diluted to 350 ppm). Cuttings usually rooted in 3 to 4 weeks and developed shoots several weeks thereafter.

Inoculation of American Elm Trees in Pots and in Field Plots

Survivor American elm trees from Illinois, Indiana, Ohio, and Michigan were clonally propagated between 2006 and 2010 and planted in replicated plots at the U.S. Department of Agriculture Forest Service (USDA FS) Delaware, Ohio laboratory. In 2015, rooted cuttings from each tree were generated and transplanted into 3.8 liter (1 gal) pots. Results of inoculation experiments on planted and potted trees of the same genotypes will be compared to determine whether potted trees can be used in an early screening for DED. The trees generated from clonal propagation and crosses from New England survivor elm trees, described in the earlier sections of this paper, will be screened using these techniques in 5 to 7 years.

American elm trees, including susceptible and tolerant controls, were inoculated with a 50/50 mixture of *O. ulmi* (PG442 strain) and *O. novo-ulmi* (H961 strain) spores on June 7 and 8, 2016. The inoculum was prepared a week in advance as follows: frozen cultures of *O. ulmi* (strain PG442) and *O. novo-ulmi* (strain H961) were thawed and spread on separate potato dextrose agar plates, 50 µl/plate, and nine plates/isolate. The plates were kept dark and at room temperature. Fungal spores were harvested after 11 days of growth by addition of sterile deionized water to the plate surface. The surface was scraped gently with a bent glass rod and the spores of each isolate were removed to a separate sterile 50 ml conical tube. Fungal spore concentrations for each isolate were determined using a hemocytometer. The final 50/50 concentration of spores was adjusted to a volume appropriate for the inoculation of trees. Trees in field

plots received either 6×10^5 or 1.2×10^6 *O. ulmi* and *O. novo-ulmi* spores and potted elms were inoculated with a total of 2.8×10^4 spores. A cordless drill containing a 0.5 cm (3/16 inch) diameter brad point bit was used to drill a 1.3 cm (1/2 inch) deep hole 30 cm (12 inch) from the base of trees located in field plots, and the fungal spores were pipetted into the hole. A 0.2 cm (1/16 inch) diameter bit was used to drill a 0.6 cm (1/4 inch) deep hole 15 cm (6 inch) from the base of potted trees and the fungal spores were pipetted into the hole.

Canopy Decline Measurements

The canopies of field-grown elms were cleared of any dead branches at the time of inoculation. As such, all trees had baseline measurements of 0 percent canopy decline. Each tree was re-measured 4 weeks post-inoculation (early July). Canopies were rated at 5 percent decline classes (i.e., 0, 5, 10…95, 100 percent) for DED symptoms. Typical DED symptoms consist of foliar yellowing, wilting (flagging), and eventual browning as the branch dies.

Analysis of Polyamines

Foliage was collected from several DED-tolerant and -susceptible elms in two replicated field plots 7 days before inoculation with DED and processed as described previously (Minocha and Long 2004, Minocha et al. 2000). Undamaged leaves from the sun-exposed upper canopy were sampled between 8:30 am to 5:30 pm. Both treatment plots were sampled at the same time for each clone. Samples (100 mg) collected in 5 percent perchloric acid (PCA) were extracted by three freezing and thawing cycles using procedures described previously (Minocha et al. 1994, Minocha et al. 2000) and were stored at -20 ˚C until they were analyzed. For analyses of PAs, the supernatant of the PCA extracted samples was subjected to dansylation and quantitation by HPLC (PerkinElmer Inc., Waltham MA) using previously published protocols (Minocha and Long 2004, Minocha et al. 2000). We have used these procedures for polyamine analysis in over 30 species including algae, fungi, plant cell cultures, herbaceous plants, young and old trees, and animal cells.

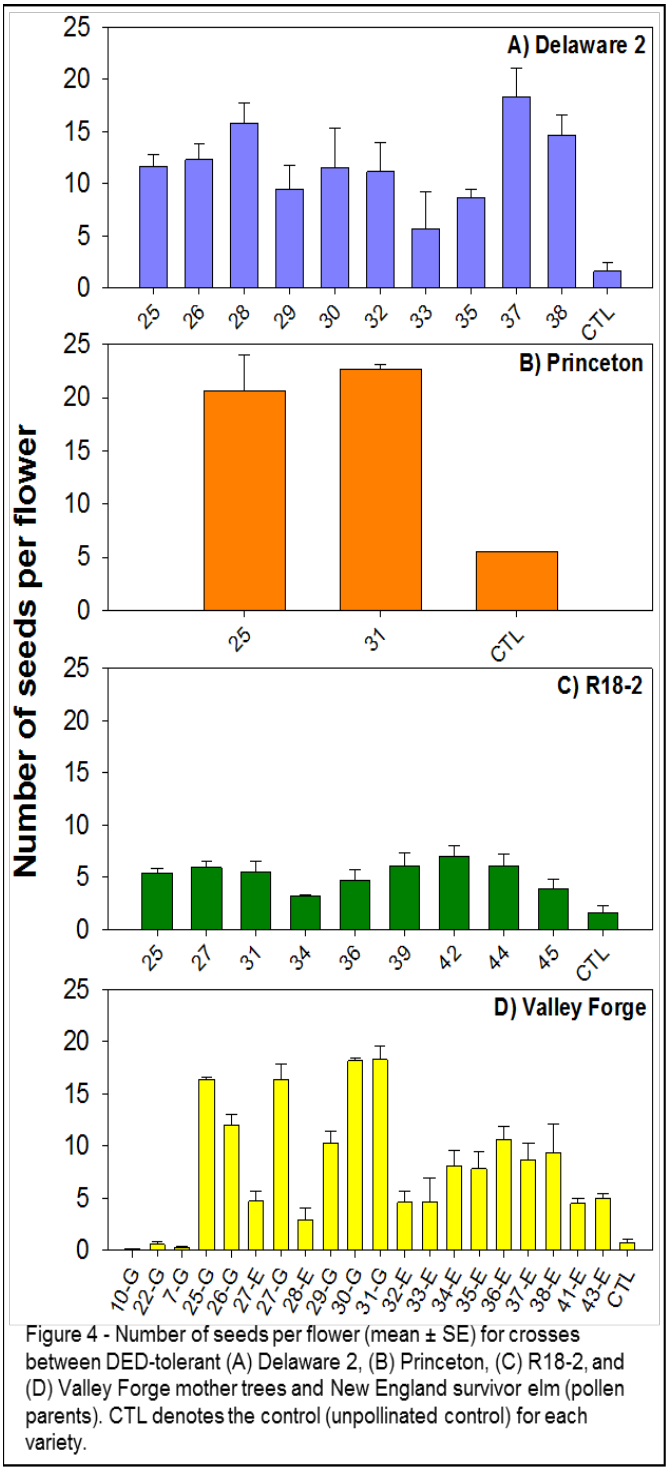

Figure 4 - Number of seeds per flower (mean ± SE) for crosses between DED-tolerant (A) Delaware 2, (B) Princeton, (C) R18-2, and (D) Valley Forge mother trees and New England survivor elm (pollen parents). CTL denotes the control (unpollinated control) for each variety.

Results and Discussion

Pollen Collection, Controlled Pollinations, and Seed Production

Between 0.5 and 8 mL of pollen was collected from cut branches of each of the 21 survivor trees. A total of 37 crosses made with four DED-tolerant mother trees and 20 pollen parents yielded approximately 42,000 seeds. The number of seeds produced by 'Delaware 2' × New England survivor tree crosses ranged from about 5 to18 seeds per flower. In contrast, unpollinated bagged controls averaged 1.6 seeds per flower. Each of the crosses performed with 'Delaware 2' (with 10 New England survivor elm pollen parents) was successful and produced viable seed (fig. 4A). The 'Princeton' × New England trees crosses produced between 20 to 23 seeds per flower, the greatest of all performed crosses (fig. 4B). The high number of seeds produced by these crosses was likely due to placement of the pollen bag over the branches a day late as evidenced by the high seed count in the control bags. The number of seed produced by the R18-2 × New England survivor tree crosses was in general lower than the other crosses (fig. 4C) except a few of the 'Valley Forge' × New England survivor tree crosses (7, 10, and 22; fig. 4D). The low seed production for those crosses was likely due to the use of pollen that was 3 years old. In previous years we have successfully used 2-year-old pollen that was stored in the refrigerator in a tube with desiccant. Seed generated by the 'Valley Forge' mother tree growing at the G site generally yielded more seed compared to 'Valley Forge' trees at the E site (fig. 4D).

Clonal Propagation of Trees Using Grafting and Cuttings

Thirteen out of 21 survivor tree selections were successfully grafted. Some of the unsuccessful grafts had poor quality scion wood, including small diameter twigs, dead twigs, and scale insect infestation (which we removed with floss). Grafts of unsuccessful trees will be attempted again in future years. All grafting techniques produced some successful grafts. Grafting success with different techniques varied among those doing the grafting. Overall, top grafting and veneer grafting were the most successful techniques (table 2). These two grafting techniques had also produced the largest number of cuttings per graft by July 21 (table 2). The bud grafts were initially slower to produce cuttings, but by mid-July they were growing rapidly and will likely produce cuttings as successfully as top graft and veneer graft techniques by later in the season. The bottle graft technique was not as successful as hoped. We were unable to get as good a graft union with this technique, and the water in the bottle may have grown fungus or bacteria that rotted the scion.

Table 2—Success of different grafting techniques attempted on survivor elm selections

	Bottle graft	Bud graft	Top graft	Veneer graft
Number of grafts attempted	33	338	93	44
Number of grafts that took	4	20	20	13
Grafting success rate	12%	6%	22%	30%
Number of cuttings	7	121	168	96
Average cuttings per graft	1.8	6.1	8.4	7.4

Grafted scions were at the right stage to begin taking cuttings by mid-May to early June for most selections (table 3). We produced rooted cuttings from all 13 of the survivor tree selections that were successfully grafted. A total of 581 cuttings were made and placed in mist beds. By August 2, 380 of these had produced roots, and 160 of the rooted cuttings had produced shoots (table 3). We expect that many more of the cuttings will produce roots and shoots by the end of the growing season. Some of the selections have produced many more cuttings than others because these selections had more successful grafts or because their grafts grew faster.

Metabolic Marker

Preliminary data from the Minocha laboratory on the effects of biotic stress on polyamine metabolism indicated that relatively higher disease/environmental stress tolerant aspen (*Populus*) clones (fig. 1A) as well as ash (*Fraxinus*) (fig. 1B) and hemlock (*Tsuga*) species (fig. 1C) had higher concentrations of foliar putrescine. This encouraged us to further investigate the relationship between foliar putrescine and biotic stress tolerance.

Table 3—Status of cuttings from 13 selections of survivor trees as of August 2 2016

Selection	Cuttings stuck (N)	Date of first cuttings	8/2 rooted (N)	8/2 shooted (N)
26	27	26-May	13	5
28	100	26-May	44	5
28a	74	26-May	42	2
30	37	23-May	30	2
32	11	9-Jun	11	10
33	23	2-Jun	16	3
34	47	26-May	38	22
35	79	12-May	47	34
36	5	17-Jun	5	1
39	25	23-May	20	12
41	9	26-May	5	2
42	37	26-May	15	15
44	57	17-Jun	56	13
45	50	17-Jun	38	34
TOTAL	581		380	160

Preliminary data from the pre-inoculation samples analyzed for putrescine show differences in the cellular concentrations of Put among different clones (fig. 5). However, there were no significant differences among trees sampled from control or DED designated plots at time zero. The samples for a few clones were taken from replicated plots for two DED-tolerance studies (known as cross progeny and Lesser) but these data were pooled since there were no significant differences by location of the trees within a single treatment (data not shown). Further analyses of samples collected each week post-inoculation will reveal whether these inherent differences among clones are further modified with exposure to DED or remain the same as shown in fig. 5. At this point, however, there seems to be some relationship of these data with observed percent decline in these clones at week 4 post-inoculation, indicating that there may be a correlation between homeostatic levels of cellular Put and disease tolerance (fig. 5). Although only four selections were examined in this study, results suggest that disease tolerance may be correlated with higher putrescine concentrations.

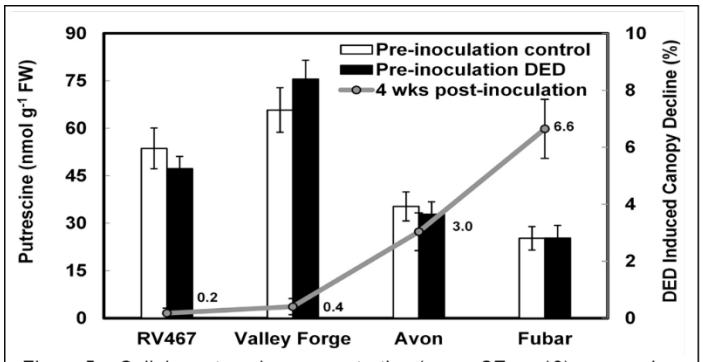

Figure 5 – Cellular putrescine concentration (mean SE; n=10) one week pre-inoculation in 4 different American elm clones. Overlaying this bar graph is the % canopy decline observed in these clones at 4-weeks post-inoculation. Whereas Fubar is known to have low tolerance to DED exposure, Valley Forge and RV467 have high tolerance. Avon's tolerance level in the long-term is yet to be determined.

Canopy Decline Measurements

Dutch elm disease-induced canopy decline results 4 weeks post-inoculation show considerable variation in the susceptibility of the different elm varieties under cultivation at the USDA FS laboratory in Delaware, Ohio (fig. 6). Several varieties (RV141, ND104, RV467, 'Sunfield', RV65, NR405, RV16 and ND1) perform comparably to known DED-tolerant varieties ['Princeton' (PRN) and 'Valley Forge' (VF)]. 'Sloan', 'Kuhar 2', 'Charlotte' and 'Braun' selections had higher canopy symptoms than the susceptible control used in this study (Amer. 57845; fig. 6). Additional readings will be obtained at 8 weeks and 1 year post-inoculation. Preliminary results from potted elm inoculations indicate that there are no differences in height or root collar diameter growth between DED-tolerant and susceptible elms, nor between elms inoculated with DED or water. Measurements of xylem discoloration suggestive of DED-infection will be collected later this growing season and may be more informative regarding potential DED tolerance.

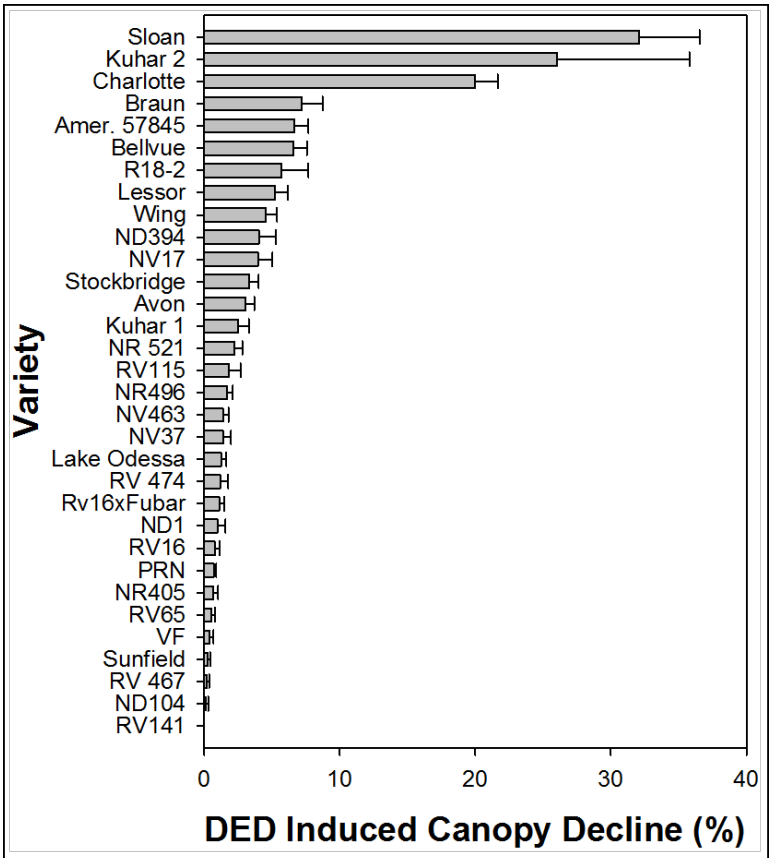

Figure 6 - DED-induced canopy decline (%) across different elm varieties (mean ± SE).

Acknowledgments

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Using Genetic Information to Inform Redbay Restoration in Laurel Wilt Epidemic Areas[1]

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Abstract

Laurel wilt disease is incited by the exotic fungus *Raffaelea lauricola* and transmitted by the Asian redbay ambrosia beetle (*Xyleborus glabratus*). The disease has spread from Savannah, Georgia in 2002 across the coastal southeast as far south as the Everglades, and in 2014 was discovered as far west as Texas. Mortality is severe, with locations in Florida reporting more than 90 percent loss of redbays, 7.6 to 10.2 cm (3 to 4 inches) in diameter and greater. Surviving redbays from coastal maritime forest ecosystems have been collected and propagated for the study of disease resistance and ultimately restoration planting. Disease severity of artificially inoculated parental trees and their open pollinated offspring will supply evidence for whether resistance is inherited simply as a dominant versus recessive trait, or as a complex, quantitative trait. These data will be used to identify and guide deployment of resistant (or tolerant) materials in areas where redbay has been decimated by laurel wilt disease. Additionally, in order to confirm parentage and potentially access population structure and diversity, simple sequence repeat (SSR) genotyping is underway in this population. Primer sequences were obtained from the Hardwood Genomics Project public resource (http://www.hardwoodgenomics.org/content/redbay-gssrs) and preliminary results, presented here, appear promising.

Introduction

Laurel wilt disease has spread with alarming speed and severity since its introduction in 2002. This relatively new disease has moved through redbay (*Persea borbonia*) and swampbay (*Persea palustris*) populations across the southeastern United States and is threatening the avocado (*Persea americana*) industry in Florida. The causative fungus, *Raffaelea lauricola,* is vectored by the redbay ambrosia beetle, *Xyleborus glabratus*. There is strong concern for the spread of laurel wilt beyond the range of the favored beetle host, redbay. The fungus can incite disease on a wide range of species within the Lauraceae (Hughes et al. 2015) and *Xyleborus* beetles other than *X. glabratus* (six identified so far) can pick up the deadly fungus by infesting the same tree (Carrillo et al. 2014). The disease poses widespread ecological threats to the large number of *Lauracae* species that contribute to canopies throughout the tropics and subtropics. In addition, these populations can act as reservoirs for the beetle and the fungus, threatening commercial avocado growers in Mexico and California.

Some of the fundamental questions surrounding how redbay, as well as other *Persea* species, can best be restored remain unanswered. Simple sequence repeat (SSR) markers are enormously useful tools for the study of population genetics and mapping. The preliminary data presented here demonstrates that the use of SSR markers in redbay has excellent potential. We tested SSR primers in a population of redbay trees that survived laurel wilt disease outbreaks.

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Methods

DNA was extracted from freeze-dried leaf tissue using the Qiagen DNeasy Plant Mini kit (69104) or using the Macherey-Nagel NucleSpin 96 Plant II kit. Grinding was done with a SPEX Sample Prep Mini-G genogrinder and steel balls.

The microsatellite loci in this study were amplified using primers flanking tetranucleotide repeats and were designed from Illumina high-throughput sequence data provided by Hardwood Genomics, a National Science Foundation-funded project (http://www.hardwoodgenomics.org/). Primer sequences were modified as follows: M13(-19) universal primer sequence was added to the 5' end of forward primers to avoid the need for fluorescently direct-labeling individual primers (Schuelke 2000) and the sequence GTTTCTT was added to the 5' end of reverse primers to avoid non-template adenylation during PCR (PIG-tailing, Brownstein et al. 1996).

Amplifications of PCR were performed in 10-µl reactions containing 20 ng genomic DNA, 0.04 µM and 0.16 µM of forward and reverse primers, respectively, 0.16 µM FAM labeled M13 primer, 2.64 µM dNTPs, 1X Invitrogen PCR buffer, 2 mM $MgCl_2$, and 0.5 U ''hot start'' Platinum Taq DNA polymerase (Invitrogen, 10966026). The PCRs were completed using the following touchdown protocol: 2 min at 94 ºC; followed by 20 cycles of 30 s at 94 ºC, 30 s at X, and 1 min at 72 ºC where X = 65 ºC in the first cycle decreasing by 0.5 ºC every cycle thereafter; followed by 25 cycles of 30 s at 94 ºC, 30 s at 55 ºC, 1.5 min at 72 ºC; followed by a 15 min extension at 72 ºC. The resulting PCR products were separated on an ABI 3710 Genetic Analyzer (Applied Biosystems, Foster City, California) as recommended by the manufacturer. GeneMapper Software 5 (Applied Biosystems) was used to size the peaks in base pairs and to score alleles, with LIZ600 as an internal size standard.

Results and Discussion

In order to confirm the feasibility of using SSRs as a genetic tool in redbay, we tested 96 primer pairs using seven trees collected from five locations (Cumberland Island, Georgia; Fort Clinch, Florida; Huntington Island, South Carolina; Fort George Island, Florida; Edisto Island, South Carolina). Of the 96 primer pairs, seven failed to amplify and three had weak amplification; 25 were monomorphic in all seven genotypes; 36 could not be scored because of one or more criteria (poor amplification, inconsistent peak heights, hedgehog peaks, peaks with large shoulders, stray peaks, peaks too close together); 35 amplified cleanly and were scored. These 35 primer pairs produced nearly 100 alleles, including a primer pair producing seven alleles in the sample of seven trees. This preliminary result of 35 of 96 (37 percent) loci easily scored, a large number alleles and the large number of redbay SSR primer pairs publicly available (table 1), indicate that it would be relatively efficient to obtain enough loci for fingerprinting or genetic mapping. This seems especially likely considering the genetic map of closely related *P. americana* consists of 25 SSR loci, that are contained in 10 of 12 mapped linkage groups and were obtained by screening only 92 primer pairs polymorphic at the population level (Sharon et al. 1997).

Table 1—Publically available redbay ssr primer pairs, Hardwood Genomics Project

Repeat Type	Available	Tested	PCR positive	Polymorphic/ scored
Dinucleotide	16,010	-	-	-
Trinucleotide	1,886	-	-	-
Tetranucleotide	271	96	92	35

A subset of six primers were selected for further testing based on ease of scoring and allele number. They were scored in 57 additional trees, collected as laurel wilt disease outbreak survivors from six locations (additional location: St. Catherine's Island, Georgia). The original seven trees were tested again and the original genotype data were confirmed. In redbay, there were a total of 56 alleles scored, 17 of which were from a single locus and an average of 9.3 alleles per locus. With this high level of polymorphism, we obtained unique genotypes for all trees in the population. In addition, three of the six

primer pairs were tested in *P. palustris*, *P. podadenia* (Mexican species), *P. americana*, and *Cinnamomum porrectum* (a southeast Asia species) and all amplified scorable alleles in each species.

Because SSRs are codominantly inherited and highly polymorphic, they are a good choice for population genetics studies. In the case of redbay, the choice is even more persuasive given the public availability of thousands of primer sequences. The information provided from comparisons between populations can help to ensure gene conservation goals are met in replanting efforts. Because these primer pairs work well in a variety of species, including swamp bay, the same loci should be useful to inform research in other species threatened by laurel wilt disease. In addition, SSR markers can be easily located in genomic sequence as resources become available in the future.

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Dynamic Genetic Conservation in the Presence of Invasive Insect and Pathogen Threats to Forest Tree Species of the United States[1]

J.L. Koch[2] and R.A. Sniezko[3]

Ex-situ genetic conservation focused on collection and storage of seed can play an important role in conserving the genetic diversity of species under grave threat by biotic organisms or a changing climate. However, *ex-situ* genetic conservation is primarily a static activity and does not allow for evolution of the species under a continuing, persistent impacting agent. Invasive insects and pathogens, once established, usually become permanent components of the ecosystem, continuously interacting with the target species. Dynamic genetic conservation seeks to actively harness the genetic variation within the species to develop a new equilibrium under which species restoration can proceed with a realistic chance of at least partial recovery and persistence of the affected tree species. The iterative process of traditional tree improvement has a long history of utilizing genetic variation to increase population level resistance to insects and diseases, and (when done right) is highly compatible with and promotes the primary goal of dynamic genetic conservation of maintaining evolutionary processes. This approach is being taken to achieve dynamic genetic conservation of several threatened species including species of ash (*Fraxinus* spp.) that are gravely threatened by emerald ash borer (*Agrilus planipennis*). Given that the introduction and establishment of invasive insects and diseases will likely continue, we suggest that the time is right to strengthen, expand, and invest in forest genetics and tree improvement programs in order to implement dynamic genetic conservation programs for the ever increasing number of threatened forest tree species. Biotechnology and genomics may provide tools to potentially accelerate breeding programs, but such tools are not necessary for, nor can they replace the need for, successful breeding programs. To maximize the likelihood of operational application of such tools, they should be developed within the context of existing breeding programs. As the development and application/deployment of these tools are completely dependent on integration with a breeding program, if funding is limiting, the breeding program should be the priority.

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Conservation and Restoration of Forest Trees Impacted by Non-Native Pathogens: the Role of Genetics and Tree Improvement[1]

R.A. Sniezko[2] and L.A. Winn[2]

North American native tree species in forest ecosystems, as well as managed forests and urban plantings, are being severely impacted by pathogens and insects. The impacts of these pathogens and insects often increase over time, and they are particularly acute for those species affected by non-native pathogens and insects. For restoration of affected tree species or for their continued presence in managed forests and urban plantings, genetic resistance will be key. Often, however, little or nothing is known about genetic resistance to these invaders. The U.S. Department of Agriculture Forest Service is a world leader in the applied development of resistance to diseases of forest trees. One of these programs, based at Dorena Genetic Resource Center (DGRC), Cottage Grove, Oregon, has been active for 50 years. It provides an example of the role of genetics and tree improvement in the conservation of affected tree species. The DGRC has active programs to develop genetic resistance to white pine blister rust (caused by *Cronartium ribicola*) in several white pine species, and Port-Orford-cedar root disease (caused by *Phytophthora lateralis*). One of the white pine species, whitebark pine (*Pinus albicaulis*), has been proposed for listing under the Endangered Species Act (ESA) in the United States and is now listed as endangered by COSEWIC, the Committee on the Status of Endangered Wildlife in Canada. The development of genetic resistance from seedling screening trials at DGRC is well underway, and seed collections from resistant parents are now being used for restoration efforts. Special regional or national units such as DGRC provide the capability to organize and respond to these invasive agents. The work at DGRC is integrated with basic research performed elsewhere, and coupled with restoration programs of land managers who work with these species. Collaboration between programs such as DGRC and land managers, other agencies, and non-government organizations provides the best chance of retaining species threatened by rapid biotic and abiotic environmental change.

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Accelerating Dynamic Genetic Conservation Efforts: Use of FT-IR Spectroscopy for the Rapid Identification of Trees Resistant to Destructive Pathogens[1]

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A strong focus on tree germplasm that can resist threats such as non-native insects and pathogens, or a changing climate, is fundamental for successful genetic conservation efforts. However, the unavailability of tools for rapid screening of tree germplasm for resistance to critical pathogens and insect pests is becoming an increasingly serious bottleneck. Here we present the development of a new technique that can potentially revolutionize genetic conservation efforts. Fourier-transform infrared (FT-IR) spectroscopy is a chemical fingerprinting technique that has been recently shown to be suitable for the rapid identification of oaks resistant to *Phytophthora ramorum* (cause of sudden oak death) prior to infection (Conrad, A.O.; Rodriguez-Saona, L.E; McPherson, B.A.; Wood, D.L.; Bonello, P. 2014. Identification of *Quercus agrifolia* (coast live oak) resistant to the invasive pathogen *P. ramorum* in native stands using Fourier-transform infrared (FT-IR) spectroscopy. Frontiers in Plant Science. 5: 521.). The aim of this study was to determine if FT-IR spectroscopy can be used for the rapid identification of resistant trees in other pathosystems as well, such as Port-Orford-cedar (*Chamaecyparis lawsoniana*)/root disease (caused by *P. lateralis*), and whitebark pine (*Pinus albicaulis*)/white pine blister rust (*Cronartium ribicola*). For both pathosystems, we collected and analyzed plant material that had been previously characterized in terms of resistance/susceptibility to its specific pathogen. Soft independent modeling of class analogy was used to discriminate between resistant and susceptible trees, while partial least squares regression was used to predict mortality rates or severity of symptoms in the progenies. Preliminary results strongly indicate that FT-IR can discriminate between different phenotypes, and predict resistance-associated traits in the progenies of sampled trees in these pathosystems. Our results also suggest that this technique can be expanded to the rapid phenotyping of hosts in many other pathosystems, including tree crops, e.g., cacao, coffee, or eucalypts. This technique could also be developed for rapid identification and separation of morphologically similar tree taxa, further contributing to genetic conservation efforts worldwide.

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Status and Future of Breeding Disease-Resistant American Chestnut[1]

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The American Chestnut Foundation (TACF) has worked since 1983 to introduce genetic resistance to the chestnut blight fungus (*Cryphonectria parasitica*) into an American chestnut (*Castanea dentata*) population. As part of a broader goal for species restoration, TACF seeks to instill within that population sufficient diversity so as to enable the species to reproduce on its own in forests throughout its native range. Dr. Charles Burnham proposed introgressing blight-resistance from Asian chestnut species into a predominantly American chestnut genetic background with backcross breeding. Hybrids of American chestnut and Chinese chestnut (*C. mollissima*) were backcrossed to American chestnut over three generations. Third backcross trees (BC3s) selected for resistance to chestnut blight were intercrossed to generate a segregating population of BC3-F2 trees. Currently, TACF has advanced two sources of resistance, derived from two first backcross trees with different Chinese chestnut grandparents to the BC3-F2 generation. Over 60,000 BC3-F2s from these sources have been planted in seed orchards at TACF's Research Farms in Meadowview, Virginia since 2002. After artificially inoculating seed orchards with the chestnut blight fungus and culling individuals with significant canker expansion, 5,000 trees remain from which to make the final selections of the 500 most resistant trees.

While TACF has achieved successes toward the creation of a disease-resistant American chestnut, uncertainties about the genetic control of blight resistance remain after 30 years of backcross breeding under the Burnham plan: the number of loci that control blight resistance is not known with confidence; it is uncertain whether alleles that confer blight resistance are segregating at the same or different genetic loci across sources of resistance; it is uncertain whether blight resistance alleles are lost through backcrossing; and it is uncertain whether there are genetic interactions between host resistance and pathogenicity in different strains of *C. parasitica*. Future work at TACF will seek to resolve these uncertainties and improve the efficiency and efficacy of traditional breeding for disease-resistant American chestnuts.

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Genetic Conservation

Assisted Diversification for an Era of Habitat Extinction[1]

Charles H. Cannon[2]

Abstract

How do we conserve tree diversity in a rapidly changing world, dominated by intensive human impact on the landscape? The Anthropocene is a useful term to describe a new era in Earth's history, where we dominate the globe's resources so completely that our activities alter basic nutrient, water, climate, and energy cycles. These rapid environmental changes and the substantial decline in available and appropriate habitat for many organisms has led to predictions of a sixth global extinction event, where a large fraction of the world's species are lost. These mass extinctions clearly impacted animal species more than they did plant species. Recent studies suggest that plant speciation may have actually increased at the end of the Cretaceous Period when the dinosaurs largely vanished. Plants must have life histories and reproductive strategies that allow them to persist through times of rapid environmental change.

In addition to being autotrophic and capable of remaining dormant as seed for many years, most plants in diverse genera remain inter-fertile among closely related species. Early evolutionary botanists described these suites of inter-fertile species that retain the ability to exchange genes at a diminished rate as a syngameon. Numerous examples have been identified and documented in the scientific literature and this reproductive strategy has also been termed "diversification with gene flow". Oaks (*Quercus* spp.) are famous for being promiscuous across species boundaries and numerous examples of hybrid offspring, genetic introgression, and cytoplasmic organelle capture exist. I would argue that oaks are not at all unusual among trees, but instead are representative of diverse tree genera, particularly in the tropics. The oaks are one of the few examples of a temperate tree group that has diversified in the same way that many tropical genera have. I would further suggest that participation in a syngameon is a critical aspect for trees to adapt to environmental change and novel environments.

The most troubling part of the Anthropocene is that habitats themselves will become extinct. How does one conserve a species for which the natural and preferred habitat no longer exists? The Anthropocene presents an essentially unpredictable and unprecedented challenge to trees over the last 5 million years. We cannot accurately predict what climate and land-use will exist in any one location over the next century. So, as tree breeders, geneticists, and land managers, how do we choose a 'winner' in this situation? Do we invest in a particular breeding program or germplasm stock? Can we be assured that these narrowly-related trees will thrive in the Anthropocene? Environmental change has always happened in Earth's history. The major difference now is the pace of change, which has accelerated substantially. How do we assist trees to accelerate their ability to adapt? We should exploit the strategies they employ naturally. One of these strategies is the participation in a syngameon and the exchange and capture of advantageous genetic material from closely related species.

A carefully designed program of assisted diversification should be explored and the living collection of an arboretum is the perfect setting to conduct this work. Trees of the same type, often in the same genus, are brought together from around the world. These interactions allow cross-pollination and hybridization across considerable phenotypic and biogeographic difference. Seedlings from these crosses could be screened and selected, both by phenotype and genotype, to represent the broadest possible diversity of variation and combinations. These diverse set of hybrids could then be grown in a common-garden experiment and their growth and performance followed. The production of novel phenotypes which combine the best of both progenitors is standard practice among plant breeders, often to overcome invasive disease or pests. This 'diversity grove' could act as a fail-safe resource of genetic and trait variation for future use and allow 'winners' to naturally emerge from the stock.

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Why Pollen-Atmosphere Interplay Matters to Forest Gene Conservation[1]

Claire G. Williams[2]

Forests are thought to adapt too slowly to anthropogenic climate change, making them highly vulnerable to large-scale loss. Losses can accrue swiftly because generations are lengthy, particularly at higher latitudes (>23□ to 73□) where wind-pollinated forest species are commonly found to mature slowly. Losses incurred during adaptation to climate change translates into less allelic richness, or genetic diversity, and one can expect some resilience on this point because forests have more genetic diversity than other seed plants and this feature has shaped the forest fragmentation paradox debate (Bacles and Jump 2011, Kramer et al. 2008, Lowe et al. 2015). These great reservoirs of genetic diversity in forest trees have an overlooked dimension: temporal layering.

To explain temporal layering of genetic variation, consider that a given pollen pool is available to any year's cohort of ovules is shaped by weather conditions during pollen release, transport and deposition (Box 1). Seed and pollen dispersal occurs on far greater distances than once thought (e.g., Ehrlich and Raven 1969, Williams 2017). Seed from that one ovular cohort will thus have an allelic composition distinct from other cohorts. Shaped by weather conditions occurring pollen release, transport and deposition, the pollen pool is a function of certain atmospheric events[3] (Lanner 1966).

Atmospheric turbulence is the prevalent delivery system for wind-delivered forest tree pollen. Turbulence refers to a continuous succession of gusts, swirling eddies and lulls accompanied by swift changes in wind direction or advection. Turbulence is a product of atmospheric motion systems which wax and wane with the seasons. Examples of these systems include low- and high-pressure weather systems, turbulent large-scale eddies and land-sea circulations (Liu 2007 pages 3 to 5). Together these converge into unique atmospheric events during a given year's pollen season which in turn disperse pollen grains vertically and horizontally through the atmosphere. This is simply described by Lanner (1966) who wrote: …"forest trees and other perennial seed plants have genetic diversity patterns shaped by annual meteorological events."

Box 1. The pollen-atmosphere model proposed by Lanner (1966)
The pollen-atmosphere interplay model proposed by Lanner (1966) considers the pollen pool for each annual seed cohort, not adult populations. For example, one year's pollen pool might be composed of many long-distance pollen parents. The next year's pollen pool might be composed of a few local pollen parents. The third year's cohort might have a large yet equal composition of local and long-distance pollen parents. This year-to-year variation corresponds to specific set of atmospheric events. One year had dry gusting winds which favored long-distance pollen transport. The next year had steady winds punctuated by afternoon rain showers which scoured pollen out of the atmosphere. The third year had gusting winds with a rare rainstorm. This is part of the reason that the magnitude of gene flow between any two populations is poorly correlated with distance.

Together with pollen phenology, atmospheric events specific to the interval of pollen release shape genetic diversity from one year to the next for a given year's seed cohorts (Box 1). These temporally-layered seed

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[3] A forest tree population's entire life cycle, not only standing forest, is the unit of *interactive* response to rapid climate change. Considering the diplohaplontic life cycle of these long-lived perennial seed plants shapes how general circulation models (GCM) can be linked to forest ecosystems for climate change forecasting.

cohorts contribute to the next sporophyte generation. Any one seed cohort is shaped by that year's pollen pool, not spatial distances between standing adult populations (Box 1). Taking this further, we can hypothesize that a paternity analysis of one single year's seed cohort will reflect more closely the true genetic structure than measuring the horizontal distance between two adult populations.

Next, let us consider how this cohort-level concept behind the pollen-atmosphere interplay model fits with traditional population genetics models. Traditionally, genetic properties of a population are assigned to a group of reproducing adults, i.e. a population or a collection of populations. In our new model (Box 1), genetic properties are ascribed instead to each annual seed cohort.

First, each year's seed cohort from a forest population is assigns its own level of genetic variation. Second, effective population number is now indeterminate, changing from one year to the next (Lanner 1966). Third, dispersal and potential gene flow could be correlated with regional atmospheric events occurring during pollination. Might this explain the structure of genetic variation more than geographical separation between two adult populations? Testing this hypothesis for gene conservation programs of higher-latitude wind-pollinated forest species is the next step for testing the pollen-atmosphere interplay model.

In closing, temperate forest species are long-lived, perennial and wind-pollinated, all of which are life history features distinct from the short-lived animal and plant model species used to develop Sewall Wright's isolation-by-distance theory (Wright 1943). This dimension can be envisioned as a temporal layering of genetic diversity into temperate forest species. Could genetic variation within a forest population have an overlooked temporal dimension which is shaped by year-to-year atmospheric events during pollination? Implicit to the pollen-atmosphere interplay model is how much depends on how much effective gene flow actually takes place. To this end, one must test patterns of genetic variation and gene flow among annual seed cohorts for a given set of populations.

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Conserving Genetic Diversity in Ponderosa Pine Ecosystem Restoration[1]

L.E. DeWald[2]

Abstract

Restoration treatments in the ponderosa pine (*Pinus ponderosa* P. & C. Lawson) ecosystems of the southwestern United States often include removing over 80 percent of post-EuroAmerican settlement-aged trees to create healthier forest structural conditions. These types of stand density reductions can have negative effects on genetic diversity. Allozyme analyses were used to evaluate potential impacts of restoration treatments on genetic diversity of five ponderosa pine populations located 2 to 3 km apart within the U.S. Department of Agriculture Forest Service Fort Valley Experimental Forest, located north of Flagstaff, Arizona. Results revealed allele frequencies varied significantly among pre-settlement clusters of trees within and among populations, and trees within clusters had lower than expected heterozygosity levels. These results indicate the clumpy stand structure typical of reference stand conditions represent "genetic neighborhoods". A combination of limited seed movement created by seeds dropping below parent trees within existing tree clusters along with kin-structured clusters created by scatter-hoarding animals likely created the genetic neighborhoods within the clumpy stand structure in Fort Valley pre-settlement populations. Gene flow among clusters in Fort Valley is not sufficient to overcome the family structure created by half and full siblings and parent-progeny kin relationships existing within individual clusters and suggests restoration prescriptions for clustered versus more evenly dispersed trees might be needed to protect evolutionary genetic patterns. Post-settlement trees averaged 150 years younger and were likely offspring of pre-settlement trees, since the pre- and post- trees sampled were interspersed. Compared to pre-settlement populations, post-settlement trees had slightly greater heterozygosity and allelic richness, and allele frequencies between these two age groups varied significantly. Post-settlement trees did not contain unique alleles, and genetic difference between age groups could be explained by different microclimate and thus selective conditions under which the two age groups became established. Simulated removal of 50 percent of post-settlement trees did not reduce genetic diversity, but 75 percent removal resulted in decreased allelic richness in the thinned population, particularly among rare and low frequency alleles. The loss of these alleles could be disadvantageous if they have adaptive significance to changing environments such as experienced between the two age groups. Maintaining some post-settlement populations with higher tree densities across the landscape could conserve low frequency alleles. Overall results of this study provide evidence of rapid evolution in ponderosa pine and indicate restoration treatments must consider genetic diversity to ensure adaptive potential is conserved.

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Genetic Conservation and Management of the Californian Endemic, Torrey Pine (*Pinus torreyana* Parry)[1]

Jill A. Hamilton,[2] Jessica W. Wright,[3] and F. Thomas Ledig[4]

Torrey pine (*Pinus torreyana*) is one of the rarest pine species in the world. Restricted to one mainland and one island population in California, Torrey pine is a species of conservation concern under threat due to low population sizes, lack of genetic variation, and environmental stochasticity. Previous research points to a lack of within population variation that is unprecedented among conifer species, although a few fixed genetic differences between the populations contribute to subspecies classification. Given this, development of best conservation practices requires a combination of genetic and trait evaluation tools to conserve this keystone species. To evaluate phenotypic differences between populations, a provenance trial was established in 2007 at the Santa Barbara Botanic Garden. The trial consists of seeds established from cones collected within a mainland progeny trial of mainland and island individuals and includes mainland, island, and hybrid individuals, the result of natural hybridization within the progeny trial. Genetic ancestry of individuals was evaluated using allozyme markers as fixed genetic differences were observed between populations. We evaluated phenotypic differences between mainland, island and hybrid individuals, comparing early germination traits and annual fitness metrics height and fecundity following establishment. Preliminary results indicate admixed individuals exhibit increased fitness relative to mainland and island individuals at all development stages, suggesting a potential role for genetic rescue via intraspecific hybridization in this genetically depauperate species. However, unidirectional hybridization within the F_1s indicates extrinsic or intrinsic barriers to reproduction have evolved between these populations, indicating between-population crosses may not represent a viable option to conserve evolutionary potential. This long term dataset provides an invaluable resource to test predictions regarding the use of genetic rescue in rare, long-lived species.

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Genetic-Environment Associations Across the Range of *Pinus strobus*[1]

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Because of rapid global warming, it is critical for us to better understand the capacity of forest trees to adapt to a changing climate, especially species such as five-needle pines that are particularly at risk to threats because of fire suppression, population fragmentation, and pests. In this study, we used several methods to disentangle the effects of local adaptation (isolation by environment, IBE) from those of isolation by distance (IBD) and isolation by colonization (IBC) in *Pinus strobus*. For this reason, 153 SNPs from 103 genes, including 44 candidate genes for growth and phenology, were genotyped in 133 populations across the range of *P. strobus*. IBD and IBC were found to be significant drivers of population structure. STRUCTURE analyses identified two distinct southern and northern genetic groups that likely originated from different glacial lineages. IBE did not significantly explain population structure when controlling for IBD and IBC. However, genetic-environment association (GEA) methods and F_{ST} outlier tests detected 33 (21.6 percent) outlier SNPs, indicating that local adaptation took place in the presence of high gene flow. We combined results across GEA and F_{ST} outlier methods and identified six highly supported candidate genes for local adaptation. Local adaptation was further tested by a dendrochronological analysis on a subset of mature *P. strobus* trees representative of the species range and established in a provenance trial. Cumulated radial growth decreased with increasing difference in mean annual temperature between the population origin and the trial location. Many of the highly supported SNPs identified by GEA and F_{ST} outlier tests were also associated with growth tolerance to summer drought and heat constraints in the provenance trial.

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Genetic Differences Between Yellowwood (*Cladrastis kentukea*) in Wild Populations Versus Urban Forests[1]

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Yellowwood (*Cladrastis kentukea*) is an uncommon, relict, tree species with a disjunct distribution primarily in the Central Hardwoods region. Most common on rocky, sheltered slopes of the Cumberland Plateau in Tennessee and Kentucky, isolated populations occur on appropriate sites throughout the southern and central United States. In Illinois, Louisiana, and South Carolina, yellowwood is listed as endangered by state conservation agencies, but it is not federally protected. Yellowwood has increased in popularity as a street tree and is planted in or predicted to be suitable for urban forests in every continental state and even parts of Canada. We investigated the genetic diversity of a sample of yellowwood from the midwestern urban forest and compared it to the genetic diversity of yellowwood populations sampled in Kentucky, Indiana, Arkansas and Missouri. We found that wild yellowwood populations are characterized by high levels of genetic differentiation and the presence of large numbers of unique alleles. Pollen movement, which is probably mediated mostly by bees, appears to be local, and migration out of current habitats unlikely. Urban yellowwoods we sampled were strongly differentiated from sampled wild populations, indicating they were not drawn from them as seed sources; rather, they may be primarily derived from yellowwood populations in Tennessee that were not sampled. Urban populations had higher heterozygosity, a much larger number of alleles, and a large number of alleles not found in any sampled natural population, indicating that they may represent a mixture of genetic material from several long-isolated wild subpopulations. Landscape and street trees could contribute to conservation and restoration of species with desirable horticultural traits, particularly when local wild populations are extirpated or suffer from depleted genetic diversity and inbreeding due to genetic isolation.

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Conservation Strategies (Species Restoration)

Applied Genetic Conservation of Hawaiian *Acacia koa*: an Eco-Regional Approach[1]

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Abstract

Koa (*Acacia koa*) 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. Producing seeds with genetic resistance to *FOXY* is vital to successful koa reforestation and restoration. 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. The population genetics of koa are poorly understood across the broad range of habits that koa occupies and seed zones have not been sufficiently established. Thus, HARC estimates seed zones based on biogeographic variables and has selected wilt resistant koa populations for six ecological regions (eco-regions) in Hawaii. This conservative approach, based on planting locally sourced germplasm, is often a requirement of many restoration programs in the state. We further consider population genomic (single-nucleotide polymorphism) data in relation to the proposed eco-regions. Preliminary analyses suggest genetic differences among and within islands that are broadly consistent with eco-regions, but also suggest additional population differences that should be considered in genetic conservation of koa.

Koa Significance

Acacia koa (koa) is a highly valuable timber tree species endemic to the Hawaiian Islands. Koa is a dominant canopy tree and keystone species in native forests where it provides critical habitats for endangered native birds and epiphytic plants. Koa is also a nitrogen-fixing tree legume that forms both root and canopy nodules in association with *Bradyrhizobium* (Leary et al. 2004). Under ideal conditions, koa grows to heights of over 30 m and lives several 100 years. This tree is of immense cultural importance to native Hawaiians, as its wood is used for a range of traditional applications. Most notably, it is the preferred wood for construction of traditional Hawaiian voyaging canoes. Koa timber is used for producing musical instruments, specialty furniture, and other high value craft goods. The very limited supply of commercial quality trees is a significant limiting factor to the Hawaiian forestry industry, with the total annual value estimated at $20 to $30 million (Yanagida et al. 2004).

Koa Distribution

Owing to its topographic and oceanic island position, the Hawaiian archipelago contains a wide range of terrestrial ecological zones that include alpine, subalpine, montane, lowland, and coastal (Juvik and Juvik 1998). Within each ecological zone, three general moisture regimes are recognized, dry (<1,200 mm), mesic (1,200 to 2,500 mm), and wet (>2,500 mm) of annual rainfall (Mueller-Dombois 1992). As a

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consequence, Hawaii's small land base supports a high level of ecosystem richness and diversity. However, Hawaii's forests are species poor, similar to many remote oceanic islands. In Hawaiian forest ecosystems, koa is a conspicuous component occupying a wide range of environments (Baker et al. 2009). Noted for its environmental plasticity, koa occurs in subalpine, montane, wet, and lowland forest eco-zones across the four main islands (Hawaii, Maui, Oahu, and Kauai) (fig. 1).

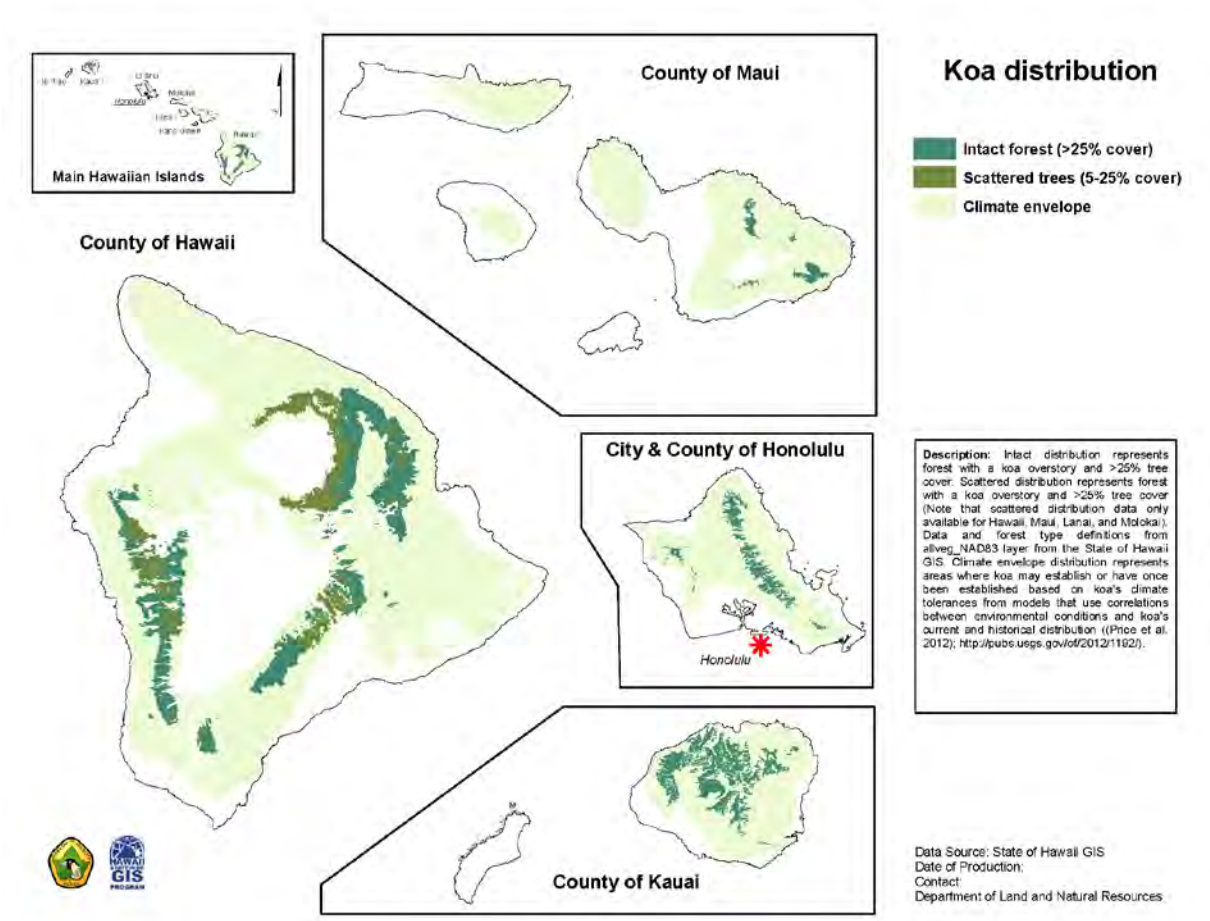

Figure 1—Current koa concentrations in the Hawaiian Islands (Price et al. 2012).

Koa Wilt Disease

Koa wilt disease is a vascular wilt caused by the fungal pathogen *Fusarium oxysporum* f. sp. *koae*. This soil pathogen invades susceptible plants through the root system, enters the xylem and restricts water transport, eventually leading to tree mortality. In Hawaii's remaining koa forests, koa wilt disease has caused further decline and is considered one of the greatest threats to the resource (Anderson and Gardner 1998, Anderson et al. 2002, Dudley et al. 2007, Gardner 1980, James 2005). Koa wilt disease severely restricts koa reforestation in most low to mid-elevation locations (sea level to approximately 1,000 m elevation) with mortality rates commonly exceeding 75 percent. As low elevation sites provide the greatest growth potential for this tropical species, there is significant incentive to develop wilt resistant populations for commercial reforestation.

The virulence of *Fusarium oxysporum* in relationship to soil temperature is well studied in many host species, with increased virulence at higher temperatures (Clayton 1923, Landa et al. 2006, Scott et al.

2001). While these temperature effects have not been studied directly in koa, the cooler temperatures at higher elevations may explain the current lower disease severity found at these locations. However, global climate change and the subsequent increase in soil temperature threatens to increase the disease severity in areas where it is currently limited. Developing and conserving wilt resistant high elevation seed sources will provide a level of biosecurity to mitigate the predicted effects of increased soil temperatures and ensure that koa remains a keystone species in Hawaii's remaining native forests.

Koa Genetics

Koa is primarily a cross-pollinating species, or outcrossing (i.e., mating among unrelated individuals) species, which is important to maintain genetic diversity within populations. Unlike most *Acacia* spp., koa is a tetraploid species and contains four sets of chromosomes within the cell nucleus, facilitating high levels of genetic variability (Atchinson 1948, Carr 1978, Le Roux et al. 2014, Shi 2003). Koa's polyploidy complicates analysis of genetic studies, and the literature conflicts regarding koa's tetraploid origins. Most previous literature claims koa to be an allotetraploid with disomic segregation and genetic studies were analyzed based on this assumption (Adamski et al. 2012, Brewbaker 1977, Shi 2003, Sun 1996). Nevertheless, no strong evidence supports this claim. Recent work provides evidence to support that koa is indeed an autotetraploid, derived from *A. melanoxylon*, with the species diverging over 4.9 million years ago (Brown et al. 2012, Le Roux et al. 2014).

Variation

A large degree of phenotypic variation has been documented as early as the 19th century and has led to varying taxonomic classifications (Adamski et al. 2012, Daehler et al. 1999, Hillebrand 1888, St. John 1979, Sun 1996, Wagner 1990). The most commonly described variations occur in: phyllode shape, pod shape, seed shape and arrangement, inflorescence and flower structure, growth form and rates, wood characteristics, and disease resistance (fig. 2). Isozyme study on populations from across Hawaii revealed a large degree of diversity (Conkle 1997). The variable genes studied had between three to seven alleles and an average expected heterozygosity of 0.41. The study utilized the variable genes to calculate genetic distance and found Hawaii Island populations were distinct from Kauai, Oahu and Maui populations. Recent research confirmed a large degree of genetic variation in koa, but found no correlation between genetic and geographic distance (Adamski et al. 2012), and 73 percent of genetic variation was partitioned among individuals within a population, compared with 6.5 percent for the whole taxa and 21 percent among populations. Several common garden trials examine the relationship between genetic and phenotypic variation were planted during the 1990s as a joint effort between the University of Hawaii and HARC (Daehler et al. 1999, Shi 2003, Sun 1996). While these trials had high rates of mortality (>70 percent) from koa wilt disease, several key observations were made: estimated family heritability for height and diameter at breast height (1.37 m) approached 0.75; genetic variations were observed for disease resistance; tree form, seed size, seed shape, seed weight, seedling growth, juvenile growth, phyllode development, phyllode shape, nectary, flowering pattern and duration of vegetative stage; and Oahu and Hawaii Island trials showed a significant genotype by environment interaction.

Figure 2—Phenotypic differences in pods, seeds, and phyllodes. (D. Adamski)

The multiple uses of koa justify a robust genetic conservation program sensitive to the needs of the various stakeholders while reversing the genetic degradation of this iconic species. Koa's apparent high degree of genetic and morphological variation makes it a strong candidate for genetic improvement. Beyond commercial forestry, koa is a keystone species in Hawaiian native forests and koa forest restoration is a primary objective for numerous local conservation and community groups.

Establishing Seed Zones: an Eco-Regional Approach

Sustainable forestry emphasizing restoration or reforestation requires choosing the proper seed source of high genetic quality. It is critical that the seed be ecologically well adapted, productive, and healthy (Morgenstern 1996), and this will have a significant impact on the planted forest. Knowledge about the geographic variation of any species aids in selection of the most appropriate provenance for reforestation and restoration (White et al. 2007, Wright 1976, Zobel and Talbert 1984).

Accordingly, many landowners/managers and restoration groups are reluctant to plant koa originating from outside their eco-region. The current recommendation is to plant locally sourced seed to ensure that seedlings will be well adapted to site conditions (Baker et al. 2009). This is because seed or planting zones (a seed zone is a single geographical or ecological unit within the range of the species based on ecological and genetic criteria) for koa in Hawaii are not well defined, due in part to the limited information on koa population genetics. Planting seedlings from seed collected and planted within the same established ecological zone would be considered a "local" source of seed (White et al 2007).

The HARC—in collaboration with the U.S. Department of Agriculture Forest Service, the State of Hawaii, Department of Land and Natural Resources, and County of Maui, Department of Water Supply—has an on-going program to identify and conserve Fusarium wilt resistant koa populations. The lack of established koa seed zones necessitated utilizing the best currently available information to estimate seed zones and to develop wilt resistant populations from those preliminary zones. This preliminary demarcation and delineation of seed zones for koa is based on ecological data (Morgenstern 1996).

Landscape classification variables or zonation can be an efficient means for quantification of biological diversity (Ferrier and Smith 1990). Our goal is to provide a framework to guide genetic conservation and more precisely deploy koa seed sources for restoration and reforestation efforts. This is a conservative approach seeking to ensure that planted trees are well adapted to local conditions and similar genetically to local populations with the objective of dynamic genetic conservation of fragmented koa populations at low and mid-elevation sites across the state (Campbell 1975, Dudley et al. 2015).

Basis for Seed Zones

At the outset of this project, owing to the impact of Hawaii's complex biogeography on koa population structure, we proposed the following framework to utilize (the currently best available) geographic and genetic variation data to delineate preliminary/provisional koa seed zones (Hamann et al. 2005, White et al. 2007, Ying and Yanchuk 2006). This is a synthesis of geographic, climatic and vegetation patterns, we describe as geo-climatic, or ecological regions (eco-regions) (Pojar et al. 1987). HARC's preliminary eco-regions for koa populations are delineated as follows:

- By island, discontinuous by island and within island.
- By aspect within island, primarily windward (wet) and leeward (dry) zones.
- By elevational sub-zone within each zone: sea level to 600 m (low); 600 m to 1200 m (mid); 1200 m to 1800 m (high).
- By special situation (*A. koa'ia* in Kohala, Hawaii; low-elevation *A. koa*, west Maui).

The lack of more robust genetic data, the multiple uses for koa, and the ecological and cultural significance force a conservative approach to genetic conservation and seed zone delineation. Therefore, the HARC koa improvement program is primarily based on developing *in-situ* seed orchards for local koa populations in the different geographic zones, referred to as eco-regions. This approach allows for increased flexibility once koa population genetics and the relationship between genetic variation and adaptability to Hawaii's numerous ecological zones are better understood. Thus, these preliminary seed zones are expected to evolve over time with increases in knowledge of genetics and ecology of koa, and as new analytical approaches arise. This will assist in further elucidation and delineation of these seed zones.

Refining Koa Seed Zones: Incorporating Genomic Data

To better understand these preliminary proposed seed zones for koa in relation to genetic variation, we analyzed genomic data consisting of 11,002 diallelic single-nucleotide polymorphisms (SNPs) derived from genotyping by sequencing (GBS; Elshire et al. 2011) for 311 individual *A. koa* trees collected across Hawaii as part of an ongoing study (Gugger, Liang and Wright, unpublished data). We used SNP allele frequencies to calculate pair-wise F_{ST} values among eco-regions modified according to observed population genetic structure (Wright 1965). Although it is problematic to calculate F_{ST} in tetraploid populations using SNP data that were coerced to be "diploid" because assumptions can about allele frequency calculations are not necessarily met, our goal is to compare the relative differences among islands and proposed seed zones.

For the eco-region seed zones, we compared trees from Kauai that are understood to be from the native population of the island with Kauai trees that potentially originated from other islands as a result of reforestation efforts. On Maui, we compared two windward sources from opposite ends of the windward zone (see fig. 3 showing the seed zones). On Oahu, we did not have enough samples to compare the Koolau and Waianae (two different mountain ranges) sources, and only show data for the Koolau population. On Hawaii Island, four eco-region seed zones were proposed (fig. 3); however, we can only compare three, as we do not have enough sources from the *A. koaia* zone.

Figure 5 shows a dendrogram of hierarchical clustering based on Euclidean distance of pairwise F_{ST} among eco-regions. Eco-regions connected closer together on the tree have more similar pairwise F_{ST} values, suggesting they are more similar genetically. The results suggest genetic differences among all

four islands, with comparisons between the most geographically distant islands (Kauai and Hawaii) showing the highest levels of differentiation. Within islands, differences were much lower, with nearly no difference between the native and potentially introduced trees on Kauai. The two Maui seed zones showed the greatest differences, while there were smaller differences among the Hawaii seed zones.

As an exploratory analysis of genetic structure in relation to eco-regions, we modified the ecological seed zones in Maui and Hawaii to genetic seed zones (fig. 4, 6). To preliminarily define genetic seed zones, genetic clusters were estimated with Admixture 1.3 (Alexander et al. 2009) using the SNP data. Individuals from Maui and Hawaii were generally assigned to the cluster from which they derived their largest ancestry (highest *Q*). We recalculated the F_{ST} values using these genomic seed zones, and found a higher level of differentiation within those two islands (fig. 6) in comparison to the zones based purely on eco-region. Interestingly, the new Maui Low elevation-wet seed zone is very similar to the Oahu Koolau seed zone, with little differentiation between them (less than between the two Maui seed zones; low elevation trees from Maui are more similar to trees on Oahu than to high elevation trees on Maui).

The genetic seed zones presented here are preliminary only, and further analysis is needed to better define the zones. However, our goal with these alternative genomic zones is to show that there is genetic differentiation within island populations of *A. koa*, particularly on Hawaii Island, and to suggest that further refinements of the proposed seed zones may be necessary to account for these genetic differences. Indeed, the origin of koa trees within islands appears to be associated with genetic differentiation.

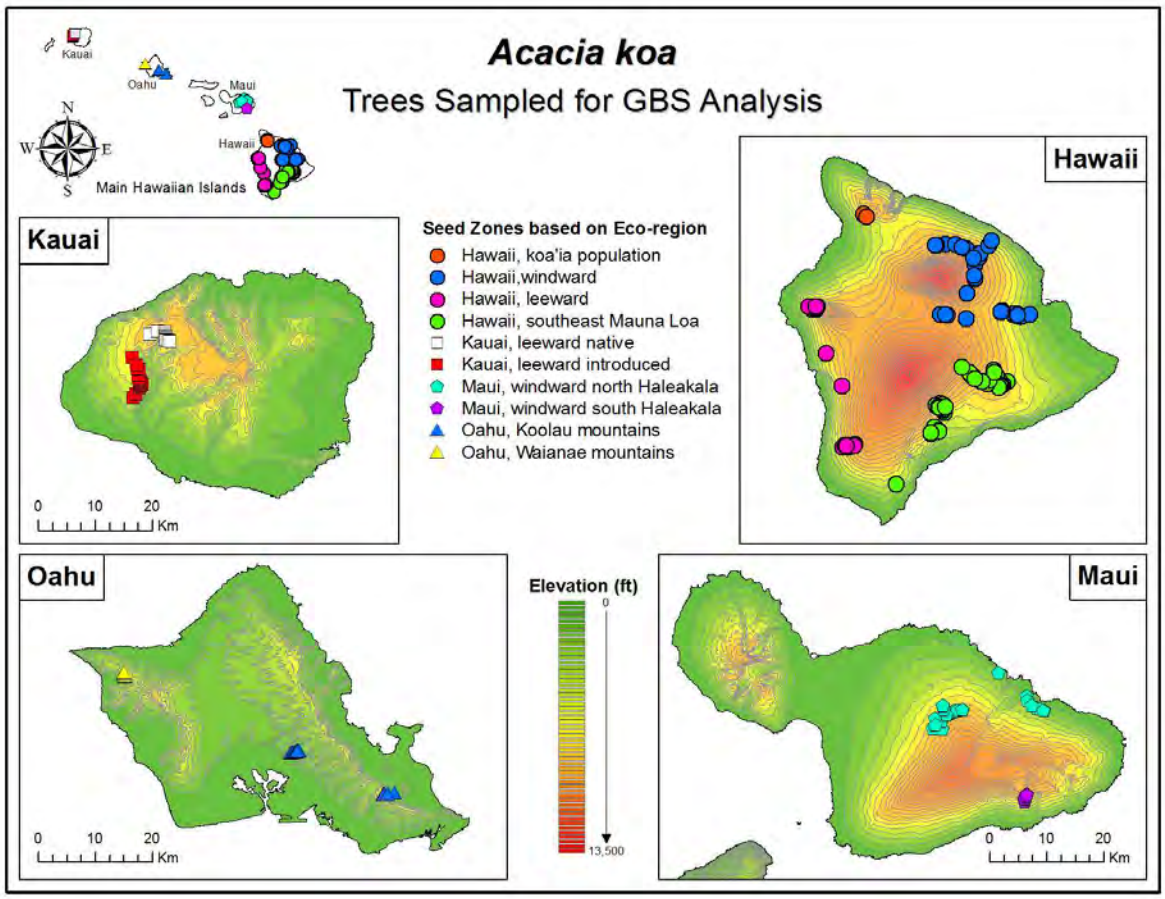

Figure 3—Seeds zones of 311 sample trees used in genomic analyses based on eco-region.

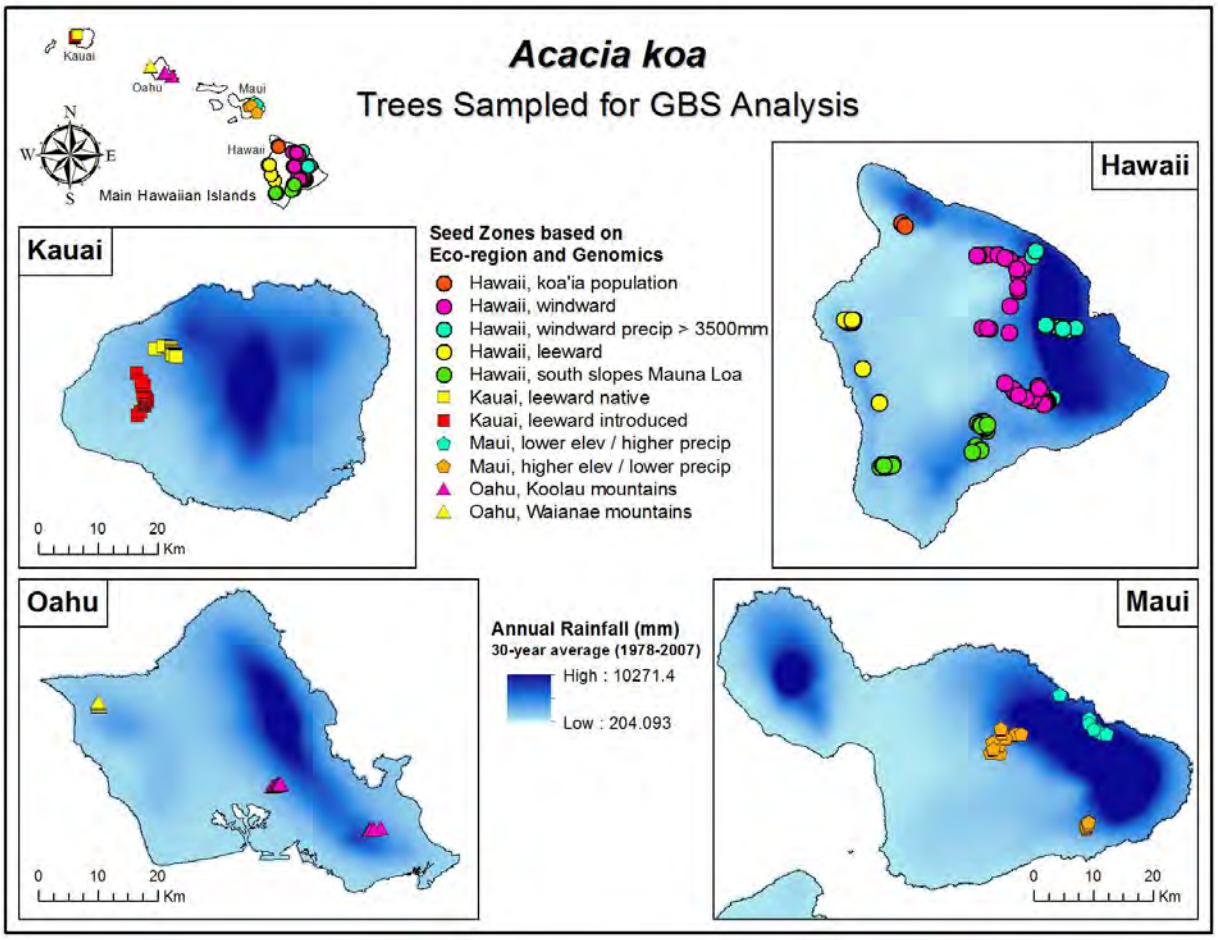

Figure 4—Seed zones of 311 samples used in genomic analyses based on a combination of eco-region and genomic data.

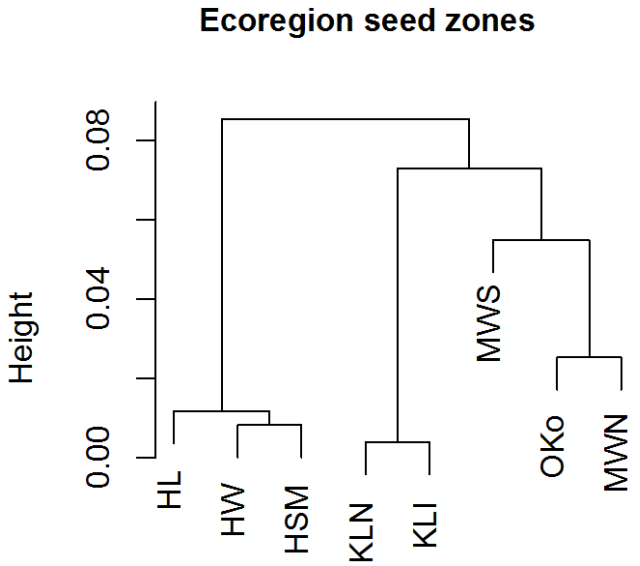

Figure 5—Hierarchical clustering based on Euclidean distance of pairwise F_{ST} among eco-regions, as implemented in R 3.3.0. HL- Hawaii Leeward, HW- Hawaii Windward, HSM- Hawaii South Slope of Mauna Loa, KLN- Kauai Leeward Native, KLI- Kauai Leeward Introduced, MWS- Maui Windward South, OKo- Oahu Koolau, MWN- Maui Windward North.

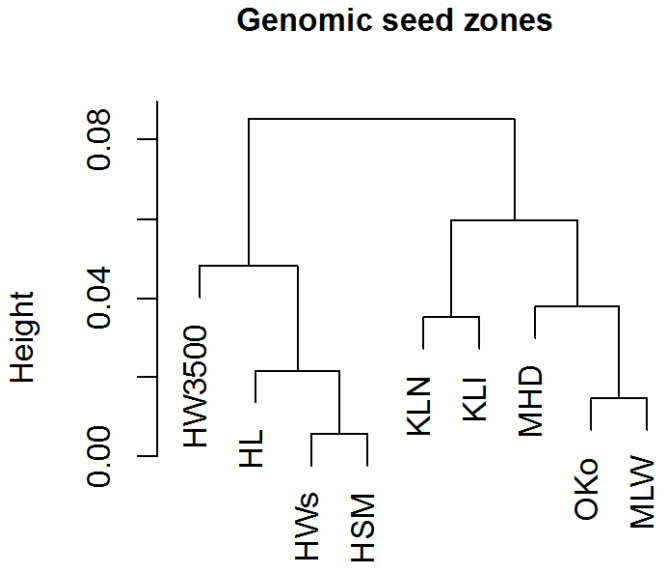

Figure 6—Hierarchical clustering based on Euclidean distance of pairwise F_{ST} among eco-regions modified according to genomic data, as implemented in R 3.3.0. HW3500- Hawaii Windward, annual precipitation >3500 mm, HL- Hawaii Leeward, Hawaii Windward Slopes, HSM- Hawaii South Slope of Mauna Loa, KLN- Kauai Leeward Native, KLI- Kauai Leeward Introduced, MHD- Maui Higher Drier, OKo- Oahu Koolau, MLW- Maui Lower Wetter.

Application of Seed Zones to HARC Fusarium Wilt Resistant Koa

Wilt Resistance Screening

Koa wilt disease is caused by a pathogen of unknown origin found throughout Hawaii and is a major impediment to successful koa reforestation and restoration. HARC utilizes an artificial inoculation test to select half-sib families with an increased frequency of genetic resistance to the causal agent, *F. oxysporum* f. sp. *koae* (Dudley 2013, 2015). Highly virulent isolates are used to inoculate very young koa seedlings from half-sib families. Family survival ranges from 0 percent in the most susceptible to over 80 percent in the most resistant, with an average survival of approximately 40 percent. This rapid and reliable screening method enables HARC to quickly screen koa families collected from various eco-regions across the state. Wilt resistant families are then out-planted in field trials/seed orchards located within the mother tree's originating eco-regions to monitor the durability of resistance, produce wilt resistant seed and maintain *in-situ* genetic conservation (fig. 7).

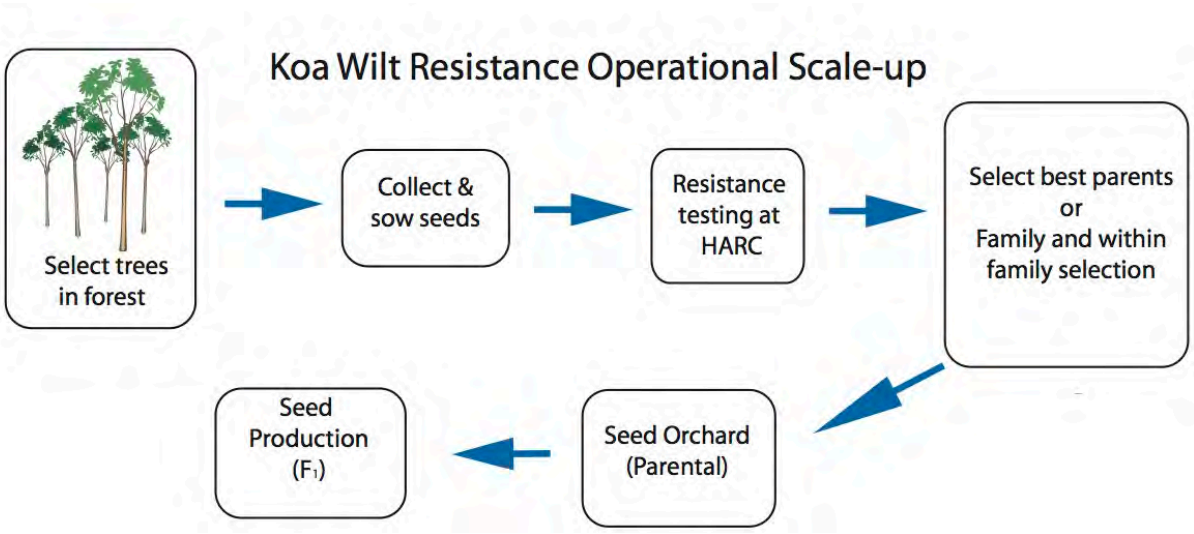

Figure 7—*Acacia koa* wilt resistance tree improvement (1st cycle).

Seed Collection Methods

Koa seeds are collected from individual dominant, or co-dominant mother trees with vigorous canopies within specific eco-regions. These candidate trees are identified by collection location and geo-referenced. Care is taken to avoid collecting from isolated trees with potentially elevated levels of selfed offspring. This permits the capture and study of genetic variation within and between populations (Willan 1985). Following seed collection, a database was developed from seed collection data.

HARC's standard practice is to collect a target population size of at least 50 to 100 mother trees per eco-region (Nikles 1974). Ideally, samples trees are at least 50 m apart to avoid relatedness among seed lots from different mother trees, and equal amounts of seed are collected from different quadrants of the canopy. The goal is for open-pollinated offspring from each selected mother tree, to be the result of numerous male pollen parents. Approximately 500 viable seeds are collected from each mother tree for wilt resistance screening and genetic conservation effort. Koa seed is a non-recalcitrant with a hard outer shell, permitting seed to be stored for decades under proper conditions.

In-situ Seed Orchards

Since 2011, koa populations from five eco-regions have been screened for wilt resistance; Koolau Mountain (Oahu), Southeast Mauna Loa (Hawaii Island), windward Hawaii Island, windward Haleakala (Maui), and Kokee (Kauai) (table 1). A leeward Haleakala (Maui) population is currently scheduled for

screening by the end of 2016, but samples were not collected from this region for the genomic portion of this project.

Field sites were selected for planting wilt resistant families selected from greenhouse screening trials. In 2012 to 2016, HARC planted seed orchards for the Koolau, Southeast Mauna Loa and windward Haleakala eco-regions. Wilt resistant families have been selected for the windward Hawaii Island and Kokee eco-regions and seed orchard establishment is scheduled for late 2016 to 2017. Early survival data indicates a significant improvement over control families, particularly at the Oahu site, where pathogen pressure is highest. Continued monitoring and data collection are critical to understand the durability of resistance. If survival remains high, thinning will be based primarily on growth characteristics such as stem form and volume. It is anticipated seed production will commence is 3 to 5 years after outplanting.

Table 1—HARC Fusarium wilt resistant seed orchards

Eco-region	Island	Germplasm number of families	Planting year	Seed zone of mother trees[a] (GBS)
Southeast Mauna Loa	Hawaii	12 families	2012	HSM & HW.s
Windward Haleakala	Maui	15 families	2013	M.HL
Koolau	Oahu	34 families	2012	OK
Kokee	Kauai	25 families	2016[2]	KLN
Leeward Haleakala	Maui	~ 25 families	2016[2]	not tested
Windward Hawaii	Hawaii	20 families	2017[2]	HW3500

[a] HSM- Hawaii South Slope of Mauna Loa; HW.s- Hawaii Windward Slopes; M.HL - Maui Higher elevation, Lower precipitation; OK- Oahu Koolau; KLN- Kauai Leeward Native; HW3500- Windward, annual precipitation >3500 mm.

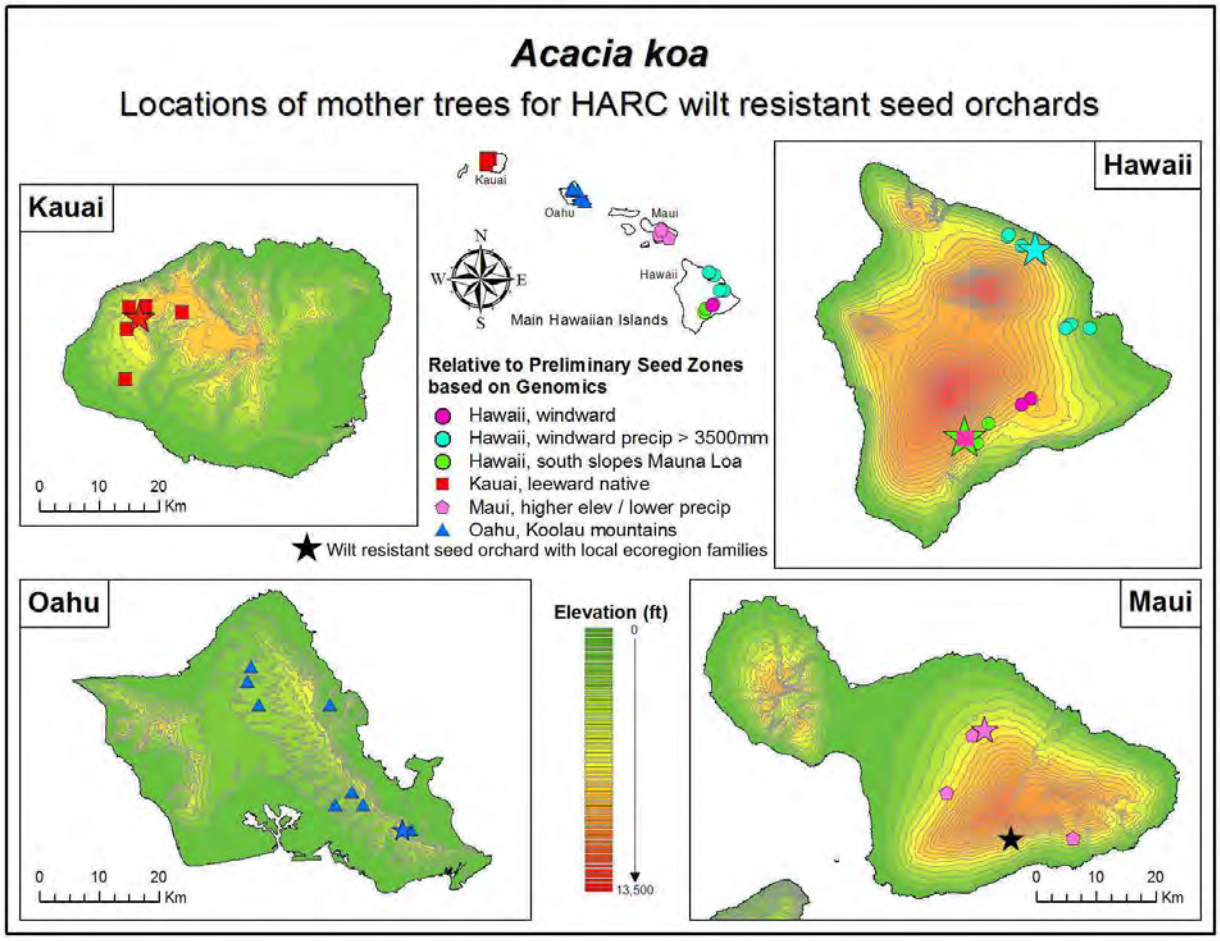

Figure 8—Locations of HARC koa wilt resistant seed orchards and mother trees. Color of icons indicates from which preliminary seed zone the mother trees originate based on available genomic data.

The location of the selected mother trees from which the seedlings included in the seed orchards was plotted and compared to the preliminary seed zones based on genomic data (table 1 and fig. 8). The eco-region approach to seed zone delineation is relatively consistent with the preliminary genomic delineations. All orchards exclusively contain trees from the local zone, with the exception of the South Mauna Loa orchard, which utilized mother tree from both the local, and the Hawaii windward zone. Overall, genomic data suggests that the eco-region approach to seed zone delineation was appropriate and should continue, as genomic-based approaches are refined.

Summary and Discussion

Developing koa planting stock that is resistant to the wilt-causing fungus *F. oxysporum* is a critical step to meet the overall objectives of conserving Hawaii's remaining koa forests, restoring koa to its native range and ensuring a sustainable supply of koa timber for future generations. It would significantly reduce risk and uncertainty associated with growing koa in plantations below approximately 1,000 m in elevation. The rapid disease screening methods developed during the early phases of this project gives koa improvement programs the ability to accomplish this goal in a timely and efficient manner. It should be noted that some continuing effort is always needed to obtain new, virulent strains of *F. oxysporum* and to maintain virulent strains for use in these screening operations.

Koa's significance to the ecology, economy and culture of Hawaii mandate conserving wilt resistant koa populations while maintaining genetic differentiation within the species. Therefore, the HARC koa

improvement program is primarily based on developing disease resistant koa seedling seed orchards for local koa populations in the different eco-regions by island. Other than wilt resistance, these koa families approximate the wide range of genetic variation and diversity found in natural populations. This approach allows for flexibility as koa population genetics and the relationship between genetic variation, population structure and adaptability to Hawaii's numerous ecological zones become better understood.

The opportunity to further refine koa seed zones recently emerged by the characterization of genomic data based on statewide sampling. This has resulted in further refinement and verification of koa seed zones. However, these genetic seed zones are preliminary in nature, and further analysis is needed to refine and better delineate these zones. A landscape genomic analysis is currently underway using the single-nucleotide polymorphism data described here, which raise some interesting questions that merit further investigation. Additional sampling and genomic sequencing will contribute data to address these and other questions, and allow for further refinement of the proposed seed zones.

The goal of establishing wilt resistant koa seed orchards comprised of locally sourced germplasm in numerous ecological regions throughout the state is now underway, with several sites established and three additional sites scheduled to be installed by mid-2017. In the future, additional sites are planned in eco-regions that are currently under represented.

The result of this endeavor will be locally adapted, eco-region specific koa seed that allows for the restoration of this iconic species and commercial reforestation opportunities. It is envisioned this will include further development of a distribution and seed banking network for the release of improved koa seed for non-industrial and forest landowners and managers across the state. This will allow for the efficient distribution of improved (locally adapted, genetically diverse, disease resistant) koa seed, permitting the reduction of risk and increased confidence in future reforestation and restoration efforts.

Acknowledgments

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Kentucky Coffeetree, *Gymnocladus dioicus* (L.) K. Koch: Current Abundance in Nature and Prospective Persistence[1]

J.D. Carstens[2] and A.P. Schmitz[3]

Recently, a collaboration between The Brenton Arboretum and the North Central Regional Plant Introduction Station (NCRPIS) was initiated to assemble comprehensive *ex situ* germplasm collections of Kentucky coffeetree, *Gymnocladus dioicus. Gymnocladus dioicus* was selected due to its adaptation to poor soils common to urban conditions, extreme drought tolerance, and no reported serious insect or disease problems. These factors make it a promising candidate among diverse tree genera to replace ash trees in urban environments affected by the emerald ash borer (*Agrilus planipennis*). Targeted germplasm collections will eventually represent material from various habitat types and Omernik Level III Ecoregions within the native range of *G. dioicus*.

Gymnocladus dioicus was sampled and surveyed at 80 sites across the Midwest from Minnesota south to Arkansas and from Oklahoma east to Kentucky. Our observations in sampling *G. dioicus* across circa 95 percent of the species native range would indicate the species is rare not because of an obvious or direct threat of insect or disease, but rather because of indirect, often overlooked, ecological changes. Observations in nature indicate *G. dioicus* recruitment is poor likely due to the absence of an effective seed dispersal agent, intolerance to shading, and the requirement of scarification to germinate. Observations at NCRPIS have determined that *G. dioicus* is androdioecious, yet this androdioecy is non-functional, confirming xenogamous pollination is required for fruit production.

The Endangered Species Act was passed by Congress in 1973 to protect and recover imperiled species and the ecosystems upon which they depend. Specific factors are typically met for listing a species as endangered or threatened, and these factors are generally obvious and/or recent. However, in the case of *G. dioicus*, historical ecological changes have reduced the potential of this species to persist, warranting evaluation for potential protection.

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Conservation Genetics of the European Beech in France[1]

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European beech (*Fagus sylvatica*) is one of the most abundant tree species in Europe. Its genetic structure and diversity have been investigated using both molecular markers and adaptive traits as assessed in field and laboratory experimental tests looking at adaptative traits. A great deal of information also exists on the Quaternary history of the species and on plant communities associated with this keystone species. In France, the conservation of its genetic resources relies on both *in situ* and *ex situ* approaches. Some outlying populations at the margin of its distribution, that are known to have acted as populations directly descended from glacial refugia, have been selected as gene conservation units. Because these populations are under a particular type of pressure and because of their disproportionate importance for conservation, they are the focus of more detailed investigations. Such relict populations tend to occur in environmentally unusual areas characterized by highly stable mild climates, which have allowed them to persist *in situ* through both glacial and interglacial episodes. This results in a complex genetic structure. This climate stability has also favored populations of associated rare species, making these areas important zones not only for the conservation of *F. sylvatica* genetic resources, but also for the conservation of associated biodiversity. The integrated strategy used to preserve these populations and the associated communities, focusing on both research and action, includes establishment of *ex situ* plantations, citizen science for promoting the establishment of plantations using locally sourced seeds, and the identification and mapping of the most important risks faced by these populations (i.e., land use changes and concurrence with invasive tree species) to guide their management and restore the ecosystem.

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Hybridization and Management of Oak Populations[1]

Oliver Gailing[2]

Abstract

Hybridization can result in the transfer of adaptations among species and may contribute to speciation processes. On the other hand, hybridization can also result in a loss of species diversity due to asymmetric gene flow between species (genetic swamping) and in low hybrid fitness. An understanding of the outcomes of interspecific hybridization is crucial for the management and conservation of tree populations. As a result of warming climates, it is expected that distribution ranges of species will shift, resulting in new zones between species and potentially new or increased hybridization.

Oaks (*Quercus* spp.) are the dominant species in many hardwood forests of North America. Hybridization is common in oaks and species boundaries are fuzzy as a result of large within-species variation, phenotypic plasticity and interspecific gene flow. While morphological identification of species can be difficult, DNA markers such as nuclear and genic microsatellites can be used to assign individuals to species and hybrids. We have developed a set of 44 microsatellite markers that distinguishes between closely related red oak species with different adaptations to drought. While most markers in the set showed low to moderate interspecific differentiation, one genic microsatellite marker showed pronounced interspecific differentiation as result of strong divergent selection and may be associated with adaptive species differences. By using these new microsatellite markers, we will be able to assess interspecific gene flow and introgression of adaptive alleles. Results on the frequency of hybridization and hybrid fitness will be important for the management of oak populations in the face of climate change.

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Gene Conservation of *Pinus aristata*: a Collection with Ecological Context for Management Today and Resources for Tomorrow[1]

A.W. Schoettle[2]

Pinus aristata, Rocky Mountain bristlecone pine, has a narrow geographic and elevational distribution and is threatened by rapid climate change, the introduced pathogen *Cronartium ribicola* that causes white pine blister rust (WPBR), and bark beetles. The core distribution of *P. aristata* is near and at treeline in central and southern Colorado and extends into northern New Mexico with a disjunct population in northern Arizona. The combination of low genetic diversity, moderate population isolation, and a protracted regeneration dynamic puts populations at risk for extirpation by novel stresses highlighting the need for *ex situ* gene conservation.

Populations range-wide are still healthy and offer the opportunity to sample the genetic diversity of the species. An efficient range-wide gene conservation sampling design of seed and tissue was developed and executed. Ten populations within each of six collection areas corresponding to the observed genetic substructuring were identified; 10 individual tree seed collections and a bulk collection were targeted from each of the 60 populations. Sample trees are georeferenced and sampled for seed and needle tissue; each stored for a working collection and gene conservation at -18 °C. Ecological data (physical site characteristics, stand density, species composition, disturbance history, regeneration capacity) on each population complement the genetic collection. The collection has been used to assess geographic and source-climate variation in genetic disease resistance to WPBR and other adaptive traits. The collection serves (1) to guide further collections and support proactive planting efforts to increase population resilience in the field and (2) to provide conservation and research material of the range-wide diversity for the species before constriction due to directional selection by climate change and WPBR.

More details can be found in: Schoettle, A.W.; Coop, J.D. 2017. Range-wide conservation of *Pinus aristata*: a genetic collection with ecological context for proactive management today and resources for tomorrow. New Forests. 48(2): 181–199. http://link.springer.com/article/10.1007/s11056-017-9570-z.

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A Holistic Approach to Genetic Conservation of *Pinus strobiformis*[1]

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Pinus strobiformis (southwestern white pine) is threatened by both a rapidly changing climate and the tree disease white pine blister rust, caused by an introduced fungal pathogen, *Cronartium ribicola*. We began a proactive program in ~2009 to sustain *P. strobiformis* that includes genetic conservation, research, and management strategies. Research is related to the silvics, ecology, genetics, and future climate profile of the species. The results of these investigations will be used to develop refined seed transfer zones and silvicultural approaches to managing southwestern white pine in a changing climate.

We began collecting *P. strobiformis* seed and foliage in 2012, and have collected from over 80 sites across Arizona and New Mexico. Seeds of 233 individual parent trees from 53 sites have been archived at the National Seed Laboratory in Fort Collins, Colorado for genetic conservation. Collections from 20 sites across Mexico were completed and will be included in the ongoing research. Foliage is being utilized for genomics and leaf trait (e.g., stomatal density) research; extra foliage is in storage at Northern Arizona University.

Research objectives in this program are broad and multi-faceted, but all lead to better management of this species. An initial common garden study determined population differentiation under stressful conditions and defined gradients of growth and drought tolerance across sampled populations and seed source climates (Goodrich, B.A.; Waring, K.M.; Kolb, T.E. 2016. Genetic variation in *Pinus strobiformis* growth and drought tolerance from southwestern United States populations. Tree Physiology. 36: 1219–1235.). We are expanding the initial greenhouse common garden to field trials with a series of sites and an elevation gradient in northern Arizona. These common gardens will test for population and family structures and genotype-by-environment interactions. Extensive disease resistance trials are ongoing at the U.S. Department of Agriculture Forest Service (USDA FS) Dorena Genetic Resource Center in Cottage Grove, Oregon to investigate both complete and partial gene resistance to the pathogen in populations from across the species range. Outplanting of families into field trials will complement and verify resistance trials. Genetic conservation is ongoing.

Results will be disseminated to resource managers by the research team in conjunction with USDA FS Forest Health Protection staff with the intent that the results be used to proactively and adaptively manage *P. strobiformis*. These results add to the knowledge base related to *P. strobiformis* silvics, regeneration ecology, and population genetics. Reducing maladaptation to seedling transfer will be a priority because the species may have genetic resistance to *C. ribicola* in a few populations. Both genetic resistance to disease and drought tolerance are desirable for *P. strobiformis* sustainability.

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Tools for Tree Genetic Conservation

Forest Service Access to and Use of the Germplasm Information Network (GRIN-Global) Database and Security Backup at the National Laboratory for Genetic Resource Preservation[1]

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Abstract

The U.S. Department of Agriculture Forest Service (USDA FS) National Seed Laboratory (NSL) began long term seed storage for genetic conservation, in 2005, for USDA FS units and cooperators. This program requires secure storage of both seeds and the data documenting the identification of the seeds. The Agricultural Research Service (ARS) has provided both of these services to the USDA FS through the NSL. Security backup, of all samples, is provided at the ARS National Laboratory for Genetic Resource Preservation. Forest Service seed sample data is stored in the Germplasm Resource Information Network (GRIN-Global).

Introduction

Successful genetic conservation of native trees through most of United States history has been through care of the natural forest and by maintaining broadly adapted sources for reforestation seedlings. In recent decades, that strategy has been stressed by catastrophic wild fires, climate change, and exotic invasive pests and diseases such as emerald ash borer (*Agrilus planipennis*) and white pine blister rust (*Cronartium ribicola*). Now, the World Conservation Union estimates that one in three plant species in the United States is threatened with extinction. Because losses of genetic resources were occurring faster than could be addressed by ongoing programs, conservation strategies have been expanded to include long term seed storage. This approach permitted for a relatively rapid response to changing conditions as long as species possessed seeds that met the requirements for long term storage and a seed crop was available. Fortunately, most North American trees and other native plants meet these requirements and do have seeds that are desiccation tolerant and are capable, following an adequate drying period, of remaining alive in freezer storage for decades. The administration of a long term seed storage program requires a seed laboratory facility which can test the seeds for viability prior to storage and at periodic intervals during storage to make sure the resource is remaining alive and to determine when stored seeds might need replacement or regeneration. The U.S. Department of Agriculture Forest Service (USDA FS) National Seed Laboratory (NSL) is the only national facility in the USDA FS with capability to perform this work. Therefore, long term seed storage for genetic conservation was formally added to the NSL mission by the Chief of the USDA FS, in 2005. As part of that mission, it was necessary to partner with the USDA Agricultural Research Service (ARS), National Plant Germplasm System (NPGS) for access to the National Laboratory for Genetic Resource Preservation (NLGRP) security backup of seed collections and the Genetic Resources Information Network (GRIN-Global) database in which all the conserved seed collections could be documented and displayed for potential users of the seed lots. This paper describes how samples can be submitted at the NSL, what data is entered into the GRIN-Global data base, and how to access information on samples held in the USDA FS collection. All USDA FS units and cooperators can submit samples for preservation. A material transfer agreement, renewable at 5 year intervals, defines

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the relationship of the NSL and the NLGRP. Seed samples remain the property of the USDA FS or the cooperator even though they are entered into the GRIN-Global database and a security backup sample is placed in the ARS storage vaults. The NSL is responsible for all maintenance activities such as periodic testing, sample distribution, and any increase of seeds in the event that viability begins to decrease.

Organization

The NPGS is part of the ARS in the USDA. It is composed of over 25 clonal and seed repositories and maintains over 500,000 accessions. These materials are made available for research and breeding on crop plants. As all crop plants have relatives in the wild, wild plants are also an important part of these collections. The efforts are a cooperative work among state, federal, and private organizations. To document the collections of plant materials and communicate the status and availability of the collections, the GRIN-Global was created. This database is the primary vehicle through which the NPGS interacts with the international germplasm community and the scientific public.

The collection of plant resources is divided into two parts. The first is working collections from which propagules are distributed to breeders and researchers. The second mirrors the working collection by holding a duplicate of what is in the working collections in secure facilities. This second part is called the security backup. This security backup is held at the National Laboratory for Genetic Resource Preservation at Fort Collins, Colorado.

The USDA FS has access to the database and the security backup facility and in turn makes them available to conservation communities. The USDA FS brings to the partnership an extensive network of contacts as well as the unique expertise and capacity of the NSL for quality testing of wild plant seeds and preparing them for storage. The NSL also has extensive history in sharing forest tree germplasm, having been a central point of contact to distribute seeds to international forest scientists since 1972. The USDA FS manages and conserves approximately 780,000 km^2 (193 million ac) of wild plants and assists in managing many millions more of private forests. Therefore, the agency brings an enormous working collection of plant material to the NPGS.

Genetic Resource Information Network (GRIN-Global)

GRIN-Global is a flexible, open source information management system designed to enable genebanks to store and mange germplasm information and deliver that information globally. The USDA ARS Database Management Unit at Beltsville, Maryland maintains and enhances the GRIN-Global.

The flexibility of GRIN-Global allows genebanks to tailor the system for optimum performance. The system is made up of multiple tables. Each table has required fields but you can add as much optional information as needed to adequately describe your accession. Typically, the USDA FS stores taxonomy data, collection id, material type, origin of sample, latitude and longitude, habitat, cooperator data, viability and inventory (number of seeds).

The Public Website - Requesting Seeds

Many types of information are available from the GRIN-Global public website, including a wealth of information on taxonomy, rare and endangered plants, and noxious weeds. It is also here that queries are made to find what materials are being curated in the NPGS. Simple searches allow the user to enter search criterion, such as a species name, in the search box. To view materials curated by the NSL it is necessary to check the block for “historic” and “unavailable.” More complex searches can be used to restrict the query by more than one criterion. Again, it is necessary to check the block for “historic” and “unavailable” in order to view materials curated by the NSL. The link to the query page is: https://npgsweb.ars-grin.gov/gringlobal/search.aspx? To perform a complex search follow the “Advanced Search Criterion” link found on the simple search page. Contact the NSL at 478-751-3552 for information on entering material into GRIN-Global or assistance in searching the GRIN-Global database.

Accession Information

As of December 31, 2016, the NSL long term seed storage program list 7,600 accessions, 37 genera, 107 taxa of 103 species. The full list of the accessions can be viewed at https://npgsweb.ars-grin.gov/gringlobal/site.aspx?id=32. Figure 1 shows five at-risk genera preserved in the program, and for each genus, its proportion of the total.

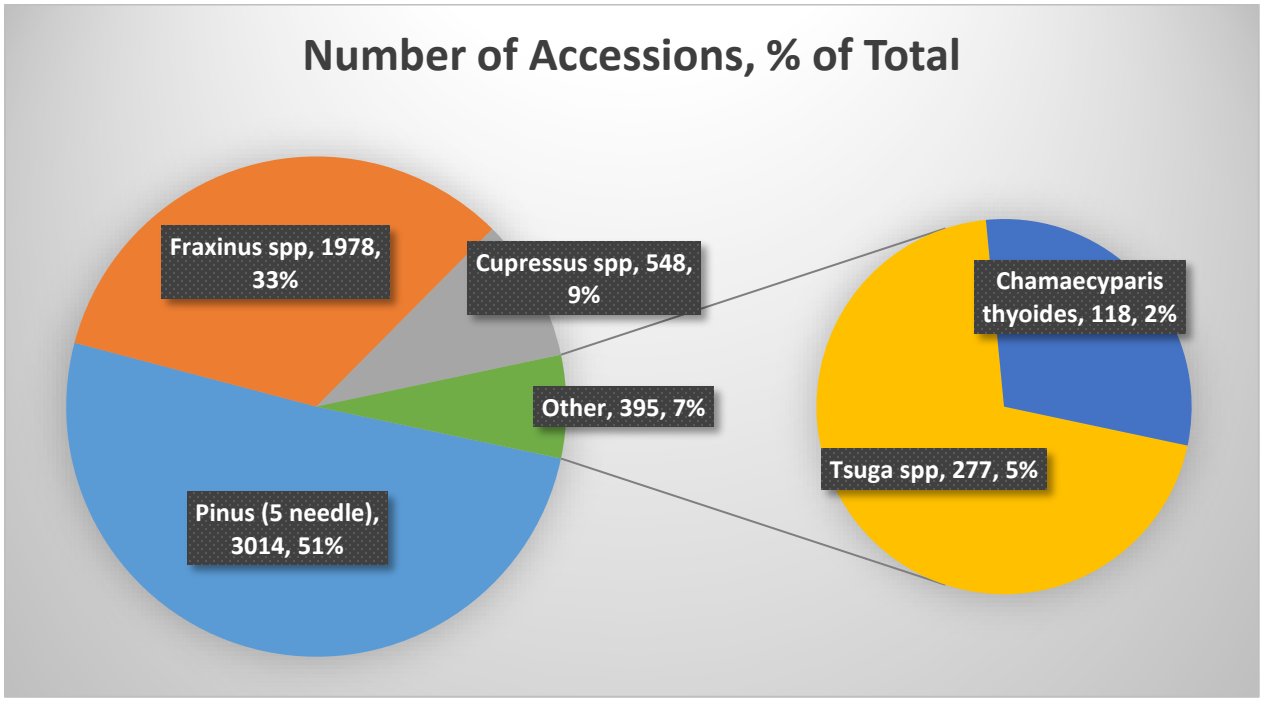

Figure 1—The five most collected genera in the National Seed Laboratory long term seed storage program.

The total number of tree accessions is 7437. The herbaceous accessions number 163.A tabulation of tree species and the number of accessions in the NSL long term seed storage program follows:

Species	Accessions
Pinus albicaulis	1032
Fraxinus americana	838
Fraxinus pennsylvanica	759
Pinus lambertiana	732
Pinus longaeva	441
Pinus sylvestris	441
Pinus flexilis	429
Pinus ponderosa	405
Pinus pungens	290
Fraxinus nigra	276
Tsuga canadensis	222
Pinus monticola	148
Pinus strobiformis	147
Chamaecyparis thyoides	118
Cupressus macnabiana	104
Pinus balfouriana	85
Fraxinus profunda	80
Juniperus scopulorum	74
Cupressus sargentii	71
Cupressus abramsiana	70
Pinus cembra	66
Cupressus guadalupensis var. *forbesii*	60
Tsuga caroliniana	55
Pinus palustris	33
Pinus echinata	30
Cupressus arizonica var. *nevadensis*	29
Fraxinus quadrangulata	25
Cupressus pigmaea	21
Pinus taeda	18
Juniperus virginiana	16
Pinus clausa	10
Pinus rigida	10
Pinus resinosa	9
Picea breweriana	8
Pinus nigra	8
Pinus peuce	8
Cupressus abramsiana subsp. *butanoensis*	6
Pinus parviflora	6
Robinia pseudoacacia	6
Pinus elliottii	5
Pinus strobus	5
Fraxinus spp.	4
Ulmus pumila	4
Juniperus spp.	3
Pinus densiflora	3
Pinus pumila	3
Pinus virginiana	3
Juniperus communis	2
Juniperus oxycedrus	2
Pinus ayacahuite	2
Pinus hartwegii	2
Pinus nigra subsp. *Laricio*	2
Pinus nigra subsp. *Pallasiana*	2
Juniperus macrocarpa	1
Juniperus phoenicea	1
Pinus arizonica var. *cooperi*	1
Pinus douglasiana	1
Pinus engelmannii	1
Pinus greggii	1
Pinus nigra subsp. *nigra*	1
Pinus patula	1
Pinus pseudostrobus	1
Pinus roxburghii	1
Pinus tabuliformis	1
Pinus teocote	1
Taxodium distichum	1

Conclusion

In the 11 years since the establishment of the NSL long term seed storage program, large numbers of seed lots have been placed into security backup and recorded in the GRIN-Global for centralized and accurate communication of the progress. The database is well-suited to the needs of the NSL and it is well maintained. Some distributions, to researchers, have already been made. The centralized nature of GRIN-Global has made data on conservation efforts very easy to share among USDA FS offices and users.

Saving Green Ash[1]

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Abstract

The emerald ash borer (EAB, *Agrilus planipennis*) continues to kill ash trees in North America at an alarmingly fast pace. Although EAB is a threat to all species of ash (*Fraxinus*) in the United States, green ash (*F. pennsylvanica*) is among the most susceptible. Among the most commonly planted landscape trees in the United States, green ash is also an important species in riparian forests, rural agricultural systems and urban woodlands. Within 4 to 6 years from the time of first detection, the damage caused by EAB larval feeding can kill 100 percent of the green ash trees in a stand. Green ash is genetically incompatible with EAB-resistant Asian ash species, ruling out a simple backcrossing program to transfer resistance from species. However, a small number of green ash trees (~0.05 percent) have survived long term EAB attack. Careful testing of grafted clones of these "lingering ash" selections provides compelling evidence of a defensive response against EAB. The defenses lingering ash trees possess enable longer survival, but do not prevent premature death. Individual lingering ash trees employ different types of defense responses. Combining these defenses through breeding is expected to produce progeny that combat EAB more effectively than the original parent trees and presumably allow for long-term survival. However, if we do not act now to prevent the death of lingering ash, we will risk losing this invaluable genetic variation forever. An interdisciplinary strategy that combines long-term monitoring to identify lingering ash, wise application of genomic tools, and an EAB resistance breeding program will rescue an irreplaceable genetic resource and provide an accelerated route to the restoration of this important species.

Introduction

Emerald ash borer (EAB, *Agrilus planipennis*) poses an acute threat to the *Fraxinus* species in North America (Herms and McCullough 2014). Green ash (F. *pennsylvanica*) and white ash (*F. americana*), attractive and fast-growing, were widely planted in urban forests and suburban landscapes to replace the American elm, *Ulmus americana* (Poland and McCullough 2006). Native *Fraxinus* species are widely used for shelterbelts in northern climates and for riparian buffer zones. Although EAB attacks all the major species of ash in the United States and Canada, green ash appears to be more susceptible (Anulewicz et al. 2007). The EAB was first detected in the region around Detroit, Michigan and Windsor, Ontario in 2002 and has since spread to 27 states in the United States and three Canadian provinces (http://www.emeraldashborer.info/). EAB kills 99 to 100 percent of green ash trees in forest stands within 4 to 7 years of first detection and kills urban green ash plantings as fast or faster (Knight et al. 2013, Kooster et al. 2014). The near synchronous loss of green ash across broad areas is having a cascade of negative impacts, including direct financial losses to industry, billions of dollars in tree removal cost to local governments, and the rapid loss of naturally occurring riparian forests comprised mainly of green ash (Gandhi and Herms 2010a, 2010b; Hausman et al. 2010, Knight et al. 2013, Kovacs et al. 2010). Without effective and timely intervention, EAB invasion threatens the survival of one of the most widely distributed hardwood in the riparian forests of eastern North America.

Accidentally imported exotic pests and diseases have adversely affected a host of native forest trees over the last several hundred years. In the case of the EAB, doing nothing will mean that green ash will likely become extinct over a large part of a vast native range. We propose an approach that implements a breeding plan based on intensive phenotyping, rapid deployment of improved trees, and wise use of genomics tools. This approach will help conserve the rapidly disappearing green ash gene pool, provide

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source materials for early testing, and provide the basis for breeding for resistance. An approach like this can be successful, as evidenced by the white pine blister rust breeding program in the northwestern United States (Liu et al. 2016; Sniezko et al. 2014, 2016), and many others programs worldwide (FAO 2015).

A Consideration of Strategies

Containment and Control Are Not Sufficient

The response to EAB initially focused on eradication, prevention of further dispersal, and diminishing EAB pressure through insecticides and biocontrol (Poland and McCullough 2006). As containment efforts around the point of introduction in Michigan were defeated, management strategy shifted from extirpation to slowing ash mortality through the reduction of EAB populations using selective removals of ash trees, insecticide treatments, and other containment strategies (McCullough et al. 2015, Mercader et al. 2016, Mercader et al. 2011). Containment strategies may spread out financial impacts, but do not change the outcome or restore ash to native ecosystems.

Currently four Asian parasitoids are being released in the United States in an attempt to control EAB population levels. Although studies show successful establishment of these parasitoids in some areas, this approach alone will not save green ash. The release range of one parasitoid is limited by its lack of cold tolerance (Duan et al. 2010). Another parasitoid is limited to attacking larvae only in young ash trees due to its inability to oviposit on thick barked trees (Abell et al. 2012). Recent studies report parasitism rates in the United States similar to those reported on North American ash species growing in Asia (Duan et al. 2015, Liu et al. 2007). Despite the high level of parasitism of EAB in Asia, North American ash species in Asia still experience mortality due to EAB infestation. Biocontrol alone will not provide a solution in the absence of host resistance (Duan et al. 2015, Duan et al. 2012, Herms and McCullough 2014). However, increasing population level of EAB resistance in green ash, in conjunction with biocontrol of EAB populations, could allow the establishment of a new equilibrium that ensures the survival of North American ash. Implementation of a resistance breeding program can achieve this by rescueing the ash gene pool before it is lost.

Technology in Absense of Breeding Program will Not Save Ash

Many people assume that cutting edge genetic technology will be the key to saving green ash. While high throughput genotyping has spurred the development of insect-resistant high-value row crops, this success is due to heavy reliance on long-term traditional breeding programs, made more efficient with high throughput genotyping, high throughput phenotyping, and better statistical tools. In the absence of a breeding program that will produce well-characterized phenotypes, appropriate germplasm, and replicated testing, the latest technologies will not save the ash of North America.

Evidence That Natural Resistance Exists

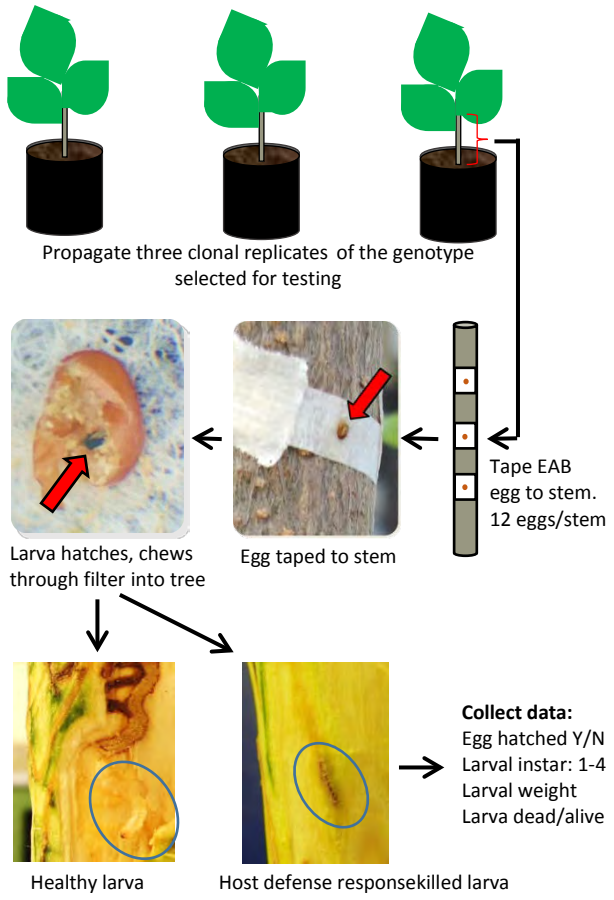
Certain species such as Manchurian ash (*F. mandshurica*), tolerate EAB without sustaining major damage. This immediately suggests employing the classic plant hybrid breeding strategy of crossing with a resistant species. Unfortunately this is not feasible because green ash and Manchurian ash are genetically incompatible. However, there is another approach that shows promise.

A small number of green ash trees (<1 perecent) survive EAB attack many years longer than nearby trees of the same species. These ‘lingering ash’ show evidence of less severe EAB infestation, often accompanied by evidence of vigorous wound healing, and maintain a healthy crown for years after local conspecifics have died (Knight et al. 2013). Knight and her colleagues (Klooster et al. 2014) identified trees in or near a subset of permanent plots established in southeastern Michigan and northeastern Ohio based on two criteria: 1) a healthy canopy 2 years after the mortality rate of the stand exceeded 95 percent, and 2) a minimum diameter at breast height (DBH, 1.37 m from the ground) of 10 cm, indicating they were over the minimum size preferred by EAB (>3 cm), when the infestation was at peak levels (Wei et al. 2007). Some of these plots continue to be monitored yearly (Knight et al. 2012). In another

study, a helicopter fly-over was done in natural areas just outside the core infestation zone to identify ash trees that were still alive (Marshall et al. 2013). The criteria for selection of these trees were a healthy canopy and a minimum DBH of 24 cm, to exclude the possibility of trees that may have re-sprouted after the main bole was killed. The status of these trees was verified by examination from the ground.

EAB egg bioassays (fig. 1) were done on grafted replicates of four 'lingering green ash' genotypes along with one susceptible wild green ash tree (PE-36) and the susceptible green ash cultivar 'Summit'. An EAB-resistant Manchurian ash was included as well. Dissections were performed to determine the outcome for each of the 12 eggs placed on each grafted plant, whether larvae lived or died and if they died how far they developed (larval instar L1, L2, L3, or L4). Larval outcome was modeled as a multinomial response using a general linearized mixed model. Differences among ash genotypes were significant ($p = 0.0002$). When larvae killed by host defense response were modeled as an outcome, one lingering ash clone (PE-L19) killed significantly more larvae ($p < 0.0001$) than the susceptible control 'Summit'. When larval weight was modeled as an outcome, the larvae in the clone PE-L22 had significantly lower larval weights ($p = 0.0163$) than the susceptible control. These initial results suggested that lingering ash employ different mechanisms to enable longer survival in the field (Koch et al. 2015). Although lingering green ash genotypes permitted more EAB larval development in additional egg bioassay experiments than the resistant Manchurian ash, some lingering ash clones consistently killed more early instar larvae (35 to 50 percent) than the susceptible green ash controls (0 to 10 percent). While lingering ash trees are clearly not as resistant as Manchurian ash, they do possess a partial resistance that permits longer survival. Given this preliminary evidence for multiple mechanisms, lingering ash (once EAB phenotypes have been confirmed), could form the basis of a breeding program for 'stacking' or pyramiding the multiple allelic variants that may be responsible for the multiple mechanisms of partial resistance. Phenotypic confirmation is essential, as past work has shown that ~20 percent of lingering ash identified in a forest setting show no evidence of partial resistance when clonal replicates are tested by egg bioassay. Other mechanisms of resistance have been shown to exist (e.g., EAB prefer to avoid some trees) so these clones are retained for later tests (Koch et al. 2015, Peterson et al. 2015). Preserving lingering ash by grafting and confirming the EAB phenotype rescues the genes and gene variants that confer these defenses before they are permanently lost. Conservation of this valuable resource is a major step towards the long term goal of producing trees that can withstand EAB long enough to maintain populations in a forest setting.

Figure 1—Egg bioassay protocol. The grafted genotype is preserved in untreated grafted clones.



There are other examples of infrequently occurring individuals of native tree species having genetic resistance to non-native invasive insects. Some American beech trees (*Fagus grandifolia*) are resistant to the nonnative beech scale insect (*Cryptococcus fagisuga*). The insect creates entry wounds permitting

infection with *Neonectria* spp. This insect-disease complex results in beech bark disease. After Koch and colleagues demonstrated that scale resistance is heritable (Koch et al. 2010), scale-resistant clones were planted in regional seed orchards to enable beech restoration of state and national forests severely impacted by this disease. Other investigators have recently shown that some eastern and Carolina hemlocks (*Tsuga canadensis* and *T. caroliniana*) have resistance to the non-native hemlock wooly adelgid (*Adelges tsugae*) (Oten et al. 2014).

A Strategy to Save Green Ash

We propose a breeding plan based on intensive phenotyping to identify the best lingering ash trees, establishing orchards planted with polycrossed progeny (resulting from many different combinations of the best lingering ash trees as parents), managing such orchards to encourage early flowering, and using genomic tools to serve the program, rather than drive it. This plan will save the lingering ash gene pool, establish a sound breeding program, insure genetic diversity, and lay the foundation for functional genomics studies. The plan we outline below could be fully implemented in the same amount of time (8 to 10 years) it would take for the development of a full set of genomics tools for green ash (sequenced and assembled genome, deep transcriptome and functional annotation). A 'genomics only' approach would not deliver breeding populations, would not provide immediately useful information in the absence of such populations, and would fail to rescue the existing lingering green ash gene pool. We propose an integrated strategy that included functional genomics as well as traditional breeding. We argue that a breeding program based on the phenotyping approach outlined here (visual identification of "candidate" lingering ash followed by confirmation with the egg bioassay) can proceed without any genomics other than the development of DNA "passports" that uniquely identify each individual. A genomics approach alone produces nothing that saves the lingering ash gene pool.

Goals, Strategies, and Objectives

The proximate goal of our plan is to save and characterize the lingering ash gene pool. The ultimate goal is to save green ash. The strategy is to 1) save naturally occurring resistance alleles from extinction by finding lingering ash trees, grafting them and planting them in containers for testing and breeding; 2) combine the unique alleles of each lingering ash selection by using them to produce progeny in controlled cross pollinations with at least two other lingering ash parents; establish the sets of progeny from each of these lingering ash families in a "polycross" seed orchard where they can naturally cross pollinate with members of many different families, producing many different combinations of alleles, and 3) maintain high genetic diversity within these polycross populations and manage for early flowering. We have four objectives:

1. Using container-grown lingering ash parents, generate at least two different full sibling families per parent and establish these progeny in a planting as a polycross population.
2. Confirm our preliminary result supporting the hypothesis that cross-pollination between parents with different lingering phenotypes produces some progeny with EAB defensive responses more effective than either parent.
3. Generate scalable, transferrable DNA passports for all lingering ash germplasm in the project.
4. Develop regional programs to identify additional lingering ash parents and establish polycross orchards.

A Testable Hypothesis

If there are multiple genes with allelic variants that act additively to inhibit the growth of or kill EAB, then individuals having partial resistance could have some, but not all, of the "optimum" variants that contribute to resistance (fig. 2). If this is true, then among a large number of full sib progeny of two individuals having presumably different mechanisms of partial resistance, there are likely to be some individuals who have higher levels of resistance than either parent and some individuals who have lower levels than either parent. These transgressive phenotypes, if verified in independent tests of clonal

replicates, would support the hypothesis of multiple genes with allelic variants that act additively to inhibit the growth of or kill EAB. Intercrossing or polycrossing lingering ash would 'stack the deck' to produce some individuals capable of more effective defensive responses than their parents.

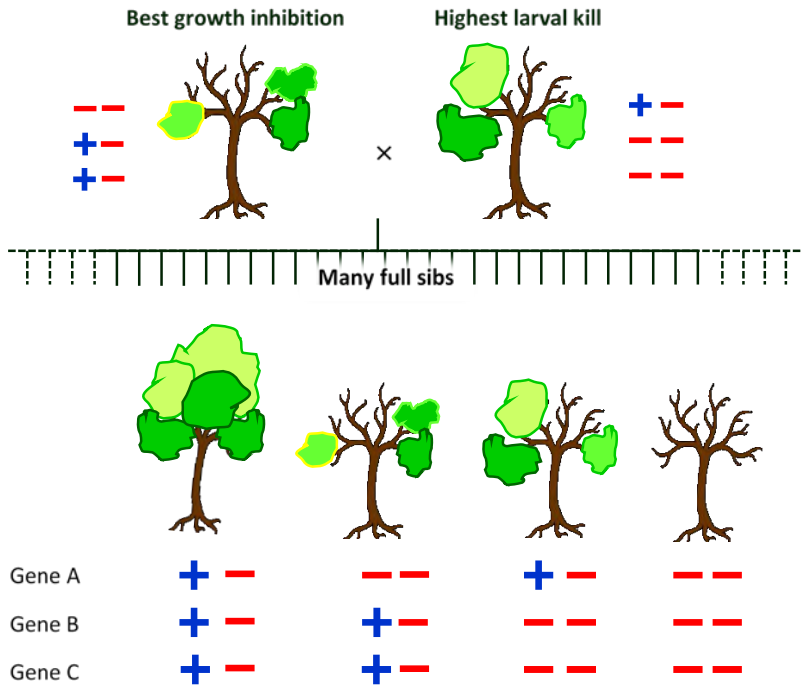


Figure 2—Transgressive phenotypes, as detected by replicated egg bioassays, indicate allelic variants at multiple genes. Highly simplified cartoon with progeny drawn as if they were mature trees.

The Next Step

The next step in our plan is to remove individuals from the polycross population who have lower levels of resistance than their parents, and allow the remaining trees with the best defensive responses to intercross with each other.

Seedlings produced by the intercross and polycrosses can be outplanted for early testing in EAB-infested areas. As polycross orchards mature, seed could be distributed to cooperators. Many of these seeds will have little EAB resistance, but some will have much higher levels of resistance than the original parent populations. This approach does not involve marker-assisted selection (MAS) or genome-wide association (GWAS). MAS and GWAS are premature at this stage of a breeding project and may be ineffective in this system. Functional studies do have value, but only if identification of superior phenotypes has been done in replicated tests that include susceptible controls. Indirect selection using DNA markers works only in well-characterized systems, where the variation in the phenotype due to environmental influences is well understood (Bian et al. 2014, Muranty et al. 2014). Genomic selection, using a high density collection of markers distributed across the genome also works, but only in those systems where 1) dense genotyping tools exist for the species of interest, 2) the phenotype is thoroughly characterized and accurately measured, 3) extremely large pedigrees exist, 4) a long term breeding program exists, and 5) funding is available to back the effort over the long term (Isik 2014).

Transferrable and Scalable Marker Systems

The cost-effective integration of a tree breeding program with wise use of DNA markers requires a marker system that has two features that most high-throughput genotyping approaches lack: scalability and transferability. Our proposed breeding program will have a DNA- passport (a genetic fingerprint) for every individual, to insure that the relationship of phenotypic data to genotypic data is not compromised. These specific sets of sequences need to be scalable so that they can be generated again and again, for any number of trees, from one to thousands. In species where interspecific hybridization occurs naturally, the markers need to be transferrable, i.e., likely to be informative across species within sections and even across all the species in a genus. This will enable leverage of the information gained in one species to other species within the genus and in the case of using markers from expressed genes, may provide relevant functional information. Previous work has shown that the informative EST-SSRs we developed

from the green ash transcriptome are informative in other Meloides in the *Fraxinus* genus and most are informative in sections *Ornus* and *Fraxinus* as well (Noakes et al. 2014). For our plan, these markers have a dual purpose: to enable monitoring of genetic diversity in polycross orchards to limit narrowing of the genetic base and to serve as DNA passports.

A DNA-based genetic passport would consist of those sequences that capture the most polymorphism with the least number of sequences while at the same time uniquely identifying every individual in the project. A DNA passport must be easily verifiable. The verification technology must be scalable (i.e., a passport can be generated for only one tree at one time, or for many trees, as needed). A DNA passport must also be designed so that any instrument capable of DNA sequencing can generate it, i.e., platform-independent. The methods most frequently employed for sequencing or genotyping do not meet both of these requirements. Reduced representation sequencing (RRS) technologies, high-throughput exome or whole genome sequencing and SNP chips enable discovery of unique DNA-based fingerprints, but lack scalability. Genomic SSR and EST-SSR markers, amplified a few at a time then size fractionated using capillary electrophoresis, are highly scalable and in the case of EST-SSR markers, highly transferrable (Noakes et al. 2014). However, genotyping is slow and labor-intensive. Amplicon size estimation, while highly accurate within the same technology platform, can vary across platforms.

A targeted sequencing approach (capture by hybridization or bait-capture) has the potential to meet both requirements. Bait-capture using microsatellite-containing EST sequences is scalable, transferrable, and platform independent, in that each tree has a fingerprint consisting of a set of sequences, any or all of which may be generated from any number of trees using the sequencing technology of choice. Capture by hybridization (Sun et al. 2013, Zhou and Holliday 2012) in which *Fraxinus* EST-SSR sequences are the baits is a promising approach. The *Fraxinus* genus has two publically available transcriptomes: the *F. pennsylvanica* transcriptome (http://www.hardwoodgenomics.org/node/68249), and the *F. excelsior* transcriptome (http://www.ashgenome.org/transcriptomes). However, other approaches that are just as good or better, and cheaper, will almost certainly be available soon. A caveat is that the expense of generating a DNA passport in not limited to the cost of sequencing, which is almost trivial. The expense lies in the DNA library prep kits and the salary cost for entering data into a usable, secure, and easily retrievable form.

Collaboration, Cooperation and Partnership

Green ash is the most wide-ranging hardwood tree in eastern North America. A serious effort to save green ash and the other North American *Fraxinus* species will require regional cooperation. Confirming that defensive responses of some of the progeny in a first generation cross are superior to either parent, without destroying these genotypes (which is technically possible, but requires space for many grafted clones), will help attract funding and collaborators.. The program we envision will be amenable to a participatory breeding approach with partners that may include private citizens, state and federal agencies, and nonprofit organizations.

Conclusion

The ultimate goal of this plan is restoration of a green ash resource that can survive long enough to ensure self-sustaining populations with minimal loss of genetic diversity in the riparian forest ecosystems of eastern North America. The plan we have outlined here is dependent on securing funding to support a sustained effort that may take 3 or more years before the first results are analyzed and published. Involving the public gardens in this effort could greatly assist in garnering public support for a longer term project. Almost everyone who lives in the suburbs or a city in the upper Midwest has seen dying ash trees as a result of EAB. The partnership of The American Public Gardens Association with the U.S. Department of Agriculture Forest Service includes provisions for conservation education programs. “Saving green ash” could become a part of those programs.

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USDA Forest Service Southern Region – It's All About GRITS[1]

Barbara S. Crane[2] and Kevin M. Potter[3]

Genetic resource management programs across the U.S. Department of Agriculture Forest Service (USDA FS) play a key role in supporting successful land management activities. The programs are responsible for developing and providing plant material for revegetation, seed management guidelines, emergency fire recovery assistance, genetic conservation strategies, climate change guidance, and partnership opportunities. The primary objective of the USDA FS genetics programs is to provide the genetically most appropriate plant material to support diverse, sustainable and resilient forests. These key concepts are captured in the USDA FS National Genetics Strategic Plan (Forest Service internal document, 2004; unpublished). The Southern Region (R8) National Forest System (NFS) Genetic Resource Management Program (GRMP) is engaged in following these concepts. By working collaboratively with the national forests and other partners, the GRMP is a leader in meeting current and future needs. By developing and integrating new ideas into our management strategies, the Genetic Resources In TranSition (GRITS) philosophy succeeds in supporting future healthy forests.

The Southern Region is home to some of the most biodiverse forests in the United States. Over 140 tree species occupy coastal, piedmont and mountain landscapes. The southern ecosystems are increasingly vulnerable to forest health issues and climate change impacts. For example, over 30 species of pests and disease pathogens affect forests in R8. Our forest landscapes are changing, so monitoring the state of our tree species is critical. Furthermore, we need to plan for healthy and productive forest structure and forest composition in 100 years. Though locally adapted and regionally appropriate seed sources are meeting current goals, will the same sources be adapted in 100 years? How to match species to future sites affected by climate change (e.g., fire, drought, excess moisture) requires careful considerations. Seed zones need to be revised, similar to the updated USDA Plant Hardiness Zone Map. Planting in the right areas, for now and for the future, will support resilient forests. For those species currently imperiled, or residing in vulnerable areas (e.g., high elevation or low coastal), assisted migration may be an option for their conservation. The bottom-line: trees will adapt, migrate or die, so actions are necessary to take to preserve species diversity across the landscapes, promote adaptation and support future forest resiliency.

To manage multiple issues and multiple tree species across diverse landscapes, and to continue to be effective in supporting successful reforestation and restoration, a prioritization strategy had to be developed. In 2010, the Eastern Forest Environmental Threats Assessment Center (EFETAC) and R8 GRMP developed an assessment tool entitled "Forest Tree Genetic Risk Assessment System" (FORGRAS) (Potter and Crane 2010). This assessment system is a flexible framework, utilizing factors such as life history trait data, climate change and pest and pathogen threat information, to categorize, rank and prioritize our many tree species for conservation, monitoring, management and restoration. The assessment factors included intrinsic attributes *(*e.g., population structure, fecundity, seed dispersal ability, crop frequency, range, density, rarity, regeneration capacity); external threats to genetic integrity (e.g., pest, diseases, loss of habitat, fragmentation, drought); and conservation factors (e.g., evolutionary distinctiveness, regional responsibility).

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FORGRAS has identified at least 12 tree species that are currently imperiled. The R8 GRMP has increased tree conservation efforts, engaged more external partners, and initiated more seed collections for a variety of these species. The focus was initially on glacial refugia in the Southern Appalachian Mountains, but we have expanded our efforts to encompass other ecoregions where tree species are threatened. Currently, we are working with Carolina and eastern hemlocks, several pines (Table Mountain, pitch, longleaf, shortleaf), red spruce, Atlantic white cedar, balsam and Fraser firs, American chestnut, Ozark chinquapin, butternut, oaks (Boynton, Oglethorpe, Maple-leaf, Arkansas, Georgia, Florida, Lacey) and ash (Texas, blue, pumpkin, Carolina).

Partnerships are critical to R8, and we work with numerous internal and external groups. All agencies and organizations have limited resources, so collaborations facilitate achieving multiple goals. Our internal partners include Southern Research Station units, National Genetics Lab (NFGEL), National Seed Lab, Forest Health Protection, and National Forest units. Our primary external partner is the Central America and Mexico Coniferous Resources Cooperative (CAMCORE), North Carolina State University. Others include the Longleaf Alliance, Shortleaf Initiative, Atlantic White Cedar group, Southern Appalachian Red Spruce Initiative, The American Chestnut and the Ozark Chinquapin Foundations, American Public Gardens Association members, universities, tree improvement and nursery cooperatives, and state and private nurseries.

Safeguarding and maintaining the genetic resources and genetic variation across multiple species will require tailoring of conservation, management, monitoring and restoration measures for each. Strategies and guidance are in development or being implemented in R8, including (1) developing new or updated seed zones, (2) establishing *in situ* and *ex situ* seed production areas, (3) mixing seed lots to match updated or new seed zones, (4) designing new planting range maps and (5) writing field protocols on how to establish living tree conservation banks and restoration tree seed reserves (Echt et al. 2011) within seed orchards or in general forested areas on the national forests. R8 GRMP seed orchards have already begun work on establishing living tree conservation banks using the imperiled tree species seed collections.

In summary, a variety of threats, most importantly climate change and insect and disease infestations, will increase the likelihood that forest tree species could experience population-level extirpation or species-level extinction during the next century. Region 8's FORGRAS tool has provided a list of species to target for monitoring efforts and for proactive gene conservation and management activities. GRITS is essential to support and maintain healthy, sustainable, resilient and productive vegetation on the National Forests, for now and into the future.

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Somatic Embryogenesis and Cryostorage for Conservation and Restoration of Threatened Forest Trees[1]

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Threats to North American forest trees from exotic pests and pathogens or habitat loss, make it imperative that every available tool be employed for conservation and restoration of these at risk species. One such tool, *in vitro* propagation, could greatly enhance conservation of forest tree genetic material and selection and breeding of resistant or tolerant genotypes for restoration. *In vitro* propagation approaches include standard micropropagation (axillary shoot multiplication), organogenesis (adventitious shoot production) and somatic embryogenesis (SE), a process by which structures (somatic embryos) resembling seed embryos are produced asexually. The SE systems, in particular, are well-suited for conservation and restoration purposes, due to the high multiplication rates and the amenability of embryogenic cultures to cryostorage. Examples of threatened forest species for which we have developed SE systems that are already being applied for conservation and restoration efforts include American chestnut (*Castanea dentata*), eastern and Carolina hemlocks (*Tsuga canadensis* and *T. caroliniana*), Atlantic white cedar (AWC, *Chamaecyparis thyoides*), and green and white ash (*Fraxinus pennsylvanica* and *F. americana*). We have also developed an adventitious shoot-based propagation system for Franklinia (*Franklinia alatamaha*), which has been extinct in nature for over 200 years.

American chestnut, once one of the most important trees in eastern North America, was devastated by chestnut blight, caused by *Cryphonectria parasitica*, which was accidentally introduced from Asia in the late 1800s. Breeders in the American Chestnut Cooperators Foundation (ACCF) have been crossing large surviving American (LSA) chestnut trees to produce progeny with enhanced resistance, while The American Chestnut Foundation's (TACF) breeding program has focused on a hybrid backcross breeding program to introgress genes from the blight resistant Chinese chestnut (*C. mollissima*) into the American chestnut background. Over the past 25 years, we have developed an embryogenic culture system for American chestnut using immature seeds as explants (fig. 1A). Employing a suspension culture-based system, the cultures can be scaled-up to produce hundreds of clonal somatic seedlings from a given culture line (Andrade and Merkle 2005). The cultures are also highly amenable to cryostorage and recovery (Holliday and Merkle 2000). In collaboration with ACCF, we have used SE to propagate germplasm derived from crosses between LSAs so that clones can be tested for blight resistance. The first somatic seedlings from these cultures were planted on National Forest land in Virginia starting in 2012 and are growing well. We have also collaborated with TACF to implement clonal testing of accessions from their breeding program for resistance to chestnut blight. Control-pollinated BC3F3 seeds from selected BC3F2 seed orchard parents were used to initiate embryogenic cultures from which chestnut somatic seedlings have been produced for field testing (Holtz et al. 2017, Merkle et al. 2013). Copies of the BC3F3 cultures will be held in cryostorage until the clones with the best field performance are identified. These clones can then be recovered from cryostorage and scaled-up for production of planting stock. For restoration purposes, multiple, chestnut clones of different parentage will probably need to be developed to ensure genetic diversity and adaptation to regional conditions. Elite clones showing the fastest growth rates and/or superior wood quality, in addition to disease resistance, may someday be

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deployed by landowners for timber production. We have also applied our chestnut SE protocol to produce the first embryogenic cultures of Ozark chinquapin (*Castanea pumila* var. *ozarkensis*), which like the American chestnut, has been severely affected by chestnut blight (Merkle et al. 2017).

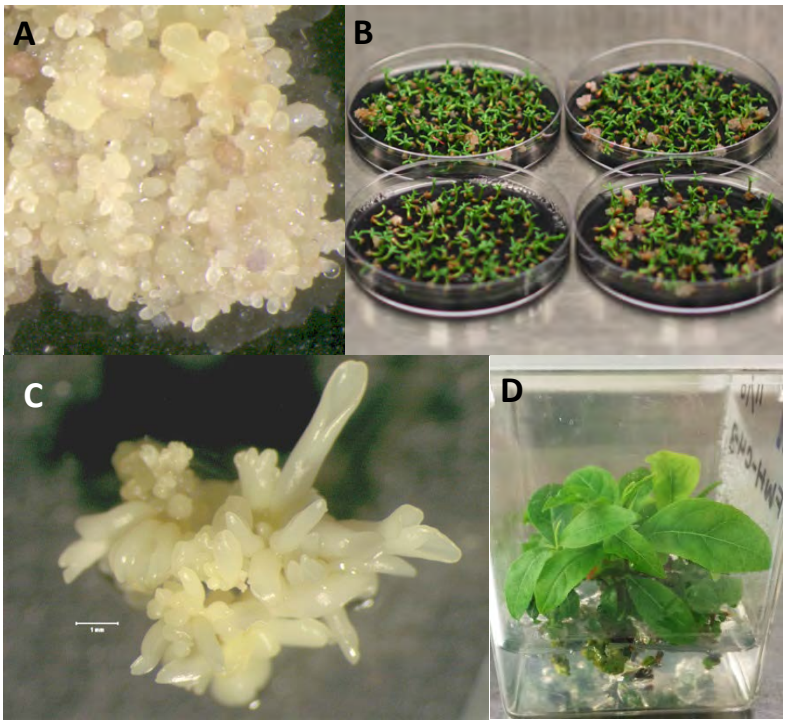

Figure 1—*In vitro* propagation of threatened and rare forest trees. A. American chestnut somatic embryos. B. Germinating Carolina hemlock somatic embryos. C. White ash somatic embryos. D. Franklinia shoot proliferation.

Both eastern hemlock and Carolina hemlock have suffered huge losses from hemlock woolly adelgid (HWA, *Adelges tsugae*) attacks, and genetic diversity is being lost in these species. While seeds of many forest tree species are amenable to long-term storage, this has not been demonstrated with seeds of hemlocks, different seed lots of which showed highly variable retention of viability when refrigerated for 2 to 4 years (Olson et al. 1959). Primarily as a means of conserving hemlock germplasm, we developed embryogenic and cryostorage systems for these two species by applying standard protocols that were successfully applied to other Pinaceae species (Merkle et al. 2014). More recently, we worked with breeders at North Carolina State University and the Forest Restoration Alliance (FRA) to apply SE to clonally propagate hybrids between Carolina hemlock and HWA-resistant Asian hemlock species and putatively HWA-resistant eastern hemlocks, with the goal of producing HWA-resistant hybrid hemlocks. Starting with immature seeds derived from hybrid crosses, we have produced multiple embryogenic cultures of hybrids between Carolina hemlock and Chinese hemlock and between Carolina hemlock and southern Japanese hemlock (*T. sieboldii*). Somatic seedlings have been regenerated from the cultures (fig. 1B) and transferred to FRA collaborators to grow up for eventual resistance screening. Some putatively HWA-resistant eastern hemlock individuals have been identified in the past few years. Working with collaborators from the New Jersey Department of Agriculture and the University of Rhode Island, we recently initiated cultures from seeds collected from one such tree growing in the "bulletproof" stand in New Jersey, so-called because the hemlocks in the stand have survived HWA infestation while surrounding hemlocks have all succumbed. The first somatic seedlings from these cultures should be produced soon.

While AWC populations in the eastern United States have not been attacked by exotic pests or pathogens, they have suffered dramatic declines due to over-harvesting, fire suppression, hydrologic alteration, and conversion of coastal bogs to agriculture and development. To aid in restoration efforts, we

have collaborated with Camcore personnel to develop a SE-based propagation system. Recently, using seeds collected by a Camcore collaborator in North Carolina, as well as trees planted on the University of Georgia, Athens campus, we produced the first AWC embryogenic cultures and showed that these cultures can be cryostored and recovered (Ahn et al. 2016). To date, a small number of AWC somatic seedlings have been produced, acclimatized and grown in the greenhouse, but as of yet, there are no plans to expand production of trees for field testing.

The valuable landscape and wood products trees white ash and green ash are under threat of extirpation from their native ranges by the emerald ash borer (EAB, *Agrilus planipennis*), an exotic wood-boring beetle that has already destroyed millions of ash trees in 15 states and Canada. Similar to the case with eastern hemlock, putative EAB-resistant ash individuals have been identified in native populations. Since these trees are found in areas where over 90 percent of the ash trees have been killed by EAB, they are called "lingering ash" trees. Applying a highly prolific SE system that we originally developed for green ash (Li et al. 2014), we are collaborating with Ohio State University scientists to initiate embryogenic cultures (fig. 1C) from seeds collected from multiple "lingering" white ash trees in Michigan. The first somatic seedlings from some of these "lingering ash" cultures have been produced and have grown rapidly in the greenhouse and shade house. We hope these will reach sufficient diameter in the next few years to be screened for EAB resistance, and may become the basis for new EAB-resistant varieties to be planted by landowners.

Franklinia, a member of the tea family discovered growing in a single population in Georgia by John and William Bartram in the 1700s, has not been seen in nature since 1803. Its reintroduction to the wild, or even as a landscape tree in the southeastern United States has been hindered by its extreme susceptibility to Phytophthora root rot (PRR), caused by *Phytophthora cinnamomi*, which is endemic throughout the southeast. As a first step to developing Franklinias that can survive PRR infection, either using mutagenesis or transgenics, we have developed an adventitious shoot-based regeneration system for the tree. Adventitious buds form on cultured immature zygotic embryos and rapidly elongate into shoots (fig. 1D) that are rooted in vitro and hardened off to greenhouse conditions.

All of the SE and other in vitro propagation systems we have developed for threatened or rare forest trees have benefitted greatly from collaboration with scientists in different organizations. Their continued development and eventual application similarly will rely on such collaborations. In fact, we contributed this paper to the Gene Conservation of Forest Trees meeting primarily in hopes of forging new collaborations with scientists working with threatened forest tree species. These systems are potentially very powerful conservation and restoration tools, but they are only of use when we have selectors, breeders, pathologists, entomologists, silviculturists and other scientists who will collaborate with us to test and apply the products of our work to addressing forest health challenges.

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TreeGenes and CartograTree: Enabling Visualization and Analysis in Forest Tree Genomics[1]

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Association studies integrating environmental, phenotypic, and genetic data are key in understanding forest tree resilience to climate change and disease. As genomic resources increase, both in terms of complete reference sequences and magnitude of individuals genotyped, researchers are better equipped to identify correlations between genetic variation and adaptive or commercial traits. Computational resources designed to integrate and distribute high quality genotypic, phenotypic, and environmental datasets remain insufficient for the task.

TreeGenes is a web-based resource dedicated to the forest tree community. The database hosts genetic data from >1700 tree species, collected from primary databases such as GenBank, genomes and transcriptomes from dozens of species, genetic maps and phenotypic/association study data submitted by users. Additionally, TreeGenes hosts custom-developed tools that allow researchers to make the most of our genetic offerings. Through the website, users can access a custom Laboratory Information Management System (LIMS), download bulk datasets from the database, and visualize genetic and genomic data via GMOD tools (e.g, CMAP, GBrowse).

CartograTree is a web-based application hosted through TreeGenes that allows researchers to identify, filter, compare, and visualize geo-referenced biotic and abiotic data. Its goal is to support numerous multi-disciplinary endeavors including phylogenetics, population structure, and association studies. These goals are supported and enabled through Simple Semantic Web Architecture and Protocol (SSWAP), which leverages high-performance computing and data storage. Development on CartograTree will expand the available datasets and analytical capabilities. The map interface will include new layers such as forest fragmentation and climate shift predictions. TreeGenes' upcoming transition to Tripal, a Chado- and Drupal-based web content management system, will allow access to more data through connections to the Hardwood Genomics Project and Genome Database for Roseaceae, and to powerful analytical pipelines and computational resources through the Tripal module connection to the Galaxy Project.

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Conservation Program Case Studies

The National Program for Long Term Seed Storage for Ash Germplasm Preservation[1]

R.P. Karrfalt[2]

Abstract

The U.S. Department of Agriculture Forest Service (USDA FS) began ash (*Fraxinus*) germplasm preservation in 2005, through seed collections for long term seed storage. The work was coordinated with the Agricultural Research Service (ARS). Collections have been accomplished through many cooperators. Various methods of outreach were deployed to solicit cooperators. No method stood out as better than another. However, the level of interest in genetic conservation held by the cooperator seemed much more important in determining if they chose to participate rather than how they were contacted. The most effective collectors were conservation professionals. Collections were made according to a plan written jointly by ARS, USDA FS, and Bureau of Land Management. About 4000 seed lots have been collected by the ARS and the USDA FS. Seed samples have been supplied to USDA FS research and to the Animal and Plant Health Emerald Ash Borer parasitoid rearing program.

Introduction

The Emerald ash borer (*Agrilus planipennis*) was first discovered in the United States in 2002 in the Detroit Michigan area. Initial response to the EAB focused on eradication and containment by the state of Michigan and federal agencies. In the course of a few years it became apparent that these efforts were not effective at containing the EAB. With the loss of ash (*Fraxinus*) in infested areas at almost 100 percent, it became imperative that conservation efforts be initiated to preserve genetic resources for any future restoration or resistance breeding work. Of the options available for conservation, seed collections seemed a good choice. Ash species are regular and abundant seed producers and there was good evidence that the seed would store for long periods in freezers. The storability of a seed is directly related to its desiccation tolerance and degree of dormancy. Nursery experience had demonstrated ash seeds to have moderate to strong dormancy, requiring at least 30 days of cold moist treatment and many cases 60 or 90 days of such treatment for germination to occur. Barton (1945) reported good storability of ash seeds when they were dried to 7 to 10 percent moisture content and kept at 5 ºC. All available evidence pointed to ash being a good candidate for long term seed storage.

Maintaining seed collections is also a much less expensive conservation option than methods such as a clone bank that require long term access to suitable land and regular annual maintenance such as controlling competing vegetation and protecting the trees from the borer. Seeds on the other hand are stored in existing seed storage freezers, and do not require annual attention. A viability test is necessary at some interval to know that the seed resource is able to produce seedlings. If decreases in viability are detected it becomes necessary to produce a replacement seed lot. The frequency of viability testing and when to regenerate the seed lot are decisions made by the curator of the seed collection.

Initially three federal agencies took an interest in the seed collections: the Natural Resource Conservation Service (NRCS) at Rose Lake Plant Material Center, the Agricultural Research Service (ARS) both at the Northern Woody Ornamental Collection at Ames. Iowa and the National Arboretum in Washington, DC, and the Forest Service (FS) National Seed Laboratory. Long term seed storage for genetic conservation was added to the National Seed Laboratory (NSL) mission in 2005. The three agencies had different initial approaches and resources for the collections, but within a few years a common protocol was adopted. When it became apparent that the seed collections would require range

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wide efforts and capacity to store seeds for decades the NRCS transferred their collections to the FS NSL and discontinued direct efforts to make collections. The Bureau of Land Management (BLM) also joined the effort in 2009 as they had opportunity to make collections through the Seeds of Success program as they collected other native plants from BLM lands.

Methods and Materials

Collection Locations/Numbers of Trees

Collections were focused initially on the areas being infested as this is where the resource was fast disappearing. Five ash species were in the immediate path of EAB: white ash (*Fraxinus americana*), green ash (*F. pennsylvnica*), black ash (*F. nigra*), blue ash (*F. quadrangulata*), and pumpkin ash (*F. profunda*). These five species of ash have very broad ranges, with white and green ash ranges covering much of the eastern United States. Making collections of every local population was not practical nor even necessary to capture most of the genetic resource. Therefore, a systematic and scientifically sound method was needed to divide the full range of a species into smaller areas, seed zones, from which seed collections would be made. A seed source trial had been conducted for both white and green ash, but these only gave general overviews of the genetic variation among populations and had not been developed into seed zones. As surrogates to seed zones, Omernik level III ecoregions were adopted. Seed was to be taken from 50 trees evenly spread over the ecoregion. These trees could either be at 50 individual locations or 10 locations with five trees each. This was a minimum and more trees could be included if convenient. A minimum of about 1.9 to 2.8 liters (2 to 3 quarts) of seeds was to be taken from each tree. White and green occur well dispersed over the landscape while the occurrence of blue, pumpkin, and black is more clustered. Therefore, white and green were more likely to be collected from 50 individual collection sites and seeds of the other three species were collected in small populations of five trees. The protocol stipulated that when seeds were collected from clusters of five trees, the five trees needed to be at least 30.5 m (100 ft) apart (to avoid collecting from trees too closely related), and not over 1.6 km (1 mile) apart (to put an upper limit on the size of a local population). These procedures were expected to provide seed collections containing at least 90 percent of the species' genetic variation. Trees were all to be naturally occurring trees and not planted landscape trees. A tree near a residence was acceptable as long as it could be determined that the tree was part of the natural vegetation. Ash, a pioneer species, is a frequent and successful invader of road sides and property boundaries. The full collection plan is found at http://nsl.fs.fed.us/Fraxinus_Germplasm_Preservation_Plan_March_2010.pdf.

Translating the plan into action required maps to indicate where to collect seeds. Maps were assembled using ArcGIS with layers for geography, ecoregions, and species ranges. Fifty dots were then manually placed evenly across each ecoregion that contained part of the species range. Each dot was then to be used as a general guide to a potential collection site. Not uncommonly, potential collections sites landed in the middle of a large agricultural field which meant the site had to be moved at least to the edge of the field. Because there was no information as to where an ash tree might actually be growing, the distance from the potential collection site to an ash tree was, in some cases, several km. However, there was enough distance among potential sites that collecting several km away from a potential collection site was easily accommodated. The GPS coordinates for each potential site were transferred to a spreadsheet from which collectors could identify proposed sites. Neither the ArcGIS maps nor the spreadsheet of coordinates were effect tools to communicate with cooperating seed collectors. The most easily understood and most universally applicable method of directing collectors to potential collection sites was to provide a list of towns that corresponded to GPS coordinates and instruct collectors to find a tree as close to that town as possible. Then either a paper road map or automobile GPS could be used to find the collection sites.

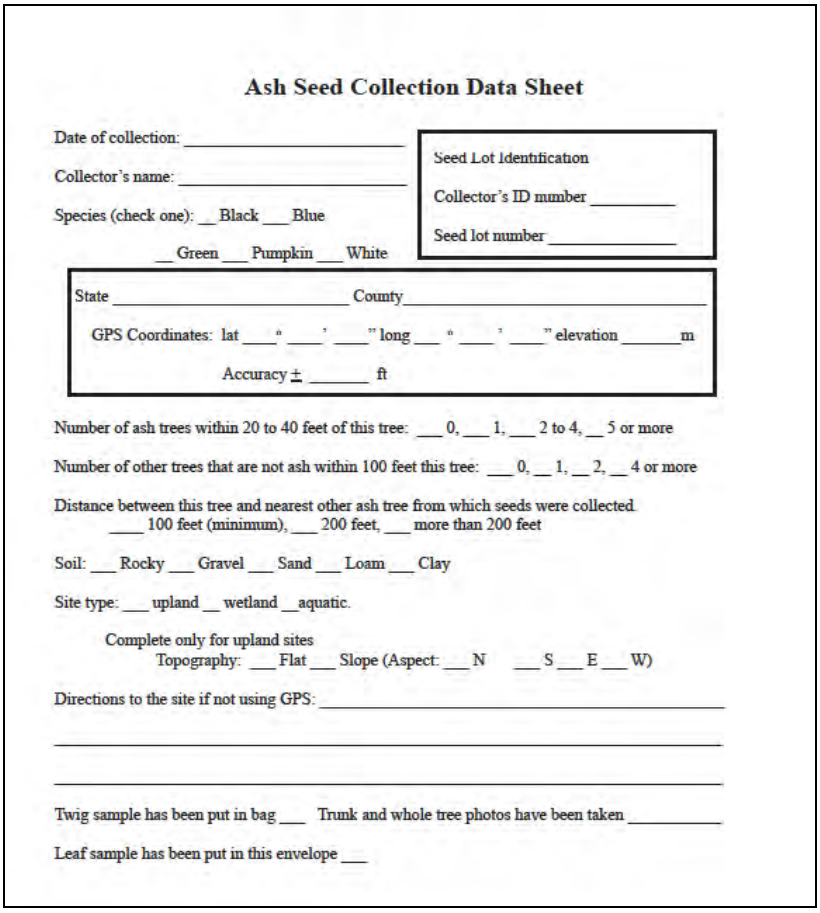
Access to Trees

Most land in the eastern United States is privately owned, which meant that permission to access trees had to be considered. Simply speaking to the property owner proved sufficient to gain approval to make a

collection. No land owner ever denied access once the program was briefly explained. Most collection sites were located along road ways which meant they were in a public right of way where the public is accustomed to seeing workers trimming vegetation or performing other tasks. Therefore, by wearing a safety vest the seed collector was just another road side worker and collections were made without inquiring with a landowner. Should a tree occur in front of a residence or back from the road by more than 0.3 or 0.6 m (10 or 20 ft), collections were not made unless the land owner could be contacted. Road side trees were easy to access and 10 to 20 trees could be collected each day. Access to trees in natural areas required greater effort resulting in collections of four or five trees per day. When trees were found on public lands the local manager was contacted to grant permission. No managers denied permission to collect and all were enthusiastic to participate in the program.

Collection Procedure

The first steps were to determine the species identity and open a few seeds to determine if the embryos were matured adequately and the seeds free of insects. If the seed was good, the data sheet (fig. 1) was completed and the paper collection sack marked clearly with the accession number. To speed the data recording, a check system was used. Next a healthy twig section 15.2 to 22.9 cm (6 to 9 inches) long and including a terminal bud was taken and placed into the collection sack. Photos of the trunk and the whole tree were taken next. Finally the seeds were collected and the bag stapled shut. The twig, seeds, and the two photos were used to make up the voucher specimen for documenting the species identity. Detailed instructions to the procedure are available at http://nsl.fs.fed.us/GeneticConservation_Ash.html. These instructions were also used to train collectors.



Ash Seed Collection Data Sheet

Date of collection: _____

Collector's name: _____

Species (check one): __ Black ___Blue
__ Green ___ Pumpkin ___ White

Seed Lot Identification
Collector's ID number _____
Seed lot number _____

State _____ County_____

GPS Coordinates: lat ___° ___' ___" long ___ ° ___ ' ___" elevation _____m
Accuracy ± _____ ft

Number of ash trees within 20 to 40 feet of this tree: ___ 0, ___ 1, ___ 2 to 4, __ 5 or more

Number of other trees that are not ash within 100 feet this tree: ___ 0, __ 1, __ 2, __ 4 or more

Distance between this tree and nearest other ash tree from which seeds were collected.
____ 100 feet (minimum), ___ 200 feet, ___ more than 200 feet

Soil: ___ Rocky ___ Gravel ___ Sand ___ Loam ___ Clay

Site type: ___ upland __ wetland __aquatic.

Complete only for upland sites
Topography: ___ Flat ___ Slope (Aspect: ___ N ___ S ___ E ___ W)

Directions to the site if not using GPS: _____

_____

_____

Twig sample has been put in bag ___ Trunk and whole tree photos have been taken _____

Leaf sample has been put in this envelope ___

Figure 1—The data collection sheet used in the USDA Forest Service ash seed collection program.

Harvesting the seeds was accomplished in several ways. The easiest was simply to hand pick the seeds directly from the branch when the seeds were low to the ground. Generally this was not possible. A pole pruner gave access to the seeds up to a height of about 6.1 or 7.6 m (20 or 25 ft), and occasionally to 9.1 m (30 ft). These techniques worked well for road side trees and black ash which often had seed bearing branches within reach of the pruner. Seeds above 9.1 m (30 ft) required different techniques. The technique most easily deployed and most affordable was to use a large sling shot to throw a rope over seed bearing branches and then to shake them to knock the seeds down onto a tarp spread below the tree (Knight et al. 2010). This technique required great patience as even the slightest breeze would cause the seeds to miss the tarp and fall into the ground litter, but it did prove very effective in natural areas were access was primarily by foot. Climbing and bucket trucks were also very successful for collecting seeds high in the crown but not used extensively because of the skills and cost required.

Collectors

Collections were largely made by volunteers and persons who could work the collection activities into their regular work schedules. Outreach to collectors was made through direct email to colleagues in and outside government agencies, through presentations at meetings, and via the NSL website. Several workshops were presented with cooperators who in turn recruited additional cooperators. The most successful collectors were persons who had plant experience of some sort, including ecologists, foresters, nursery personnel, and the like.

Laboratory Procedures

Seeds were sent by overnight parcel service to the NSL were they went through a series of steps to prepare and evaluate them for long term storage. The first step was to put the seeds into the cold room for at least a few days. This caused any weevil larvae to exit the seeds (fig. 2). Removal of the larvae is important if the seeds were ever to be provided to another country as international phytosanitary requirements would likely require the seeds be insect free. Next seeds were equilibrated to 30 percent equilibrium relative humidity as measured by a hygrometer (Karrfalt 2014) (fig. 3). Thirty percent eRH was found to correspond to approximately 7 percent seed moisture content which was reported by Barton (1945) to be a suitable moisture to store ash seeds. With species of seeds able to remain alive at moisture contents below 10 percent, 25 percent to 30 percent eRH is the point where maximum viability is preserved. Stems and large sticks were removed from the seed lots by hand. Viability of the seeds was determined by both x-ray (fig. 4) and embryo excision test (fig. 5). A seed lot is accepted for long term storage if 80 percent of the seeds were full. A full seed is one that has a good embryo, a fully formed endosperm, and no insect damage. A lower percentage of full seeds was accepted for some collections that were more difficult to obtain. For seed lots meeting the full seed requirement, an excised embryo test was made on 10 undamaged embryos excised from full seeds. Again, an 80 percent germination of the embryos was desired; however, seed lots with as little as 50 percent germinating embryos are sometimes kept. A precise estimate of viability was not obtained in this test, but it did provide complete assurance that the seed lots had a useable number of viable seeds. Viability and eRH are both evaluated at 10 year intervals beginning at year 10. The final storage preparation steps included checking the eRH, drying as needed to put the seeds at an eRH of 30 percent, sealing the seeds in storage bags, and filing in the freezer. Seeds are kept in two separate collections. One is the working collection kept at the NSL and the second is the security backup collection at the National Laboratory for Genetic Resource Preservation. Working collection samples are used to fill requests from persons with a good justification to have some of the seeds. Security backup samples are not used for distribution, but are solely to provide one more assurance that the germplasm is not lost in the event of a catastrophe with the working collection. Working collection samples are stored in 6 mil poly bags; while security backup samples are stored in foil laminate bags. Close to 99 percent of all samples received make it through the evaluation process and are entered into the collections. This is a testament to the quality of the work done by the collectors. All data are recorded into the Germplasm Information Resources Network database called GRIN Global

(https://npgsweb.ars-grin.gov/gringlobal/search.aspx). This database is maintained by the ARS for all materials entered into the National Plant Germplasm System. Once accepted into the collection, seed lots are assigned a final accession number. A photo is made of every voucher specimen (fig. 6). The photos will ultimately replace the actual physical voucher specimens as there is insufficient space to store the physical specimens.

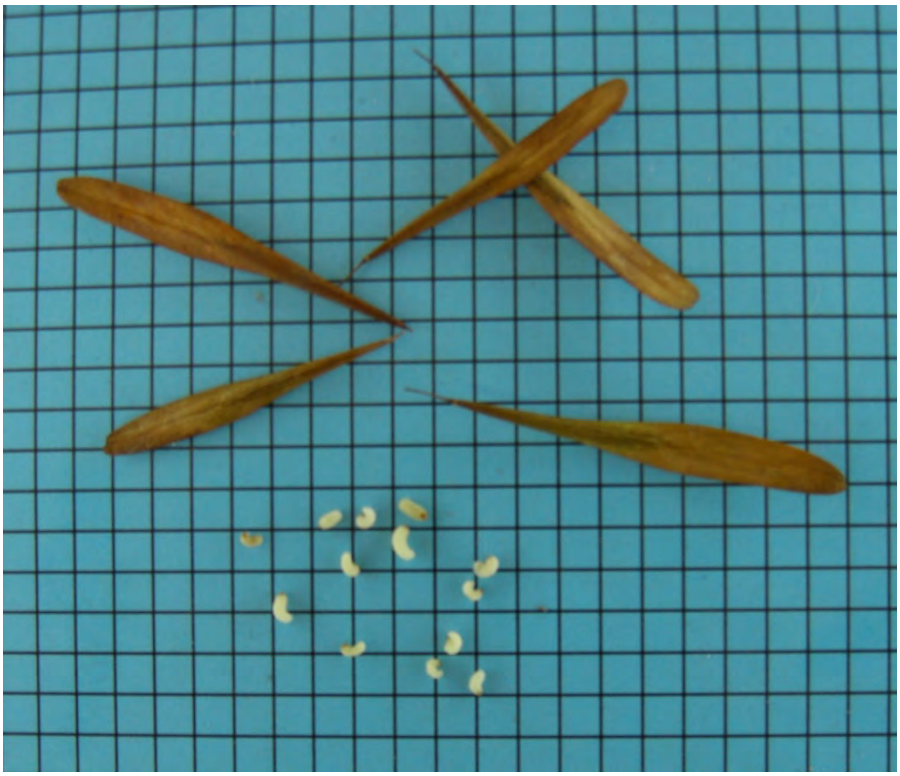


Figure 2—Weevil larvae exit the ash seeds after placing the seeds in a cooler at approximately 3 °C for a few days. The grid is composed of 5 mm squares.

Figure 3—A hygrometer is used test whether the seeds are sufficient dry for long term storage.

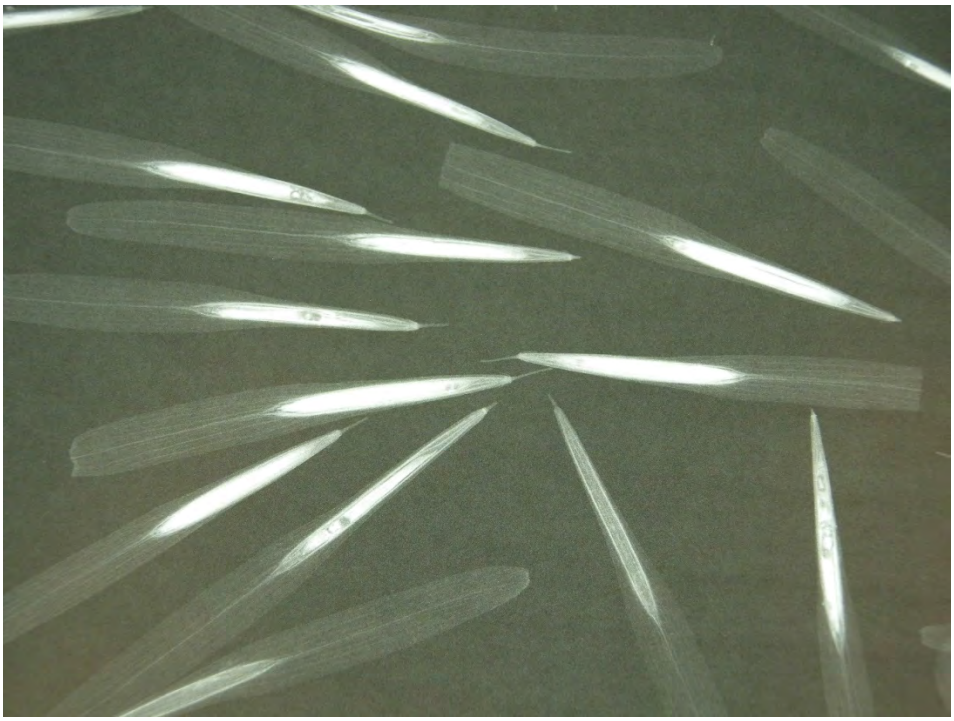


Figure 4—This radiograph quickly and accurately shows the number of good seeds, the number with a weevil larvae, and the number that are empty in a sample of ash seeds.

Figure 5—This excised embryo test is a good way to estimate the viability in ash seeds.

Figure 6—Photo made to electronically document seeds and twig portion of the voucher specimens. The number shown is the permanent accession number assigned to the seed lot. The grid is 5 mm squares.

Results and Discussion

Over 4000 single tree collections of ash species were accomplished among all agencies. Most collections were of white and green ash. Black, blue, and pumpkin ash were also collected. A full listing of collections held by USDA agencies can be viewed at https://npgsweb.ars-grin.gov/gringlobal/search.aspx. The oldest samples in the collection are now 10 years old and viability tests on these samples show that viability is remaining high and the storage is successful. Many collections remain to be made and in no ecoregions are collections completed to the original target of 50 trees evenly spread across the region, although some are very close (Karrfalt et al. 2013). The EAB continues to spread across the country and collection efforts will continue as resources become available and interest is shown by cooperating agencies and individuals.

Conclusions

The genetic resources of ash, despite ongoing loss in the wild, are being successfully preserved through long term seed storage. Mortality caused by EAB continues to erode the germplasm base in new areas, so many more samples remain to be collected.

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The Vallarta Botanical Garden's Advancements in Conserving the Diversity of Native Mexican Oaks and Magnolias[1]

N.A. Gerlowski[2] and M.A. Muñiz-Castro[3]

Mexico is both an oak (*Quercus*) biodiversity hotspot (over 160 described species) and the western hemisphere's leader in magnolia (*Magnolia*) diversity (36 described species). In the face of myriad threats to these groups, including climate change, habitat loss/fragmentation, overharvesting, and plant pests/pathogens, the imperative to preserve the genetic diversity of these trees has become a high priority of the Vallarta Botanical Garden (VBG). In collaboration with researchers from the University of Guadalajara, the VBG has several new initiatives underway to acquire diverse and well-documented plant materials from these taxa. Their goals are the enhancement of the *ex-situ* collections of the VBG and to continue to research and monitor *in-situ* populations. Because *Quercus* and *Magnolia* seeds are recalcitrant, *ex-situ* collection is currently the most viable strategy to safeguard the genetic diversity of these trees beyond their native distribution, which in the tropics is often limited to very small and vulnerable stretches of forest.

The VBG is also trialing and documenting successful horticultural practices to launch satellite community collections in both rural and urban landscapes. These efforts seek to multiply the overall potential *ex-situ* collection holdings and to engage local communities in the importance of protecting their forests and the valuable resources they harbor. While the United States is rich in botanical gardens with strong conservation programs, their southern neighbor boasts a greater floristic biodiversity (roughly 26,000 species of vascular plants in Mexico compared to approximately 17,000 in the United States) over a much more concentrated landmass (about 1/5 the size), and has few gardens with active conservation programs beyond their grounds. United States gardens with missions to conserve threatened tree species regardless of geopolitical boundaries have incredible opportunities to collaborate with counterparts south of the border to realize their objectives. Since many conservation programs in Mexican gardens are in their formative stages, there are also ample opportunities for advising these institutions' strategies for the best chance of success.

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Important Hawaiian Tree Species in Need of Genetic Conservation[1]

Robert D. Hauff[2]

Abstract

Resource managers in Hawaii face unique forest conservation challenges. Invasive species continue to inundate the remote island archipelago, directly threatening its forest resources. Hawaii has the largest number (> 400) of endangered plants in the United States, and managers use genetic approaches to preserve these small populations which are often island endemics. Many of the common forest tree species that grow throughout the islands face threats from pests and disease, but in most cases, little is known about their genetics and whether breeding resistance is a viable option. This presentation will highlight three important native Hawaiian forest trees that are currently threatened by disease or insect pests. While one species, *Acacia koa* A. Gray has been the focus of a 10-year long program for breeding disease resistance, others are still only in the conceptual phase. The recent outbreak of *Ceratocystis fimbriata* on the most common Hawaiian tree species, *Metrosideros polymorpha* Gaud., has managers struggling for solutions, and genetic approaches are urgently needed to restore affected forests. A thrips insect (*Klambothrips myopori*) that causes morality in *Myoporum sandwicense* (A. DC.) A. Gray cannot be managed through classical biological control, and trials looking for resistance in local populations are planned. The resources needed for genetic approaches to tree conservation are limited locally, and national and international partnerships will be vital for the success of any project.

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How New York State Saved its Ash[1]

C.L. Holmes,[2] M. Marquand,[2] and E.M. Toth[2]

Across the United States, forest communities are faced with the prospect of extirpation of *Fraxinus* (ash) species owing to mortality caused by invasion of the emerald ash borer (*Agrilus planipennis*). However, with the advancement of *ex situ* seed conservation practices, we have the opportunity to conserve the ecoregional-based genetic variability of *Fraxinus* species before they are lost from the wild. Genetic variability is critical to resistance research and to potential future reintroductions. Established in 2009 by the U.S. Department of Agriculture Forest Service (USDA FS), the Ash Genetic Resources Conservation Plan developed a seed collection protocol that maximizes genetic diversity to meet the conservation goals for these species. In 2014, the Mid-Atlantic Regional Seed Bank (MARSB) received funding from the USDA FS's Northeastern Area State and Private Forestry to train volunteers on this protocol in order to make 150 collections from the three *Fraxinus* species found in New York State. The grant period spanned 3 years to increase the program's chances of coinciding with a mast year. In 2015, a major mast year throughout the Northeast, MARSB accelerated and expanded its outreach campaign through traditional means and social media, as well as through targeted outlets such as the New York State Forest Owners Association, Partnerships for Regional Invasive Species Management, and the New York State Department of Parks. By the end of the year, we had trained over 350 workshop participants and made over 200 collections contributed by over 70 dedicated volunteers. This marks perhaps one of the most successful statewide efforts to mobilize the community to conserve a species ahead of extirpation. Furthermore, this model is easily adaptable to other states and similar efforts. This presentation will discuss the methods used to build this cadre of volunteers, where and how collections were made, and ways to expand this effort to related causes.

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Designing Seed Collections

Collecting Genetic Variation on a Small Island[1]

S. Kallow[2] and C. Trivedi[2]

Abstract

Genetic variation is the most powerful factor in ensuring the long term success of trees and forests in times of change. In order to protect against loss of genetic variation from threats, including pests and diseases and climate change, the Royal Botanic Gardens, Kew, is developing a national tree seed collection for the United Kingdom.

This paper describes the authors methodologies and experiences of developing the national collection: firstly, forming species target lists using plant health risk and conservation assessments; then, developing national sampling strategies based on the distribution of target species in biogeographic zones; and, finally, how collecting strategies were designed to capture and preserve genetic variation within populations.

Additionally, we discuss social and ecological factors taken into account when developing and implementing the sampling program. These include phylogeography; the history of woodland management, ownership and fragmentation; and introgression risks from plantation and garden escapees. Finally citizen participation is discussed.

Introduction

The Royal Botanic Gardens, Kew launched the United Kingdom National Tree Seed Project (UKNTSP) in 2013 as an *ex situ* seed conservation initiative. The UKNTSP's stated aim is: 'To provide a national repository of plant material and associated knowledge, for the purposes of long term conservation, and to make these resources available to users, in order to better understand and manage tree and shrub species in the United Kingdom landscape.'

In this paper we set out our key considerations and experiences in targeting, sampling and collecting seed for the project.

Developing Target Lists

Native Species

The United Kingdom woody flora consists of 139 native species, 11 archeophytes, (naturalized non-native species introduced prior to 1500), and 57 neophytes (naturalized and introduced after 1500). Native species are considered to be those that recolonized the British Isles between the last ice age and the separation of the British Isles from mainland Europe, around 8 to 10,000 years ago; or those that speciated in the United Kingdom after this period. Re-colonization occurred from glacial refugial populations from Spain, Italy and the Balkans, in the main (Newton et al. 1999).

Prioritization

To prioritize native species for seed banking in the project timeframe, a target list was developed, using a scoring and ranking system. Impacts from invasive species, including pests and diseases, is one of the key drivers for change affecting forest genetic resources (FAO 2014). Plant health, therefore, was a primary factor in our prioritization. We used the United Kingdom Plant Health Risk Register (DEFRA 2014) to prioritize species most at risk. This tool adopts a risk assessment approach to potential plant health threats to hosts, by assessing impact and likelihood and setting out mitigation approaches. 'Unmitigated risk'

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assessments for plant health risks were totalled for each host, which were then ranked accordingly. Wider threats to species were scored using Great Britain Red List status (Cheffings et al. 2005). Species were again ranked, those with a higher 'Extinction Risk' threat status received greater priority. Finally, as a measure of potential ecological impact at a landscape-scale, 'prevalence in the landscape' was also used to rank targets, using the PLANTATT database (Hill et al. 2004); species that had wider distributions received greater priority. For each ranking 'Plant health risk' was used as a secondary factor in ranking if species received the same score, for example both *Pinus sylvestris* and *Acer campestre* have a Red List status of 'Least Concern', but *P. sylvestris* received a higher rank in this category because there is a greater 'Plant health risk' for this species. See table 1 for a list of target taxa and prioritisation data.

Table 1—Prioritization list of target taxa

Taxon name	Unmitigated risk total score	Risk rank	Red list GB[a]	Red list rank	No. 10 km² in GB	Distri-bution rank	Native status[b]	Native rank	Total rank
Acer campestre	745	25	LC	68	1389	37	N	62	192
Alnus glutinosa	420	46	LC	85	2478	8	N	80	219
Betula nana	591	45	LC	84	125	77	N	79	285
Betula pendula	591	44	LC	83	2293	21	N	78	226
Betula pubescens	651	30	LC	72	2399	15	N	66	183
Buxus sempervirens	54	111	DD	13	2	112	N	108	344
Carpinus betulus	281	51	LC	90	1488	35	N	84	260
Cornus sanguinea	182	56	LC	95	1179	41	N	89	281
Crataegus laevigata	153	58	LC	97	597	59	N	91	305
Crataegus monogyna	233	54	LC	93	2496	7	N	87	241
Erica vagans	16	115	LC	116	6	99	N	110	440
Fagus sylvatica	280	52	LC	91	2397	16	N	85	244
Fraxinus excelsior	407	47	LC	86	2459	11	N	81	225
Ilex aquifolium	183	55	LC	94	2353	17	N	88	254
Juniperus communis	147	59	LC	98	1020	46	N	92	295
Juniperus communis subsp. *hemisphaerica*	87	65	CR	1	2	107	N	98	271
Lonicera periclymenum	0	120	LC	119	2622	1	N	115	355
Malus sylvestris	1175	19	LC	62	2023	26	N	56	163
Pinus sylvestris	2114	2	LC	46	65	83	N	39	170
Prunus avium	2225	1	LC	45	2136	24	N	38	108
Prunus padus	1901	4	LC	48	1089	44	N	41	137
Prunus spinosa	1901	3	LC	47	2308	20	N	40	110
Pyrus cordata	642	31	VU	32	9	94	NA	128	285
Rubus idaeus	914	22	LC	65	2425	13	N	59	159
Sambucus nigra	4	118	LC	118	2457	12	N	113	361
Sorbus admonitor	72	87	EN	24	1	122	NE	22	255

Sorbus anglica	72	99	NT	41	12	93	NE	34	267
Sorbus aria	72	106	LC	109	341	67	N	103	385
Sorbus arranensis	72	96	VU	36	1	130	NE	31	293
Sorbus arvonicola	72	67	CR	3	1	113	NE	3	186
Sorbus aucuparia	*72*	103	LC	106	2472	9	N	100	318
Sorbus bristoliensis	72	88	EN	25	1	123	NE	23	259
Sorbus cambrensis	72	84	EN	21	2	109	NE	19	233
Sorbus cheddarensis	72	68	CR	4	1	114	NE	4	190
Sorbus cuneifolia	72	89	EN	26	1	124	NE	24	263
Sorbus devoniensis	72	101	LC	104	32	89	NE	36	330
Sorbus domestica	72	76	CR	12	4	101	N	99	288
Sorbus eminens	72	77	EN	14	9	95	NE	12	198
Sorbus eminentiformis	72	85	EN	22	2	110	NE	20	237
Sorbus eminentoides	72	69	CR	5	1	115	NE	5	194
Sorbus evansii	72	90	EN	27	1	125	NE	25	267
Sorbus greenii	72	91	EN	28	1	126	NE	26	271
Sorbus herefordensis	72	92	EN	29	1	127	NE	27	275
Sorbus lancastriensis	72	100	NT	42	9	96	NE	35	273
Sorbus leighensis	72	93	EN	30	1	128	NE	28	279
Sorbus leptophylla	72	81	EN	18	3	104	NE	16	219
Sorbus leyana	72	66	CR	2	2	108	NE	2	178
Sorbus margaretae	72	79	EN	16	5	100	NE	14	209
Sorbus minima	72	97	VU	37	1	131	NE	32	297
Sorbus parviloba	72	70	CR	6	1	116	NE	6	198
Sorbus porrigentiformis	72	102	LC	105	30	90	NE	37	334
Sorbus pseudofennica	72	98	VU	38	1	132	NE	33	301
Sorbus pseudomeinchii	72	71	CR	7	1	117	NE	7	202
Sorbus richii	72	86	EN	23	2	111	NE	21	241
Sorbus rupicola	72	107	LC	110	98	81	N	104	402
Sorbus rupicoloides	72	72	CR	8	1	118	NE	8	206
Sorbus saxicola	72	73	CR	9	1	119	NE	9	210
Sorbus spectans	72	94	EN	31	1	129	NE	29	283
Sorbus	72	82	EN	19	3	105	NE	17	223

stenophylla									
Sorbus stirtoniana	72	74	CR	10	1	120	NE	10	214
Sorbus subcuneata	72	95	VU	35	4	103	NE	30	263
Sorbus torminalis	*72*	105	LC	108	*573*	60	N	102	375
Sorbus vexans	72	80	EN	17	4	102	NE	15	214
Sorbus whiteana	72	83	EN	20	3	106	NE	18	227
Sorbus wilmottiana	72	75	CR	11	1	121	NE	11	218
Taxus baccata	57	110	LC	113	1881	28	N	107	358
Tilia cordata	72	104	LC	107	896	51	N	101	363
Tilia platyphyllos	72	108	LC	111	84	82	N	105	406
Ulmus glabra	659	29	LC	71	2338	18	N	65	183

[a] Red list Great Britain (GB): Not Evaluated (NE), Data Deficient (DD), Least Concern (LC), Near Threatened (NT), Vulnerable (VU), Endangered (EN), Critically endangered (CR).
[b] Native status: Native (N), Native endemic (NE), Native or alien; Native status doubtful (NA).

Practical and Technical Considerations

The Millennium Seed Bank Seed Information Database (Royal Botanic Gardens, Kew 2008) was used to identify the seed storage behavior of target species. Recalcitrant *Quercus* species, and short lived Salicaceae were removed from the target list, as these cannot be dried and stored under conventional seed bank conditions or require careful post-harvest handling, not considered possible when using volunteer seed collectors for much of the collecting. Similarly, taxonomically complex woody taxa including most *Rubus* and *Rosa* species were removed to reduce problems of identification. The *Sorbus* micro-species were agreed as targets as, while taxonomically complex, they are well-recorded in the United Kingdom, have limited distribution, and there was an expert available to collect them. All *Ulmus*, apart from *U. glabra*, were removed because of the on-going effects of the Dutch elm disease (caused by species of *Ophiostoma*) outbreak which limits the ability of elm trees to reach maturity. *Ulmus glabra* is less susceptible than other *Ulmus* to Dutch elm disease, and has a greater propensity for sexual reproduction and therefore seed production. The resulting ranked target list was used as the basis for a project target list of 70 taxa which was agreed upon following further discussions by the project advisory group, a group of professionals from both the forestry and conservation sector brought together for oversight and technical advice.

Sampling Strategy

National Level

Unfortunately, most published research dealing with the genetic diversity and structure of United Kingdom woody species is only at the European scale, providing an insufficient basis for a national sampling strategy. The Forestry Commission (the United Kingdom government department responsible for forestry), has developed a seed zone map to guide seed transfer under the Forest Reproductive Material (Great Britain) Regulations 2002 legislation implemented under the EC Directive 1999/105. The map divides Britain into four broad regions of provenance and 24 smaller native seed zones; each zone is further divided by elevation above and below 300 m (Herbert et al. 1999) (fig. 1). Zones are based on biogeographic factors, such as watersheds and climate as well as man-made barriers which may influence dispersal and gene-flow, such as major roads. We decided to use the seed zone map as a framework for our sampling strategy, aiming for a seed collection in each seed zone where there is a native population for each of our target species, and an additional collection over 300 m, where this is possible. This standardised approach was adopted across all target taxa.

The native distribution of each target taxa was mapped against each seed zone using the Atlas of the British and Irish Flora (Preston et al. 2002), excluding the introduced distribution, based on historical pollen records and archaeological findings. Unfortunately, elevation analysis is not provided by the Atlas. The open access data from the Shuttle Radar Topography Mission (Jarvis et al. 2008), and records from the Botanical Society of Britain and Ireland database (BSBI 2016) were used to estimate the elevation of specific records. Estimates of occurrence over 300 m in seed zones were then confirmed on the ground by partners in seed zones 105, 108, 109 and 204, to validate the desk-based analysis, in order to produce a target list which is both vertically and horizontally distributed. This resulted in a total of 680 target seed collections.

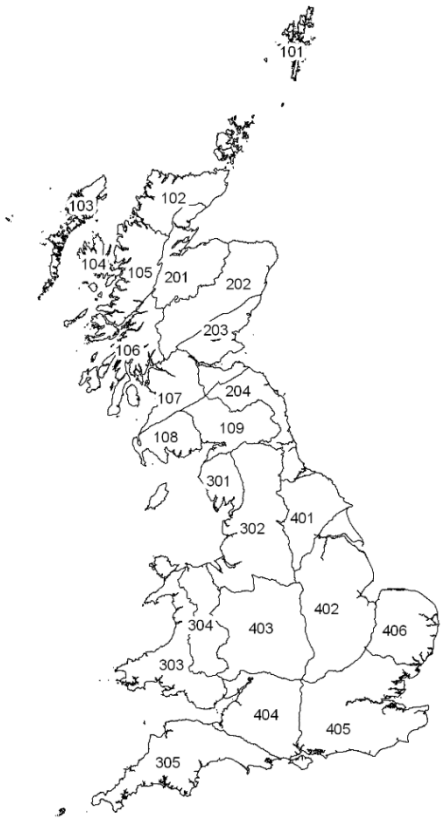

Figure 1—Native seed zones in Great Britain (Herbert et al. 1999).

This sampling strategy is a highly-effective way of sampling both common and rare locally adapted alleles (Hoban and Schlarbaum 2014), which are ecologically and economically important for conservation. Additionally, this form of sampling allows for conservation of the spatial structure of populations, which provides important data for research and restoration use of the collections. However, as we are using a standard strategy for all species which is based upon assumptions of genetic diversity and structure, rather than actual data, the strategy risks missing important species-specific factors and potentially over or under sampling.

One further implication of our sampling approach is that some of the populations collected from are at the northwestern edge of their ranges. Range-edges have profound implications on seed collecting programs, for example, marginal population may have decreased population density or reduced seed production (Garcia et al. 2000, Rasmussen and Kollmann 2004). Anecdotally, both the number of trees and number of seeds collected for the project have been less at range edges. Such peripheral populations may be adapted to the extremes possible for the species, and are, therefore, important sources of evolutionary potential, especially in the context of climate change (Fady et al. 2016, Havens et al. 2015).

The north and west are also the most deforested regions of the United Kingdom; therefore, also reducing seed sample sizes. The project is therefore re-assessing how to maximize sampling in these regions.

Sampling From Appropriate Sites

To meet our project aim, it is important that seeds are sampled from autochthonous, locally-adapted populations. As the United Kingdom is one of the least forested countries in Europe, with around 12 percent forest cover in contrast to averages in Europe of 45 percent and the United States of 40 percent (World Bank 2016a), this can be a challenge. Average woodland size (including exotic plantations), is only 7.9 ha in the United Kingdom overall, and only 4.9 ha in England (Watts 2006). The forest which once covered Britain was cleared for agriculture, mainly in the Bronze and Iron Age (Rackham 2001). The remaining 'ancient semi-natural woodland' (defined as having a continuous woodland cover since the earliest maps of 1600), is highly fragmented and now accounts for only 2 percent of land cover (Atkinson and Townsend 2011). The remaining woodlands also have a long history of management, usually by coppicing and favouring 'useful' species (Rackham 2001). There is, therefore, a high probability that any sampled population is not strictly autochthonous. There is an inevitable risk, therefore, that seed collected for the project may result from planted or naturalized trees originating from other parts of the United Kingdom or abroad, and in some cases form hybrid progeny. Indeed, the challenge is so great that even when we hand-pollinated native black poplars (*Populus nigra* subsp. *betulifolia*) and excluded pollen using bags, DNA analysis showed several resulting progeny had been pollinated by exotic, but abundant hybrid poplar (*Populus* x *euramericana*) (Gargiulo, personal communication, 2016). Resources such as the Native Woodland Survey of Scotland (Forestry Commission Scotland 2016), the Ancient Woodland Inventory (Natural England 2015), and sites protected under the Wildlife and Countryside Act (1981), and the EC Habitats Directive have been invaluable in guiding seed collectors to autochtonous populations. While every effort has been made to collect from such populations, we recognize that sampling is of the current genepool, which includes introduced material.

Sampling Strategy at the Seed Zone

As a citizen science project working with a wide range of, mostly voluntary sector, partner organizations, we had to be realistic and pragmatic when setting an achievable sample size. Standard population sampling guidelines advise collection from either 30 randomly chosen individuals in a fully out-crossing species, or 59 random individuals in a self-fertilizing species (Brown and Marshall 1995) in order to collect 95 percent of the non-rare alleles present in a population. For many of our targets, collecting from this number of individuals in a population is not possible, because of the limited numbers of accessible seed-bearing plants. Even when populations were large enough, practical experience in the first year of the project demonstrated that collecting from 15 individuals in a day represented good effort. We therefore agreed to aim for sampling 10,000 seeds from at least this many individuals, where possible. This number of seeds is enough for some to be used for standard MSB germination tests, and allows for others to be available for distribution to researchers and conservation projects, while maintaining a sizeable sample in the seed bank.

In order to maximize the genetic diversity of the sample, collections were taken throughout the population, and over 50 m apart where possible; and seeds were collected from across the canopy at various places, to maximize the number of pollen sources. Individual mother trees were georeferenced using a GPS and were also physically tagged with a project aluminium tree tag. The seed from each mother tree was collected and stored separately in order to maximize the potential use of the collections for research and breeding. On every occasion, herbarium specimens accompanied a collection, for identification. In some cases tissue samples were collected for dry storage in silica gel, these can be used for DNA extraction and genomic analysis.

Building a Network of Seed Collectors

Although the United Kingdom is one of the least forested and most densely populated countries in Europe, with 267 people per km^2 compared to an European Union average of 116, and United States of 35

people per km^2 (World Bank 2016b), many people have easy access to local woodlands. In fact, 65 percent of the population live within 4 km of a 20 ha woodland (The Woodland Trust 2010). The link between health and wellbeing and access to natural environments, such as woodlands, is increasingly being valued (DEFRA 2012). It was important for the project, therefore, to develop an approach which capitalised on local community engagement in woodlands and forests, by working with conservation non-governmental organizations (NGOs). Working with land-owning NGOs also increased access to collecting sites, particularly useful as state ownership of woodlands and forests in the United Kingdom is also relatively low, at around 28 percent (DEFRA 2012).

Since the project began in 2013, seed collecting partnerships have been developed with over 30 organizations. Small local groups, for example Cree Valley Community Woodland Trust in Dumfries and Galloway, Scotland, or Suffolk Wildlife Trust, England, have collected the full target list in their respective seed zones. Locally-based NGOs, with access to woodland and existing experience in seed collecting with a team of engaged volunteers, are an incredible resource for the UKNTSP. Partners like this are able to monitor the availability and maturity of seed for collection without having to travel long distances. We also worked with the Forestry Commission, which led on collecting ash (*Fraxinus excelsior*), yew (*Taxus baccata*) and juniper (*Juniperus communis*) right across the United Kingdom.

In order to communicate the sampling strategy and collecting methodology to NGOs and volunteers, we produced a seed collecting manual (Kallow 2013), and ran training sessions across the United Kingdom each year. We developed a standard memorandum of collaboration and grant agreement in order to provide funding to partners and to agree the terms of ownership and transfer of seed collections, herbarium vouchers and data. We devised a new landowner consent form for use across the project partnership and made consent agreements with statutory agencies for collecting from protected sites. Working with these NGOs has also provided an opportunity to raise public awareness of forest conservation and *ex situ* conservation.

To conclude, the UKNTSP took into account scientific knowledge, existing national infrastructures, and engaged with local communities to build a seed repository which provides an important resource of genetic material and knowledge to help the conservation and management of trees and forests for the future.

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Saving Seeds: Optimally Planning Our *Ex Situ* Conservation Collections to Ensure Species' Evolutionary Potential[1]

Sean M. Hoban[2,3]

In the face of ongoing environmental change, conservation and natural resource agencies are initiating or expanding *ex situ* seed collections from natural plant populations. Seed collections have many uses, including in provenance trials, breeding programs, seed orchards, gene banks for long-term conservation (live plants or seeds), restoration, reforestation, and scientific study of plant germination or other plant ecology studies. Well-known examples of *ex situ* collections include the Millennium Seed Bank Partnership, Australian Seed Bank Partnership, United Kingdom National Tree Seed Program, United States National Plant Germplasm System, and South African Regional Seed Bank. Some collections focus on rare species, species with relevance to agriculture or forestry, or regional flora. Other collections are in response to immediate threats, such as damaging insects and pathogens (e.g., emerald ash borer). In this talk I will discuss how to sample seeds to most optimally conserve the evolutionary potential of a species to ensure its long-term survival.

A useful seed collection captures as much phenotypic and genetic diversity from natural populations as possible. Choices for a collector include how many populations, maternal plants, and seeds per plant to collect. A collector wishes to achieve efficiency—to not waste limited time, resources, personnel, and storage space, but also to achieve effectiveness—to be as complete as possible in case important genetic variants are lost from natural populations.

In a series of papers starting in 1975, Brown and Marshall (1975) proposed some solutions to this general sampling problem. They used simple mathematical equations to derive a minimum number of samples needed to capture allelic variants that occur in a population at a given minimum frequency. For an arbitrary minimum frequency of 0.05, the recommended minimum sample size was 30 individuals from a fully outcrossing species or 59 individuals from a fully self-pollinating species. A 'rule of thumb' of 50 samples was suggested for practical use. This 50 sample guideline has since been integrated into many protocols for sampling seed, and is still common 4 decades later. Hoban and Strand (2015) found this guideline cited in 60 percent of protocols from major seed collecting or natural resource management organizations.

Though widely used, these guidelines may be suboptimal for genetic representation for several reasons. Principally, this approach assumes that there is no genetic structure in a population. Specifically, the assumption is that all populations have completely different sets of alleles present – no sharing of alleles among locations. An alternative approach, that of Lawrence et al. (1995), assumes all populations have the exact same allele frequencies. The corollary assumption is an absence of spatial genetic structure such as clines, barriers, or disjunctions. Most real species, however, have a substantial amount of shared alleles among populations, and feature spatial patterns such as a decline in genetic similarity between populations due to geographic or environmental distance (Sork and Smouse 2006).

The Brown and Marshall approach also assumes that every seed chosen within a population will contain a random sample of the population's genetic variation; thus, every seed has an equal chance to add new variation to the sample. In reality, however, sampling many seeds from a maternal plant does not

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add significant variation but rather largely duplicates variation because factors such as limited pollen dispersal and dominance of nearby fathers result in typically small paternal gene pools.

It has not yet been evaluated whether the Brown and Marshall assumptions are too simplifying, whether they truly capture the expected amount of genetic variation in the seed sample for real world species. Some authors have proposed that collection protocols should be based on a species' biological characteristics, such as population size, rarity, degree of self-pollination, and level of habitat fragmentation (CPC 1991, Guerrant et al. 2014, Hoban et al. 2015). As yet, there is no quantitative advice for how to tailor a collection protocol to a species' traits. Plant traits are incorporated into conservation tasks such as quantitative assessments of invasion or extinction risk, however (Bland et al. 2015. Murray et al. 2014), suggesting that plant traits could be incorporated, quantitatively, into collection protocols.

With a group of colleagues, I have been using a new approach to designing *ex situ* seed collections. It is based on population genetic simulations that incorporate plant traits. This approach is similar to the method of Bataillon et al. (1996), who used simulations to sample from existing seedbanks to create a 'core collection' that would contain most of the genetic variability of the whole collection. In my approach, simulations are used to create in silico datasets representing the species or set of populations of interest. These datasets are then sampled from with different potential sampling protocols. Then the genetic diversity captured in each protocol can be compared, and the protocol capturing the most variation in the most efficient manner can be chosen. Throughout, this work assumes that the goal is to calculate a minimum sample size- the fewest number of seeds or samples to capture a given amount of genetic variation. Note that this approach assumes that all seeds are viable and all will grow to a reproductively mature individual, i.e., no attrition or loss during seed storage and use. It has been shown that attrition can vary from a few percent to nearly 95 percent (Cochrane et al. 2007; Hoban and Way, unpublished data). In addition, loss of genetic variation can occur through multiple cycles of seed increase (sowing the collected seed to then gather larger amounts of seed) and other processes (Basey et al. 2015). Minimum sample sizes such as those discussed here should be adjusted upward to allow for expected losses.

In a series of studies, colleagues and I used simulations and real datasets to determine the influence of several factors on the amount of genetic variation captured: different plant traits, degree of range wide population structure, varied local sampling patterns (transect, random, etc.), and size of the paternal pollen pool. I also calculated the return on investment (ROI, defined as new alleles captured per sampling effort) of sampling from a new plant, or sampling more seeds from plants that were already sampled.

In one simulation experiment, we quantified the influence of dispersal kernel, self-pollination rate, and life history (annual or perennial habit) on genetic capture in a collection. This experiment demonstrated that each of these biological factors does influence genetic variation captured, and that simulations can be used to calculate how minimum sample sizes should differ in plants with different traits (Hoban and Strand 2015). For example, a species with high selfing rates and low dispersal required seed lots up to five times as large (about 40 seeds each from 40 plants, or 1600 total seeds) as species without those characteristics (about 10 seeds each from about 30 plants, or 300 seeds), to achieve a reasonable goal of conserving 90 percent of the rare alleles. I confirmed this general trend by sampling from existing datasets of three tree or shrub species with different traits, and found drastically different allelic capture in each species for a given sampling effort. The results of this experiment underscore that the same sampling strategy applied to different species will capture different amounts of genetic diversity, for each species.

Another characteristic that influences the amount of variation captured by sampling is the level of population fragmentation, which can range from well-connected to scattered populations. We simulated one common model of range wide population structure, the ecoregional model, in which migration among ecoregions is lower than within them. We compared two differing approaches to sampling- sampling one population from each of four regions (dispersed) and sampling four populations all in one region (constrained). This is the same number of populations but different spatial coverage. Our work showed (as predicted by theory) that it is more important to sample from each ecoregion for less well-connected populations than it is for more well-connected populations. Also, the amount of variation captured when sampling in a constrained fashion was as little as half the variation captured by dispersed sampling, depending on the type of allele of interest (Hoban and Schlarbaum 2014). This information is important

because sampling may be constrained by logistical, financial or political borders. In such cases, the genetic variation collected may be less than desired.

We also showed that population structure had a previously unrecognized effect on sampling: the minimum number of samples recommended by Brown and Marshall (50 sampled plants) may sometimes be a conservative number when sampling many populations in a many-population system. This is due to allele sharing among populations; in real populations many alleles occur in multiple populations, albeit at different (sometimes low, sometimes high) frequencies. Thus, sampling multiple populations will increase the cumulative probability an allele will be captured from at least one population. Therefore in a real system of populations, the Brown and Marshall suggestion of 50 samples per population will capture more than the amount of genetic diversity predicted by their models. In one situation we examined, sampling just 25 samples per population from many populations in a many-population system captured 97 percent of all alleles, while sampling 50 samples captured 99 percent. Sampling from many populations in a well-connected system clearly gives multiple chances to capture alleles and may allow lower minimum sample numbers per population.

We then showed that the better choice is almost always to collect from a new plant rather than to collect more seeds from the plants already visited. Indeed the return on collecting from a new plant is relatively constant across collections sizes, while the return rapidly drops to near zero when sampling more seeds on plants already visited. This is because all the maternal alleles will be collected by a small sample (half of each seed is maternal genetic material) and a small number of fathers that typically contributes to the seed set on a given plant (in trees, often less than 20 fathers, e.g., Grivet et al. 2009). Hoban and Schlarbaum (2014) found that, in several situations examined, allelic capture appears to begin to plateau after 16 seeds are taken per maternal plant, though more situations need to be examined. Hoban et al. (unpublished data) showed that sampling 1000 seeds from a plant may capture negligibly more genetic variation than 200.

Next, we examined how to sample spatially at local scales within populations. I tested this because collectors often sample opportunistically, at accessible trails or roadside patches, or by straight line transects. It would be expected that a random or systematic approach covering equal parts of the population would be best, while covering small areas would capture less genetic variation, but it has never been quantified how each strategy performs relative to the others. We showed that random and grid sampling are statistically indistinguishable, that opportunistic sampling captures about half the variation of random sampling, and that sampling a transect produced results intermediate between restricted and random sampling (Hoban and Strand 2015). With this information, a sampler can decide whether it is worth the extra effort to cover more ground.

There are interesting future directions for this type of work. Other aspects of plant biology may also be expected to influence gene distribution on the landscape and thus the effectiveness of different sampling strategies. Population density, animal vs. wind pollination, and clonality are three such aspects (Vekemans and Hardy 2004). The location of seeds within the canopy of a single plant, especially large trees, may also be important because different parts of the canopy will receive pollen from different sources. Another unexplored aspect of the distribution of genetic diversity in plant populations is the influence of location within the species' range, e.g., edge vs. center (see Gapare et al. 2008). Lastly, population history likely influences the amount and type of variation available, and thus how to sample optimally. It will be important to determine how to sample species subject to recent colonization or bottlenecks, or large disjunctions.

Of course, the degree to which these aspects can be incorporated into sampling strategies will depend on the amount of information available for a species. If little information is available, desk studies or surveys of a species may help gain information and develop an effective strategy. For an emergency collection, the Brown and Marshall guideline remains a starting point, though for a species or population under immediate threat such as destruction from development, collecting all available seed has been recommended.

The simulation approach can also be applied to real species' collections. Thus far, my investigations have been into species archetypes or broad categories as defined by traits. I can also build demographic

genetic simulations tailored to particular taxa. Along with colleagues at the United Kingdom National Tree Seed Project, I have been working to test sampling protocols for European ash (*Fraxinus excelsior*) in the United Kingdom. We will estimate how much genetic variation has been captured in the samples taken so far (three seasons of seed sampling), where to sample next, what is the relative benefit of sampling on the edge vs. the center and in the north vs. the south, and what are optimal numbers of plants per population and seeds per plant to collect. This simulation experiment will use a realistic demographic model based on observed tree abundances at fine scale (10 km grid), and a genetic model fine-tuned to produce F_{ST}s similar to those observed in a recent microsatellite study of this species.

In summary, I explained and demonstrated a new approach to optimize sampling protocols for a conservation seed collection. I used spatial, demographic and genetic data from empirical and simulated data under an individual-based model, to lead to tailored collections that maximize diversity while minimizing collection size when a collector knows some basic biological characteristics of the species. I showed that characteristics of plant reproduction and dispersal, as well as logistical factors, significantly influence the genetic diversity captured in seed collections. As one example, a highly self-pollinating, low dispersal species likely needs sample sizes five times larger than current guidelines. Results show that minimum collection protocols should be customized for the target species, rather than commonly implemented "rules of thumb" like 50 samples. There is not a single minimum sample number suitable for all species, but we can derive minimum numbers for archetypes defined by plant traits. Overall, my work shows that it is possible to improve the value of seed collections by quantitatively integrating current knowledge of plant biology, spatial distribution, and genetics into collection design. This work is important and timely knowledge for managers and policy makers because limited conservation resources demand effective, efficient investment.

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Synthesizing Genetic Divergence and Climate Modeling to Inform Conservation Planning for Ponderosa Pine[1]

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Geological, climatological and ecological processes partially or entirely isolate evolutionary lineages within tree species. These lineages may develop adaptations to different local environmental conditions, and may eventually evolve into distinct forms or species. Isolation also can reduce adaptive genetic variation within populations of a species, potentially compromising their ability to respond to climate change. Dramatic climate changes during the Pleistocene, for example, caused species ranges to contract and fragment into isolated glacial refugia before expanding and reconnecting. The genetic signals of these processes remain in several species, and may be useful in guiding gene conservation strategies. Such within-species evolutionary differences should be considered when predicting species responses to climatic conditions. We considered within-species evolutionary differences within a climate context for ponderosa pine (*Pinus ponderosa*), applying results from range-wide molecular marker assessments and nonparametic multiplicative regression climate models. In this widespread western North American species, we detected and mapped 10 mitochondrial (mtDNA) haplotypes from 3,100 trees across 104 populations. Each haplotype is an evolutionarily distinct unit that may be evolving separately and responding differently to climate change. Our analyses, in fact, indicate strong relationships between genetic lineages and climate. Most important were differences in seasonal precipitation regimes between the Rocky Mountain and Pacific evolutionary lineages, but other precipitation differences were also apparent among haplotypes. This synthesis of phylogeography, population genetics, and climate modeling should assist management and conservation planning for this widespread and ecologically important forest tree species in the face of climate change.

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From Forest to Freezer: a Comprehensive Seed Collection of the Kentucky Coffeetree, *Gymnocladus dioicus* (L.) K. Koch[1]

A.P. Schmitz[2] and J.D. Carstens[3]

Kentucky coffeetree, *Gymnocladus dioicus*, is a picturesque shade tree adaptable to urban conditions and drought, with no serious insect or disease problems. These traits make *G. dioicus* a promising candidate among diverse tree genera to replace ash (*Fraxinus*) trees affected by the emerald ash borer (*Agrilus planipennis*) or oaks (*Quercus*) being affected by oak wilt (caused by the fungus *Ceratocystis fagacearum*) within our cities. In nature, Kentucky coffeetree comprises only a small percentage of forested systems across the Midwest, warranting the assembly of an *ex situ* germplasm collection.

Collaboration between The Brenton Arboretum and the North Central Regional Plant Introduction Station has resulted in the acquisition and preservation of one of the most comprehensive *ex situ* woody plant seed collections represented in the United States National Plant Germplasm System and an extensive *G. dioicus* living plant collection at The Brenton Arboretum. To date, 80 georeferenced sites in 12 of the 14 states within the core native range of *G. dioicus* have been sampled. Targeted sites were systematically selected focused on Omernik Level III Ecoregions followed by geographic gaps. This targeted approach along with the sampling of multiple genets within a population strives to ensure the assembly of a genetically heterogeneous collection adaptable to a wide-range of climatic factors. Our efforts have documented the natural occurrence of this species along with its habitat and associated vegetation, soil type, plant health, rarity, and regeneration.

These collections will provide a foundation for research on genetic diversity, the potential to select elite lines for use in managed landscapes, and allow selection of specific seed sources to be utilized in restoration projects. Our firsthand knowledge of these genetic resources will help with restoration and management efforts of *G. dioicus* in forested ecosystems, strengthen risk assessment surveys, and demonstrate the importance of gene banks and arboreta in the conservation of tree genetics.

[1] A version of this paper was presented at the Gene Conservation of Tree Species – Banking on the Future Workshop, May 16-19, 2016, Chicago, IL.

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Ex Situ Conservation

Ex-situ Conservation of *Quercus oglethorpensis* in Living Collections of Arboreta and Botanical Gardens[1]

Matthew S. Lobdell[2] and Patrick G.Thompson[3]

Abstract

Quercus oglethorpensis (Oglethorpe oak) is an endangered species native to the southeastern United States. It is threatened by land use changes, competition, and chestnut blight disease caused by *Cryphonectria parasitica*. The species is distributed sparsely over a linear distance of ca. 950 km. Its range includes several disjunct populations potentially harboring unique genetic diversity or adaptive variation. Protected populations in the Bienville National Forest (Mississippi), Oconee National Forest (Georgia), and Sumter National Forest (South Carolina) are regularly monitored and managed through a combination of techniques including burn management and selective clearing. Recently, several additional populations were discovered in Alabama, primarily along rights-of-way or on private land where they should be considered vulnerable or at risk of extirpation. One documented population in Sumter County, Alabama has already been lost to land clearing or logging activities. Traditional techniques such as seed banking are insufficient for *ex-situ* conservation of *Q. oglethorpensis* because it has recalcitrant seeds. It has been demonstrated, however, that the species is suitable for cultivation in much of the United States, allowing for the possibility of *ex-situ* conservation in the living collections of arboreta and botanical gardens.

In 2015, through a joint venture between the U.S. Department of Agriculture Forest Service and American Public Gardens Association, seed and/or samples of scion wood were collected from populations of Oglethorpe oak in Mississippi, Alabama, Georgia, and South Carolina and propagated at The Morton Arboretum (Lisle, Illinois). From there, they will be distributed to five arboreta and botanical gardens: Chicago Botanic Garden (Glencoe, Illinois), Starhill Forest Arboretum (Petersburg, Illinois), Holden Arboretum (Willoughby, Ohio), Donald E. Davis Arboretum of Auburn University (Auburn, Alabama), and Moore Farms Botanical Garden (Lake City, South Carolina). Through cultivation in the Nationally Accredited Collections™ of these arboreta and botanical gardens, genetically diverse and representative germplasm of *Q. oglethorpensis* will be preserved and potentially utilized in future reintroduction efforts.

Introduction

Quercus oglethorpensis (Oglethorpe oak) is a species of conservation concern occurring in sparse, relatively isolated populations located within the southeastern United States. When put in the context of other eastern North American tree species, *Q. oglethorpensis* is a rather recent discovery, remaining undescribed until 1940. Originally mistaken for a southern disjunction of *Q. imbricaria*, closer inspection revealed *Q. oglethorpensis* to be a distinct species in the white oak group (*Quercus* sect. *Quercus*). The species was named for Oglethorpe County, Georgia, and thus indirectly named for James Oglethorpe, the founder of the State of Georgia (Coombes and Coates 1997). It can generally be distinguished from associated *Quercus* spp. by its entire to sparse or irregularly lobed leaves lacking bristles or awns (fig. 1), though confusion with *Q. durandii* may be possible without examination of reproductive material.

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Figure 1—Foliage of *Quercus oglethorpensis* as observed in Bienville National Forest; July 2015.

Subsequent floristics (Haehnle and Jones 1985, Marx and Thomas 1975) revealed a disjunct population of *Q. oglethorpensis* in Caldwell Parish, Louisiana, as well as additional populations in Georgia and South Carolina. Once the Caldwell Parish site was documented, an effort to locate the species on similar soil types in Mississippi was initiated, and three populations were discovered in Bienville National Forest (Wiseman 1987). In 1998, a population of the species was discovered in Sumter County, Alabama, with additional Alabama populations (fig. 2) located in Marengo and Wilcox counties in 2013 (Keener et al. 2016).

Figure 2—Habitat of *Quercus oglethorpensis* west of Catherine, Alabama (Marengo and Wilcox counties); July 2015.

Although the distribution of *Q. oglethorpensis* was found to be broader than initially realized, it still exhibits a fragmented distribution such that it is locally uncommon. It exhibits some susceptibility to chestnut blight disease, however the greatest threat facing the species is likely land clearing, particularly that which occurred prior to its description (Coombes and Coates 1997). It is listed as Endangered B1+2ce on the IUCN (International Union for Conservation of Nature) Red List, defined as a species

which is severely fragmented or known to exist at no more than five locations, with continuing decline inferred, observed or projected in area, extent and/or quality of habitat, and number of mature individuals (Nixon et al. 1998). It is not federally ranked as endangered, though is considered threatened in the state of Georgia.

Populations located in Sumter National Forest (South Carolina), Oconee National Forest (Georgia), and Bienville National Forest (Mississippi) are conserved and managed *in situ* by the U.S. Department of Agriculture Forest Service (USDA FS). Management includes prescribed burning or "release," in which competing, rapidly growing woody species such as *Liquidambar styraciflua* and *Nyssa sylvatica* are cut back from the vicinity of *Q. oglethorpensis* saplings to allow the latter to establish (D. Elsen, Bienville National Forest, personal communication, 2015).

Interest in horticultural cultivation of *Q. oglethorpensis* has been minimal. Several United States arboreta and botanic gardens began growing the species in 1980, following distribution of seed collected in Greenwood County, South Carolina by the Clemson University Forestry Department. Cultivation of Oglethorpe oak at The Morton Arboretum (Lisle, Illinois) demonstrated unexpected cold-tolerance for a species native to the United States southeast (fig. 3). It may be propagated either by seed or by grafting onto a compatible rootstock such as *Q. alba, Q. bicolor*, or *Q. robur*.

Figure 3—*Quercus oglethorpensis* in cultivation at The Morton Arboretum (Lisle, Illinois); fall 2007.

Quercus oglethorpensis has never been common in the nursery trade, though it has been available from specialty providers such as Woodlanders, Inc. (Aiken, South Carolina) and Heritage Seedlings (Salem, Oregon). The species has also been trialed in Europe, with an introduction occurring at Hillier Nurseries in 1978 (Hillier and Lancaster 2014). Its performance in Britain has been poor, likely due to insufficient summer heat for hardening of growth (Coombes and Coates 1997). All plants in cultivation worldwide appear to trace their lineage to Georgia or South Carolina populations, with those in Louisiana, Mississippi, and Alabama apparently unrepresented.

As with many *Quercus* taxa, *ex-situ* conservation through seed banking is not currently feasible for this species because its seed (acorns) are recalcitrant. Conservation through living collections of botanical gardens and arboreta is likely to provide more success, particularly when considering demonstrated success of cultivation in several regions of the United States.

Summary of Fieldwork

A concerted effort to collect seeds and/or scion wood from populations of *Q. oglethorpensis* for the purpose of *ex-situ* conservation was initiated in 2015 as a pilot project of the American Public Gardens

Association and USDA FS's Tree Gene Conservation Program. Populations in Mississippi, Alabama, and South Carolina were targeted and visited in late July and early August for verification and observation of seed production, and again in mid to late October for seed collection when applicable.

Populations in Bienville National Forest (Mississippi) were located and documented with herbarium vouchers (fig. 4). All individuals located appeared to be in good health with no significant threats noticed save competition from associated species. Fruit production was not observed, so a return visit that fall for seed collection was deemed unnecessary. Later that winter, USDA FS staff collected and sent scion wood to The Morton Arboretum for propagation by grafting.

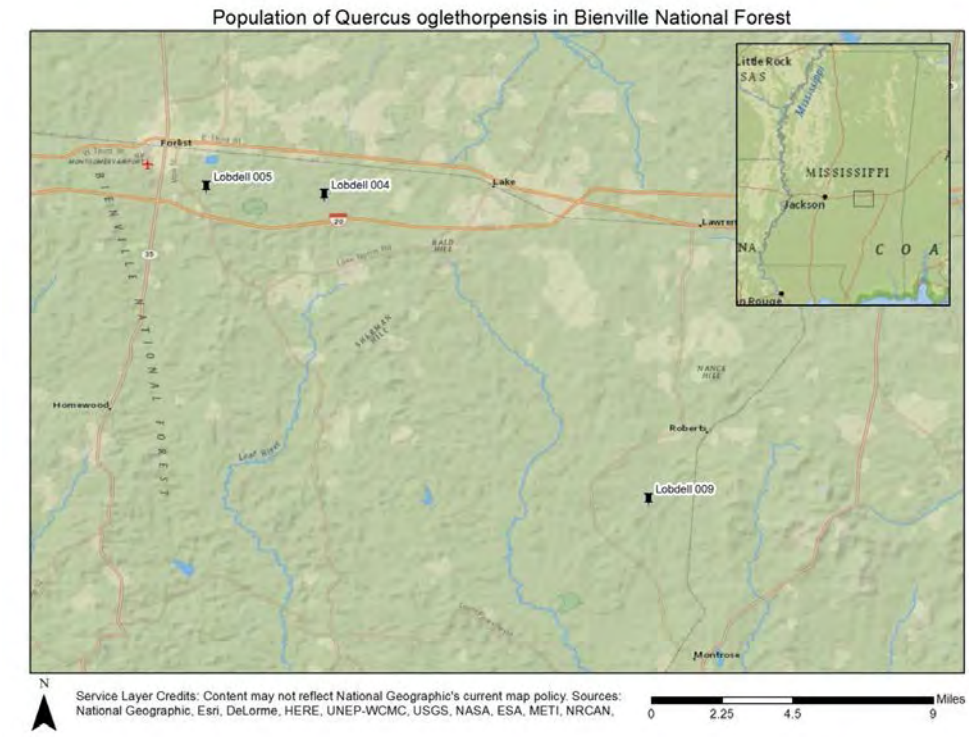

Figure 4—Map depicting *Quercus oglethorpensis* locations in Scott and Jasper counties, Mississippi.

Quercus oglethorpensis was first documented to occur in Alabama at a location identified by Al Schotz in Sumter County along the bottomlands of the Alamuchee Creek. These trees, which were also visited in summer, 2015, were apparently extirpated by logging activity in the area. Attempts by Al Schotz to relocate the original specimens or outlying members of the population failed, as did attempts by Wayne K. Webb in 2012 and 2013. The population west of Catherine, Alabama spanning Marengo and Wilcox counties is much more robust, consisting of at least 60 individuals. Multiple individuals were located and documented with herbarium specimens (fig. 5). Many were also observed producing suitable quantities of fruit. These were revisited in the fall for seed collection. Despite prolific seed production, seedling recruitment was virtually nonexistent in this population.

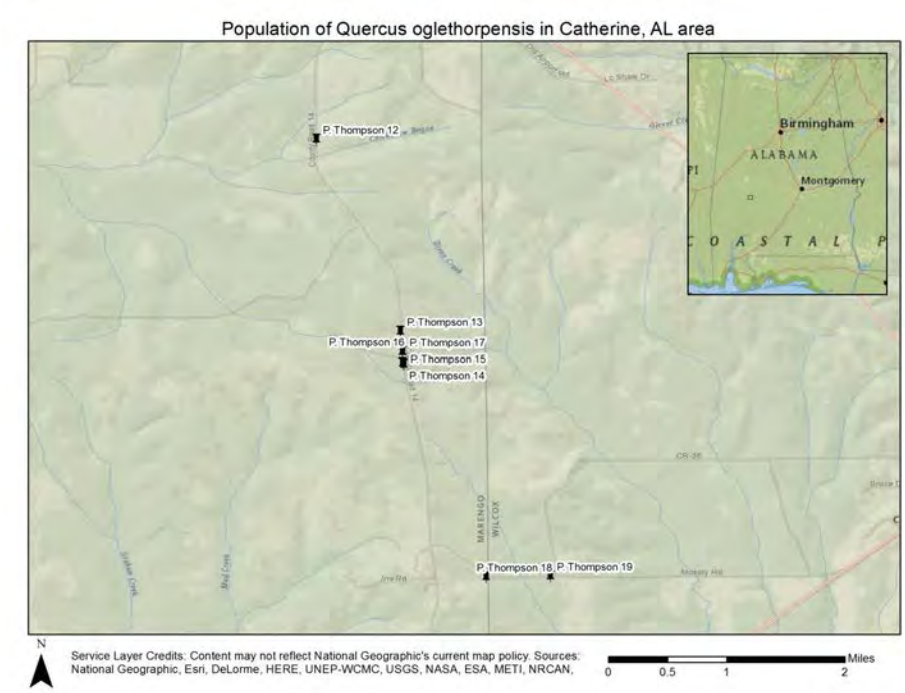

Figure 5—Map depicting *Quercus oglethorpensis* locations in Marengo and Wilcox Counties, Alabama.

Across the Sumter National Forest in South Carolina, the species appeared to be in good health and was more locally common than in Mississippi or Alabama. Several locations were visited throughout the forest and documented with herbarium vouchers (fig. 6), but only one was observed with sufficient fruit production to justify a second visitation in the fall.

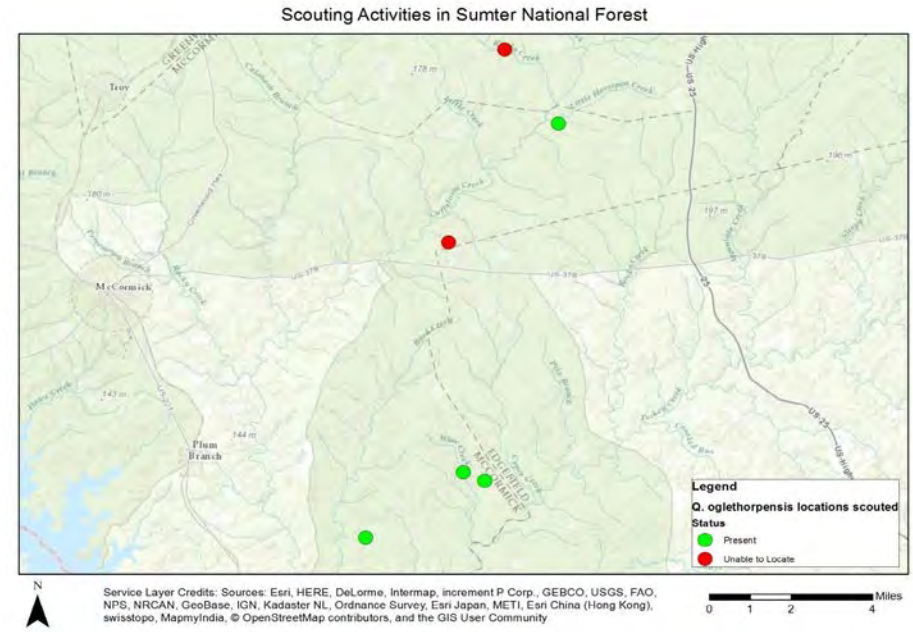

Figure 6—Map depicting *Quercus oglethorpensis* sites scouted in Sumter National Forest.

A search was also undertaken for a reported population in York County, South Carolina likely representing the most northern distribution of the species. It could not be located after several hours of searching. The population is likely extirpated (fig. 7).

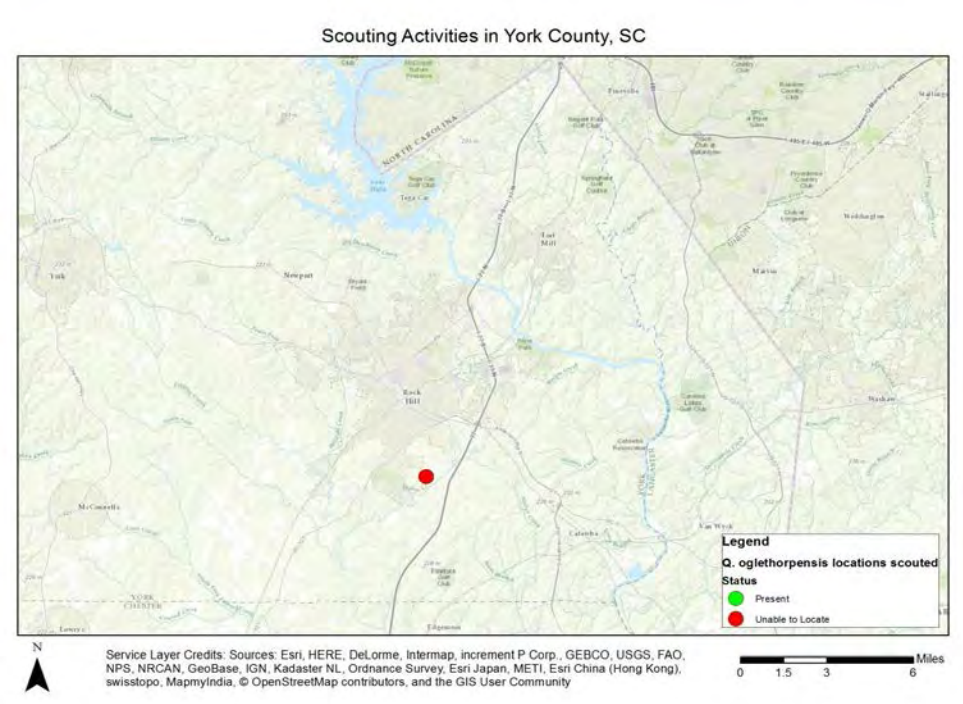

Figure 7—Map depicting potentially extirpated *Quercus oglethorpensis* site scouted in York County, South Carolina.

A total of 18 herbarium vouchers were collected during this project. They have been deposited in the United States National Arboretum Herbarium, with the duplicates held in The Morton Arboretum Herbarium. Though poor fruit production in Bienville and Sumter National Forests prevented extensive sampling in those locations, 281 seeds were collected as part of this effort, largely representing the population west of Catherine, Alabama (table 1). In January, 2016, USDA FS Staff from the Bienville National Forest sent cut stems from four individuals in Scott County, sufficient for a total of 35 scions to be grafted.

Table 1—Collections made from *Q. oglethorpensis* individuals (#V and #S indicate number of vouchers and seeds collected from each individual)

Project #	Collection #	#V	#S	State	County	Latitude	Longitude
MS-Sco-A-1	M. Lobdell 4	2	0	MS	Scott	32.33903	-89.40681
MS-Sco-B-1	M. Lobdell 5	2	0	MS	Scott	32.34278	-89.46128
MS-Jas-A-1	M. Lobdell 9	2	0	MS	Jasper	32.19828	-89.25672
SC-McC-A-1	M. Lobdell 14	2	0	SC	McCormick	33.97656	-82.103
SC-McC-B-1	M. Lobdell 16	2	0	SC	McCormick	33.83528	-82.18683
SC-McC-B-1	PCC15-SEUS086	0	7	SC	McCormick	33.83511	-82.18689
AL-Mar-A-1	P. Thompson 12	1	36	AL	Marengo	32.23148	-87.54363
AL-Mar-A-2	P. Thompson 13	1	36	AL	Marengo	32.20761	-87.53332
AL-Mar-A-3	P. Thompson 14	1	13	AL	Marengo	32.20372	-87.53307
AL-Mar-A-4	P. Thompson 15	1	36	AL	Marengo	32.20354	-87.5329
AL-Mar-A-5	P. Thompson 16	1	36	AL	Marengo	32.20512	-87.53304
AL-Mar-A-6	P. Thompson 17	1	9	AL	Marengo	32.20504	-87.53304
AL-Mar-A-7	P. Thompson 18	1	36	AL	Marengo	32.17713	-87.52282
AL-Wil-A-1	P. Thompson 19	1	36	AL	Wilcox	32.17725	-87.51493

Propagation and Distribution

Collected seeds were placed in germination flats containing 50 percent germination mix and 50 percent potting mix. Seed was warm stratified for 2 months (November 2015 to January 2016), then cold stratified for 4 months (January 2016 to May 2016). Germination was as low as 29 percent (two of seven seeds) for acorns collected from the ground in McCormick County, South Carolina, to as high as 100 percent for acorns collected from one individual west of Catherine, Alabama. A total of 218 out of 281 seeds germinated, with a mean germination percentage of 78 percent when considering all seed collections (table 2).

Table 2—Germination rates of acorns collected during project (accession numbers are of The Morton Arboretum)

Accession #	Project #	Sown (N)	Emerged (N)	Germination (%)
644-2015	AL-Mar-A-1	36	30	83
645-2015	AL-Mar-A-2	36	23	64
646-2015	AL-Mar-A-3	13	13	100
647-2015	AL-Mar-A-4	36	35	97
648-2015	AL-Mar-A-5	36	33	92
649-2015	AL-Mar-A-5	36	25	69
650-2015	AL-Mar-A-6	9	3	33
651-2015	AL-Mar-A-7	36	31	86
652-2015	AL-Wil-A-1	36	23	64
717-2015	Sc-McC-B-1	7	2	29
Total		281	218	78

In March, 2016, scions were grafted via the side-veneer method onto *Q. oglethorpensis* understock received from Heritage Seedlings (fig. 8). Grafts were waxed and callused in a tube for approximately 5 weeks. Upon removal from the tube and subsequent transplanting 1 month later, 28 of 35 attempted grafts (80 percent) appeared to have taken (table 3).

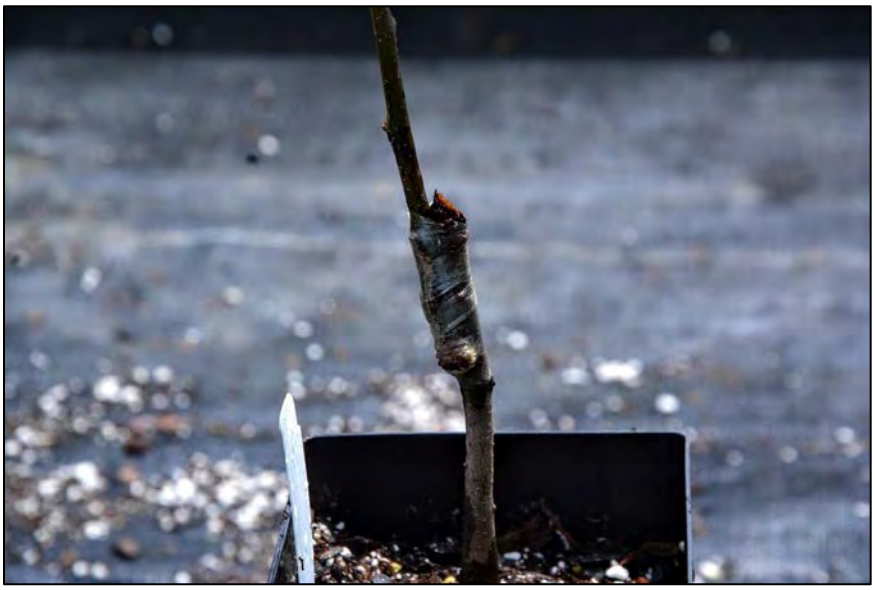

Figure 8—Side veneer graft of *Q. oglethorpensis* scions onto rootstock of the same species. The Morton Arboretum; June 2016.

Table 3—Success of side veneer grafts made in 2016; all scions from Scott County, Mississippi (accession numbers are of The Morton Arboretum)

Accession #	Attempts (N)	Takes (N)	Success (%)
1-2016	5	4	80
2-2016	7	7	100
3-2016	14	10	71
4-2016	9	7	78
Total	35	28	80

Seedlings and scions from this project will be grown in the collections of The Morton Arboretum (Lisle, Illinois), Chicago Botanic Garden (Glencoe, Illinois), Starhill Forest Arboretum (Petersburg, Illinois), Donald E. Davis Arboretum (Auburn, Alabama), Moore Farms Botanical Garden (Lake City, South Carolina), and Holden Arboretum (Willoughby, Ohio). Shipment of seedlings to these institutions will occur in early 2017 (fig. 9).

Figure 9—Seedlings of *Quercus oglethorpensis* in early production at The Morton Arboretum in June, 2016.

Conclusions

Despite low fruit production by many populations in 2015, this project was largely successful in terms of acquiring propagules of *Q. oglethorpensis* for *ex-situ* conservation. The populations West of Catherine, Alabama and in Bienville National Forest will likely be adequately represented in cultivation barring production failure. Additional seed collections from the latter population may be desirable if the plants grafted during this project exhibit graft incompatibility later. Collection of seed or scion wood from the population in Caldwell Parish, Louisiana would also be desirable, as germplasm from there does not appear to be represented in cultivation at all.

Of further interest is the long-term performance of *Q. oglethorpensis* in a horticultural setting. By cultivating and evaluating the species in the collections of botanical gardens and arboreta, a better understanding of its preferred growing conditions will be gained, and the success rate for *Q. oglethorpensis* in cultivation will likely increase. The potential also exists for cultivated plants to be utilized for reintroduction efforts where appropriate. Furthermore, interpretive and other educational activities occurring at botanical gardens and arboreta could increase awareness of the species, ultimately supporting further conservation efforts.

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Restoration Seed Reserves for Assisted Gene Flow Within Seed Orchards[1]

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Changing climate and declining forest populations imperil the future of certain forest tree species. To complement forest management and genetic conservation plans, we propose a new paradigm for seedling seed orchards: foster genetic mixing among a variety of seed sources to increase genetic diversity and adaptive potential of seed supplies used for forest restoration. This new type of seed orchard, a restoration seed reserve (RSR) targeting imperiled species, would incorporate into seed production the seed transfer concepts of assisted gene flow and composite provenancing (Aitken and Bemmels 2016, Broadhurst et al. 2008). The RSRs can be considered a secondary restoration gene pool under the restoration gene pool concept (Jones 2003). They are a hedge against future climate uncertainty by providing seed that minimizes adaptive constraints by maximizing genotypic diversity within restored stands (Lefèvre et al. 2014). While this approach may appear risky, we view it as a responsible strategy to augment, not replace, ongoing National Forest seed programs. The goal of RSRs is to provide range-wide, restoration-ready, seed that has increased adaptive diversity beyond what is available from native local seed sources.

Operationally, RSRs differ from standard seed orchards because no attempt would be made to select for production forestry traits, surmise which adaptive traits are needed, or adhere to strict seed zones, although options remain flexible with respect to policy arising from newly drawn seed zones. At production age, a properly designed RSR would contain about 200 trees grown from seed collected from 20 or more distinct populations across the species' range. RSRs avoid inbreeding by retaining no related individuals (no clones or family structure) and containing accessions selected only for seed production traits (by rouging an initial planting of 2000 to 2400 seedlings). Such a design assures gene flow among genotypes sampled from dispersed populations. For most tree species, especially those in the southern United States, outbreeding depression from intercrossing among diverse provenances would not be an issue (Frankham et al. 2011).

While not all RSR seed is expected to be fully adapted to any particular restoration site, the idea is that, as a restoration stand becomes established, a high enough proportion of individuals will be naturally selected to survive and successfully reproduce; any maladapted offspring would be selected against or comprise a low fraction of a restored stand. RSRs obviously do not follow the "local is best" approach to genetic conservation. When a species' local seed sources are from small, isolated, or inbred populations, however, their genetic diversity may be insufficient for future restoration needs (Jones 2013). Because gene flow can promote adaption and not degrade local adaption that may exist in neighboring populations (Tigano and Friesen 2016), RSRs can redress genetically depauperate local seed sources and provide supplemental material for forest restoration of sensitive species. Further, the RSR design is economically feasible for seed collection and orchard management, and can capitalize on the range-wide collections done to date.

RSRs would be established on current National Forest System seed orchard property to produce seed in support of restoration activities. Alternatively, for arboretums, botanical gardens, and other organizations with a restricted land base, there are opportunities to meet their species conservation goals, with RSRs or other strategies, by partnering with U.S. Department of Agriculture Forest Service geneticists to share expertise and gain access to seed orchard and experimental forest resources.

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A Multi-state Collaborative Effort to Conserve Butternut *Ex Situ*[1]

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Butternut (*Juglans cinerea*), a native riparian forest tree, has suffered significant decline throughout most of its range due to a fatal fungal disease, butternut canker (caused by *Sirococcus clavigignenti-juglandacearum*). It has also been subject to natural hybridization pressures from Japanese walnut (*J. ailantifolia*), first introduced to North America ~1850. Butternut is now listed as a "species of concern" in Canada and similarly classified in several states of the United States. Previous experience indicates site-related shifts in fitness for Japanese walnut × butternut hybrids compared to "pure" butternuts. The presence of at least putative "tolerance" to butternut canker in some butternuts resulted in the establishment of a multi-state effort to restore this species by establishing a series of *ex situ* germplasm collections in the northeastern United States. Both nuclear and chloroplast markers developed at the University of Notre Dame were used to define the hybrid status of 1400+ individuals across 48 sites, representing 17 states plus two Canadian provinces. The proportion of hybrids found in this survey averaged 17 percent, but this percentage varied greatly across sites, with more hybrids occurring near farms and/or agricultural fields than in forested areas. From 2009 to 2014, non-hybrid individuals that also exhibited some level of tolerance to butternut canker were identified in Iowa, Missouri, Pennsylvania and Vermont. Dormant scions were collected and grafted on black walnut (*J. nigra*) seedling rootstocks at the University of Missouri. Similar propagation efforts were also undertaken in Indiana using locally-sourced scions. Successful grafts have been outplanted as clonal gene banks/seed orchards in Indiana, Missouri, Pennsylvania and Vermont, established by the Indiana Department of Natural Resources, University of Missouri, Pennsylvania Bureau of Forestry, and Vermont Department of Forests, Parks and Recreation, respectively. In addition, a clonal population representing accessions from five states was established by the Connecticut Agricultural Experiment Station near Windsor, Connecticut to serve as a source of diverse germplasm for use in future butternut canker screening trials.

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Germplasm Conservation for Species Restoration: Examples From Efforts to Restore the American Chestnut[1]

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American chestnut (*Castanea dentata*) was once a foundational species in much of its native range, especially in the Appalachian Mountains of the eastern United States. Unfortunately, the species was driven to functional extinction by the accidental importation of an exotic fungal pathogen (*Cryphonectria parasitica*), the causal agent of chestnut blight disease. Efforts to restore the American chestnut have been ongoing since the early 20th century and include three primary techniques: classical plant breeding, genetic modification, and reduction of fungal virulence.

While restoration of the American chestnut focuses primarily on incorporating disease resistance into a founding population, it has also facilitated the *ex situ* and *in situ* conservation of remnant populations. Conservation of American chestnut germplasm significantly increases genetic diversity and local adaptation among remaining populations, which in turn can contribute genes to disease resistant founder populations. Any plan to achieve complete species restoration in the long-term must also conserve diverse and locally adapted sources of American chestnut in the near-term.

Through a combination of traditional plant breeding methods, efforts of citizen scientists, and other means, preservation of genetic diversity of remaining American chestnut populations has become a priority for restoration work. Decades-long improvement programs have incorporated range-wide diversity and adaptations into the species' disease-resistant stock, developed germplasm conservation orchards and collections, and inventoried and monitored remaining wild individuals and small populations. With chestnut reintroduction trials now underway, the focus on understanding the diversity of the species, as well as the pathogen, has gained new momentum. Improvements in genomic technologies have provided new tools for assessing species diversity and guided restoration efforts.

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Science in Support of Conservation

The Role of Seed Analysis in Genetic Conservation[1]

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Abstract

Long term storage of seeds at freezing temperatures is one strategy for genetic conservation of tree species. It can be used to preserve species that produce seeds that remain viable after drying to a low seed moisture content. The U.S. Department of Agriculture Forest Service (USDA FS) National Seed Laboratory (NSL) began long term seed storage for genetic conservation in 2005. The program is mostly focused on five-needle pines (*Pinus*) and ash (*Fraxinus*) species. The genetic resources of both groups of species are eroding rapidly in the wild because of attack from invasive exotic pests for which there are no effective control measures. Seeds on the other hand, once placed in a freezer, are quite safe and require comparatively little maintenance. Part of the maintenance required for long term stored seed is to know the seeds are viable and sufficiently dry going into storage and that they remain alive and dry while in storage. To gain this information it is necessary to use seed analysis. The various seed analysis methods used at the National Seed Laboratory are described.

Introduction

Long term seed storage is a major tool for preserving the genetics of crop plants and perhaps the most famously reported example of this activity is the Svalbard Global Seed Vault in Norway for which the reader can find many references on the internet. But underpinning this magical arctic hideaway tunneled into a remote frozen mountain, and other collections of seeds held long term around the world, is seed analysis and seed technology. Before seeds are placed into storage they must be tested for viability and moisture. Viability must be assured because only living seeds are of any value in preserving the genetics, and moisture is tested because only seeds that can be and are dried to low moisture status can be kept long term in the freezer. These tests are conducted as the seed is first entered into the collection and then periodically after a specified number of years in storage. If viability is found to be declining then some seeds are planted to produce a fresh batch of seeds to replace the one that is declining in viability. Herbaceous plants produce seeds in 1 or 2 years generally, but trees typically have a comparatively long period of maturation before producing seeds. Because trees can take a number of years to mature and produce seeds and require large parcels of land to reach maturity, the practice of long term seed storage for trees was not practiced very extensively.

However, in 2005 the Chief of the U.S. Department of Agriculture Forest Service (USDA FS) expanded the role of the National Seed Laboratory (NSL) to include long term seed storage for genetic conservation. This was primarily in response to the loss of five-needle pines (*Pinus*) and ash (*Fraxinus*) species. Both groups of species are disappearing in nature because of attack from invasive exotic pests for which effective affordable control measures do not exist. Long term seed storage was an available cost effective method to preserve the genetic resources for both groups. This paper is a general overview of the testing conducted on seeds stored for genetic conservation and how those tests are used at the NSL. Extensive detail on seed testing can be obtained from the rules and handbooks published by the Association of Official Seed Analysts (www.aosaseed.com) and the International Seed Testing Association (ISTA, www.seedtest.org).

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The Testing Methods

Testing Seed Moisture

Seed moisture is the most important factor in preserving the viability of stored seeds (Justice and Bass 1978). Therefore, before a seed is placed in freezer storage, it is dried in air with a stable relative humidity in the range of 25 percent to 30 percent. Once the seed has reached moisture equilibrium with the air around it, it is ready to seal in a moisture proof container to maintain its dry condition. To be certain the seeds are dry, a seed moisture test is required. This moisture test can be conducted using the destructive method of oven drying at 103 ºC or the nondestructive equilibrium relative humidity (eRH) test (Baldet et al. 2009, Gold and Manger 2014, Karrfalt 2014). Because most of the seed lots in the NSL collection are irreplaceable and generally expensive to produce, the nondestructive eRH test is used to preserve as many seeds as possible. As an example, the cost of putting an ash sample into the collection is about $250. These samples on average weigh about 113.4 g (4 ounces). Therefore, the per-pound cost is $1,000, substantially more than commercially produced tree seed. The cost of the pine would be even higher as these trees are in more remote mountainous areas requiring more effort to visit, while the ash are generally reached by vehicle along well traveled roadways. An eRH test is done by placing the seeds in a sealed container along with the probe of a hygrometer (fig. 1). The air inside the container will equilibrate to the moisture status of the seeds. When the relative humidity in the container is in the range of 25 percent to 30 percent we know that the seeds are sufficiently dry and ready for storage. It is critically important that the seeds be at equilibrium internally. That means that inner and outer layers are at the same water potential. Otherwise the eRH test will be representative of the outer layers of the seed only and give a false reading. Sealing the seeds in the test container 16 to 24 hours before the test aids in ensuring the seeds have had time to reach equilibrium. The hygrometer probe does not need to be attached during this equilibration period. Once the probe is attached to the test container, the eRH can be taken after about 10 minutes. The reliability of this moisture testing method is seen in that samples tested with this method have lost no viability after 10 years of storage.

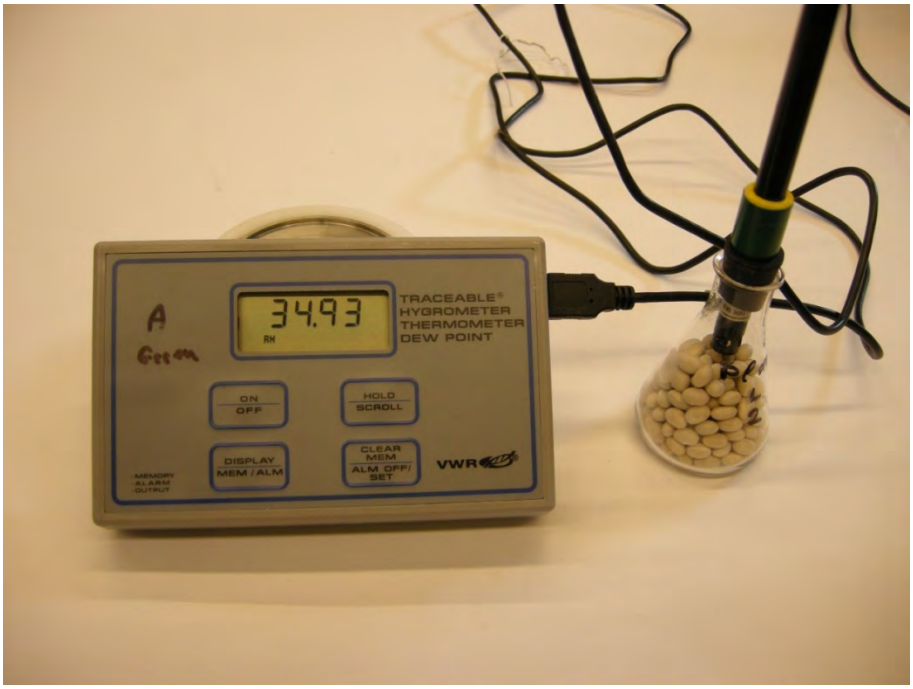

Figure 1—Testing the equilibrium relative humidity of seeds with a hygrometer.

Testing Viability

Storing dry seeds in a freezer is a good conservation strategy only if the seeds are alive. This means that seed viability must be tested before placing seeds into storage and periodically while they are in storage. Without this viability information, there is no way to know how many new plants can be produced from the seeds or if the seeds need to be replenished with a new collection.

Germination Testing

Germination is most simply defined as the emergence from the seed of a normal plant. A standard germination test for commercial purposes consumes 400 seeds, but that number of seeds is reduced for genetic conservation in order to minimize the number of seeds used for testing. This extends the time before the lot is reduced to the point where it needs to be replenished. 200 seeds or as few as 10 can be tested depending on lot size and the precision of estimate required. Germination is the gold standard for testing seeds as we actually see the full seedling produced.

Sometimes seeds do not germinate when placed in conditions favorable for germination. This result is usually referred to as dormancy. Various treatments are used to overcome dormancy, but moist chilling is most often used on trees. Some dormancy is easily overcome and only 30 to 60 days of chilling are needed to make most of the good seeds germinate. When dormancy is harder to overcome, then quick viability tests are often substituted for germination. These quick tests are described in the following sections. Even sometimes when the protocol to overcome a complex dormancy is known, a quick test is used in order to more rapidly complete the processing of seeds into the collection.

Excised Embryo (EE) and Tetrazolium (Tz) Tests

Excised embryo and tetrazolium tests along with x-ray are referred to as quick viability tests. In this way they are distinguished from germination and thought of as an estimate of what the germination value might be. For an excised embryo test the embryo is removed from the seed and placed in a controlled environment chamber where optimal temperatures, light levels, and moisture are provided (fig. 2.). Separating the embryo from the rest of the seed permits the embryo to initiate growth. Those embryos that initiate growth are said to have germinated. Seeds that contained embryos that germinate or remain sound in the excision tests are considered viable.

Figure 2—An excised embryo test of *Fraxinus* embryos. Greening and spreading of the cotyledons indicates these embryos come from live seeds.

The tetrazolium test uses a vital stain, tetrazolium chloride salt, which will stain living tissues a light pink and any dead seed tissue will not stain (fig. 3). The seeds are first imbibed with water, usually cut open slightly and then placed in a solution of the Tz salt. The whole test takes approximately 2 to 3 days. The EE test takes 7 to 10 days. The Tz test is evaluated usually under a microscope and each seed must be cut a second time to fully expose the embryo structures and food tissues. The excised embryos of some species do not germinate or do not germinate as well as the Tz test would indicate. The EE test therefore, is not used on those species. Combining germination with Tz is another strategy. Germination is first attempted and then any un-germinated seeds are tested with Tz. The sum of the Tz test and the germination test are added together for a single estimate of viability.

Figure 3—A viable *Viburnum* seed stained pink in a tetrazolium test. Note the embryo at the bottom of the seed.

X-ray Testing

X-ray testing is very fast and nondestructive. It clearly shows how many seeds are full and well-formed and how many are damaged (fig. 4). If seeds are properly handled during collection and cleaning it can be relied upon to give a good estimate of initial viability. However, x-ray has less reliability for seeds that have been stored because losses of viability in stored seeds are usually not visible morphologically. When the seeds are too rare to sacrifice any in a destructive test, initial viability is estimated exclusively with x-ray.

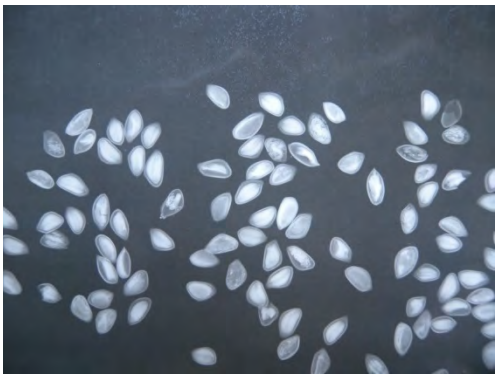

Figure 4—Radiograph of *Pinus* seeds showing many seeds that were likely damaged by insects. Seeds that appear completely dark are classified as empty and seeds with large portions that appear dark are damaged.

Purity

Purity tests indicate how much of a seed lot is pure seed and how much is trash. Genetic conservation seed samples are cleaned as close as possible to 99 or 100 percent pure to reduce the space used in the freezer. Therefore, testing for purity is not normally conducted on genetic conservation samples.

Seed Weight

The seed weight test tells the number of seeds per gram and ultimately the number of seeds in the collection. It is very important to know the number of seeds in a collection because this is part of knowing how many new trees can potentially be produced. In other words, this number says how large the genetic pool is in terms of numbers of individuals. For seed lots of the size used in the tree nursery trade, the International Seed Testing Rules require averaging the weight of eight 100-pure-seed subsamples to estimate the 1000 seed weight or number of seeds per gram. Because the genetic conservation samples are smaller and coming from one female tree they were believed to be more uniform than seed lots in the nursery trade. This potential greater uniformity suggested that possibly fewer seeds could be tested. A comparison between the standard ISTA procedure and a procedure using two subsamples of 100 seeds indicated the two procedures produce the same answer for our conservation seed lots. Therefore, seed weight determinations are made using only two subsamples of 100 seeds rather than the eight subsample ISTA method. Other aspects of the test are kept the same as the ISTA test. Reducing the number of seeds tested speeds up the process and reduces labor costs.

Summary

The seed analysis procedures described in this paper are indispensable to scientifically managing seeds stored for genetic conservation. They are needed to be sure the seeds are at a moisture level such that they remain viable during storage, to be sure the seeds are alive when entering storage, and to monitor seed viability over time.

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Facilitating Gene Conservation With Existing Common Gardens[1]

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Species and populations of forest trees stressed by a rapidly changing climate must adjust or they will not survive. Loss of species and populations could occur if they lack the genetic variability to adapt, the capacity to migrate to suitable habitats, or the ability to adjust to new environments through phenotypic plasticity. Fortunately, many forest tree provenance studies were established in the 20th century across the United States. Most of the studies were initiated or funded by the United States Department of Agriculture (USDA). Many of these plantations are now over a half-century old, and represent a valuable resource for understanding how trees respond to climate change. Although the original purpose of these provenance studies was mainly for traits of commercial interests, now they can help us understand and quantify intraspecific genetic variation in presumptively adaptive traits in response to climate change.

In this presentation, we summarize the scope and richness of USDA initiated or funded provenance studies in the eastern United States. We demonstrate that common gardens planted in multiple locations can be used as experiments in climate change, where climate has been manipulated to differ from the climate where the trees (the species or the population) originated and to which they are presumably adapted as a result of generations of natural selection. Thus, except for local controls, the trees in common gardens have been growing for many years in a different climate from the one they were adapted to at the site from which they were collected. In addition, we use two provenance tests to show (1) populations with different climatic origins also are measurably different in their growth and phenology, (2) within-generational rapid phenotypic adjustment to climate change. We advocate that data from common garden experiments can be used to study within- and among-population responses to novel climates and can serve genetic conservation objectives for tree species.

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Early Results From a Newly-Established Provenance Test in Valley Oak (*Quercus lobata*) Show Significant Population Differentiation[1]

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Valley oak (*Quercus lobata*) is a majestic, endemic California native oak, found throughout California's foothills, valleys and flood plains. It is threatened because:

- Contracted range due to housing and agriculture.
- Low recruitment in existing stands as a function of land use and increased stress on existing trees and recruitment due to recent and projected climate change.

Valley oak substantially shapes ecosystem functions and biodiversity where it occurs through above ground (e.g., provides shelter with cavities, and food with acorns) and below ground (e.g., soil stability and productivity) contributions. Valley oak is also important to California's diverse Native American cultures, including the location of historic trade routes and settlements. To create a resource for research, education and conservation, in 2015, we established a fully-replicated two-site provenance trial from a range-wide acorn collection, representing 674 uniquely identified maternal trees from 95 populations of valley oak from across California now growing at the U.S. Department of Agriculture Forest Service's Institute of Forest Genetics in Placerville, California, and the Chico Seed Orchard in Chico, California. Provenance tests, such as the one described here, are powerful research tools, allowing for the comparison of trees from a diverse range of climates in common garden settings. By comparing growth and performance in two climatically different gardens, we are able to understand more about the underlying genetics of traits.

While the trees were growing in the greenhouse, and during the first 2 years after the provenance trial was established, we measured growth and phenology at both sites. We found genetic correlations among a variety of leaf, growth, and phenological traits expressed across families. Many of the observed differences are associated with climatic conditions where the seeds were collected. For example, trees from warmer sites (higher mean annual temperature) were taller. Although results are preliminary given the short time span since outplanting, cluster analysis based on data collected so far shows groups of populations that are beginning to show similar trends for growth and phenology.

This provenance trial represents a major scientific and conservation resource for valley oak as a species and a community as they face multiple environmental challenges. Furthermore, we are in the process of sequencing the valley oak genome, which will allow us to integrate phenotypic and genomic data to identify the genes underlying adaptive traits. Together with the data collected from the provenance test, we will be able to address questions like seed transfer distances as well as identifying populations of conservation concern, information that will further advance our potential to inform the conservation of this iconic species.

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Posters

In-situ Genetic Conservation of White Ash (*Fraxinus americana*) at the Allegheny National Forest[1]

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Abstract

The emerald ash borer (EAB, *Agrilus planipennis*) is a non-native forest pest that has been sweeping across North America causing widespread mortality of trees in the genus *Fraxinus*, which includes the economically valuable white ash (*F. americana*). The rapid spread and lethality of EAB, paired with low levels of natural resistance in ash trees, has left forest managers with few management options to slow EAB or to conserve ash trees. Here we present the initial findings of a collaborative project to pursue regional genetic conservation of white ash trees across the Allegheny National Forest. The network of white ash conservation plots consists of 29, 3.24 ha (8 ac) plots distributed across the forest, each containing a subset of 20 ash trees that received insecticidal treatment with emamectin benzoate trunk injections. This design will allow us to test for associational protection of non-insecticide treated trees with treatment levels varying from 10 to 91 percent (i.e., proportion of protected ash trees in a stand). In conjunction with the ash conservation project, we monitored ash tree canopy health from 2010 (prior to the arrival of EAB) to 2015 across 193 permanent plots in the Allegheny National Forest. Following the arrival of EAB to the Allegheny National Forest in 2013, we conducted a follow up survey of ash canopy health in 2015 and discovered further canopy decline in both upper and lower slope positions, likely caused by EAB. Furthermore, canopy traps revealed that EAB, which was first discovered in the southern region of the forest in 2013, had now spread to the northern region.

Introduction

Native and non-native invasive forest pests represent considerable threats to host species and their associated forest ecosystems (Flower and Gonzalez-Meler 2015). Long-term persistence of affected species, as well as the maintenance of forest diversity, productivity and associated ecosystem services, is predicated on conserving susceptible individuals and populations of at risk species across the landscape. Furthermore, conservation practices that maximize the genetic diversity of the residual population and optimally mimic that of the initial population are preferred.

The emerald ash borer (EAB, *Agrilus planipennis*) is an invasive beetle which was inadvertently introduced into North America from Asia in the 1990s (Siegert et al. 2014). It feeds almost exclusively on ash trees (*Fraxinus* spp.) which are widely distributed across urban and forest environments of North America (MacFarlane and Meyer 2005). The widespread distribution of ash, coupled with EAB's rapid dispersal, has contributed to its swift invasion across the United States. Larval feeding of EAB creates serpentine galleries that girdle host trees, resulting in mortality in >99 percent of trees (Flower et al. 2013, Knight et al. 2014). Tree mortality and local ash population collapses occur in as few as 2 to 5 years. Because of the high degree of ash tree mortality, the future re-establishment of ash depends on post-EAB

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seed germination and seedling recruitment. Furthermore, although EAB populations crash following host mortality, EAB populations subsist at low densities for years after canopy mortality, potentially threatening a recruited seedling and sapling cohort. Extirpation of the genus *Fraxinus* further threatens the diversity of temperate forests of the eastern United States, which are simultaneously threatened by a variety of other forest pests and pathogens. *In-situ* conservation approaches for maintaining ash genetic diversity across the landscape are essential for maintaining biodiversity and forests resilient to disturbances.

We are currently engaged in a collaborative project to examine the efficacy of insecticidal treatments of white ash (*F. americana*) trees as a conservation strategy to manage forests affected by the emerald ash borer. The goals of the project are to:

1. Provide *in-situ* conservation of ash genetic diversity on the Allegheny National Forest (ANF), Pennsylvania, through the treatment of a subset of 20 ash trees in each of 29, 3.24 ha (8 ac) plots across the forest (a total of 580 treated trees).
2. Test treatment efficacy across a range of conditions including initial tree health (ranging from healthy to some dieback), landscape positions (upper vs. lower slope), and across a range of ash densities (ranging from 21 to 201 ash trees per plot).
3. Test for associational protection of untreated ash trees in treatment plots across a range of ash densities. We hypothesize that, like herd immunity in vaccination, treating a high proportion of trees will provide some protection to untreated trees.
4. Monitor landscape-scale progression of EAB, ash mortality, and EAB population dynamics throughout the forest.

Methods

In 2010, prior to the arrival of EAB, we established a network of 193 ash health monitoring plots across the ANF. Plots were distributed across both upper and lower slope conditions allowing investigations into differential decline patterns associated with soil weathering and abiotic parameters. Using a 1 to 5 categorical scale modified for ash trees by Smith (2006), the canopy health of ash trees was assessed (post leaf expansion) to capture pre-EAB ash canopy health conditions. Canopy condition ratings were as follows: 1 represents a healthy tree with no defoliation; 2 represents a canopy with slight reduction in leaf density; 3 represents a canopy that is thinning and some of the top branches exposed to sunlight are defoliated (<50 percent dieback); 4 represents a canopy with >50 percent dieback; and 5 represents a dead tree with no leaves remaining in the trees canopy (see Flower et al. 2013 and Knight et al. 2014 for more details). The EAB was subsequently confirmed on the forest in June 2013. During the summer of 2015, the plots were re-measured to track the progression of canopy decline and its relationship with EAB.

In 2015, we began an insecticidal treatment study to conserve the genetic diversity of white ash on the ANF. We treated a subset of 20 ash trees per plot across 29 plots with emamectin benzoate stem injections, a systemic insecticide proven to provide multiple years of protection to healthy tress and those in moderate stages of canopy decline (Flower et al. 2015, Herms et al. 2014). All ash trees within each 100 m radius plot were measured, rated for canopy condition, and trees were randomly selected for insecticide treatment. Ash density in treatment plots ranged from 21 to 201 trees. Thus, treatment of 20 trees in each of these plots yielded a range of proportions of treated trees from 10 to 91 percent, allowing for a robust design to test for associational protection of untreated trees. We expect that untreated trees may benefit from the toxicity of their treated neighbors, and this design should determine what proportion of treated trees it may take to see these benefits. Finally, purple panel traps glued with tangle foot and baited with Manuka oil lures were deployed in a subset of insecticide treatment plots (n = 12 plots, two traps/plot) to track the distribution of EAB throughout the forest (fig. 1).

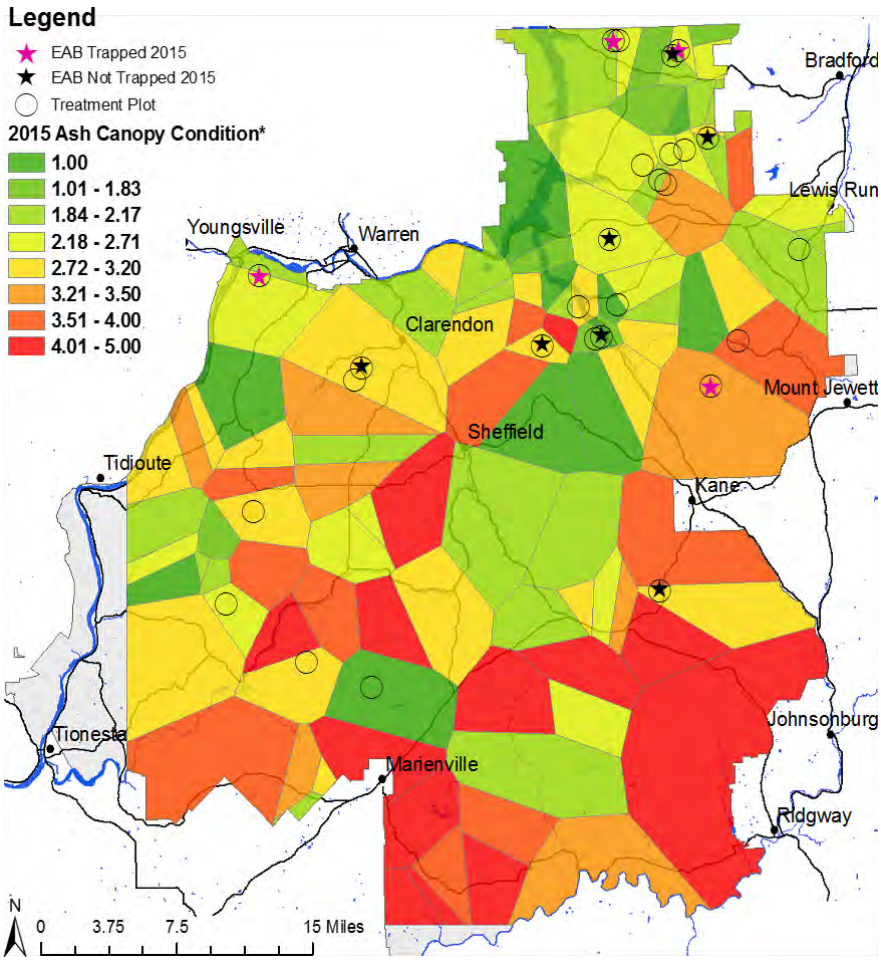

Figure 1—Map depicting the ash canopy health across the Allegheny National Forest, green to red gradient corresponds with ash canopy condition of 1 to 5 (healthy-dead). Open circles denote treatment plots and stars denote locations where emerald ash borer was trapped (pink) and not trapped (black).

In order to investigate ash canopy decline between 2010 and 2015, a repeated measures analysis of variance (RM ANOVA) was utilized with lower and upper slope positions as a main factor and ash canopy conditions in 2010 and 2015 as the repeated measure. This test was conducted using SYSTAT v. 12 statistical software (SYSTAT 2007).

Preliminary Results and Conclusions

Trapping efforts from 2015 revealed that, since its discovery in 2013 in the southern region of the forest, EAB is continuing to spread across the ANF. EAB trapping was confined to the central and northern portion of the ANF and detected EAB in four treatment plots (fig. 1). Despite the presence of EAB in these areas, ash canopies remain healthy, indicating EAB's recent arrival to the region.

Ash canopy deterioration is more severe and widespread in the southern ANF, which was expected based on the discovery of EAB prior to 2013 in counties adjacent to the southern extent of the forest (fig. 1). Additionally, it appears that canopy decline is more advanced in areas along roads and towns as predicted by the vehicle hitch-hiking spread mechanism proposed by Prasad et al. (2010). The 2010 ash survey revealed declining canopies on the upper slopes attributed to foliar nutrient deficiencies associated with base cation leaching from soils (Royo and Knight 2012; fig. 2). This difference between the canopy health of ash trees in the lower and upper canopies is consistent between time periods, with the lower

slope canopies exhibiting healthier canopies relative to upper slope positions (RM ANOVA, F = 20.527, *P* <0.001). The 2015 survey indicates continued canopy decline of ash across the ANF (RM ANOVA, F = 121.272, *P* <0.001; fig. 2).

Efforts are underway to collect ash foliage from across the ANF to estimate population genetics parameters (using genus specific microsatellite markers) and to ascertain the proportion of ash genetic diversity that the insecticide treatments are conserving. Continued insecticide applications will be conducted to conserve the genetic diversity of ash. Additionally, EAB trapping across the ANF will continue in order to monitor EAB populations. Based on these and future findings, we will make recommendations to managers regarding the efficacy of emamectin benzoate injections on trees with varying initial canopy health. These results will provide insights into regional conservation efforts of tree species in decline from invasive forest pests.

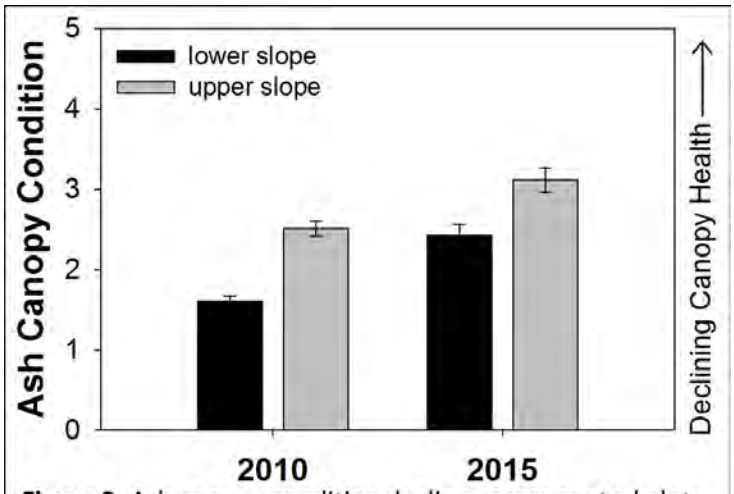

Figure 2: Ash canopy condition decline across control plots between 2010 and 2015 in both lower (black) and upper (grey) slope positions.

Acknowledgments

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Forest Gene Conservation Programs in Alberta, Canada[1]

Jodie Krakowski[2]

Summary

Provincial tree improvement programs in Alberta began in 1976. Early gene conservation focused on *ex situ* measures such as seed and clone banking, and research trials of commercial species with tree improvement programs. The gene conservation program now encompasses representative and unique populations of all native tree species *in situ*. The *ex situ* program aims to capture representative samples of each species by natural subregion combination in the provincial seed archive. Species whose seeds do not store well may be represented in clone banks, but resources limit those archives to threatened populations or those of special adaptive interest. Since most forested area in the province is public land, cooperators in tree improvement programs are legally obligated to share the responsibility for gene conservation for the benefit of all Albertans.

In Situ Conservation Status

The provincial *in situ* forest gene conservation strategy includes all 28 tree species native to Alberta. Priorities for species and populations were developed based on gap analysis interpolating species ranges, ecological regions, and expert knowledge. Ecological subregions (fig. 1) were used as surrogates for population adaptive differences as quantitative genetic data was lacking for many species. A spectrum of protection is afforded by different types of protected areas; all prohibit commercial forest harvest. National parks total 56.8 percent of Alberta's protected areas. Protection is ample in the Rocky Mountains Natural Region, but there are deficits in the Canadian Shield, Foothills, Grassland, and Parkland Natural Region (fig. 2). Climate change scenarios highlight vulnerabilities in long term genetic conservation as climates characteristic of natural subregions and species climatic envelopes are predicted to shift relatively rapidly beyond observed historic migration rates and protected area boundaries.

[1] A version of this paper was presented at the Gene Conservation of Tree Species – Banking on the Future Workshop, May 16-19, 2016, Chicago, IL.

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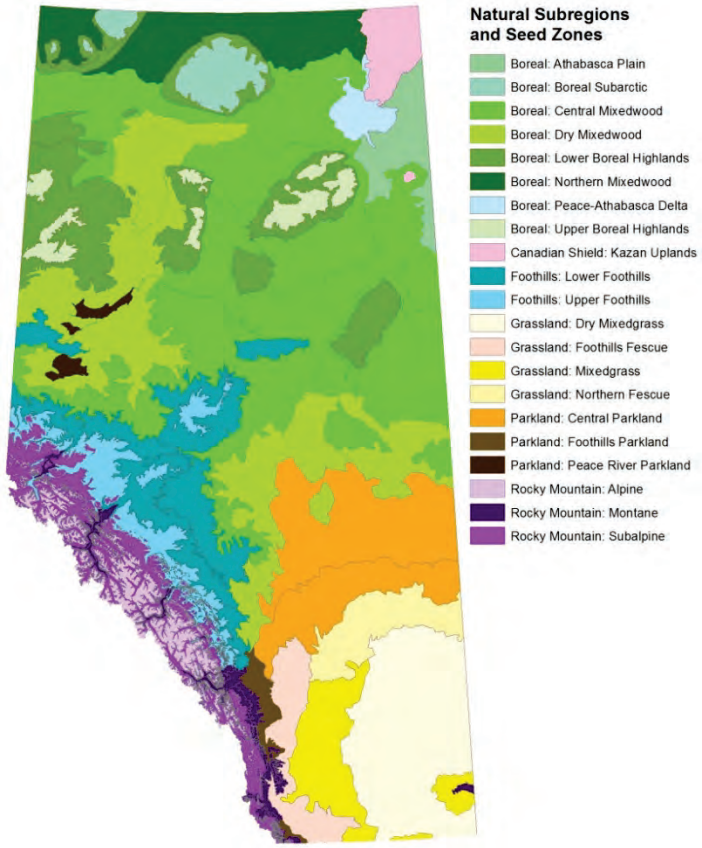

Figure 1—Natural regions and subregions of Alberta.

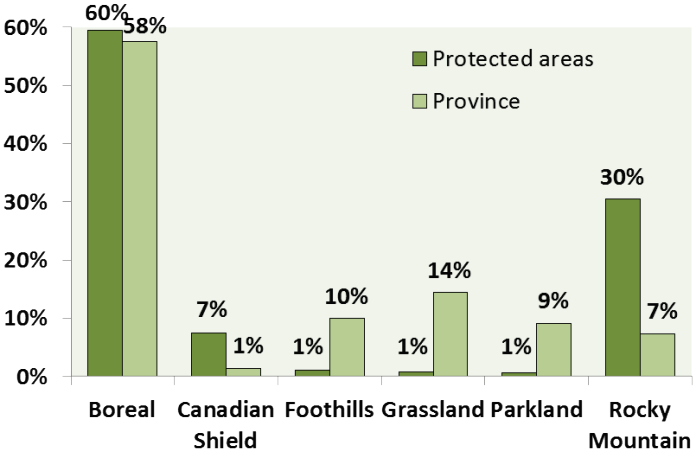

Figure 2—Distribution of natural regions by total area, and within protected areas in Alberta.

Ex Situ Conservation Status

Alberta has an *ex situ* conservation strategy for native species. Gaps and priorities were identified, emphasizing representative sampling of populations and targeted sampling of species at risk (table 1). The *ex situ* program complements provincial tree improvement programs through seed and clone bank archives. Built in 1978, a secure seed bunker stores seeds at -20 °C at the Alberta Tree Improvement and Seed Centre in Smoky Lake. Seed is stored in airtight containers, with a detailed inventory database, and

periodically re-tested for viability to identify seedlots that may need replacement. Following the recent addition of native shrubs to the provincial forest genetic standards, it is anticipated that those species will be added to the ex situ conservation program as we learn more about their seed biology.

Table 1—*Ex situ* collections in Alberta by natural region

Natural Region	Boreal		Canadian Shield		Foothills		Grassland		Parkland		Rocky Mountain	
Species	Bulk	Single	Bulk	Single	Bulk	Single	Bulk	Single	Bulk	Single	Bulk	Single
Abies balsamea	0[a]	0	-	-	1	1	-	-	-	-	-	-
Abies lasiocarpa	0	0	-	-	0	0	-	-	-	-	2	36
Betula papyrifera	4	2	0	0	1	0	0	0	0	0	-	-
Juniperus scopulorum	-	-	-	-	-	-	-	-	-	-	1	19
Larix laricina	25	89	0	0	8	1	-	-	0	0		
Larix lyallii	-	-	-	-	-	-	-	-	-	-	2	31
Picea engelmannii	-	-	-	-	-	-	-	-	-	-	9	10
Picea glauca	111	672	0	0	45	363	-	-	3	1	11	95
Picea glauca x *engelmannii*	-	-	-	-	3	0	-	-	-	-	7	10
Picea mariana	30	104	0	0	27	46	-	-	0	0	1	1
Pinus albicaulis	-	-	-	-	-	-	-	-	-	-	10	326
Pinus banksiana	35	114	0	15	-	-	-	-	0	0	-	-
Pinus flexilis	-	-	-	-	0	0	-	-	-	-	31	388
Pinus latifolia var. *latifolia*	68	113	-	-	168	576	1	0	2	0	95	216
Pinus latifolia x *banksiana*	57	70	-	-	133	658	-	-	1	0		
Populus balsamifera	4	30	0	0	1	17	0	0	0	0	0	0
Populus deltoides	-	-	-	-	0	0	1	49	-	-	-	-
Populus tremuloides	13	59	0	0	10	22	0	0	1	0	0	9
Pseudotsuga menziesii var. *glauca*	-	1	-	-	-	-	0	0	1	0	10	30

[a] Underlined cells have adequate representation, dashes indicate absence or very peripheral presence, and other cells have gaps.

A Shared Responsibility

Regulatory requirements for managing forest genetic resources on public land, with specific quantitative genetic targets, are established in the Alberta Forest Genetic Resource Management and Conservation Standards (2016 revision). Agencies with tree improvement programs for reforestation on public land must implement a forest gene conservation program for each species and region, with *in situ* and *ex situ* components that comply with the standards. Seedlots from seed orchards need an effective population size (Ne) of at least 18 to be eligible for deployment. Wild seedlots must contain at least 30 well-spaced

parents from a seed zone. Clonal collections are from 10 to 120 different clones, depending on the planned deployment on the landscape.

Case Study: Whitebark and Limber Pines

Special gene conservation measures are required by provincial recovery plans for whitebark (*Pinus albicaulis*) and limber (*Pinus flexilis*) pines, whose endangered status cannot be mitigated by *in situ* conservation alone. *Ex situ* collections include both legacy un-selected trees, and selected trees with putative genetic resistance to white pine blister rust (WPBR) caused by *Cronartium ribicola*. The current program focuses on selecting resistant trees for *ex situ* collections, identifying, and protecting high value *in situ* trees and stands. Candidate trees are being screened for WPBR resistance in British Columbia (Kalamalka Research Station), Oregon (Dorena Genetic Resource Center), and Idaho (Coeur D'Alene Forest Nursery). Scions of selected trees will be grafted in regional clonal archive that can be developed for seed production.

Conclusion

Forest genetic resource management in Alberta has been progressing for 40 years. Quantitative targets to regulate and maintain genetic diversity and adaptation of reforestation material on public lands are established in legal standards. Both *in situ* and *ex situ* conservation of native forest tree populations are ongoing, with priorities set by a provincial gene conservation strategy for native tree species.

Joining Forces for Genetic Conservation[1]

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Facing a Challenge

Non-native diseases and insects as well as a changing climate pose serious threats to native trees in North America. Genetic variation in a species is key to its enduring persistence in the face of these abiotic and biotic threats. Efforts to conserve genetic diversity of North American tree at-risk species will ensure the genetic resources of a species are available to help foster management activities to preserve tree species in native and urban forests, retain their invaluable roles in providing ecosystem services, and commercial and cultural uses.

One species facing such challenges is whitebark pine (*Pinus albicaulis*), an ecologically important and much beloved tree warranted for listing under the Endangered Species Act (USFWS 2011). White pine blister rust (WPBR) (caused by the non-native fungal pathogen *Cronartium ribicola*), mountain pine beetle (*Dendroctonus ponderosae*), and climate change are severely impacting the health of whitebark pine stands across the range of the species (USFWS 2011). Collaboration between groups such as public gardens and the U.S. Department of Agriculture Forest Service (USDA FS) can bolster efforts to conserve whitebark pine and other threatened tree species by offering opportunities for sharing resources, conservation education, and genetic conservation. The USDA FS is a leader in mitigating whitebark pine decline through its support of personnel and active programs that evaluate the frequency and level of genetic resistance to WPBR (Sniezko et al. 2011a) and the development of protocols to initiate restoration activities. Successful restoration of whitebark pine will require collaboration with and the contributions of many other groups as well.

Public Gardens' Role

Public gardens (including botanic gardens and arboreta) offer a unique and highly accessible set of sites for research, public education, and detailed monitoring of abiotic and biotic events that affect threatened tree species like whitebark pine. Many gardens hold well-documented plant collections, including data that track wild origin, propagation protocols, weed risk assessments, phenology, health/hazards, permits, images, and more. The professionalism with which many public gardens managed the diversity of trees and other plants in their care provides a wealth of opportunity to learn about and study a wide range of species in a controlled setting. Public gardens are also experts at educating the public about the challenges facing tree species and the ways in which the public can help, an important component to the success of

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[1] A version of this paper was presented at the Gene Conservation of Tree Species – Banking on the Future Workshop, May 16-19, 2016, Chicago, IL.

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any dynamic genetic conservation program. More information and further details about online data systems can be found on each public garden's website.

Communication within a network of professionals in the field of public horticulture is also essential to strengthening collections and the conservation of plant material. One particular organization serving as a link across public gardens is the American Public Gardens Association (APGA). Through collaborative efforts in preservation, education, research, and various management practices, the APGA contributes to the conservation of at-risk plants.

USDA Forest Service's Role

The USDA FS is the federal natural resource agency that serves as the steward of 780,000 km^2 (193 million ac) of National Forest System lands and provides technical and financial assistance to state and private entities for forest health and conservation related activities. USDA FS geneticists have been at the forefront of genetic conservation activities for many decades. For example, the nine white pine species, including whitebark pine, native to the United States are highly susceptible to WPBR. They are all currently being screened for genetic resistance to WPBR at regional USDA FS facilities. The first resistance trials of whitebark pine indicated higher than expected levels of genetic resistance to WPBR, at least in some Pacific Northwest populations (Sniezko et al. 2007, 2011a), and the USDA FS and partners have an active program to conserve its genetic diversity and restore the species and across its range (Mangold 2011, Sniezko et al. 2011b). Seed from over 1,000 individual tree collections is now stored for long-term genetic conservation at National Laboratory for Genetic Resources Preservation, a USDA Agricultural Research Service facility in Fort Collins, Colorado (documented in the Germplasm Resources Information Network (GRIN-Global) database). Successful seed storage for >25 years has been documented in some whitebark pine seedlots (Sniezko, unpublished data).

Joining Forces

There are many organizations that have a wealth of knowledge concerning genetic conservation. In an effort to take advantage of these riches to improve conservation outcomes and to improve communication among scientists and between the scientific community and the public, the USDA FS formally joined forces with public gardens in 2016. The USDA FS's Dorena Genetic Resource Center (DGRC) in Cottage Grove, Oregon is actively involved in WBPR resistance screening in whitebark pine. In 2015, DGRC had surplus seedlings of whitebark pine representing a range of populations in Oregon and Washington (fig. 1). The DGRC sent out informal inquiries to gauge potential interest in accepting the seedlings through APGA. Eight botanic gardens and arboreta across the country expressed interest (fig. 2). By the spring of 2016, 138 whitebark pine seedlings from 17 families (family = offspring from one parent tree) were distributed to participating institutions: Hoyt Arboretum, Minnesota Landscape Arboretum, Denver Botanic Gardens, Cornell Botanic Gardens, Morton Arboretum, Missouri Botanical Garden, Arnold Arboretum, and the United States National Arboretum. The number and source of the seedlings varied, but seedlings of two common families were shipped to seven out of the eight public gardens (one to two seedlings per garden), creating a linkage across the gardens. This core collection of whitebark pine seedlings came from two source populations found on the east side of the Cascade Range (Wallowa Whitman National Forest (NF) and Colville NF). Seedlings from the remaining 15 families were distributed amongst the gardens with some overlap (table 1). In addition, seedlings of *Pinus lambertiana* (sugar pine), *Pinus strobiformis* (southwestern white pine), *Pinus flexilis* (limber pine), and *Chamaecyparis lawsoniana* (Port-Orford-cedar, whose populations are threatened by the non-native *Phytophthora lateralis* pathogen) have also been distributed to some public gardens (fig. 3).

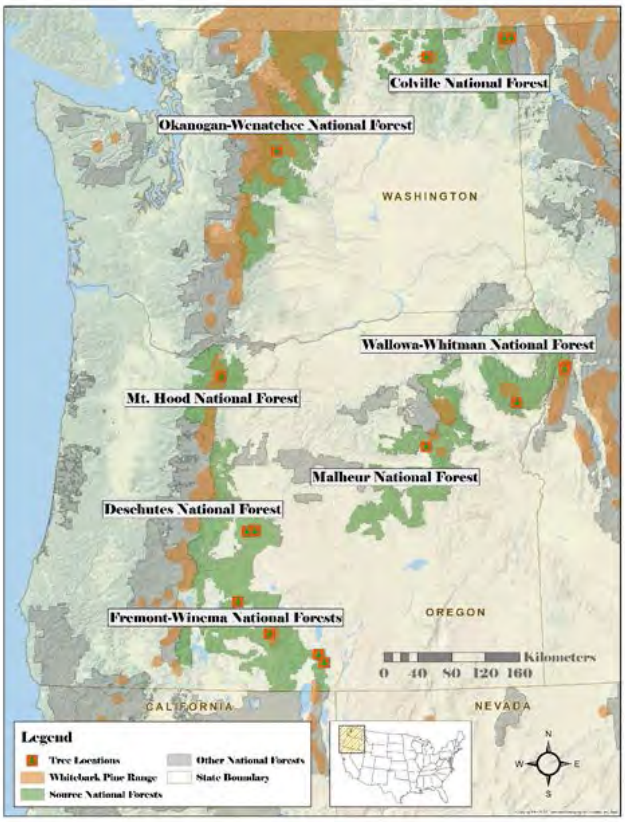

Figure 1—Geographic origins of whitebark pine seedlings sent to public gardens.

Figure 2—Participating public gardens.

Figure 3—Port-Orford-cedar, sugar pine, and southwestern white pine await transplant at Cornell Botanic Gardens. (Photo credit Phil Syphrit)

These plantings will serve to monitor each species' adaptability in different climates, as sentinels for new biotic and abiotic threats, and as resources to initiate research. Public gardens have excellent public visibility, and this partnership between the USDA FS and the public gardens will assist in conservation education.

A joint Memorandum of Understanding (MOU) was signed in May 2016 between the USDA FS and the North American Plant Conservation Initiative. The MOU will help foster a wider array of cooperation between the USDA FS and the groups (the American Public Gardens Association, the Botanic Gardens Conservation International, the Center for Plant Conservation, and the Plant Conservation Alliance non-federal cooperators) involved in the conservation initiative. As native tree species are facing increasing and serious threats such as climate change, invasive pests and pathogens, and changing fire regimes, further collaboration between the USDA FS and public gardens on genetic conservation of forest trees will pay enormous dividends for conserving trees and protecting the public's forest resources.

Table 1—Number and source location of whitebark pine seedlings shipped to each botanical garden and arboretum

National Forest, State	Accession #	Cornell Botanic Gardens	Hoyt Arboretum	Denver Botanic Gardens	Minnesota Landscape Arboretum	U.S. National Arboretum	The Arnold Arboretum of Harvard University	Missouri Botanical Garden	Morton Arboretum
Colville, WA	214738	2	1	2	2	2	2	1	
	223206	3		3			4		
	223223						2		
Deschutes, OR	011176	2		2					
	011251	3	2	3	2	2			
Fremont-Winema, OR	005107		2						
	005062				2				2
	005153					2	7		
	005081							1	
	005045	2		2					2
Malheur, OR	044664	3		3		2			
Mt Hood, OR	066016	1		2					
Okanogan-Wenatchee, WA	170835	3	2	3	2	2		1	
Wallowa-Whitman, OR	160956	5		5					
	160960	2	2	2	2	2	2	2	
	160963	2	2	2	2	2	4		5
	166260			4					5

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Genetic Conservation and Restoration of *Chamaecyparis lawsoniana* (Port-Orford-cedar) in the Face of a Non-native Pathogen and Changing Climate – On the Road to Success[1]

Richard A. Sniezko,[2] Erin Hooten,[2] Chuck Frank,[3] Rich Cronn,[4] Jim Hamlin,[5] and Peter A. Angwin[6]

Challenge

Port-Orford-cedar (POC) (*Chamaecyparis lawsoniana*) is a long-lived conifer native to southwest Oregon and northwest California, occurring from sea level to above 1500 m (5085 ft). It is a foundational species in its native ecosystems and is used world-wide horticulturally. A non-native pathogen, *Phytophthora lateralis*, cause of Port-Orford-cedar root disease, has caused high mortality in native forest ecosystems and ornamental plantings (Betlejewski et al. 2011). The presence of *P. lateralis* has limited the use of POC in managed plantations and urban forests. There is concern about the future viability of the species which has a 'Near Threatened' status on the International Union for Conservation of Nature and Natural Resources (IUCN) Red List (Farjon 2013).

Solution

The U.S. Department of Agriculture Forest Service (USDA FS) and U.S. Department of Interior Bureau of Land Management (USDI BLM) began an applied genetic resistance program in 1997. The program is based at Dorena Genetic Resource Center (DGRC). The program has delineated 13 breeding zones within the native range of POC, and *P. lateralis* resistance screening is underway for many of these zones (Sniezko et al 2012a). A strong focus for the program has been to retain both genetic diversity within the species and its adaptability, while developing populations (not cultivars) of resistant trees. Containerized seed orchards (CSOs) and containerized clone banks (~2100 clones) have been established for most zones, providing genetically resistant seed for restoration and reforestation on federal, state, county, tribal, and private lands. Two soil-based clone banks provide additional *ex-situ* genetic conservation and potentially longer term preservation of the genotypes. Current work is focused on increasing the number of parents in the CSOs, increasing the level of genetic resistance, and roguing the clone banks and orchards as resistance information is accumulated. Field trials have been established in Oregon and California to confirm the level of genetic resistance to *P. lateralis*, and to monitor the durability of resistance in the face of a potentially evolving pathogen and a changing climate (Sniezko et al. 2012b). The field trials will also be used to examine genetic variation in adaptive traits within POC, serve as sentinel plantings with known genetic constitution, and provide *in situ* genetic conservation (Harrington et al. 2012; Sniezko et al. 2012a, 2012b). More recently, DNA-based genetic markers have been developed through a USDA FS Special Technology Development grant (Jennings et al. 2011) to help assist the operational breeding program and evaluate patterns of genetic variation throughout the range of

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POC. This tool and results from its utilization will help guide continued dynamic genetic conservation activities in POC.

Success

Resistant seed is available for several breeding zones; it is being used by various federal, state, tribal, and local agencies, as well as private organizations and individuals (Sniezko et al. 2012a). The current expectation of the IUCN is for POC to be down-listed to "Least Concern" within the next 10 years if current conservation actions are successful and maintained (Farjon 2013). The program for POC conservation represents an emerging success story in forest conservation, and leads us to be cautiously optimistic for POC's future.

Future

Genetic conservation must be dynamic to effectively retain the species in its native range and other suitable areas in the future. The DGRC POC program meets this criteria; it features both *in situ* and *ex situ* genetic conservation. It specifically takes into account a major challenge to the species—the presence of a non-native pathogen—by developing resistance and maintaining genetically diverse CSOs that can easily be updated and from which resistant seed can be collected. Restoration and operational plantings, past and planned, are occurring on a variety of land ownerships: federal (USDA FS, BLM, USDI National Park Service), state (Oregon Department of Forestry, California State Parks, South Slough National Estuarine Research Reserve, tribal (California and Oregon tribes), local (Coos County), and private (small woodlands and industrial). The maintenance of genetic diversity, along with the incorporation of natural genetic resistance, will provide POC with its best opportunity to continue to flourish in our forest ecosystems under a changing climate. Further work in developing resistance is needed, but the work to-date shows the promise in developing and deploying the resistant seedlings.

For a limited time (until roguing of parents at DGRC), the large, easily accessible population of POC available at DGRC offers a unique opportunity for scientists interested in exploring genetics and climate change of a conifer species. Because of the unique range-wide collection of parent trees (as rooted cuttings) available at DGRC, the ease with which POC can be experimentally manipulated (e.g. induction of early flowering, potential of self-pollination, vegetative propagation, etc.), and the availability of disease resistance ratings for many of the clones (qualitative and quantitative resistance), POC has the potential to be used in many ways as a model species for conifers.

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Partnerships in the Pacific Northwest Help Save an Endangered Species, Whitebark Pine (*Pinus albicaulis*): an Example of Dynamic Genetic Conservation[1]

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Whitebark pine (WBP, *Pinus albicaulis*) is a keystone species distributed widely at high elevations across western North America. It is in decline due to a combination of threats including infection from white pine blister rust (WPBR, caused by the non-native fungal pathogen *Cronartium ribicola*), mountain pine beetle (*Dendroctonus ponderosae*) predation, climate change, and altered fire regimes (Lintz et al. 2016, Smith et al. 2013, Tomback and Achuff 2010) (figs. 1, 2). Whitebark pine has been classified as Endangered on the latest IUCN Red List of Threatened Species (Mahalovich and Stritch 2013). It is a federally listed endangered species in Canada under the Species at Risk Act (SARA) (Government of Canada 2012), and was found warranted (but currently precluded) for listing under the Endangered Species Act (ESA) in the United States (USFWS 2011a, 2011b, 2015).

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Figure 1—Extensive recent whitebark pine mortality from mountain pine beetle in Crater Lake National Park. (Photo: R. Sniezko)

Figure 2—White pine blister rust resistance trial conducted at U.S. Department of Agriculture Forest Service's Dorena Genetic Resource Center in Cottage Grove, Oregon. The frame above shows family variation by row in resistance to rust. Rows 3 and 11 have resistant families from Crater Lake National Park and Deschutes National Forest, respectively. (Photo: E. Heck)

Partnerships and cooperation among organizations are essential in order to efficiently and effectively maximize the conservation needs of threatened species such as WBP. Collaborations between land managers and stewards across organizational boundaries in the Pacific Northwest portion of WBP's geographic range have set the stage for beginning the recovery of WBP ecosystems (fig. 3). In fact, the majority of WBP habitat is controlled by conservation-oriented land management agencies and tribes in the United States and Canada, presenting a unique opportunity to combine institutional research and land management objectives to support the longevity of this iconic species, and to implement government restoration policy such as U.S. Department of Agriculture Forest Service's (USDA FS) directive to "achieve landscape restoration goals by engaging the public, state and local governments and consultation with Indian Tribes (USFS 2016)." Universities and government research units provide a conduit to examine basic research issues, while the various land management groups seek to apply actions to retain or restore the species (fig. 3).

For the USDA FS Pacific Northwest Region (Oregon and Washington, Region 6), concerns about WBP began to escalate in the early 1990s, and staff of the Genetic Resources and Forest Health Protection groups initiated an information survey (Sniezko et al. 1994). Seed collections for WPBR resistance testing (from USDA FS and partners) began soon afterwards, and the first full scale test of

genetic resistance began in 2002 (Kegley et al. 2012; Sniezko et al. 2007, 2011a, 2011b, 2012). Formal surveys of the health of WBP began in 1998 (Goheen et al. 2002). Continuing concerns led to a 2005 conference, 'Whitebark pine: A Pacific Coast perspective' (Goheen and Sniezko 2007), and the 2008 publication of an initial restoration strategy for the Pacific Northwest Region (Aubry et al. 2008). Seed collections specifically for genetic conservation began in 2008 (Sniezko et al. 2011c).

Genetic conservation activities continue and tremendous progress has been made in WBP *in situ* and *ex situ* genetic conservation in the Pacific Northwest. The joint efforts between tribes, government agencies, and academic institutions have provided a chance to examine WPBR resistance, collect seed for long term genetic conservation, initiate provenance trials, examine genetic variation, plant the first restoration trials, develop new genomic resources, and educate the public (e.g., Jahn 2003; Man et al., Joining forces for genetic conservation, these proceedings). Genomic advances that have generated new tools to describe patterns of genetic variation and population structure in WBP (Gruhn 2016, Liu et al. 2016, Syring et al. 2016), research characterizing patterns of adaptive genetic variation in needle and growth traits (Bennett et al. in press; Hamlin et al. 2011, Sniezko et al. in press a, b), investigations into variation of fungal endophyte communities in needle tissue and their relation to WPBR (Bullington 2017), and recent trials to confirm the longevity of seed storage and refine stratification and germination protocols (Sniezko et al. 2017), provide crucial background information for ongoing and future restoration work, seed collections, and other genetic conservation efforts.

To date, seeds from over 1000 parent trees from the Pacific Northwest part of WBP's geographic range have been collected. The seedling families being grown from these seeds are in testing at Dorena Genetic Resource Center (DGRC) for genetic resistance to WPBR. Resistance ratings from many of these parent trees are now available and have been disseminated to the cooperating partners in the United States and Canada, and useful levels and frequencies of genetic resistance have been found in some populations of WBP (Sniezko and Kegley 2015, Sniezko et al. 2007, 2011a, 2011b, in press b) (fig 2). Seeds are being collected from the parent trees to use for restoration and long-term genetic conservation, resistant parents or progeny are being grafted and placed into seed orchards or clone banks, and field trials have been established to study the adaptive genetic variation within WBP. The first restoration plantings (and combination genetic trials) with WPBR resistant seedlings have been established in the United States and Canada to validate the results of the seedling WPBR resistance testing and in situ and ex situ genetic conservation purposes, and as a conservation education opportunity (e.g., Beck and Sniezko, in press; Cartwright et al. 2016[20]). Additional field trials, conservation plantings and seed orchards are slated for 2017. A subset of the Oregon and Washington seed collections is stored at the National Laboratory for Genetic Resources Preservation (NCGRP) in Fort Collins, Colorado, and recorded in the GRIN-Global database (GRIN-Global 2016). Additional collections and duplicates of many collections at NCGRP are also stored at DGRC.

Already there is evidence of success—in 2015 the U.S. Fish and Wildlife Service downgraded the endangered species listing priority number for WBP (USFWS 2015). This was due, in part, to the recognition that some whitebark pine show genetic resistance to WPBR; key evidence for this comes from the Pacific Northwest program (Sniezko et al. 2007, 2011b; Sniezko and Kegley 2015). The status of WBP is re-evaluated annually, however, and a decision on its listing under ESA may come in 2018. Continued cooperative effort across organizational boundaries will provide the best avenue for dynamic genetic conservation and retaining WBP as a keystone species in high-elevation forest ecosystems.

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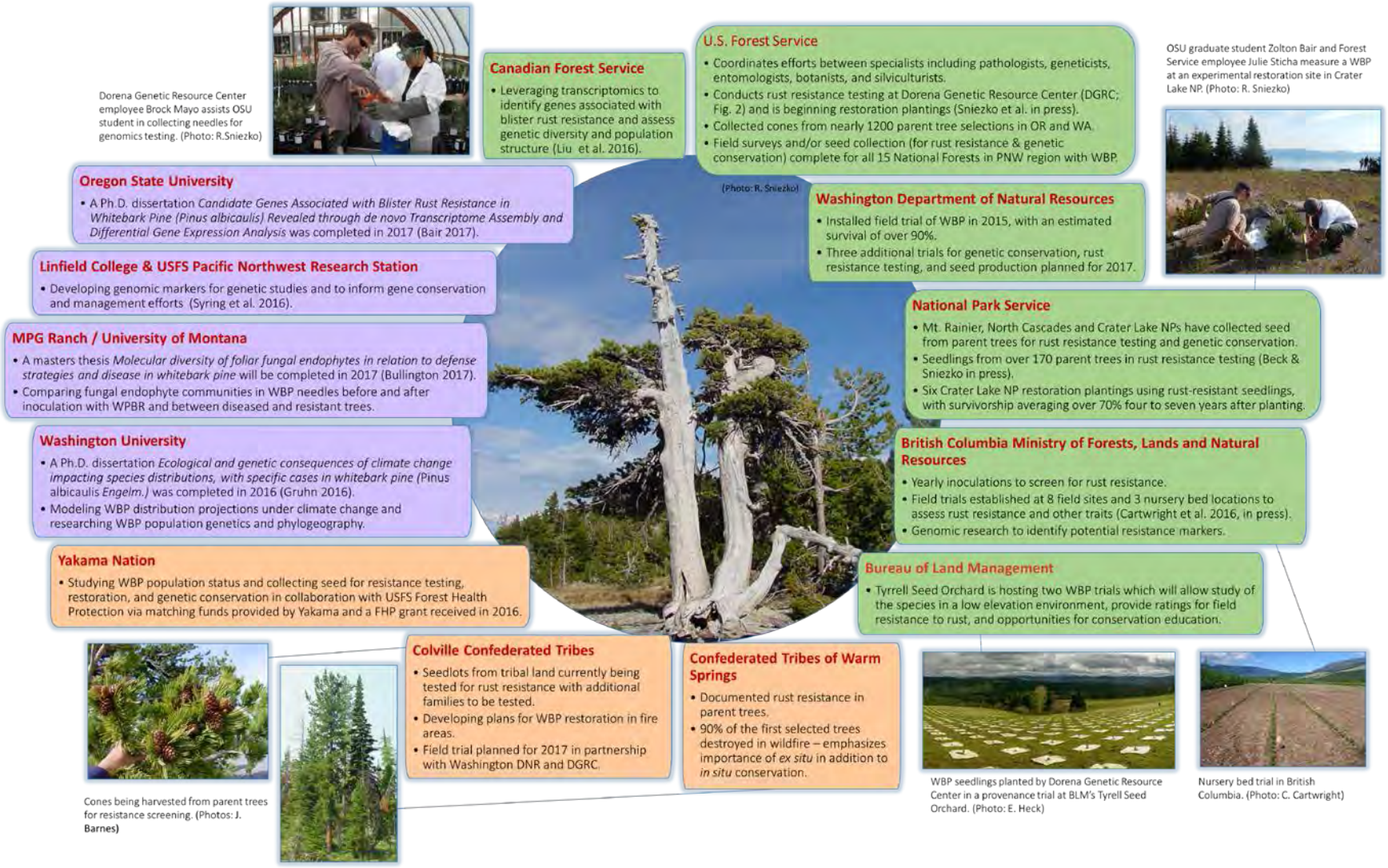

Figure 3—Contributions of partnerships and organizations instrumental to whitebark pine conservation in the Pacific Northwest. All rust resistance screening in this Region conducted at Dorena Genetic Resource Center.

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Seed Collection Success and Failure in *Fraxinus* Gene Conservation Efforts[1]

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National seed collection and gene conservation programs have expanded in recent years, especially in response to pressure from non-native pests such as the emerald ash borer (*Agrilus planipennis*). Since 2008, we have been working with the U.S. Department of Agriculture Agricultural Research Service (USDA ARS) and USDA Forest Service (USDA FS) leading seed collection efforts in the genus *Fraxinus* chiefly in Minnesota and North Dakota with smaller collections in Wisconsin and Iowa. Through 2015, we collected approximately 7.6 million seeds from a total of 1020 ash trees. The collections came from 633 green ash (*F. pennsylvanica*) and 387 black ash (*F. nigra*) trees, from which we collected an estimated 5.8 million and 1.8 million seeds, respectively. More green ash seed was collected because green ash has a larger geographic range, a broader ecological niche, and a shorter seed periodicity timeframe; green ash produces a seed crop every 1 to 3 years compared to black ash's 3 to 5 years.

The 1020 total individual-tree seed collections are a combination of collections we have made ourselves and in collaboration with natural resource professionals, students, and citizen-scientist volunteers. Natural resource professionals have been extremely helpful in identifying likely collection areas, monitoring seed crops, and making their own seed collections. Undergraduate and graduate students were involved in seed collection efforts through a Special Topics course at North Dakota State University. The course covered both the technical aspects of seed collection, transport and storage along with associated aspects of population genetics, Geographic Information Systems, and material transfer agreements. Citizen-scientist volunteers were trained either as part of a larger effort to train them as 'First Detectors', or they were directed to a web site for self-training on identifying trees and making seed collections.

Although citizen-scientists did contribute usable seed collections, overall they contributed less than 3 percent of the total collections and were inconsistent in making complete local or population level collections. Several factors may account for the low number of seed collections completed by citizen-scientists. Many may not have had the tools required for reaching up into the crown to access seed, their higher average age (estimated by authors) may limit their mobility in the field, and/or they may have received insufficient training on the value of population collections compared to single tree collections for a given locale. Utilizing volunteers as field assistants for discrete periods of time (minimum 0.5 days) may be a better approach for working with the general public rather than attempting to train them to work independently in the field.

Research at the University of Minnesota demonstrated that individual-tree collections as small as 115 seeds were adequate for collecting the vast majority of allelic diversity on that tree and that seed collected in poor seed production years were as genetically diverse as seed collected in excellent seed production years. By genetically fingerprinting a 126 seed collection from a single mother tree and simulating seed collections of 1 to 126 seeds 1,000 times each we demonstrated that collections as small as 115 seeds were sufficient to capture 95 percent of the genetic variation in a 32 allele system. Since field collections were typically 2.5 to 5.1 cm (1 to 2 inches) of sound seed in the bottom of a paper grocery bag per tree (minimum 2,000 to 3,000 seeds) these collection methods sampled virtually all of the allelic diversity available on a given tree. Despite the lack of studies on intra- and inter-population genetic variation in

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Fraxinus, life history trait analysis—ash species are wind pollinated, obligate outcrossers—indicates they would have little within-population and large amounts of among-population genetic diversity. Considering the level of genetic diversity within and among populations, as predicted by life history traits, the amount of seed collected per tree, and the population level approach to seed collection used here (2,000 to 3,000 seeds per individual; minimum 20 individuals per location; five to seven locations per state) it is highly likely that the majority of allelic variation in black and green ash that exists in Minnesota and North Dakota is found in these seed collections.

Research into genetic variation in high and low seed production years showed similar levels of genetic variation for total alleles per locus (A_T) and mean number of alleles per locus ($A_{\overline{x}}$). In addition, an index that balanced allelic richness and evenness (similar to Shannon's H) was not significantly different between high and low seed production years. This information is helpful to managers who are trying to determine the appropriateness of collecting in a poor seed year and/or those whose seed collection efforts are limited by grant award dates.

Butternut Health and Genetic Diversity in New Brunswick, Canada[1]

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Butternut (*Juglans cinerea*), a native tree species of eastern North America, is under serious threat from an introduced fungal pathogen (*Ophiognomonia clavigignenti-juglandacearum*), the agent of butternut canker disease. Butternut canker was first reported in North America in Wisconsin in 1967 and finally reached New Brunswick (NB) in 1997. The purpose of this study was to assess the health of NB butternut populations and develop a cryogenic *ex situ* conservation reserve of NB butternuts using part of the embryo isolated from nuts. We assessed a total of 425 trees in 25 populations for general health and genetic diversity. Parameters included tree vigor, crown dieback, and the presence of cankers. We use 11 nuclear microsatellite markers (gSSR), and two chloroplast CAPS markers to evaluate the genetic diversity of NB butternuts relative to butternuts in the rest of the species' native range, and to detect evidence of hybridization with Japanese walnut. To date, approximately 25,000 embryos either have been or are in the process of being cryopreserved. The results of the population survey and intended use of the *ex situ* collection will be discussed.

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Federal Conservation of Western Cypress in the United States[1]

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Abstract

It is important to identify and protect at risk and sensitive tree species before irreparable damage occurs to their genetic base. Western cypress (*Hesperocyparis* spp.) is threatened by habitat loss and fragmentation, competition from nonnative species, and susceptibility to current fire intervals and intensities. In an effort to safeguard the western cypress species through *ex situ* gene conservation, the U.S. Department of Agriculture Forest Service collaborated with the Bureau of Land Management, U.S. Fish and Wildlife Service, California Department of Fish and Wildlife, California Department of Parks and Recreation, CALFIRE, San Mateo County Parks and Recreation, University of California at Santa Cruz, Rancho Santa Ana Botanical Gardens, and private landowners. The conservation effort over the past 3 years consisted of identifying, mapping, and collecting seed from 28 native cypress populations across California and southern Oregon. The populations included Baker, MacNab, Piute, Pygmy, Santa Cruz, Sargent, and Tecate cypress. Collections yielded seed from 527 individuals (ranging from 3 to 29 individuals per population) and are now stored in the National Center for Genetic Resources preservation facility in Fort Collins, Colorado. The completed work and collaboration efforts provide a strong foundation to further expand conservation for *Hesperocyparis* spp. and other species in the Pacific Southwest.

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Ex Situ Genetic Conservation of Vulnerable High Elevation Conifer Species in the Pacific Northwest, USA[1]

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Species with small or disjunct populations, and those populations at the southern margin of a species' range, are likely to be at higher risk from climate change. Two recent U.S. Department of Agriculture Forest Service (USDA FS) documents (Devine et al. 2012, Erickson et al. 2012) have advocated for *ex situ* genetic conservation of seed and evaluation of current seed inventories (including viability of older stored seedlots) for species and habitats most likely to be negatively impacted by the effects of climate change. Devine et al. (2012) ranked 57 widespread forest canopy species according to their assessed vulnerability to predicted climate change. High elevation conifers comprised the six most vulnerable species: whitebark pine (*Pinus albicaulis* Engelm.), subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.), Pacific silver fir (*A. amabilis* (Douglas ex Loudon) Douglas ex Forbes), Englemann spruce (*Picea engelmannii* Parry ex Engelm.), subalpine larch (*Larix lyallii* Parl.), and noble fir (*A. procera* Rehder). In addition, Alaska yellow-cedar (*Xanthocyparis nootkatensis* (D.Don) Farjon & Harder), mountain hemlock (*Tsuga mertensiana* (Bong.) Carrière), and Brewer's spruce (*P. breweriana* S. Watson) were among those species most threatened by climate change and have additional conservation concerns. The USDA FS, Pacific Northwest Region (Oregon and Washington) has collected seed from over 835 individual whitebark pine trees for *ex situ* genetic conservation since 2009. Seeds are stored at the USDA Agricultural Research Service (ARS) Center for Germplasm Preservation in Fort Collins, Colorado and at the USDA FS Dorena Genetic Resource Center in Cottage Grove, Oregon. Recent *ex situ* genetic conservation activities have focused on other vulnerable high-elevation species. Collections have targeted the southern and western ranges of several species in southwestern Oregon and northern California, as well as the north Cascades in Washington State.

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Effect of Average Growing Season Temperature on Seedling Germination, Survival and Growth in Jack Pine (*Pinus banksiana* Lamb.)[1]

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Abstract

Because jack pine (*Pinus banksiana* Lamb.) is serotinous, it retains multiple years of cones until environmental conditions are favorable for releasing seed. These cones, which contain seed cohorts that developed under a variety of growing seasons, can be accurately aged using bud scale scars on twigs and branches. By calculating the average daily temperature for June through August for the past 15 years in Grand Rapids, Minnesota, and comparing them to the published 30 year average, we identified 3 years that could serve as a warmer (2005), cooler (2008) and control (2013) growing season (range = 3.4 ºC). Cones that could be dated to these 3 years were collected from three mature jack pine, their seeds extracted and sown in a greenhouse. The seedlings were grown with minimal temperature control and minimal, but equal amounts of water to maintain warmer and drier than ambient conditions. Germination rates were scored for each tree/year combination and at the end of the growing season percent survival and height were calculated. Germination rates for the three trees averaged 51.9 percent, 39.1 percent and 48.9 percent across the 3 years with a range for each tree between 10.2 percent and 11.0 percent, suggesting comparable levels of filled seed. Seedling survival under warm greenhouse conditions was 104 percent of control for seedlings from the warm summer of 2005 while seedlings from the cold summer of 2008 survived at 98 percent of control seedlings. A similar trend was observed for seedling height. The warm 2005 seedling cohort grew at 129 percent of the control while seedlings from cold 2008 grew at 98 percent of the average control height. These results suggest that there is an epigenetic effect in jack pine between growing season temperature experienced during seed development and future seedling survival and height growth. Alternatively, differential pollen contribution in warm vs cold years could have contributed to the observed results, but the genetic diversity of seeds from open-pollinated, temperate conifers is remarkably consistent from year to year. Despite being based on a single year of data in an artificial environment, these findings represent the first data that suggest that jack pine, an ecologically and economically important boreal species, possesses some inherent ability to adapt to a warming climate.

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Missouri Botanical Garden's Support of *Ex-situ* Conservation with Living Collections[1]

David Gunn,[2] Meg Engelhardt,[2] and Derek Lyle[2]

Abstract

The Missouri Botanical Garden's living collections are critical for supporting its multi-disciplinary strategy of integrated plant conservation. The Garden is increasing *ex-situ* collections of plants in need of conservation to build species diversity into its displays for visitor education. Current areas of focus include native Missouri species and International Union for Conservation of Nature and Natural Resources (IUCN) Red Listed genera from around the world. Several key projects support this initiative. These include development of a Living Collections Management System (LCMS), establishment of a seed bank, and opening of the new Oertli Family Hardy Plant Nursery in the spring of 2017. Data recorded in LCMS include field collection information, propagation and cultivation data, location on the Garden's grounds, and much more. The seed bank has two main goals: to serve as a hub for long term seed storage needs on an institutional level, and to collect and conserve Missouri's native flora. The 2.6 ha (6.5ac) Oertli Family Hardy Plant Nursery will be approximately 5 minutes south of the Garden's main campus and will include a 1, 208 m^2 (13,000 ft^2) greenhouse with climate controlled environments for seed, cuttings, and liner and pot production. Well-documented and responsibly collected plants of wild origin will be grown at the nursery for the Garden's outdoor displays and collections. As plants are propagated, data is recorded and stored in LCMS, providing propagation protocols to aid in plant conservation efforts. Through these additional capacities, the Missouri Botanical Garden will aid in conserving the world's flora and increase its impact to stem the loss of plant species.

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A New Program of Work to Conduct Research in Support of Gene Conservation, Restoration, and Proactive Deployment of Red Spruce in Light of Climate Change[1]

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Abstract

Red spruce's (*Picea rubens* Sarg.) range extends from the southern and central Appalachians north into Vermont and Maine and then to the Canadian Maritime provinces with relic populations as far west as Ontario. Due to heavy logging and resultant severe fires in the 19th and early 20th centuries, and more recent declines related to air pollution and invasive pests, the amount of red spruce is now less than five percent of its former prevalence in the southern and central part of its range and these populations are highly fragmented. As a montane species over much of its range, it also has a high potential to become maladapted with predicted climate warming in the future. Restoration and gene conservation for the species has begun in the south and central regions. Currently, seed for restoration and gene conservation is collected by non-governmental organizations and state and federal entities. Seed collections are typically ad hoc; occurring in good seed years and in areas readily accessible by roads, potentially missing valuable populations. We will conduct work to guide both seed collection and restoration activities. We will develop a GIS data base to inventory seed collections to date. Stand characteristics will be input and then climate data will be overlaid. We will use these data to fill in the gaps of seed availability in terms of latitude and elevation. Second, we will conduct genecology research to assess if red spruce is a genetic generalist or specialist. If the latter, information of its adaption with respect to climate will be critical for restoration, gene conservation, and perhaps artificial migration of red spruce in light of climate change.

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The Importance of Site Quality to Backcross Chestnut Establishment Success[1]

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Short-term studies show that American chestnut (*Castanea dentata*) grows faster on mesic compared to xeric sites. Long-term impacts of site quality and corresponding moisture and nutrient availability on backcross chestnut establishment success and resistance to the chestnut blight fungus, *Cryphonectria parasitica*, however, have not been evaluated. We report here the first year results from a study designed to evaluate the effects of three site quality treatments—mesic, xeric, and intermediate—on the establishment success and blight resistance of chestnut seedlings planted on the Allegheny Plateau in northwestern Pennsylvania. We hypothesized that long-term chestnut growth and competitive ability will be greatest on sites intermediate in resource availability, and severity of blight will be lowest on mesic sites.

In April, 2015 we planted 360 backcross (BC_3F_1 x BC_3F_2), 90 American, and 90 Chinese (*C. mollissima*) chestnut seedlings in 15 recently-harvested sites. Study sites were categorized as mesic, xeric, or intermediate using the integrated moisture index (IMI) (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 (USA). Landscape Ecology. 12(5): 331–348.), which calculates moisture ratings using digital GIS-derived topographical features and soils data. Soil characteristics for each site, including NO_3, NH_4, Ca, P, K, and Mg levels; percent sand, clay, and organic matter; and plant available water, were evaluated from soil samples. Chestnut seedlings were planted as 1-0 nursery stock, averaging 1.2 m in height and 11.6 mm in root collar diameter at planting. After 1 season, seedlings had grown an average of 7 cm (± 21) in height and 1 mm (± 2) in diameter. Growth did not differ among the IMI treatments, but differences were found among chestnut types. Chinese chestnut height growth was greatest (P = 0.009, 11 cm ± 3), with no significant differences between American and hybrid chestnuts (between 4 cm ± 2 and 9 cm ± 3). Ground level diameter ranged between 0.3 mm ± 0.3 and 1.3 mm ± 0.3 and was lowest for the American and one hybrid chestnut family (P = 0.001). Multiple regression models indicate that diameter growth was best predicted from P and K levels. Mortality (2 percent) and incidence of blight infection (<1percent) were too low to provide meaningful contrasts. Relationships between chestnut performance and site characteristics may change as the seedlings overcome transplant shock and allocate more energy to growth.

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Using American Elm in Mixed-Species Plantings to Restore Above- and Below-Ground Function to Degraded Riparian Buffers[1]

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We recently established a study to evaluate the effects of several riparian restoration treatments on degraded streambanks located on the Finger Lakes National Forest (FLNF) in western New York. A legacy of cattle grazing has led to soil compaction, invasion by non-native invasive plant species (NNIP), as well as heavy nitrogen loading and increased bacterial levels in riparian corridors on the FLNF. These characteristics slow the conversion of non-native grassland to closed-canopy forest, a FLNF management goal for these sites. We are testing two planting treatments: tree vs. mixed tree and shrub plantings, and a mulch vs. no mulch treatment. We hypothesize that plantings with tree and shrub species will be better able to competitively exclude NNIPs than plantings without shrubs included, and that the mulch treatment will reduce the reinvasion of NNIPs by decreasing the C:N ratio and increasing soil fungi diversity and abundance. Indicators of restoration success will include successful establishment of planted and naturally regenerated seedlings, reduced reinvasion of NNIP, reduced soil nitrogen and bacterial levels, and increased diversity and abundance of insects, an indicator of bird habitat quality.

The study is also designed to evaluate the establishment success of Dutch-elm disease (caused by *Ophiostoma ulmi and O. novo-ulmi*) -tolerant American elm (*Ulmus americana*) on degraded riparian sites. Before the arrival of Dutch elm disease, American elm was a dominant component of riparian corridors and floodplains throughout the eastern half of the United States. Its tolerance of soil compaction, its ease of transplanting, and its competitive ability may enable American elm, when incorporated into mixed-species plantings, to effectively reclaim riparian sites invaded by NNIP.

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Population Isolation Results in Low Genetic Variation and High Differentiation in Carolina Hemlock (*Tsuga caroliniana*), an Imperiled Southern Appalachian Conifer[1]

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Carolina hemlock (*Tsuga caroliniana*) is a rare conifer species that grows in small, isolated populations in the southern Appalachian Mountains of Virginia, North Carolina, South Carolina, Tennessee, and Georgia. The species is additionally imperiled by the hemlock woolly adelgid (*Adelges tsugae*), an invasive insect that can kill the trees in as few as 4 years. We conducted the first range-wide genetic diversity study of Carolina hemlock, using 16 highly polymorphic nuclear microsatellite loci to quantify genetic variation across 439 trees from 29 populations, representing a comprehensive range-wide sampling of most known populations. The results demonstrate that this southern Appalachian endemic has low genetic diversity, is highly inbred, and consists of populations that are highly differentiated from each other. Populations outside the core of the species range are characterized by particularly low variation and high differentiation. Most populations contained at least one unique allele. Clearly, Carolina hemlock exists primarily as a limited set of small populations with restricted inter-population gene flow. Knowledge about the population genetic structure of the species will inform ongoing management and conservation efforts, including prioritizing regions and populations for protection and seed collections. The fact that nearly all Carolina hemlock populations are highly inbred emphasizes the necessity of quickly and effectively preserving the genetic diversity of the species. The high levels of differentiation among Carolina hemlock populations, and the commonness of alleles unique to populations, underscore the importance of ensuring that ongoing gene conservation efforts represent as many populations as possible.

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Determining Genetic Erosion in Fourteen *Picea chihuahuana* Martínez Populations[1]

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Picea chihuahuana is an endemic species in Mexico and is considered endangered, according to the Mexican Official Norm (NOM-ECOL-059-2010). This species covers a total area of no more than 300 ha located in at least 40 sites along the Sierra Madre Occidental in Durango and Chihuahua states. A minimum of 42,600 individuals has been estimated, although the number of mature individuals is uncertain and could be less than 2,500. The size of the populations varies from 21 to 5,546 individuals, including trees, saplings, and seedlings. Theory suggests that small populations can be more susceptible to loss of genetic variability due to genetic drift, inbreeding depression and strong unidirectional selection. The predicted result is the total loss of genetically distinct populations, the loss of alleles or change in frequency of specific alleles within populations or over the species as a whole, or the loss of allelic combinations. Therefore, the principal aim of the present study was to determine genetic erosion in 14 populations of *P. chihuahuana* by comparing the genetic diversity among trees sorted into diameter classes (as a substitute variable for age classes). Needles were sampled from about 700 randomly chosen individuals of *P. chihuahuana* from these 14 populations, and genetic data were obtained through AFLP technology. In order to determine genetic erosion, the genetic diversity was quantified by measuring mean total differentiation (δ_T), the proportion of polymorphic fragments (pr_{poly}), *DW* (a parameter to quantify rare markers within cohorts), and genetic distance using GenAlEx 6.5. Finally, a two-sided permutation test was performed for the observed degrees of covariation (*C*). If the value of *C* is positive and *P* ($Z \geq C$) is statistically significant (i.e., P <0.025; two-sided permutation test), we can assume that genetic erosion has occurred in a defined area.

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Sampling Scheme on Genetic Structure of Tree Species in Fragmented Tropical Dry Forest: an Evaluation From Landscape Genetic Simulations[1]

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Investigating how genetic variation is distributed across the landscape is fundamental to inform forest conservation and restoration. Detecting spatial genetic discontinuities has value for defining management units, germplasm collection, and target sites for reforestation; however, inappropriate sampling schemes can misidentify patterns of genetic structure. Appropriate sampling is more critical in fragmented landscapes where patterns of genetic diversity and structure might not yet reflect the current landscape structure. Landscape genetic simulations are useful for assessing the uncertainty of sampling schemes and the statistical power of hypothesis testing under varying scenarios. Here, we explore the effects of sampling design, sampling effort, and microsatellite number on the ability to detect patterns of genetic structure in two tropical dry forest tree species. The tropical dry forest is a species-rich ecosystem in México that is experiencing fast fragmentation rates due to habitat conversion to agriculture, expansion of rangeland for livestock, urban development, and overharvesting. The decline of tropical dry forests threatens biodiversity and the livelihood of rural communities that are dependent on forest resources. We used spatially explicit landscape simulations to model gene flow in two species that vary in spatial distribution and life history traits. (*Bursera* spp.: discrete populations, dioicous, and shorter dispersal distances vs. *Acacia* spp.: continuous distribution, monoicous, and larger dispersal distances). Gene flow was modeled under isolation by distance (IBD) to ask the following: Which is the best performing spatial sampling design? Does performance of spatial sampling design vary with sampling effort and the number of microsatellite loci? Results revealed that random sampling was the best performing sampling scheme, irrespective of sampling intensity, while the cluster and the systematic sampling did not perform well for both species. The number of microsatellites affect estimation of IBD, because using a low number of loci (n = 8) underestimated IBD. Our study highlights the usefulness of computer simulations for advance planning in empirical landscape genetic studies (Rico, Y. 2017. Using computer simulations to assess sampling effects on spatial genetic structure in forest tree species. New Forests. 48: 225–243. DOI: 10.1007/s11056-017-9571-y.).

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Potential for Long-Term Seed Storage for *Ex Situ* Genetic Conservation of High Elevation White Pine Species – Whitebark Pine and Foxtail Pine Case Study[1]

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Whitebark pine (*Pinus albicaulis*) and foxtail pine (*P. balfouriana*) are conifers native to western North America. Due to several threats, including a non-native pathogen (*Cronartium ribicola*) and a changing climate, whitebark pine and foxtail pine are classified on the IUCN Red List as 'endangered' and 'near threatened,' respectively. Whitebark pine has been proposed for listing under the Endangered Species Act (ESA) in the United States and is now listed as endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). *Ex situ* genetic conservation activities are underway, including long-term seed storage for both species (Sniezko et al. 2011). However, little is known about how long seeds of these species can be stored in freezers and retain their viability. The study reported here and in more detail in a subsequent paper (Sniezko et al. 2017), examines germination of the oldest known foxtail pine seedlots from California (its native range), as well as the oldest known seedlots from different parts of the range of whitebark pine (collected and stored by different groups in the Pacific Southwest, Pacific Northwest, and Interior West United States and in British Columbia and Alberta). Results indicate that at least some seedlots of whitebark pine and foxtail pine can be stored for several decades and show very high germination (50 to >90 percent) in subsequent tests (Sniezko et al. 2017). The germination trial was conducted in Oregon at Dorena Genetic Resource Center, but more recent refinements in germination protocols for whitebark pine developed in Canada indicate that even higher levels of germination are possible. A subsequent sowing of some of the 'old' foxtail pine seedlots showed that they retained capacity for high germination several years beyond this trial. In this subsequent sowing, the foxtail pine seedlots also showed similar seedling vigor (height growth and survival) compared with more recent seedlots, when grown for disease resistance testing. We conclude that mature whitebark pine and foxtail pine seeds collected and stored under suitable conditions can retain viability for at least several decades (Sniezko et al. 2017).

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The Role of CVS (and FIA) Data and Genetic Tests in Assessing Species Vulnerability to Invasive Pests and Changing Climate[1]

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United States tree species and their associated ecosystems, managed forests, and urban plantings are increasingly vulnerable to non-native invasive pathogens and insects as well as effects associated with a changing climate. Some species, such as whitebark pine (*Pinus albicaulis*), have been proposed for listing under the Endangered Species Act. To fully assess the vulnerability of tree species and ecosystems, we need to make better use of data that shows temporal trends in mortality and forest health. With these data, forest managers and the United States public will have a greater sense of urgency, and debate over the full extent of possible management actions will be better informed. Several under-utilized types of data are available to help quantify changes over time in vulnerability of forests and tree species and the potential impacts of the biotic and abiotic agents driving change. One of these is Forest Inventory and Analysis (FIA) data, which provides a probabilistic sample across all land ownerships so that results from the analysis can be reliably extrapolated to all lands. The FIA sample design will consist of a new annual inventory which will be consistent across the United States. The first round of plot re-measurement in this program is currently underway. Continuous Vegetation Survey (CVS) data, currently available, and used here, covers primarily U.S. Department of Agriculture Forest Service land (Lintz et al. 2016). Other important sources of data include long-term progeny tests, provenance trials, and clone banks, which serve as de-facto permanent sentinel plots comprised of known genetic components, which is a distinctive benefit. We provide examples from each of these types of data and show examples of species that are on the decline (Lintz et al. 2016; Sniezko et al. 2012, 2013).

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Blending Ecology and Evolution Using Emerging Technologies to Determine Species Distributions with a Non-native Pathogen in a Changing Climate[1]

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A collaborative team of researchers from the United States and Mexico has begun an exciting new research project funded by The National Science Foundation's Macrosystems Biology program. The project will study ecological and evolutionary processes affecting the distribution of southwestern white pine (*Pinus strobiformis*), an important tree species of mixed conifer forests in the southwest United States and Mexico. Southwestern white pine sustainability is threatened by changing climate, and a non-native tree disease, white pine blister rust caused by *Cronartium ribicola*. White pine blister rust causes extensive tree decline and mortality where it occurs in North America, including an ever-expanding area where it overlaps with southwestern white pine. In addition, climate may be changing too rapidly for southwestern white pine to adapt. The dual threats of a changing climate and an invasive species make forecasting future tree distributions across continental scales an urgent challenge. The goal of our research 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 is developing 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 will use the Southwest Experimental Garden Array, a new genetics-based research platform that allows scientists to quantify the ecological and evolutionary responses of species to changing climate conditions. The research approach will provide a prototype for forecasting complex system behavior applicable to other systems, including those facing similar ecological challenges, and will contribute directly to the conservation of southwestern white pine while strengthening cross-border research and management efforts in forest conservation. This work is partially supported by the National Science Foundation under Grants No. EF-1442597, EF-1442486 and EF-1442456 and the U.S. Department of Agriculture Forest Service's Special Technology Development Program.

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Silvicultural and Integrated Pest Management Strategies for Restoring Eastern Hemlock to Degraded Southern Appalachian Mountain Ecosystems[1]

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The ecologically foundational species eastern hemlock, *Tsuga canadensis*, is being functionally eliminated from southern Appalachian forests by the hemlock woolly adelgid (HWA, *Adelges tsugae*). The management of HWA has focused on chemical and biological control, conservation of hemlock genetic resources, and host resistance breeding; however, research on the reintroduction of hemlocks to forests where it has been lost has received almost no attention. This poster presents progress made on phase one of a three phase project to develop a hemlock restoration strategy that integrates silvicultural prescriptions with biological and chemical controls for the reintroduction of eastern hemlock to southern Appalachian forests. Phase one is testing the effects of canopy structure (thinned versus canopy gap), deer exclusion, fertilization, and weed control on the establishment, survival, and growth of planted eastern hemlock seedlings receiving insecticide protection. A total of 12 research plots were established at two sites in western North Carolina in stands dominated by dead and dying eastern hemlock. Plots were established as pairs with one serving as a "high" light canopy treatment (clearcut/canopy gap) and the other as a "low" light canopy treatment (thinned to ~27.55m^2/ha). Each plot is divided into two 10x10 m subplots with one surrounded by at 2.5 m high deer exclusion fence and the other remaining open (no fence). Each subplot contains four 5x5 m treatment plots, each with 16 eastern hemlock seedlings planted at a 1x1 m spacing. The four treatments are weed control, fertilization, weed control + fertilization, and no treatment (control). Wildlife cameras were installed at each of the 12 plots to monitor deer presence. Year 1 growth data indicates superior diameter and height growth in the clearcut/canopy gap plots compared to the thinned treatment. A thorough statistical analysis has not been completed, but there are no discernable trends in growth means between the fenced and unfenced plots or the fertilization and herbicide treatments. While wildlife cameras have recorded deer presence in each of the plots, hemlock browse has not been observed. This poster will provide an overview of study establishment and preliminary results on seedling height and basal diameter following the first year of the study. This study is ongoing. A more detailed data analysis is planned when additional yearly measurements have been recorded.

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