

STRATEGY FOR CONSERVING ASH TREES IN THE NORTHEAST: COLLECTION, ANALYSIS, AND OUTREACH

Project Outcomes, 2016-2017

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Project Background

NYBG scientists, working as part of a multi-disciplinary, multi-institutional team, are investigating the genetic diversity of ash trees and will make these findings available to the scientific and conservation communities for use in efforts to protect North American ash trees from the invasive Emerald Ash Borer (EAB). The project's goal is to elucidate the relationships of North American Ash species and to study patterns of susceptibility to EAB, enabling adoption of appropriate conservation strategies. Additionally, citizen scientists will be trained in New York and New England in ash identification and data collection, allowing them to contribute scientifically to the proposed project as well as further ash conservation initiatives. The project will take advantage of the unique opportunity to obtain and employ critical data while considerable areas in this region remain un-invaded by EAB. But time is of the essence, as the EAB's range is rapidly expanding and it is known to inflict nearly 100% mortality on the ash species native to New York and New England.

To address this conservation impediment, NYBG's research team includes the Mid-Atlantic Regional Seed Bank, U.S. National Arboretum, U.S. Forest Service, Ecological Research Institute, and Bluegrass Woodland Restoration Center. The immediate goal is to develop a robust taxonomic picture of North American ash trees, focusing largely on the *Melioides* section of the genus and particularly the taxonomically complicated New York and New England species. This will be accomplished by conducting a phylogenetic study (i.e., determination of evolutionary relationships) using DNA samples, chromosome analysis, herbarium specimens, and viable seed from all generally accepted and hypothesized species. Moreover, by collecting environmental data along with field samples, we will be able to account for environmentally related variability in species' characteristics. Through outreach and coordination, our findings will be incorporated into on-the-ground conservation efforts. The project's three main elements are collection, analysis, and outreach.

Project Outcomes

This two-year conservation project (2016 – 2017) employed successfully three interrelated approaches to advance the goal of preventing the invasive Emerald Ash Borer (EAB) beetle from devastating Ash trees in the northeastern United States: collection, analysis, and

outreach. Together, these approaches synergistically set the stage for educating the public during the [Saving the American Ash Summit, Friday, October 13, 2017](#). Equally importantly, the outcomes of the project lay the foundation for all the other various teams, foresters, breeders, land managers, entomologists, and others to tackle their particular piece of the puzzle.

Collection. The overarching goal was to create a taxonomic framework for use by Ash tree breeders, land managers, and citizen scientists. The project accomplished this goal by obtaining 349 herbarium vouchers, DNA, chromosome, and anatomical samples of all putative *Fraxinus* species and varieties in the eastern United States with an emphasis on New York and New England. Field notes for each species included a description of the tree, ecological data, including the trees' exposure, and responses to EAB as well as environmental variables that might influence these responses and other relevant plant traits. The team documented the occurrence of four populations of the Critically Endangered (IUCN Red List of Threatened Species™) Pumpkin Ash in New York State. See this [Interactive Map of Ash Tree Specimens Examined 2016-2017 by NYBG Team](#). The next steps are to scale up Ash collections to provide a more complete sampling of Ash diversity across North America to inform taxonomic studies and breeding programs.

Analysis. In the NYBG's Cullman Laboratory, under the leadership of Gregory Plunkett, assisted by Sarah Hardy, DNA was extracted from 304 samples, resulting in the largest DNA data-set of American Ash species ever assembled. A subset of these samples that yielded high-quality DNA was used for analysis. The 189 *Fraxinus* samples used represented 45 species (about 95% of the genus worldwide). Three quarters of the samples are in the large, mostly American section *Melioides*—the group of Ash most at-risk from the EAB. The analysis included 133 field-collected samples dried in silica and 56 herbarium specimens. Five molecular markers were targeted for Polymerase Chain Reaction (PCR) amplification, three of which were found in chloroplast DNA and two found in nuclear DNA. The resulting sequences were assembled and edited. A phylogenetic tree resulting from maximum likelihood (ML) analysis was prepared. Preliminary results confirm previous higher-level classifications of the genus. Results within the large, American section *Melioides* suggest that the species comprising this group may have evolved very recently and are probably very closely related. A paper reporting results from this DNA analysis is in preparation.

The chromosome number of the 12 putative Ash species from the eastern United States was determined by Alan Whittemore, assisted by Zheng-Lian Xia at the U.S. National Arboretum. They were calibrated with known chromosome counts, and were used to assess genome size, and infer ploidy for 303 accessions of *Fraxinus* spp. A paper reporting the results will be published in the *International Journal of Plant Science* [Whittemore, A., J.J.N. Campbell, Z-L Xia, C.H. Carlson, D. Atha, R.T. Olsen. In press. Ploidy variation in *Fraxinus* L. (Oleaceae) of eastern North America: genome size diversity and taxonomy in a suddenly endangered genus].

Prior to this study, there was no scientific consensus on number of Ash species and varieties in the northeastern United States. Hypotheses ranged from six to nine species and none to six

varieties. In the absence of a solid taxonomic framework, these various "species" and varieties were generally categorized into informal groups or complexes: White Ash, Black Ash, Green/Red Ash, Blue Ash, Pumpkin Ash, Water Ash and European Ash.

On the basis of our results obtained from detailed field observation, anatomical study, DNA and chromosome analysis, we propose that there are eight wild species and no varieties of *Fraxinus* in the northeastern U.S., seven native and one non-native. These are described in the recently published *Field Guide to Ash Trees of Northeastern United States*. The guide is designed for scientists as well as the lay public and includes a dichotomous key to the species, short morphological descriptions, distribution maps, and color photographs of leaves, bark, twigs, and fruits for each species. The guide is available as a free [PDF download here](#), and complimentary bound copies may be obtained upon request from the Center for Conservation Strategy, The New York Botanical Garden, 2900 Southern Boulevard, Bronx, NY 10458.

The eight wild species of Ash in the northeastern United States and adjacent Canada are:

Fraxinus americana (White Ash)

Fraxinus nigra (Black Ash)

Fraxinus biltmoreana (Biltmore Ash)

Fraxinus pennsylvanica (Green/Red Ash)

Fraxinus caroliniana (Water Ash)

Fraxinus profunda (Pumpkin Ash)

Fraxinus excelsior (European Ash)

Fraxinus quadrangulata (Blue Ash)

The next steps for analysis are to collect the remaining species of *Fraxinus* in North America for anatomical, chromosome and DNA analysis. This will enable the team to produce the first complete phylogenetic assessment of North American Ash trees.

Outreach. Through a dozen workshops and conferences, culminating with the [Saving the American Ash Summit, Friday, October 13, 2017](#), the plight of Ash trees in North America was brought to the public's attention. Without minimizing the threat, the Summit presented positive measures scientists and citizen scientists could take to mitigate the impacts and advance the search for resistant trees and traits. As another outreach component of this project, the Managing and Monitoring Ash (MaMA) protocol was developed by Jonathan Rosenthal and Radka Wildova of the Ecological Research Institute to engage citizen scientists in helping to solve the Ash tree conservation crisis. MaMA is a protocol for engaging citizen scientists in conserving ash and mitigating the threat of EAB. In particular, the protocol emphasizes looking for "lingering Ash" in forests of the northeastern U.S. The product consists of a [MaMA presentation](#) and a new website MonitoringAsh.org, <http://www.monitoringash.org/>.

The next steps for outreach are to engage private landowners, who manage more than a third of U.S. forests. These landowners are on the front line of identifying EAB infestations and can be trained as citizen scientists to help find the "lingering Ashes" on their properties. With heightened awareness of Ash tree diversity in the northeastern forests will come greater chances of discovering EAB resistant trees, and a brighter future for Ashes in the region and throughout North America.

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