Building a comprehensive collection of Ash germplasm

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Abstract

This paper summarizes a presentation from the Congress Symposium, "The Introduction of the Emerald Ash Borer in North America, A Case Study of Invasive Species Epidemiology and Conservation of the Host Species." It briefly discusses the state of *Fraxinus* (ash) taxonomy, ash as a landscape and forest tree, some of its specialized uses, including those by Native Americans, and its role in supporting other organisms. The devastation caused to native, North American ash populations by the introduction of *Agrilus planipennis* (emerald ash borer; EAB) to the Detroit, Michigan area has already led to the loss of tens of millions of trees. Diverse efforts are underway to document and slow EAB's spread and develop appropriate biological controls. Scientific research on ash-EAB interactions, including the study of potential tolerance or resistance mechanisms, breeding and genetic-diversity analyses, and ash systematics, would all benefit from access to well-documented, diverse ash germplasm. To help redress this unfolding biological tragedy, a collaborative, international effort to conserve these important genetic resources has been organized. Fortunately, ash is amenable to *ex situ* conservation through seed storage and cryogenic storage of dormant winter buds. Key partners in this effort are described herein, with a focus on the coordinating organization, the USDA-Agricultural Research Service's National Plant Germplasm System, along with a summary of progress to date and future plans.

Keywords

Agrilus, Emerald ash borer, *Ex situ* conservation, *Fraxinus*, Invasive species, Seed collection

An introduction to Ash, its value, and Emerald Ash Borer

Fraxinus is a member of the Oleaceae with winged fruits, consisting primarily of temperate, deciduous trees and shrubs, including ±60 species native to the Northern Hemisphere. Ash diversity is highest in China (22 species; Wei & Green, 1996) and the United States (16 species; USDA-ARS, 2010a). In the absence of a modern monograph for *Fraxinus*, however, such information can only be gleaned from floras. Fortunately, future efforts to develop a modern monograph should benefit from the recent phylogenetic analyses of Wallander (2008), who used nuclear ribosomal ITS sequences to study 40 *Fraxinus* taxa, and the work of Guy Neson, who is preparing a treatment of *Fraxinus* for Flora of North America.

In eastern North America, there are six native *Fraxinus* species (Fernald, 1950) under threat of functional extinction by the exotic insect pest, *Agrilus planipennis* (emerald ash borer or EAB), introduced from Asia to southeastern Michigan, probably in the 1990s (Siegert *et al.*, 2007). EAB adults feed on ash leaves, with larvae feeding on cambial tissue in ash stems and trunks. Female EABs oviposit on both healthy and stressed ash trees (Poland & McCullough, 2006), and there is no documented resistance among these six ash species to larval feeding, which ultimately leads to the death of the infested trees. Larvae commonly infest and kill both mature trees and juvenile saplings (Hermes *et al.*, 2009a). This phenomenon severely reduces opportunities for the evolution of increased tolerance to EAB and may hasten these species' extinction.

Two native *Fraxinus* species, *F. americana* (white ash) and *F. pennsylvanica* (green ash), are extensively cultivated and widely appreciated as stress-tolerant landscape trees (MacFarlane & Meyer, 2005). Many communities in the north central U.S. have overused green ash along their streets, leading to situations where it forms a significant proportion of the urban forest (Raupp *et al.*, 2006). Green ash also has a long and extensive history of cultivation as one the most commonly used trees for windbreaks in the Great Plains (Hoag, 1965). In garden settings, only a few selected staminate clones of white and green ash have generally been planted, which presents special challenges for the *ex situ* conservation of these species as noted below.

In addition to white ash and green ash, *F. quadrangulata* (blue ash) and *F. profunda* (pumpkin ash) also grow to sufficient size in forests to facilitate commercial harvest for timber and wood products. But beyond ash as a general timber commodity, ash wood has a diverse range of specialized uses. White ash wood exhibits a combination of strength and flexibility that makes it especially well suited for tool handles and as the first choice for professional baseball bats (Gasner & Widmann, 1990). Ash wood is also being crafted into artistic furniture and bowls.

Ash wood has also traditionally been employed by Native American communities in the north central and northeastern U.S. and eastern Canada (Schmidt, 1990). *Fraxinus nigra* (black ash) logs are laboriously pounded, and long thin strips of wood, known as splints, are removed layer-by-layer and trimmed. The splints are then woven to make a wide range of both utilitarian and decorative baskets.

These ash species occupy a wide range of ecological niches in eastern North American forests. Green ash has an especially wide geographic range from the Atlantic Coast west to the foothills of the Rocky Mountains and from the Gulf of Mexico north to the Canadian Prairie Provinces. Its wide distribution is mirrored by its broad ecological amplitude, occurring in seasonally flooded, floodplain and lakeside habitats, all the way to dry upland forests. White ash has nearly as wide a native range, but is generally restricted to fairly well-drained, mesic forests. Black ash is found in northern wet or boggy forests, often associated with *Picea* (spruce) and *Larix* (larch). Blue ash is associated with alkaline or calcareous soils, in a more limited geographic range in the central United States with outliers in Ontario, typically occurring in rocky, limestone woodlands. Pumpkin ash and *F. caroliniana* (Carolina ash) are typically found in or near standing water in the southern United States, growing with *Taxodium* (bald cypress) and *Nyssa* (tupelo).

In addition to the general ecological services that native ash trees contribute by providing food and shelter for wildlife, they also support a suite of at least 70 native specialist arthropods (Gandhi & Herms, 2010). Such species include *Tethidia barda* (brown-headed ash sawfly), *Lignyodes* sp. (ash seed weevil), *Aceria fraxinifolia* (flower gall mite), and 21 species of North American butterflies and moths (Wagner, 2007) affected by EAB's spread and the resulting death of millions of trees.

The spread of Emerald Ash Borer and potential controls

Since its North American introduction, EAB has been expanding via both natural dispersal and human assistance. Of the two, human-mediated dispersal is the more serious, in that it facilitates long-distance movement and the establishment of new infestations beyond the primary detection network (Poland & McCullough, 2006). This initially happened via the transport of nursery stock, wood products, and firewood. Today, firewood movement remains the most serious concern, as it is difficult to control through regulation. The spread of EAB is being diligently tracked by an extensive network of traps and regularly documented through the online publication of maps (Emerald Ash Borer, 2010).

In the wake of EAB, tens of millions of ash trees have already been lost (Smith *et al.*, 2009), with billions of dollars invested in tree removal, disposal (to prevent EAB reproduction), and replanting. But potential future economic losses are even greater, considering that the estimated number of remaining ash trees is as high as 8 billion (USDA-APHIS, 2010). When facing these huge costs, it is clear that investments to slow the spread of EAB serve two functions. First, they limit the annual economic burden to landowners and governmental agencies (Sharov, 2004; Poland & McCullough, 2010). Second, they buy time towards the effective deployment of biological control strategies, new treatments, and the potential development of resistant/tolerant ash trees.

There are many EAB-response strategies being implemented; a detailed enumeration is well beyond this paper's scope. Some of the most important, especially for the near-term, include the refinement of effective EAB trapping (Francese *et al.*, 2009; Marshall *et al.*, 2009) and monitoring systems (Careless *et al.*, 2009), the use of quarantines (USDA-APHIS, 2006) on the movement of ash nursery stock, timber products and firewood, accompanied by extensive public-education campaigns such as Don't Move Firewood (2010), the development of insecticide treatments to protect high-value trees (Herms *et al.*, 2009b), and research to identify, test, and introduce biological control agents (Bauer *et al.*, 2009; Gould *et al.*, 2009; USDA-APHIS, 2010). From a more long-term perspective, studies on the evaluation, mechanisms, and genetic control of host-plant resistance in Asian ash (Eyles *et al.*, 2007; Rebek *et al.*, 2008; Mason *et al.*, 2009), and on the potential tolerance of surviving native ash in highly infested zones (Koch *et al.*, 2010) are critical, as are tests of interspecific ash hybridization (Koch *et al.*, 2009), genetic transformation in ash (Pijut *et al.*, 2010), and investigations of genetic diversity, breeding systems and structure in extant populations (Hausman *et al.*, 2010). These efforts hold the promise of an eventual revival in planting ash as a landscape tree and of the re-introduction of ash populations to native forests.

The need for Ash germplasm

Research is focused on the host plant should advance more quickly with reliable access to well-characterized ash germplasm. *Ex situ* germplasm collections, if well designed, can provide a wealth of genetic diversity for economically and ecologically important traits, and supply known sources of clones and populations to serve as scientific controls or checks. But as we entered this crisis, *ex situ* ash germplasm collections in the U.S. were poorly developed. In 2002, there were no recognized ash collections among North American botanic gardens in the North American Plant Collections Consortium; ash provenance collections previously assembled by foresters were neglected or entirely abandoned (Steiner & Lupo, 2010); and the U.S. National Plant Germplasm System (NPGS) conserved only a few ash collections.

At that time, among other curatorial functions, I served as the NPGS curator for *Fraxinus*. As EAB spread and losses mounted, I began to consult other researchers and agencies concerned about this gap in *ex situ* conservation. At that time, the USDA-Natural Resources Conservation Service's Rose Lake Plant Materials Center began to mobilize volunteers to collect ash seeds in Michigan (USDA-NRCS, 2010). The U.S. Forest Service National Seed Laboratory initiated seed collections within its agency and with numerous partners (USDA Forest Service, 2010). The Canadian Forestry Service expanded efforts to collect native ash seeds for the National Tree Seed Centre (Natural Resources Canada, 2010). And within the NPGS, I began planning a series of domestic seed-collection expeditions and established contacts with the Morton

Arboretum and Beijing Botanic Garden to plan Chinese collection trips, designed to sample potentially EAB-resistant ash populations. In addition to the parties noted above, other botanic gardens, state forestry and natural resource agencies, and Native American communities became involved in the collection effort. It was quickly apparent that clear communications and interagency coordination were needed. At a 2009 meeting of interested parties held in Annapolis, Maryland, I agreed to serve as coordinator. In March 2010, I chaired a second meeting at "The Symposium on Ash in North America" in West Lafayette, Indiana, to prepare for the 2010 field season.

Taking on this role was a logical move in that the NPGS is the lead organization within the U.S. for the *ex situ* conservation of genetic diversity of economically important plants and their wild and weedy relatives (Widrlechner, 2009). It has extensive collections and supports considerable research on germplasm conservation protocols. NPGS collections are freely available for research and educational purposes world-wide, and information about the collections is widely disseminated online through the Germplasm Resources Information Network (GRIN) database (USDA-ARS, 2010a).

Assembling and conserving Ash germplasm collections

Fortunately, ash populations can be effectively conserved as seeds with orthodox storage characteristics (UK Forestry Commission, 2010), and clones cryogenically preserved as dormant buds (Volk *et al.*, 2009). Field plantings can also be maintained, if sufficient land is available and trees can be protected from EAB by geographic isolation and/or the effective use of systemic insecticides. Our initial focus has been on the assembly of comprehensive seed collections. To accomplish this, we must ensure good initial seed quality (embryo development and weevil infestation are important limitations, see seed-collection guidelines by Knight *et al.* (2010)), proper taxonomic identity (supplemented with herbarium vouchers), complete passport data, and effective sampling strategies. Ideally, samples should be diverse enough to allow us to preserve genetic profiles over future generations without inbreeding depression. To maximize overall diversity and the capture of genes conferring local adaptation, it is crucial to avoid areas with significant numbers of cultivated, staminate ash trees that can bias local pollen deposition and focus on sites with established natural populations.

On a broad scale, our sampling strategy is conducted on an individual-species basis, focusing on regions that are being colonized by EAB, stratified by plant-community diversity as reflected in Omernik Level III Ecoregions (Omernik, 1987; US Environmental Protection Agency, 2010). A new website for this conservation project has recently been developed (USDA-ARS, 2010b), which details the sampling strategy and presents current protocols.

Beginning in 2007, the USDA-ARS Plant Exchange Office has supported yearly ash seed-collection trips. Successful trips were conducted in New England in 2007, Missouri and southern Illinois in 2008, and Wisconsin and northern Illinois in 2009. Trips to Minnesota and Wisconsin and to Kansas, southern Missouri, and northern Arkansas are planned for 2010. Because of wide year-to-year fluctuations, summer reconnaissance trips to assess variation in local ash seed production and identify optimal populations for fall collection have proven quite helpful.

These collections and those of many collaborators are being incorporated into the NPGS. All these collections are being conserved at the National Center for Genetic Resources Preservation in Fort Collins, Colorado, with many of the best documented and representative samples being incorporated into the active collection at the North Central Regional Plant

Introduction Station in Ames, Iowa, which I curate. Table 1 lists the number of *Fraxinus* accessions in the active collection by species and continent.

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Table 1. Current *Fraxinus* accessions (by species and continent) conserved at the North Central Regional Plant Introduction Station, Ames, Iowa, USA, as of 1 July 2010.

Species	North America	Asia	Europe	Africa
F. americana	61			
F. angustifolia		1	7	
F. anomala	6			
F. bungeana	1	7		
F. chinensis		23		
F. excelsior			14	
F. “hybrid”	2			
F. insularis		1		
F. latifolia	1			
F. mandshurica		10		
F. nigra	15			
F. ornus	1		10	
F. paxiana		3		
F. pennsylvanica	77			
F. profunda	6			
F. quadrangulata	12			
F. raibocarpa		1		
F. sieboldiana		2		
F. “sp.”	6			1
F. stylosa		2		
F. xanthoxyloides		1		
Total	188	51	31	1