PROCEEDINGS OF SYMPOSIUM ON ASH IN NORTH AMERICA

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Abstract

Proceedings of the Symposium on Ash in North America held March 9-11, 2010, in West Lafayette, IN. Includes 5 papers and 30 abstracts covering topics related to the biology and ecology of the ash species, ash utilization and management, and emerald ash borer.

Cover Photos: ***Ash basket***, Les Benedict, St. Regis Mohawk Tribe; ***street trees***, Mary Carol Hunter, University of Michigan; ***lumber***, Daniel Cassens, Purdue University; ***seeds***, Robert Karrfalt, U.S. Forest Service. Photos of basket, street trees, lumber and seeds all used with permission.

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THE SIGNIFICANCE OF BLACK ASH AND SPLINT BASKETRY TO THE AKWESASNE KANIENKEHAKA (MOHAWK)

Les Benedict

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Black ash basketry is one of the many ways in which the Akwesasne Kanienkehaka identify with and express their rich heritage. The Kanienkehaka have faced multiple cultural and social challenges since their first contact with European society. These challenges have sometimes had negative social, psychological, lifestyle, and health consequences for the Kanienkehaka.

Black ash basketry directly connects the Kanienkehaka to their past and is linked to language and ceremonies. For many, it expresses their cultural identity. Black ash basketry is considered a form of medicine, providing healing through the Kanienkehaka's expression of the natural world and of their cultural heritage.

Without black ash, the Kanienkehaka will lose a resource that defines their culture, provides a link to their heritage, and serves as an important medicine.

PRESENT STATE, USE, AND PERSPECTIVES OF THE GENUS *FRAXINUS* IN MEXICO

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The genus *Fraxinus* is found in the main mountain areas of Mexico and, like many other Holarctic genera, reaches its southern limit in Central America. The Comisión Nacional para el Estudio de la Biodiversidad reports 21 species of *Fraxinus*, but a detailed assessment of their status still needs to be done. If synonymous names are removed, there are probably 13 species and one variety of *Fraxinus* in Mexico: *F. anomalla, F. berlandieriana, F. cuspidata, F. dipetala, F. dubia, F. gooddingii, F. gregii, F. papillosa, F. pringlei, F. purpusii* (var. *purpusii* and var. *vellerea*), *F. rufescens, F. udhei,* and *F. velutina.* The presence of two of them (*F. anomalla* and *F. dipetala*) in Mexico still needs to be confirmed, and the status of *F. pringlei* is doubtful and needs revision.

Many of the species found in northern Mexico are shared with the southwestern United States (*F. anomalla, F. berlanderiana, F. dipetala, F. gooddingii, F. gregii, F. papillosa*, and *F. velutina*). Three other species from central/south Mexico are also in Guatemala or even Honduras (*F. dubia, F. purpusii*, *and F. uhdei*). Only *F. rufescens* (and perhaps *F. pringlei*) is endemic to Mexico, where it grows in the central states.

Most species of *Fraxinus* are found in protected humid slopes in temperate (*Quercus-Pinus*) forests and as part of riparian vegetation, but species such as *F. uhdei* are also elements of moist forests. Still others (such as *F. rufescens*) are found in the transition between temperate and tropical deciduous forests and even in xeric shrublands. *F. uhdei* is the only species that has a widespread distribution in Mexico, and therefore it is the only one that has been relatively well studied. It is a common urban tree, very abundant in Mexico City and Guadalajara, where it shows a weedy behavior. Because of its fast growth and high survival rate, it is a commonly cultivated species for reforestation programs. Ecological and forestry studies of other species present in Mexico still need to be done.

The main pest of *Fraxinus* in Mexico is *Hylesinus aztecus* Wood (Coleoptera, Curculionidae), a bark beetle, which has caused large declines in urban ashes. The emerald ash borer (*Agrilus planipennis*) has not been reported in Mexico, but its rapid spread through the United States makes it very probable that it will be able to reach Mexico in the near future.

BLACK ASH SILVICULTURE PROJECTS IN NEW YORK AND MAINE

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Silvicultural activities on stands of black ash (*Fraxinus nigra* Marsh.) are limited by the quality of the site, which is usually poorly drained, and by the low commercial value of slow-growing, small-diameter trees. Silviculture recommendations for black ash focus on its role as the primary raw material in the manufacturing of hand-crafted baskets.

This paper reports the success of artificially establishing two black ash stands in northern New York using planted seedlings. Gas exchange and stomatal conductance of planted seedlings were studied to determine the impact of site quality on seedling physiology. Results of thinning projects in northern New York and Maine are also reported.

Planted seedlings should be protected with tube shelters, both to minimize deer browsing and to escape weed competition with increased early height growth. These shelters, however, decrease seedling photosynthesis rates to about one-third that of seedlings growing in full sunlight. Stomatal conductance rates are similarly decreased.

A black ash stand in the Brasher Falls State Forest in New York was thinned to three residual densities, using a randomized block design. Four years after treatment, stand density showed no effect on either crop tree diameter growth or understory seedling development.

A black ash stand near Presque Isle, ME, was cruised and marked for thinning using stocking charts developed by Erdmann and others (1987). The simulator models, NE Twigs, SILVAH, and FIBER, were used to predict future growth rates. NE Twigs and SILVAH yielded similar results, but FIBER, which is more applicable to spruce/fir forests and uneven-aged stands, predicted much higher annual increment rates. Simulation of the plots thinned to 50-, 75-, and 100-percent crown cover, as opposed to the control plot (no cutting), showed consistently increasing volume production rates and diameter growth for black ash over the 20-year simulated period.

WOOD CHARACTERISTICS OF AND USES FOR ASH LUMBER

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Ash is one of about 16 commercial hardwood lumber groups and accounts for about 5 percent of total hardwood lumber production in the eastern hardwood region. Ash wood has several interesting characteristics and applications. This presentation will briefly review the lumber characteristics of the different species. Then the quality/color characteristics, physical properties, mechanical properties, grading and pricing, log and lumber weights, and important applications will be outlined. Numerous photographs illustrating details will be presented.

ASH, THE EMERALD ASH BORER, AND PRIVATE FOREST LAND MANAGEMENT

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Forest management through emerald ash borer (EAB) will be a dynamic process that will change based on the best information available at the time. Management decisions will depend on the anticipated time of EAB arrival; the diameter and number of ash present in the forest stand; the diameter and number of other desirable and undesirable species present in the stand (especially exotic invasives); the current and anticipated future ash markets; and, most importantly, the long-term economic and biological impact that the loss of ash will have on the residual stand in terms of future stocking level and natural regeneration.

As a consulting forester for more than 25 years in northeastern Indiana, I was first introduced to EAB in a woods near Quincy, MI, in 2003. It was obvious how devastating this insect would be. In addition, because EAB is difficult to identify (initially), management will need to be proactive. In early 2004, EAB was found in my primary work area. Based on very strong ash markets at that time and the potential for heavy mortality before the next scheduled harvests (typically 10-year intervals; most woods are <20 acres in size), my recommendation was to harvest all merchantable ash when conducting a harvest for a landowner. Financially, this approach worked well for about 2 years. However, ash regeneration thrives on disturbance; because of more aggressive harvesting, ash was more dominant in the understory than ever.

In late 2005, EAB was found in Huntington County and, soon after, the price plummeted, at least locally. In addition, new locations of EAB were being identified and subsequent quarantines made it difficult to market a large amount of ash, particularly during the summer (generally a desirable time to harvest ash due to low moisture content). In quarantined areas, the decision at that time was to include as much ash as possible and still attract a competitive price. In areas that were not quarantined, I continued to sell all merchantable ash.

I have serious concerns that ash (now undesirable) and other undesirable species will further dominate stand regeneration. Observations over the years have shown that many older timber harvests failed to regenerate acceptable species (even following timber stand improvement and completing openings). Observations also indicate that recent harvest openings include a high percentage of undesirable seedlings, such as ash, elm (vulnerable to Dutch elm disease), ironwood, blue beech, buckeye, and box elder, due to the abundant seed bank present immediately prior to the timber harvest. The presence of any invasive species only compounds that problem. Attempts to attain better natural regeneration have led to my current management philosophy: to increase management of the seed bank within the forest stand. My philosophy applies to management of any stand, with or without EAB. The goal is to minimize the amount of undesirable regeneration to improve the regeneration of desirable species and the long-term economic viability of the woodlands. Understory treatments (timber stand improvement) are done to eliminate or reduce the number of less desirable seed prior to a timber harvest. For example, treatment of any exotic invasives is mandatory. Harvests are delayed until EAB results in moderate to heavy mortality, usually 2 to 5 years, with the accompanying loss of seed bank.

EAB is now observed almost daily in my work area. The price of ash is now relatively stable, although considerably lower than 4 or 5 years ago. It is also anticipated that the current price is not likely to improve significantly. The current price appears to be relatively unaffected, except for the best trees, even when the trees have been dead for less than a year or two. The current timber markets in my area are primarily handles and pallets with some grade and sucker-rod.

As a general rule, each woods is treated on a case-by-case basis. In woods that have a considerable amount of high quality ash or a very small component of ash, I still recommend proceeding with the timber harvest as soon as possible, prior to mortality. Regarding tree planting, ash has not been a component since 2005.

FOREST HOST MAPPING: IMPLICATIONS FOR PEST SURVEY AND MODELING

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The Forest Health Technology Enterprise Team has developed forest-health risk maps for the past decade. Each subsequent rendition has improved upon previous renditions by acquiring and developing better data. Part of the improvement has focused upon host data of individual tree species. Knowledge of a host species' extent and density is critical to the success of an integrated pest management endeavor. The purpose of this presentation is to highlight developments pertaining to the different ash species modeled for the forest risk mapping project. The developed products can be used by land managers, policymakers, and resource specialists for strategic planning, monitoring, and resource allocation for a given forest-health concern. A scale assessment of alternative data for host mapping will also be demonstrated.

COMPARING METABOLOMIC PROFILES OF ASIAN AND NORTH AMERICAN ASH SPECIES (GENUS *FRAXINUS*) TO INVESTIGATE THE BASIS FOR RESISTANCE TO EMERALD ASH BORER

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At present, North American ash (*Fraxinus* spp.) are under attack by the emerald ash borer (*Agrilus planipennis* Fairmaire; EAB), an invasive species native to eastern Asia. Interestingly, Asian ash species are comparatively resistant to this phloem-feeding insect. Most researchers currently working on EAB are focused on improving detection, monitoring, biological control, and eradication methods in an attempt to protect our population of North American ashes against the advancing infestation front. To date, however, little work has been done to determine why our North American ash species seem to be so markedly different from the Asian ash species in EAB susceptibility. If we can determine that the difference is due to metabolites produced by one species but not by another, we might be able to exploit those differences to engineer ash trees with elevated resistance to EAB. One objective of this research is to compare the metabolomic profiles of North American and Asian ash species. Seedlings of susceptible North American ash species (white, green, black) are being grown in a greenhouse alongside resistant Asian ash species (Chinese, Manchurian), plus two European ash species that have unknown resistance. Seedlings will be challenged with methyl jasmonate root drenches to simulate insect feeding; some will be left untreated to serve as controls. Phloem, root, and leaf samples will be harvested from each seedling at various times and subjected to high performance liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry analyses. Additionally, high-throughput sequencing is being used to compare the transcriptomic profiles of infested and uninfested green ash trees in an attempt to determine genes that are up- or down-regulated in response to EAB feeding. Once candidate genes have been identified, a set of molecular experiments will be conducted to investigate the function of various genes in the jasmonate pathway in tissue harvested from these same trees.

UNDERSTANDING AND INTEGRATING NATIVE KNOWLEDGE TO DETERMINE AND IDENTIFY HIGH QUALITY ASH RESOURCES

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Black ash (*Fraxinus nigra*) is spiritually, economically, and culturally connected to Native American tribes throughout its range. Considered a cultural keystone species, black ash can be pounded and split along its growth rings to produce exceptionally strong and pliable strips to weave into baskets. Black ash harvesters and basketmakers (subsequently referred to as "experts") report increasing difficulty obtaining basket-grade wood. Emerald ash borer will contribute to this problem. We report here on a study that seeks to address this concern by documenting the traditional ecological knowledge (TEK) of harvesting basket-grade black ash. We will attempt to combine TEK with U.S. Forest Service Forest Inventory and Analysis (FIA) data to model tree and site characteristics associated with basket-grade wood and estimate the current supply and spatial distribution of basket-grade trees. Ongoing consultation with tribes and Native American experts is a central component of the study design.

During the summer of 2009, we conducted interviews with experts in the Wabanaki tribes of Maine and New Brunswick, Canada. Interviewees were asked about the wood characteristics needed for basketmaking and the environmental cues used to identify sites and individual trees, as well as any observations of changes in quality, supply, and spatial distribution of basket-grade trees. Preliminary results suggest that site characteristics, genotype, and life history interact to determine whether an individual tree will be basket-grade quality. We hope to consult with experts in the Mohawk Nation during the spring of 2010. Interview findings will then be validated through fieldwork, in preparation for identifying and analyzing relevant variables in the FIA data set. Results will contribute to the identification of strategies for management and/or restoration.

IMPACT OF EXTENSIVE STREET TREE LOSS ON URBAN DWELLERS' SENSE OF PLACE

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I took advantage of a natural experiment that occurred in southeast Michigan when an exotic insect pest caused the death and removal of more than 10,000 urban street trees within several years. Where dead street trees were clustered, the ecological and aesthetic integrity of neighborhoods changed dramatically, and the restorative benefits provided by these trees were lost. This paper will consider the impact of street tree loss on a community's sense of place and resultant interest in environmental stewardship.

In 2007, 1,300 households were selected to receive a four-page survey about their reaction to the loss of street trees and their attitudes toward urban nature. Households were selected based on physical, social, and economic criteria and the property's location on a street with either great (>70 percent) or limited (<30 percent) street tree loss. Forty-three percent of the sample population responded.

The results are described in relation to these categorical topics: sense of place (a psychological measure of security, attachment, contentment), degree of engagement in urban nature as experienced from home and neighborhood, degree of engagement or interest in helping the ecological recovery process, likelihood of physical participation in habitat creation (spending time, getting dirty), intellectual participation (using educational materials that may alter perception and action), economic participation (spending money on tree installation and habitat improvement), and community participation (planting new trees or enhancing habitat in the neighborhood and nearby park). Eighty-one percent of the survey participants said that street trees contribute to a sense of pride in the neighborhood; 68 percent said they believe that street trees enhance the sense of well-being. Of those who lost street trees in front of their home, 13 percent use their front porch less, 17 percent close the blinds or drapes more often, 11 percent spend more time gardening, and 56 percent report getting less enjoyment from driving down their home street.

The results of this scientific inquiry are being used to inform design options and suggest management strategies for urban nature in residential areas ranging along a continuum of environmental stewardship. This guidance is being shared on the Web site http://natureforcities.snre.umich.edu/, developed as an outreach component of this research.

THE U.S. FOREST SERVICE NATIONAL SEED LABORATORY AND *FRAXINUS* EX SITU GENETIC CONSERVATION

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The U.S. Forest Service's National Seed Laboratory (NSL) has as part of its mission the conservation of genetic resources for the Forest Service and Forest Service cooperators through long-term seed storage. The Forest Service recognizes ash as one of four priority species for genetic conservation. The NSL is in charge of the Forest Service ash preservation plan. NSL cooperates with the Natural Resources Conservation Service (NRCS) Rose Lake Plant Materials Center and the Agricultural Research Service North Central Plant Introduction Station. The main activities of the NSL are described as follows:

Planning – Collections were principally made in areas where emerald ash borer (EAB) was active or areas near active EAB infestations. Some collections have also been made to provide a wider geographic spread of the genetic material for research. As a general rule of thumb, seeds are collected from 50 individual mother trees evenly spread over an Omernik level III ecoregion. Every ash species existing in an ecoregion is collected. In 2010 Geographic Information Systems (GIS) mapping will start.

Cooperative Collections – The NSL staff was able to make some collections directly, but many more seeds were gathered by cooperators. Many persons are in the right place to collect seeds, and the program tries to encourage collection by providing training and supplies. It was found that without training, volunteers had difficulty in making any collections at all. Cooperators are contacted through a variety of avenues. Seeds collected through the NRCS Rose Lake Plant Materials Center are tested, stored, and distributed by the NSL.

Documenting collections –To the fullest extent possible, the following information is taken as a minimum on every seed tree: Name of seed collector, date of collection, GIS coordinates (or other locator information), a 6- to 9-inch twig sample with a healthy terminal bud, a leaf, and a photo of both the bark and the whole tree or stand in which it was growing.

Handling seed collections – All seed lots are given a cold treatment for at least 2 weeks to force the exit of seed weevils. This procedure makes removal of weevil-damaged seeds easier in the seed-cleaning equipment. Then the seed lots are dried thoroughly with air at 30-percent relative humidity, and empty seeds and trash are removed. X-ray and excised embryo tests are done to ensure that only seed lots of acceptable viability are placed in freezer storage. A security backup sample of each lot is placed at the National Center for Genetic Resource Preservation in Fort Collins, CO. All seed lots are listed in the Genetic Resource Information Network and made available for research and breeding projects when researchers contact the NSL directly.

EMERALD ASH BORER AFTERMATH FORESTS: THE DYNAMICS OF ASH MORTALITY AND THE RESPONSES OF OTHER PLANT SPECIES

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The effects of emerald ash borer (EAB) (*Agrilus planipennis*) on forest ecosystems are being studied through a collaborative research program involving the U.S. Forest Service's Northern Research Station and The Ohio State University. We are monitoring the decline and mortality of >4,500 ash trees and saplings, EAB population density, changes in understory light availability, responses of both native and invasive plant species, changes in species composition and forest structure, and effects on other organisms and ecosystem processes in more than 250 monitoring plots (and subsets thereof) in forests in Ohio, Michigan, and Pennsylvania along a gradient of EAB-infestation duration. The plots are located in forest stands representing different ages and habitat types to include all five ash tree species native to the region (*Fraxinus americana, F. pennsylvanica, F. nigra, F. profunda,* and *F. quadrangulata*). Yearly monitoring began in 2004 and is continuing.

Our results suggest a dismal future for ash in these ecosystems. Pre-EAB patterns of ash canopy health are functions of landscape position and nutrient availability. With the arrival of EAB, however, ash mortality in all areas reaches nearly 100 percent--regardless of initial ash density, size, habitat, or diversity. A forest stand can progress from nearly all healthy trees to nearly all dead ash trees within 6 years. EAB populations increase rapidly, peak, and then decline as the infestation progresses through the landscape. They then persist at low densities and kill small ash saplings as they reach susceptible size (3-cm diameter at breast height). Invasive plant species are present in these ecosystems and have the potential to increase as canopy gaps open, and distributions of both invasive plants and ash species depend on land use history and habitat. Indirect impacts of EAB on other organisms are likely.

COST OF POTENTIAL EMERALD ASH BORER DAMAGE IN U.S. COMMUNITIES, 2009-2019

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Emerald ash borer (EAB; *Agrilus planipennis* Fairmaire), a phloem-feeding beetle native to Asia, was discovered near Detroit, MI, and Windsor, ON, in 2002. As of March 2009, isolated populations of EAB have been detected in nine additional states and Quebec. EAB is a highly invasive forest pest that has the potential to spread and kill native ash trees (*Fraxinus* spp.) throughout the United States. There is little scientific literature on the number of ash trees in developed areas, the cost of treating trees to prevent infestation, and the cost of removing trees in response to infestation. We estimate the discounted cost of ash treatment and removal on developed land defined by the 2001 National Land Cover Database within communities defined by the U.S. Census in a 25-state study area centered on Detroit. We used 100 simulations of EAB spread and infestation over the next decade (2009-2019).

Results indicate more than 37 million ash trees occur on developed land in communities. The simulations predict an expanding EAB infestation that will likely encompass most of the 25 states and warrant treatment or removal of more than 17 million ash trees. The mean discounted cost of treating and removing those trees is $9.9 billion. States with the highest proportions of ash trees treated or removed (>90 percent) are those known to have established outlier populations present in 2009 that are predicted to expand and quickly spread across their entirety (West Virginia, Maryland, Delaware, Wisconsin, and Vermont).

Expanding the land base to include developed land outside, as well as inside, communities nearly doubles the estimates of the number of ash trees treated or removed and the associated cost. The estimates of discounted cost suggest that a substantial investment might be efficiently spent to slow the expansion of isolated EAB infestations and postpone the ultimate costs of ash treatment and removal.

NATIONAL INSECT AND DISEASE RISK MAP (NIDRM) —CUTTING EDGE SOFTWARE FOR RAPID INSECT AND DISEASE RISK MODEL DEVELOPMENT

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The Forest Health Technology Enterprise Team (FHTET)of the U.S. Forest Service is leading an effort to produce the next version of the National Insect and Disease Risk Map (NIDRM) for targeted release in 2011. The goal of this effort is to update spatial depictions of risk of tree mortality based on: (1) newly derived 240-m geospatial information depicting the distribution of tree hosts, pests, and associated factors influencing risk, and (2) modifications of risk models, including emerald ash borer, used in the 2006 NIDRM (www.fs.fed.us/foresthealth/technology/nidrm.shtml) with improved national consistency and precision.

Streamlining the construction of a higher resolution NIDRM that contains hundreds of spatial models by pest and host requires the development of a Windows desktop application that can facilitate rapid model assemblage. FHTET is in the final stages of building a desktop Risk Modeling Application (RMAP) using Microsoft Visual C# and ArcGIS Engine (ESRI Inc., Redlands, CA) technology. This application allows users to weight, rank, and overlay a wide range of geographic information system layers to model the potential susceptibility and vulnerability of forested landscapes to attack by individual insects and diseases. Although RMAP is run as a stand-alone application outside of ArcGIS, it does require an Arc/Info and Spatial Analyst (version 9.3) license. A database, such as SQL Server 2005, is also required for file and data management utilities within RMAP.

SILVICS AND SILVICULTURE OF ASH IN MIXED HARDWOOD FORESTS OF THE SOUTHERN BOTTOMLANDS AND LOESSIAL HILLS

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This presentation describes the silvics of green ash (*Fraxinus pennsylvanica*), pumpkin ash (*F. profunda*), Carolina ash (*F. caroliniana*), and white ash (*F. americana*). Green ash is the primary ash species in southern bottomlands. Pumpkin ash and Carolina ash are relatively minor species with limited ranges that occur on very wet bottomland sites. White ash is the primary ash species in the loessial hills and on other upland sites across the South. The natural range, distribution across site types, and associated forest cover types within the southern United States are outlined for each species. Silvical characteristics addressed in the presentation include (1) site requirements, (2) reproductive characteristics, (3) shade tolerance, (4) flood tolerance, (5) drought tolerance, (6) fire tolerance, (7) competitive ability, (8) growth rate, and (9) damaging agents. Timber values and wildlife uses of each of the four species are discussed as well. Silvicultural guidelines are presented for green ash and white ash in natural, mixed-species, southern hardwood forests. Topics addressed include (1) recommended regeneration methods in both even-aged and uneven-aged stands, and (2) recommended thinning prescriptions under the guiding principle of stand quality management.

TRENDS IN DOMESTIC AND INTERNATIONAL MARKETS FOR ASH LOGS AND LUMBER

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While ash is a "minor" commercial hardwood species relative to oak, poplar, and maple, it still accounts for roughly 3 percent of all hardwood lumber produced, with an estimated kiln-dried value exceeding $150 million annually. More than 35 percent of all ash grade lumber produced is exported. We will examine decadal trends in domestic and international markets for ash logs and lumber and forecast market conditions for the coming year.

THE DISTRIBUTION OF ASH IN NORTH AMERICA

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Ash trees have been important to the people of North America for thousands of years. Of the nine ash species, white ash (*Fraxinus americana* L.) and green ash (*F. pennsylvanica* Marsh.) are the most widely distributed. These ashes are common yet not dominant in most forests of the eastern United States and into Canada. They also make up a large proportion of trees in the riparian lands and shelterbelts of the Midwest and in urban areas across the country. Due to the importance of ash for water protection, durable products, shade, and shelter, the ecological and economic value of ash is huge. A variety of stressors impact the health of ash trees. The most important stressors are the emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) and ash yellows (Mycoplasmalike organisms), both of which threaten the future of the species. We use Forest Inventory and Analysis (FIA) plot data from across the eastern United States to analyze patterns in the distribution and health of ash trees, saplings, and seedlings across forest types, stand sizes, crown classes, and ownerships. We also assess the current and future trends in the distribution of ash through the use of EAB range and spread maps. Even though EAB has been active in the forests of several states for >5 years, ash volumes continue to increase across most of the eastern United States as forests continue to mature.

LARGE-SCALE REINTRODUCTION OF ASH

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No strategies currently exist for reintroducing ash; progression of emerald ash borer (EAB) through the eastern United States is likely to be a decades-long process, and extirpation of ash from this area is likely to take even longer. Reintroduction of ash into areas where it has been extirpated by EAB will require addressing technical issues as well as social and political issues. Technical issues include what type of material will be introduced, for example, EAB-resistant (if any) or non-resistant native species, resistant interspecific hybrids, or resistant genetically modified material; and how and by whom the material will be produced for reintroduction. Social and political issues include how the development, production, and establishment of this material will be funded; and, perhaps, how this material will be accepted and used by public agencies, private landowners, and the general public.

Resistant ash developed by either standard breeding methods or genetic modification is likely to be used first by the ornamental horticulture industry, where small numbers of clonal varieties (relative to natural populations) are commonly deployed. Successful restoration of ash into natural ecosystems will require material with the capability of successfully competing and regenerating in perpetuity. These characteristics will require genetically diverse populations that are also genetically well-adapted to local climatic conditions. Availability of diverse sources of native ash germplasm will be essential for producing a genetically diverse and well-adapted population of material for reintroduction in both urban areas and natural ecosystems, so current germplasm conservation efforts need to be continued and expanded as EAB infestations spread.

Once suitable material has been developed, it will need to be increased before commercial production can begin. Both the reforestation and conservation nursery industry and the ornamental nursery industry have sufficient capacity to produce commercial amounts of material for deployment.

While there are several possible alternatives for reintroduction of ash, actual development and deployment of material will depend on the resources available. The most successful U.S. tree restoration programs to date involve American chestnut and the white pines in the western United States. Both of these programs feature tree species of significant economic, cultural, and ecological value, i.e, "charismatic megaflora." Funding and support for these programs have been sufficient to maintain a moderate level of activity over several decades, resulting in slow but steady progress on resistance to diseases that were introduced more than 100 years ago.

Billions of ash trees in the eastern United States are likely to be killed by EAB. The value of street trees, the cost of removing them, and the timber value of ash likely to be killed in the eastern United States will amount to billions of dollars. However, it remains to be seen whether ash will have the "charisma" needed to generate public support for funding large-scale restoration programs. If it does, the germplasm conservation efforts now underway for ash will provide a more diverse and well-adapted source of material than has any other U.S. species restoration program to date.

GENETIC TRANSFORMATION OF *FRAXINUS* SPP. FOR RESISTANCE TO THE EMERALD ASH BORER

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The emerald ash borer (EAB; *Agrilus planipennis* Fairmaire) (Coleoptera; Buprestidae) is a wood-boring beetle that poses substantial risk to the ash resource in North America. Ash species native to the United States and known to be susceptible to EAB are *Fraxinus pennsylvanica* (green ash), *F. americana* (white ash), and *F. nigra* (black ash) trees, which are major components of the landscape; and blue ash (*F. quadrangulata*) and pumpkin ash (*F. profunda*), which are less common species. Ash timber is valued for applications requiring strong, hard wood. Green ash is used both for solid wood applications, such as crating, boxes, and tool handles, and for fiber in the manufacture of fine papers. White ash is the primary commercial hardwood used in the production of baseball bats; it also is made into furniture, flooring, doors, and cabinets. Black ash is typically used for interior furniture and cabinets, and Native Americans require this species for the art of basketry. Beyond manufacturing, ash trees play an important ecological role by providing aesthetic value, and shelter and food for wildlife. EAB threatens these resources and may permanently alter forest ecosystems throughout North America. The development of transgenic *Fraxinus* spp. exhibiting resistance to EAB attack will have great economic and ecological benefits for landowners, the nursery and forest products industries, and the U.S. foreign trade of ash lumber and logs.

This research will result in the development of an *Agrobacterium*-mediated transformation system for *Fraxinus* spp. with recovery of transgenic plants resistant to EAB. In addition, this research will initiate studies of effective deployment strategies for transgenic ash trees. Data generated will be used for risk assessment, which is necessary for commercial release. Another major focus of future studies will be to examine the use of sterility genes to further modify transgenic ash to alter flowering and prevent gene escape.

An adventitious shoot regeneration and rooting protocol was first developed for green ash and is being optimized for black and white ash. The best regeneration medium for freshly isolated green ash hypocotyls was Murashige and Skoog (MS) medium supplemented with 13.3 µM 6-benzylaminopurine (BA) plus 4.5 µM thidiazuron (TDZ). The effect of in vitro-germinated seedling age on adventitious shoot regeneration from hypocotyl explants was also studied. Results showed that hypocotyl explants from freshly isolated embryos exhibited a higher organogenic potential than 4- to 15-day-old explants. Adventitious shoots from hypocotyls were established as proliferating shoot cultures following transfer to MS basal medium with Gamborg B5 vitamins supplemented with 10 µM BA plus 10 µM TDZ. A high rooting percentage was achieved when adventitious shoots were rooted on woody plant medium containing 4.9 µM indole-3-butyric acid (IBA) plus 5.7 µM indole-3-acetic acid (IAA) with a combination of a 10-day dark culture period followed by a 16-h photoperiod. Rooted plants were successfully acclimatized to the greenhouse, and 100 percent survived after overwintering in cold storage. This regeneration system provided a foundation for developing a genetic modification protocol for green ash. We are also investigating an adventitious shoot regeneration system using leaf or internodal explants.

A genetic transformation protocol was first developed for green ash hypocotyl explants and is currently being optimized for black and white ash. Green ash hypocotyls were transformed using *Agrobacterium tumefaciens* strain EHA105 harboring binary vector pq35GR containing the neomycin phosphotransferase (*nptII*) and β-glucuronidase (GUS) fusion gene. After a 1-day pre-culture on regeneration medium, hypocotyl explants were transformed in the presence of 100 µM acetosyringone using 90-sec sonication plus 10-min vacuum-infiltration. Kanamycin at 20 mg l^{-1} was used for selecting transformed cells. Adventitious shoots regenerated on MS medium supplemented with 13.3 µM BA, 4.5 µM TDZ, 50 mg l^{-1} adenine sulfate, and 10 percent coconut water. GUS- and polymerase chain reaction (PCR)-positive shoots from the cut ends of hypocotyls were produced via an intermediate callus stage. Presence of the GUS and *nptII* genes in GUS-positive shoots was confirmed by PCR, and copy number of the *nptII* gene in PCR-positive shoots was determined by Southern blotting. Three transgenic plantlets were acclimatized to the greenhouse and then successfully survived overwintering in cold storage. Studies are underway using a construct containing the Cry8Da protein of *Bacillus thuringiensis* for genetic transformation of ash for resistance to EAB.

CLIMATE CHANGE POSES ADDITIONAL THREAT TO THE FUTURE OF ASH RESOURCES IN THE EASTERN UNITED STATES

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It is becoming increasingly clear that climate change has the potential to alter the distribution of plant species all over the world. In the United States, ash (*Fraxinus* spp.) is encountering the double threat of short-term emerald ash borer (EAB) infestation, which could decimate ash throughout the country, and longer term perturbations due to climate change. We have modeled the change in potential suitable habitats of numerous tree species in the eastern United States by relating the abundances of tree species according to U.S. Forest Service Forest Inventory and Analysis data to 38 climate, soil, and landscape predictors using decision-tree based ensemble statistical techniques (DISTRIB). We alter the climate variables in the ensemble and predict abundances according to two carbon emission scenarios (high and low) and three Global Circulation Models (GCMs). In addition to the DISTRIB model, we assess how landscape fragmentation is affecting the colonization potential of the tree species, using a spatially explicit cellular model (SHIFT). The outputs of DISTRIB and SHIFT provide a fairly reasonable assessment of the potential habitat changes that can be expected due to future climate changes predicted by GCMs. However, these models do not include biological and disturbance factors that may influence species' response to climate change. To address some of these uncertainties, we take into account 9 biological and 12 disturbance-related factors by synthesizing the literature and scoring the factors by order of importance.

Here we assess the impact of climate change on ash species according to our habitat change models and combine these results with a "risk of spread model" that we have developed by modifying the SHIFT model (to determine the risk of human-mediated long-range dispersal of EAB). We will then paint a picture of the future of ash species in the eastern United States.

ASHES TO ASHES: LARGE *FRAXINUS* GERMPLASM COLLECTIONS AND THEIR FATES

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As the emerald ash borer (EAB) threatens the survival of our ash species, measures should be taken to preserve their genetic variability in the event that we discover a way to restore populations destroyed by the beetle. As it happens, large germplasm collections exist for our most important and widely distributed eastern species of the genus, white ash (*Fraxinus americana* L.) and green ash (*F. pennsylvanica* Marsh.). The white ash collection, organized by Calvin Bey with the U.S. Forest Service, was begun in 1973 and comprised the progenies of 228 trees representing 39 presumptively native populations from throughout the species' natural distribution. Seed collections were made from an additional 16 populations, but it is unclear at this time whether these were ever out-planted. The green ash collection, organized by Kim Steiner at Penn State, was begun in 1975 and comprised the progenies of 216 trees representing 60 range-wide populations. Long-neglected as scientifically "obsolete," these provenance collections have taken on renewed significance as germplasm repositories in the face of a serious biological threat to the genus.

The white ash collections were planted at 25 locations scattered from Louisiana and Alabama to New Brunswick and Nebraska. Most plantations had progenies from 16 to 27 provenances, and one contained as many as 35. It appears that the complete collection was not represented at any one location. Each plantation contained five replicate blocks with five-tree family plots.

The green ash collections were planted at 12 locations in the northeastern and north-central United States, from Maine to Maryland and west to Nebraska. Most plantations contained progenies from 16 to 45 provenances, but all 60 were represented at one location in Pennsylvania. Most plantations contained 3 to 10 replicate blocks with four-tree provenance plots containing nested, one-tree family plots. However, one plantation contained only two replicates of provenance plots, and two plantations were designed with two- or four-tree family plots completely randomized within blocks.

Of the 25 white ash plantations, 7 are believed to still exist in a recoverable condition with >30 percent survival. All have been (or will be) remeasured and remapped. Two of the others still exist but are in very poor condition. The remaining 16 tests were failures or no longer exist because of land use conversion. The best single collection of germplasm is in Jefferson County, KS, with 44 families representing 27 provenances and >70 percent survival. A plantation at Michigan State University's Kellogg Forest still contains 32 replicated provenances, although survival is down to 33 percent. EAB has been trapped at the edge of this plantation.

Of the 12 green ash plantations, 7 are believed to have >30 percent survival and be recoverable as mapped germplasm collections. The remaining plantations have all been destroyed and the sites converted to other uses. The best single collection of germplasm is in Centre County, PA, with 216 families representing 60 provenances and 85 percent survival. Two plantations maintained by Michigan State University contain most provenances and are in good condition. EAB has been trapped near one.

In the presentation we will outline priorities for germplasm protection in the two species based on these collections of range-wide genetic material.

OBSERVATIONS ON ASHES USED IN NORTH AMERICA AND SUGGESTED ASH REPLACEMENTS

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Ten replicates of 20 taxa of *Fraxinus spp*. were planted in northeastern Toledo, OH, in 2005. Thirteen North American, three European, and three Asian taxa were evaluated, as was one purported hybrid. Most of the trees are in a wide median; a smaller number were planted in a lawn panel along the roadway. Three soil types are represented in these urban soils. Plants were mulched during establishment but were not treated with pesticides. Emerald ash borer (EAB) began to emerge from some taxa in 2007. Plant genetics, nursery propagation method, and soil type seem to impact the time required before EAB development is visually confirmed by characteristic exit holes and frass-packed serpentine galleries.

Recommended replacements were identified based on the following assumptions: The large growing ashes were appropriately sized for the site, replacements should be large in size, and the original trees were well adapted to the site and did not require a species substitution. Thirty-four species of trees were identified as potential replacements. Silvicultural characteristics such as tolerance of shade and flooding were noted for each species. This information appears in the 2005 Extension Bulletin 924, Ash Replacements for Urban and Woodland Plantings. Specific examples will be presented at the conference.

EXPLORING THE MOLECULAR AND BIOCHEMICAL BASIS OF ASH RESISTANCE TO EMERALD ASH BORER

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Larvae of the emerald ash borer (EAB) (*Agrilus planipennis*) feed on phloem of ash (*Fraxinus* spp.) trees. It is hypothesized that the resistance of Asian species of ash (e.g., Manchurian ash, *F. mandshurica*) to EAB is due to endogenous defenses present in phloem tissues in the form of defensive proteins and/or toxic secondary metabolites. We have taken a proteomic and metabolomic approach to understand the interactions between EAB larvae and ash phloem that are fundamental to elucidating mechanisms of resistance to EAB.

To identify proteins that may be responsible for resistance of Manchurian ash to EAB, we used Differential in Gel Electrophoresis (DIGE) to compare its constitutive phloem proteome with that of the closely related but highly susceptible North American black ash (*F. nigra*). Differentially expressed proteins were sequenced using nano-liquid chromatography-mass spectrometry (MS)/MS, and putative functions were assigned. Hierarchical clustering of 355 phloem proteins with concentrations that differed substantially (>twofold, $P < 0.05$) between black and Manchurian ash revealed protein expression patterns that were nearly diametrically opposed, despite the very close phylogenetic relationship between the two species. Proteins in Manchurian ash that may be associated with resistance include enzymes involved in the synthesis of lignans and flavonoids, oxidative enzymes, a PR-10 protein, and an aspartic protease. Future functional studies are needed to identify what roles these proteins may play, if any, in resistance of Manchurian ash to EAB.

Chemical characterization of phloem tissue of North American (green, white, black, blue), European (*F. excelsior*), and Manchurian ash revealed qualitative similarities in the constitutive phenolic profiles of black, European, and Manchurian ash, while individual compounds differed quantitatively. Phloem chemistry of green and white ash was similar qualitatively and quantitatively, as reported by Eyles et al. (2007). The phenolic profile of blue ash comprised mainly hydroxycoumarins and showed no similarity to the other species. The similarities and differences in phenolic profiles among species corresponded with their phylogenetic relationships (Wallander 2008).

The ability of methyl jasmonate (MeJA) (an endogenous plant stress hormone that regulates defense responses) to induce ash phloem chemistry was also investigated by applying 1 M MeJA to the outer bark of Manchurian and white ash. Treatment with MeJA increased concentrations of total phenolics in both ash species, suggesting that induced and constitutive phenolic chemistry may play a role in EAB resistance. These studies provide a foundation for ongoing investigations on the role phenolics may play in ash resistance to EAB. Discovery of resistance-related proteins and secondary metabolites that confer resistance to EAB would accelerate breeding and /or selection of resistant trees for restoration of ash to natural and urban forests.

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BUILDING A COMPREHENSIVE COLLECTION OF ASH GERMPLASM

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The U.S. National Plant Germplasm System (NPGS) has conserved seed collections of ash (*Fraxinus*) germplasm at the USDA-ARS North Central Regional Plant Introduction Station (NCRPIS) in Ames, IA, since the 1970s. The initial focus was on samples acquired from Yugoslavia but gradually expanded to include representation from other areas, including North America. At the time emerald ash borer (EAB) (*Agrilus planipennis*) was introduced into southeastern Michigan, the NCRPIS maintained a relatively small ash germplasm collection that did not encompass the range of taxonomic and genetic variation in North American species vulnerable to EAB, nor in Asian species that co-evolved with EAB. In the current NCRPIS Project Plan, this gap in the collection was recognized and plans made to address it.

As plans were refined, it became clear that proper sampling of North American and Asian ash would require a multi-institutional, collaborative effort. Fortunately, efforts were already underway within the USDA-NRCS (at the Plant Materials Center in Rose Lake, MI), the U.S. Forest Service National Seed Laboratory, and other public and tribal organizations to mobilize collection in North America. Collaborations were quickly established involving the Morton Arboretum and Beijing Botanical Garden, supported by the USDA-ARS Plant Exchange Office, to collect ash seeds in China. The NPGS recognized the value in bringing together the seed collections made by these organizations for long-term preservation at the National Center for Genetic Resources Preservation (NCGRP) in Fort Collins, CO, and for the selective integration of those collections into the ash working collection, actively curated at the NCRPIS.

In January 2009, a meeting and teleconference was held in Annapolis, MD, bringing together as many parties as possible involved with ash seed collection to develop standard collection and documentation protocols and a comprehensive plan for North America. The collection strategy centers on a stratified sampling of native populations of all North American *Fraxinus* species, based primarily on taxonomy and ecogeographic zones. This approach recognizes the urgency of collecting in regions being colonized by EAB. Since 2007, considerable progress has been made sampling native ash populations. The NCRPIS now conserves 237 accessions (78 percent of which are North American). Seed storage can also be complemented by cryogenic storage of dormant vegetative buds, with protocols recently developed in collaboration with NCGRP. Together, these efforts will create a publicly accessible, comprehensive ash germplasm collection that can serve as a foundation for research on EAB, *Fraxinus* genetics, and the eventual restoration of ash in our forests and communities.

STRIKE ONE! ALUMINUM. STRIKE TWO! MAPLE. WILL EAB BE STRIKE THREE?

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Abstract.—Baseball bats made out of white ash (*Fraxinus americana* L.) have been the standard for professional baseball since the beginning of the game in 1849. Until the mid- to late-1970s, ash was the principal material used for all baseball (and softball) bats – major league, minor league, adult, and youth. The market share of baseball bats made from wood eroded precipitously in the late 1970s and early 1980s as a result of the introduction of aluminum bats. Wooden bats now constitute less than 10 percent of bat production and sales.

Despite reduced demand for ash in bat production, the emerald ash borer's (EAB) presence became a threat to manufacturers of ash baseball blanks (termed "billets") in July 2009 with the first substantiation of the presence of EAB in southwestern New York. Billet manufacturers are having to shift their ash procurement regions, and contingency plans for using alternate species in wood bat manufacture are being formulated.

ALUMINUM BATS ... STRIKE ONE!

The aluminum bat arrived on the scene in the 1970s (although originally patented in 1924; Sheldon 2008) and quickly gained a significant share of the baseball bat market. Within a few years, the majority of youth bats, which make up about 33 percent of the overall bat market, were made out of aluminum. Likewise, the majority of adult bats—bats used by high school and college players—also are made of aluminum (or a metal alloy) today. Considering all levels of baseball and softball, non-wood bats (aluminum, metal alloys, and composites) now compose 90 to 95 percent of annual bat sales (personal communication with M. May of the Sporting Goods Manufacturers Association, Nov. 17, 2009).

The high-end and most widely recognized market for wooden bats is professional baseball–the major and minor leagues. Baseball bats manufactured for professional baseball constitute an estimated 18 percent of the wood bat market. Retail bat sales, however, represent a much larger proportion of the wooden bat market (65 percent). These sales are to youth and little league players who have aspirations of advancing to professional baseball and need to learn how to hit with a wooden bat. In addition, a few adult baseball leagues that mandate the use of wood bats can be found scattered around the country. These leagues reportedly have been gaining popularity (Wikipedia contributors 2009). Today, total annual demand for wood bats is about 1.3 million.

MAPLE BATS … STRIKE TWO!

The species of wood used for bats were quite diverse in the early years of the game of baseball, but for decades white ash has been the dominant material. Oak (*Quercus* spp.) was used by a handful of players during the early days of baseball when ball players made their own bats or had them made by a local woodworker. Babe Ruth and a few other major league players during the first half of the 20th century used hickory (*Carya* spp.), a denser, heavier wood than ash. Both hickory and oak were too heavy to swing fast and thus fell into disfavor. For major and minor leaguers, adults, and youth, ash was the material of choice for all baseball and softball bats until the mid- to late-1970s. Ash, with a specific gravity of 0.55 (green moisture content basis; Forest Products Laboratory 1999), is a strong, resilient, straight-grained wood with excellent surface hardness.

As recently as 1998, maple (specifically sugar or "rock" maple; *Acer saccharum* Marsh) bats were essentially non-existent. The maple bat's first appearance in a major league game was in 1997. By 1998 a handful of players were using the maple bat. The new bat went from being a curiosity to being a known entity in just 2 years as major league players, always seeking opportunities that might gain them a few more points on their batting average, tested maple bats made to their specifications. The use of maple bats by a couple of big-name players, Conseco and Bonds, helped cultivate interest in maple bats throughout professional baseball.

In Major League Baseball (MLB), an estimated 25 percent of the bats were maple bats in 2004. The high rate of adoption of maple for baseball bats by major league players continued for several years but appears to have leveled off since 2008. In 2009-2010, it is estimated that 50 percent of the bats used in MLB are made of sugar maple. This percentage is down slightly (perhaps 5 percent) from the peak usage rate seen in 2008.

The decline in use of maple baseball bats by major league players is associated with heightened concern about the risks associated with the number and nature of broken maple bats. During a 3-month period in 2008, all bats that cracked or broke during MLB games were collected for analysis. It was found that bats made of maple broke into multiple pieces (as opposed to just cracking or delaminating) much more frequently than ash. Analysis of these breaks showed that the grain was not straight in many of the failed maple bats, causing a high percentage of the breaks (Major League Baseball Association 2008). Nine new rules governing baseball bat manufacture were adopted by MLB before the 2009 season to reduce this risk. Early reports indicate that the incidence of broken bats during the 2009 season was reduced by 30 percent compared to 2008 (Glier 2009).

Two Strikes but Still Swinging … Ash Baseball Bats

Ash remains the dominant species used for bats in the wider bat market (75 to 80 percent of wood bats) as baseball players in the minor leagues have not adopted maple bats to the same extent as major league players have. The difference in the market share held by maple versus ash between the major and minor/adult leagues is attributable, in part, to the higher price of maple bats. Maple bat prices are 25 to 40 percent higher than ash bats of a comparable style and quality. Not only are stumpage prices for the higher grades of sugar maple twice those of white ash in Pennsylvania and New York (New York State Department of Environmental Conservation 2009, The Pennsylvania State University 2009), but the volume of baseball bat blanks, known as "billets," recovered per log is 20 percent higher for ash than for maple.

Essentially all of the white ash harvested for the baseball bat market is harvested from a fairly narrow north-south zone that runs along the state line between New York and Pennsylvania. Common belief is that the ash resource to the south of this zone is not as hard as desired, and to the north the ash is too heavy to satisfy the weight distribution specifications sought by major league players. High-quality ash of the sort that can be used to make bats for players in MLB is straight-grained, with the horizontal grain deviation along the bottom 20 inches of the bat (the handle portion, which is most susceptible to breakage) not offset more than 1 inch. This high-quality ash wood also has consistent-width growth rings with 8 to 9 growth rings per inch being preferred. Only 6 to 7 percent of the billets produced from select ash logs procured from this zone of New York and Pennsylvania are of sufficiently high quality to produce baseball bats for MLB.

Larimer & Norton: Manufacturing Billets for America's Top Producer of Wood Bats

The history of Larimer & Norton (L&N), the timber and sawmill division of Hillerich & Bradsby (H&B), manufacturer of the Louisville Slugger, mirrors the history of the ash baseball bat.

In 1954, H&B acquired L&N, a sawmill company in northern Pennsylvania, to secure its access to ash billets for the manufacture of its wood baseball bats. The acquisition also gave H&B an added measure of quality control over its raw material. The vast majority of bat manufacturers buy billets from multiple sources on the open market. H&B is one of the only bat manufacturers that make their own billets. In fact, all of the billets used by H&B to manufacture Louisville Slugger wood bats are produced by L&N.

In the mid-1970s, L&N was manufacturing 7 million ash billets per year at 11 sawmill operations, all but one of which were in the New York–Pennsylvania ash quality zone. Then aluminum entered the picture and L&N's production of ash billets dropped to about 1 million per year.

H&B did not turn its back on the aluminum bat market. In 1974 it entered the market, and in 1978 it began to manufacture aluminum bats at a plant in Ontario, CA. However, H&B has held fast to its commitment to be the premier wood bat manufacturer, and its wood bat product line is still considered the company's flagship business. While only 5 to 10 percent of all bats manufactured industry-wide are made of wood, 40 to 50 percent of the bats made by H&B are wooden.

As recently as 2004, L&N had 100+ employees, 6 sawmills, and a dimension operation that produced rough turnings, rectangular blanks, and moldings for sale to companies in other sectors of the wood products industry. Then maple, market globalization, and the recession began to complicate the marketplace and operating environment. The maple phenomenon has led L&N to shift some of its billet production away from white ash to sugar maple (ash to maple ratio is now 80:20). Today, L&N operates three sawmills in northern Pennsylvania with 35 employees and produces approximately 725,000 ash and maple billets per year.

A Field Trip to Larimer & Norton's Forest and Sawmill Operations

Ash billets are produced at Larimer & Norton's sawmills in Akeley, PA (north of Warren) and Troy, PA (north of Williamsport) while hard maple billets are produced at its sawmill in Galeton, PA (west of Wellsboro). The Akeley and Galeton mills also have dry kilns and billet finishing operations. Billets for adult and professional bats (both ash and maple) are dried and finished at the Akeley facility. The drying and finishing of billets for youth bats, which still make up approximately one-third of L&N's production, is carried out at the Galeton mill location.

L&N owns and manages 6,300 acres of forest land in the same region. The L&N forests currently provide a very small percentage of the log volume processed in the L&N mills—approximately 3 percent of L&N's annual log input. The L&N forests were heavily harvested in the 1960s and 1970s, so it will be another 10 to 20 years before the ash on these forests will be in the 15- to 17-inch diameter at breast height range, which will yield logs of sufficient size for manufacture into bats. Such logs should have small-end diameters of at least 11 inches and preferably 15 inches and larger.

L&N buys about 75 percent of its logs as "gate logs"—logs delivered directly to its three sawmills by loggers and neighboring mills that know what type and quality of ash or hard maple logs the L&N sawmills seek to buy. Stumpage sales are the source of approximately 22 percent of L&N's ash and maple logs with the remaining 3 percent obtained from company-owned forests. When L&N buys stumpage or harvests its own forests, it must resell the logs of other species. L&N has three foresters – two focused on procurement and one focused on forest land management.

Inside the Mill

If you have visited a sawmill producing lumber, your image of a sawmill is not an accurate portrayal of a sawmill producing billets for baseball bats. Logs arrive at the mill in 10- and 14-foot lengths. This is where the similarities with standard sawmill processing systems end. The logs are bucked (cut) to 40-inch lengths in the log yard. These short logs are not debarked before entering the mill, nor are they sawn. Rather, the breakdown process involves boring or turning on a lathe.

Producing billets using a lathe is the longstanding method used throughout the industry. L&N produces only 30 percent of its billets in this way. All maple billets and 60 percent of the ash billets are produced on a boring machine that is unique within the industry (Fig. 1). Overall, 70 percent of L&N's billets are produced on its four boring machines.

Figure 1.—Billet boring machine on which 70 percent of L&N's billets for baseball bats are produced.

The billets, at this point in the process referred to as "rough billets," are 3 inches in diameter (for adult and professional bats) and 40 inches in length. Depending on the diameter of the log, eight or more billets can be recovered from each of the 40-inch-long logs. Thus, on average, 24 billets are produced from 10-foot-long logs and 32 are produced from the 14-foot logs. Recoveries —the volume of usable rough billets produced, divided by the volume of the short log—are higher for ash than for maple. Ash recoveries typically range from 80 to 90 percent while maple recoveries range from 65 to 75 percent. Maple recoveries are lower because maple exhibits more internal defects than ash. This insufficient quality leads to a higher percentage of the rough maple billets being culled.

The rough billets are stacked for shipment and drying. Whereas in drying lumber, stickers are placed between layers to allow for air passage through the lumber stacks, stickers are not needed when drying billets. Maple and ash billets are dried together, but in both cases the billets are dried to a target moisture content range of 10 to 12 percent using a mild temperature schedule. Total drying time for each batch of billets, which consists of 11,000 pieces, is 28 to 30 days. The drying of ash billets is fairly straightforward as few drying- related defects arise. Maple billets can end check during drying, especially in the winter, so lower temperatures and longer drying times are necessary.

After drying, the billets are sent to a finish processing line where they are redoweled to a smaller size (down to 2.75 inches diameter for adult bats) and trimmed to 37 inches in length. At this point they are graded and weighed, then sorted into billet bundles based on these attributes (Fig. 2). The straightness of the grain is the most important quality factor. The slope of the grain cannot exceed 3 inches along the length of the billet. Grain straightness differences are evident when comparing the "Major League Quality" billet on the left and the "Low-end Retail" billet on the right in Figure 2.

All finished billets are shipped to the Louisville Slugger factory in Louisville, KY. The Galeton billet warehouse ships an average of 2 truckloads per month of billets for youth (and softball) bats. There are 10,000 billets per truckload. The Akeley warehouse ships an average of 6 truckloads of billets per month with 7,000 billets per

Figure 2.—Billet quality classes and grade-weight sorted bundles of finished billets awaiting shipment.

truckload, sized and sorted for use in manufacturing adult and professional bats.

EMERALD ASH BORER THROWS ANOTHER CURVE AT ASH BATS— WILL THIS BE STRIKE THREE?

The emerald ash borer's (EAB) presence became a real and present hazard for ash billet manufacturers in July 2009. The first substantiation of the presence of EAB in the "ash quality zone" occurred in Cattaraugus County, NY. Owing to this discovery, Cattaraugus and Chautauqua Counties in the far southwestern region of New York now have EAB quarantines in place. Ash logs can no longer be procured from these two counties by primary processors outside the quarantine zone.

For L&N, the adjustment process has begun. The two L&N mills that process ash are represented by the westernmost and southernmost star icons on the map in Figure 3. For these ash mills, the procurement zone is only 50 miles—this is the maximum economically feasible distance the sawmill can reach out for logs. For the Akeley ash mill, about 35 percent of its procurement zone is now quarantined. Consequently, the Akeley mill's procurement zone probably will have to expand out further, or the mill will have to offer a higher price for stumpage and logs available from within the 50-mile radius.

If when additional neighboring counties are affected and come under quarantine, more significant strategic adjustments will be required. A range of possibilities exists for L&N. Since L&N has three active mills and still owns one of its recently closed- down facilities, shifting maple production to the Akeley mill and ash production to one of the other facilities could be feasible for some length of time.

If EAB becomes widespread in the region and the counties in which the L&N mills are located come under the quarantine, ash logs will likely be abundant (for a few years) as forest landowners seek to harvest ash trees before they begin to die. The EAB compliance agreement into which producers will need to enter if they are to continue producing ash products will allow L&N to ship its billets (which have been debarked and dried) through other states to the Louisville Slugger plant in Kentucky.

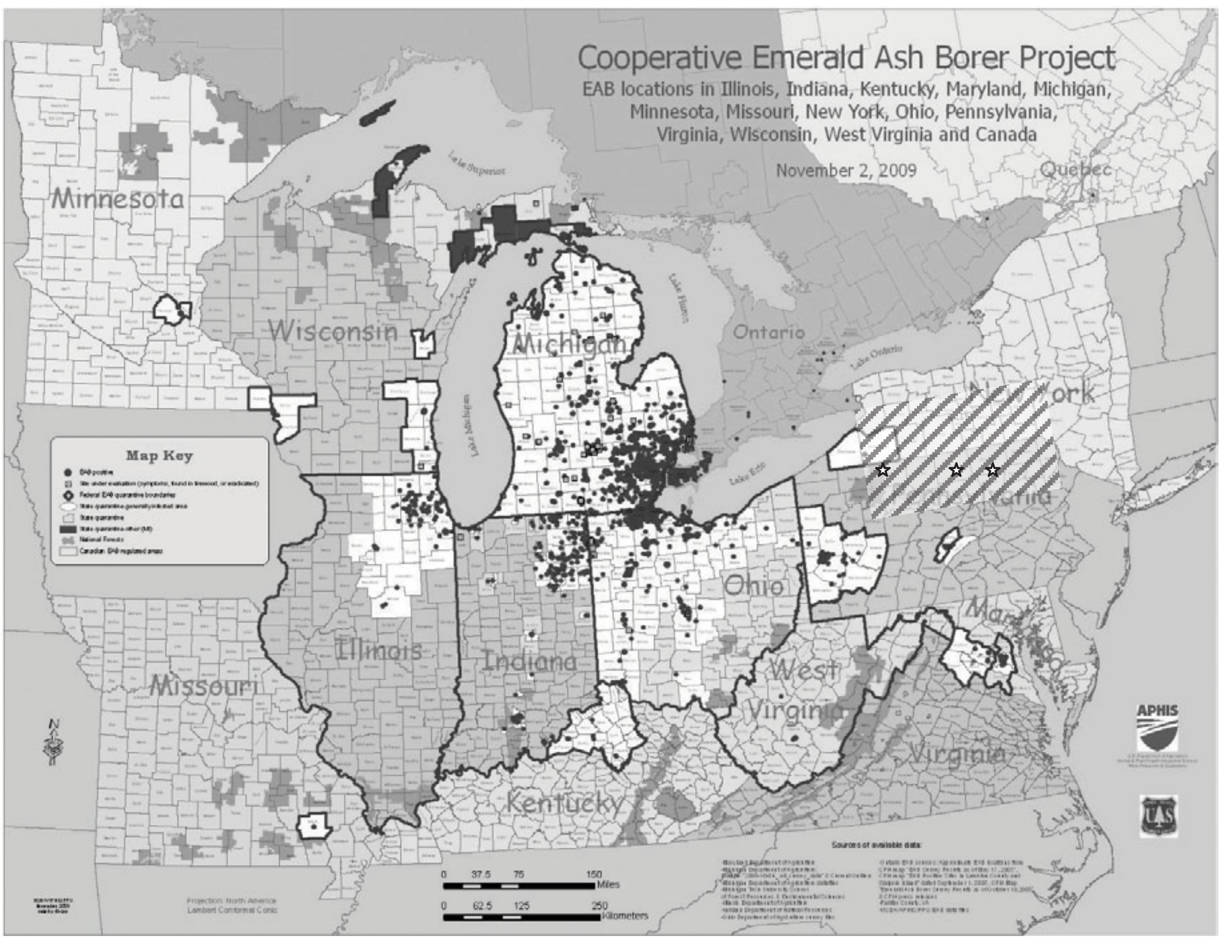

Figure 3.—Emerald ash borer infestation and quarantine map with L&N's mill locations (designated with stars) and procurement region (cross-hatched) superimposed in the northern Pennsylvania, southern New York region.

The “harvest now” behavior that some landowners will pursue is a concern for L&N as it looks to the future. This behavior will cause more ash to come on the market sooner but make ash less available a few years out. If, in the end, the EAB does not infest and kill a high proportion of the ash, the principal outcome of this preemptive harvest strategy will be to have severely distorted size and age-class distributions of the unaffected ash resource.

Ultimately, switching to other species is a strategy that could be required. Such species would include maple, of course, but other species also exist that have similar specific gravities and hardness values. Beech (*Fagus grandifolia* Ehrh.), yellow birch (*Betula alleghaniensis* Britton), and red oak (*Quercus rubra* L.) are among the top prospects. If this scenario comes to pass, it might indeed be strike three for ash baseball bats, but it will not be the end of the game for wood baseball bat manufacturers. There are more species, and more innings, left to be played.

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PATTERNS AMONG THE ASHES: EXPLORING THE RELATIONSHIP BETWEEN LANDSCAPE PATTERN AND THE EMERALD ASH BORER

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Abstract.—Landscape metrics, including host abundance and population density, were calculated using forest inventory and land cover data to assess the relationship between landscape pattern and the presence or absence of the emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire). The Random Forests classification algorithm in the R statistical environment was used to create a model relating the relative importance of landscape predictor variables to the presence/absence of EAB detected from 2003 to 2009. The dataset was then subdivided, based on quarantine year, to create two subsequent models: (1) data from 2003 to 2007 and (2) data from 2008 to 2009. Model accuracy was 85.3, 91.6, and 89.6 percent, respectively. While population density was ranked as the top predictor variable in all three models, analysis of the models separated by quarantine year showed variation among the other top predictors. Measurements of urban development and forest edge influenced the model more among counties quarantined between 2003 and 2007, and host abundance was an important predictor among counties quarantined in 2008 and 2009.

INTRODUCTION

America's forests are home to more than 8.8 billion ash trees (*Fraxinus* spp.) greater than 1 inch diameter at breast height (U.S. Department of Agriculture [USDA] Forest Service). The distribution of ash is spatially skewed. Ninety-three percent of all ash trees are found in the eastern half of the conterminous United States; 72 percent of these individuals are within the northeastern[1] region. The abundance, functionality, and utility of ash make it an ecologically and economically valuable tree species. As a prominent component of riparian forests, ash plays an important role in reducing runoff and enhancing soil stability (Goforth et al. 2002). Ash is a valuable commercial species used in the manufacturing of numerous wood products, including furniture, pulp and paper, crating, and baseball bats (USDA Animal and Plant Health Inspection Agency [APHIS] 2005). Ash was widely sold as nursery stock and planted in urban settings because of its rapid growth and high tolerance of environmental stress.

[1]Includes Maine, New Hampshire, Vermont, Massachusetts, Connectcut, Rhode Island, New York, Pennsylvania, New Jersey, Delaware, Maryland, West Virginia, Ohio, Indiana, Michigan, Illinois, Wisconsin, Missouri, Iowa, Minnesota, North Dakota, South Dakota, Nebraska, and Kansas.

In recent years, the sustainability of the Nation's ash resource has been threatened by the introduction of a wood-boring beetle identified as the emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire). Native to southeast Asia, EAB was first identified in North America near Detroit, MI, in 2002 (Cappaert et al. 2005). Throughout North America, EAB is a pest of ash. All native ash trees are susceptible to EAB infestation regardless of species, size, or vigor (Poland and McCullough 2006). Tree mortality can be rapid: trees can die within 3 or 4 years of infestation (Kovacs et al. 2009). EAB mortality estimates range in the tens of millions of trees (Poland and McCullough 2006, Kovacs et al. 2009). Thus far, EAB has been identified in 13 states and 2 Canadian provinces.

The spread of EAB in the United States has occurred through artificial and natural means. Long-range dispersal was the result of human transportation of infested firewood and nursery stock, while natural dispersal followed short-range flight of beetles to new hosts (Cappaert et al. 2005). Factors governing natural dispersal include host availability, insect flight capacity, physical barriers, and meteorological conditions (Cappaert et al. 2005).

Of particular interest in this study was the influence of physical obstacles in the dispersal of EAB. The level of forest fragmentation in EAB-infested areas makes it a challenge to understand how EAB populations respond to the arrangement and availability of suitable habitat (Schultz and Crone 2001). Therefore, the purpose of this study was to identify how well landscape characteristics (i.e., landscape pattern metrics) predict the presence or absence of EAB. To accomplish this goal, we used forest inventory data, land cover imagery, and a spatial analysis algorithm to quantify patterns on the landscape. A classification algorithm was then used to identify the relative importance of these potential predictor variables. Our motivation was to assess the role of landscape pattern in the dispersal of EAB and provide insight on habitat suitability and risk.

METHODS

Study Area

To investigate the potential effects of landscape pattern on EAB, we examined county-level data from Illinois, Indiana, Ohio, and Kentucky. Counties ranged from highly fragmented to heavily forested and represented long, short, or no EAB infestation intervals. EAB was detected in Ohio, Indiana, Illinois, and Kentucky in 2003, 2004, 2006, and 2009, respectively. Michigan was not included in the study because EAB is considered “generally” infested throughout all counties in the Lower Peninsula.

Distribution of EAB

Presence/absence of EAB and quarantine establishment was determined for all counties in the study area using state-level data (Illinois Department of Agriculture 2009, Indiana Department of Natural Resources 2009, Kentucky Department of Agriculture 2009, Ohio Department of Agriculture 2009). If EAB was present within a county, the year of detection was also recorded. Since the rules for quarantine establishment varied, the entire county was considered quarantined if any portion of the county (township or a multi-county group) was quarantined by the state. EAB has not been found in all quarantined counties. Counties were classified according to the presence/absence of EAB infestations. Quarantine year was used only as a means of splitting our dataset and was not considered in the classification process.

Landscape Metrics

Landscape metrics are measurements that quantify and describe aspects of landscape pattern (Griffith et al. 2000). In this study, 19 metrics were calculated from various data sources, including raster land-cover data and ground-level plot data (Table 1). Selected metrics describe (1) the amount and spatial arrangement of forest and host availability, and (2) aspects of non-forest landscape pattern, such as population and road density.

Data from the Forest Inventory and Analysis (FIA) Program of the U.S. Forest Service were used to calculate county-level estimates of ash trees per acre and ash basal area. Measures of urban development and supplementary measures of forest pattern were gathered from the National Land Cover Database of 2001 (NLCD) (Homer et al. 2004) and datasets by Riemann et al. (2009), where one pixel is equivalent to 30 meters, or approximately 98 feet. Additional data required to calculate airport presence/absence, population density, area of park land, and road density were gathered from the National Atlas of the USA and the Environmental Systems Research Institute (ESRI), Inc., respectively (ESRI 2008, National Atlas of the US 2009). To assess potential changes in host availability by county as EAB dispersal increased over time and space, measures of ash abundance and the percentage of forest land were compared by quarantine year.

Morphological Spatial Pattern Analysis

GUIDOS software (European Commission, Joint Research Centre, Institute for Environmental Sustainability, Ispra VA, Italy) was used to assign forest pixels from the NLCD 2001 to one of seven classes (core, islet, bridge, edge, loop, branch, and perforation). Pixel assignment was completed using morphological spatial pattern analysis (MSPA) algorithms that were based on the connectivity and geometry of the pixels (European Commission 2009, Soille and Vogt 2009). To describe the spatial pattern or degree of fragmentation of forest land, a map of the new pixel classification was produced. Pixel counts of each class were divided by the total number of pixels in each county to obtain percentages of each class by county.

Modeling the Presence/Absence of EAB

A dataset of explanatory variables was constructed from calculated landscape metrics (Table 1). The Random Forests (RF) algorithm in the R statistical software environment (Version 2.8.1 [http://www.r-project.org/]) was used to create a model relating EAB presence/absence to the input variables (Breiman 2001). Cutler et al. (2007) found that RF outperformed commonly used modeling techniques, such as logistic regression, in classifications of lichens, cavity-nesting birds, and the presence of invasive plants. RF provided diagnostics that identified the relative importance of each variable. Refer to Appendix A in Cutler et al. (2007) for a description of the Gini index and its use as an indicator of variable importance. By withholding observations (known as the out-of-bag sample, or OOB) as it builds a suite of classification trees, RF also provided an assessment of the classification accuracy.

Three iterations of the model were constructed using different county combinations in an effort to examine potential differences between these populations. The first iteration included data for all counties in the study area (ALLDATA). After the initial model was run, subsequent model iterations were completed to assess potential changes in county-level attributes. This decision resulted from analysis of mean values of percent forest and ash abundance by county and quarantine year. To accomplish this goal, a second iteration of the model was built using only a portion of the dataset: data for all non-quarantined counties and those counties that were quarantined from 2003 to 2007 (PRE-2008). The final iteration included data for all non-quarantined counties and data from counties quarantined in 2008 or 2009 (POST-2007). Year of quarantine was used to divide the datasets between the PRE-2008 and POST-2007 models; however, it was not used to determine presence or absence of EAB. Presence/absence records were used within the model to determine whether EAB had become established in a county; these records are based on confirmed infestations recorded by each state. The PRE-2008 model was used to forecast the presence/absence of EAB in counties quarantined in 2008 and 2009.

Table 1.—Calculated landscape metrics

Metric	Metric description	Data source
Ash density 1	Ash trees per acre of county land	Forest Inventory and Analysis
Ash density 2	Ash basal area as a percentage of total basal area	Forest Inventory and Analysis
Percent forest	Percent of county area that is forested	NLCD 2001
Percent branch	Percent of forest that is connected at one end to edge, perforation, bridge, or loop	NLCD 2001/ GUIDOS
Percent bridge	Percent of forest that is connected at both ends to different core patches	NLCD 2001/ GUIDOS
Percent core	Percent of forest that is the interior area of a forest patch, excluding forest perimeter	NLCD 2001/GUIDOS

Table 1.—continued

Percent edge	Percent of forest that is the outside perimeter of a forest patch	NLCD 2001/GUIDOS
Percent islet	Percent of forest that is connected and too small to contain core	NLCD 2001/ GUIDOS
Percent loop	Percent of forest that is connected at both ends to the same core patch	NLCD 2001/ GUIDOS
Percent perforation	Percent of forest that is in the inside perimeter of a forest patch	NLCD 2001/ GUIDOS
Percent agriculture	Percent of county area that is classified as agriculture	NLCD 2001 (Riemann et al. 2009)
Percent developed	Percent of county that is classified as urban development	NLCD 2001(Riemann et al. 2009)
Percent forest less than 1 pixel	Percent of forest within 1 pixel of a developed edge (urban development, agriculture, and barren land)	NLCD 2001 (Riemann et al. 2009)
Percent forest 1 to 3 pixels	Percent of forest 1 to 3 pixels from a developed edge (urban development, agriculture, and barren land)	NLCD 2001 (Riemann et al. 2009)
Percent forest greater than 3 pixels	Percent of forest that is greater than 3 pixels from a developed edge (urban development, agriculture, and barren land)	NLCD 2001 (Riemann et al. 2009)
Percent parks	Percent of county area designated as park	ESRI data and maps 2008
Road density	Miles of major roads per square mile	ESRI data and maps 2008
Airports	Presence or absence of an airport in the county	National Atlas of the USA
Population density	Number of people per square mile	National Atlas of the USA

RESULTS

The complete dataset consisted of 402 counties. During the study period (2003-2009), 120 counties were placed under state quarantine. EAB had been positively confirmed in 97 of these counties. Comparison of county-level averages for percent forest and ash density (trees per acre) by quarantine year shows that counties quarantined in 2008 and 2009 had more forest land and a higher number of ash trees per acre than counties quarantined prior to 2008 (Fig. 1).

Results from the ALLDATA model show that overall accuracy was high. Based on the OOB, the RF classifier was able to correctly assign presence/absence to 342 counties (out of a total of 402 counties) for 85.3-percent agreement. As measured by the mean decrease in the Gini index, population density provided the most explanatory power of patterns of EAB presence/absence, followed by percent core, percent forest within 1 to 3 pixels, percent developed, and road density (Table 2).

When the 2008-2009 quarantine counties were excluded from model development (PRE-2008 model), classifier accuracy increased to 91.6 percent. The model performed very well in non-quarantined counties and in highly developed areas. Misclassified counties tended to occur along the boundary between quarantined and

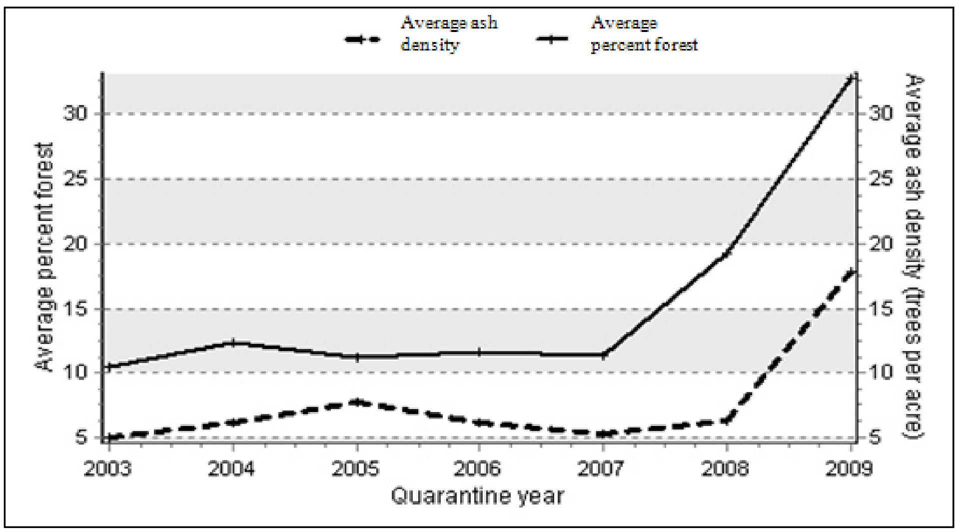

Figure 1.—Comparison of average percent forest and ash density (trees per acre) for quarantined counties by year.

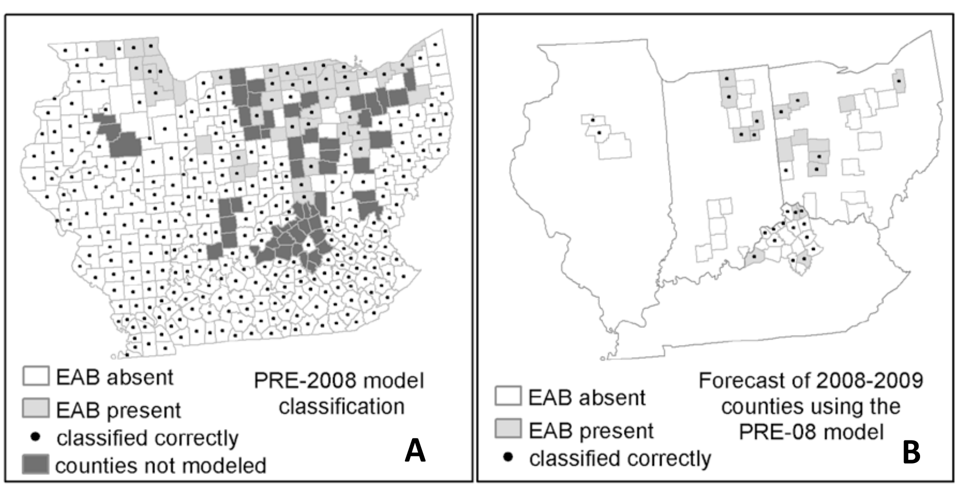

Figure 2.—Classification output from PRE-2008 model (A) and forecast of 2008-2009 counties using the PRE-2008 model (B).

non-quarantined counties (Fig. 2A). Similar to the ALLDATA model, population density was the top-ranked predictor variable; percent developed, percent forest within 1 to 3 pixels, percent core, and percent edge were also highly ranked (Table 2).

The POST-2007 model had a classification accuracy of 89.6 percent (303 out of 338 counties). As with earlier models, population density was identified as the most important explanatory variable with respect to EAB presence. Unlike the previous models, however, measures of host abundance had high predictive value; ash basal area and ash trees per acre ranked third and fourth, respectively (Table 2). Percent forest within 1 to 3 pixels and percent developed were also among the top five predictors, ranking second and fifth, respectively.

When the PRE-2008 model was used to forecast presence/absence in the 2008-2009 quarantine counties, the accuracy was 49.1 percent (27 out of 55 counties). Misclassified counties had no apparent spatial pattern, and classification errors were relatively balanced between false positives and false negatives (Fig. 2B).

DISCUSSION

Overall, models based on landscape metrics performed well in explaining the presence/absence of EAB. Population density, along with percent developed and percent forest within 1 to 3 pixels of a developed edge, were the most important variables in all modeling scenarios, indicating humans play a dominant role in EAB dispersal. Similarly, investigations by Muirhead et al. (2006) found a positive relationship between human population epicenters and the probability of EAB infestation. The PRE-2008 model, which had the highest classification accuracy, also identified percentage of core forest and forest edge as important variables. These findings indicate that forest edges may influence EAB dispersal. Interestingly, investigation of EAB in its native range shows that EAB is known to attack solitary trees and trees along edges (Chinese Academy of Science 1986). Additional research is required, but early analysis of model results indicates that EAB infestations prior to 2008 may be influenced by areas with a high proportion of forest edge.

Conversely, POST-2007 model results show that core and edge metrics, which had high importance in the PRE-2008 model, were replaced by metrics of host abundance as important indicators of EAB presence. The discrepancy between the models highlights a potential change in the relevance of explanatory variables over space and time. Infestations immediately following the initial introduction of EAB may have relied heavily on human activity. Later EAB infestations (those detected after 2007) may have depended heavily on ash abundance. The influence of host abundance is highlighted by an analysis of forestation and ash density, which indicates that the levels of these two attributes remained low and relatively stable for counties quarantined between 2003 and 2007, and then dramatically increased among counties quarantined after 2008 (Fig. 1). Presence of EAB in more heavily forested counties may be related to (1) increased awareness of EAB and a resulting change in human activities or (2) a change in EAB population dynamics in response to more continuously available habitat. Overall, results seem to suggest a change in variable importance over time and/or space and the emerging importance of the level of habitat availability.

This assessment of the relationship between landscape pattern and the presence of EAB has shown that models based on landscape metrics can help provide a measure of the importance of condition-level attributes with regard to EAB presence. However, a change in the explanatory variables across space and time makes it difficult to predict future patterns of infestation. We hope further investigation will yield more consistent results and better predictive models.

Table 2.—Ranking of the importance of explanatory variables (landscape metrics) by model and associated mean decrease in Gini index values.

Order of variable importance (high to low) by model					
ALLDATA model		**PRE-2008 model**		**POST-2007 model**	
Variable	**Δ Gini**	**Variable**	**Δ Gini**	**Variable**	**Δ Gini**
Population density	21.14	Population density	11.58	Population density	9.41
Percent core	12.11	Percent developed	9.56	Percent forest 1 to 3 pixels	5.89
Percent forest 1 to 3 pixels	12.06	Percent forest 1 to 3 pixels	8.11	Ash density 2	5.50
Percent developed	11.33	Percent core	7.74	Ash density 1	4.82
Road density	10.29	Percent edge	7.51	Percent developed	4.34
Percent edge	9.41	Percent forest greater than 3 pixels	6.14	Road density	4.27
Ash density 2	9.36	Road density	6.07	Percent forest less than 1 pixel	4.23
Percent forest greater than 3 pixels	8.65	Ash density 1	5.76	Percent core	4.17
Ash density 1	8.39	Percent forest less than 1 pixel	4.70	Percent edge	3.83
Percent forest less than 1 pixel	7.53	Percent forest	4.47	Percent forest greater than 3 pixels	3.72
Percent parks	7.52	Ash density 2	4.38	Percent agriculture	3.19
Percent agriculture	6.24	Percent parks	4.34	Percent parks	3.08
Percent forest	6.14	Percent branch	3.66	Percent forest	3.06
Percent branch	4.91	Percent agriculture	3.65	Percent branch	2.51
Percent islet	3.92	Percent islet	2.55	Percent islet	1.77
Percent bridge	2.27	Percent bridge	1.64	Percent perforation	1.12
Percent perforation	1.51	Percent loop	1.32	Percent bridge	1.08
Percent loop	1.48	Percent perforation	1.16	Percent loop	1.05
Airports	0.89	Airports	0.45	Airports	0.48

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OVERCOMING OBSTACLES TO INTERSPECIES HYBRIDIZATION OF ASH

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Tree species that share a long co-evolutionary history with insects and pathogens are likely to have developed mechanisms of resistance that allow them to coexist. When insects and pathogens are introduced to different parts of the world, high levels of susceptibility can be observed, presumably in part due to the lack of co-evolutionary history between the insect (or pathogen) and host. In such cases, use of non-native tree species as a source of resistance for introgression into native susceptible tree species can be quite helpful. Examples of interspecific hybrids include hybrid hemlocks with resistance to hemlock wooly adelgid and hybrid chestnut trees resistant to chestnut blight (Hebard 2006, Montgomery et al. 2009). If first-generation hybrids show good resistance to the insect or pathogen, then a back-cross breeding program may be initiated to produce seedlings for restoration that preserve the introgressed resistance in a progeny set with most of the characteristics of the native parent species. This approach is being used by The American Chestnut Foundation and would be the model approach used to produce emerald ash borer (EAB)-resistant North American ash species.

The success of this strategy depends on the existence of genetically controlled resistance to EAB in Asian ash species and the ability to inter-breed these resistant species with susceptible North American ash species. Evidence of EAB resistance in Asian ash species has been reported (Liu et al. 2007), but controlled testing is limited to a single horticultural selection of *F. mandshurica* (Rebek et al. 2008). Little is known about interspecies hybridization in ash, with the exception of two cultivars ('Northern Gem' and 'Northern Treasure') that have been described as hybrids between Manchurian ash and black ash (*F. nigra*) (Davidson 1999). Northern Treasure has been reported as EAB-susceptible (Rebek et al. 2008). We have begun to establish a collection of Asian ash species (and other exotic species) at the U.S. Forest Service, Northern Research Station, in Delaware, OH, to identify species with EAB resistance for use as parents in a breeding program. We have performed controlled cross-pollinations with more than 30 species combinations of ash with little success. We have identified several challenges to controlled pollinations to produce hybrids, and our program is addressing them.

Obstacles to interspecific crosses in *Fraxinus* include between-species differences in reproductive biology and genetics, such as differing ploidy levels, differing pollination systems and phenology, and potential genetic incompatibility (Wright 1957, Wallander 2001). Although the majority of ash species are 2n=46, important exceptions influence hybrid breeding programs. The exceptions include white ash (*F. americana*) 2n=46, 92, or 138; pumpkin ash (*F. profunda*) 2n=138; velvet ash (*F. velutina*) 2n=46 or 92; and Chinese ash (*F. chinensis*) 2n=92 or 138. While ploidy level differences do not always result in complete incompatiblity, the resulting F_1 progeny are often sterile. Accordingly, we need to carefully match known ploidy levels when choosing parents for initial crosses. The ploidy levels of blue ash (*F. quadrangulata*) and Manchurian ash (*F. mandshurica*) are unknown, so cytogenetic work is being conducted to establish the ploidy number of these species. Preliminary results indicate both species are 2n=46. Genetically determined incompatibility is another natural barrier to hybridization. Genetic incompatibility may be structural, such as when there are different chromosome numbers or gross differences in genome organization (major deletions or transpositions that prevent proper chromosome pairing at mitosis and meiosis), or it may be biochemical, such as the expression of S-glycoproteins (Broothaerts and Nerum 2003, Wheeler et al. 2009).

Differences in pollination system (insect, wind-pollinated) represent significant barriers to natural cross hybridization but may be relatively easily overcome by controlled cross-pollination techniques. Storing pollen and manually pollinating flowers allows the crossing of species without regard to their natural pollination system. It is not known how genes controlling the pollination system might segregate in an F_1 or F_2 hybrid population, so our initial efforts have focused largely on species pairs with similar reproductive biology. Many ash species are dioecious or androdioecious, meaning that both male and female trees must be collected and established, increasing the minimum number of trees required for breeding. Differences in phenology are closely related to differences in reproductive biology and create real barriers to natural hybridization. By maintaining known female trees as potted ramets for long periods, we are able to control the timing of flowering. The female trees are kept in a cold storage area until fresh pollen of the desired species has been collected or until local trees have shed the bulk of their pollen. It has been suggested that the large size of the genus *Fraxinus* will make crosses between sub-genera impossible (E.Wallander, pers. comm.). However, there are few to no cross-compatibility data to determine exactly what the maximum allowable phylogenetic distance is between parents that results in successful hybridizations.

Perhaps the greatest restriction to hybridization identified to date is the limited number of Asian ash accessions available in the United States. The United States has only a limited amount of ash from China, Korea, Japan, and eastern Russia, and much of it is not yet reproductively mature. Some species are represented by only one or two accessions in all the U.S. gardens and arboreta, a limitation that in some cases became apparent only after our thorough review of accession records. Continued thorough review of accession records and DNA analysis will be necessary to ensure unique material is being accessioned. Seed collections from China were distributed widely throughout the United States. For example, the North American China Plant Exploration Consortium (NACPEC) is made up of many member gardens and arboreta that all share collected seed. A single Manchurian ash seed collection in 1997 (NACPEC97048) is documented as 25 specimens under 9 accession numbers in 7 different gardens. A more recent seed collection effort by NACPEC, headed by the Morton Arboretum (Chicago, IL), included a wide range of ash species in China; it should be a valuable resource once the seeds germinate and the trees become sexually mature.

Yet another challenge is presented once successful pollinations produce potentially hybrid seedlings. To rule out pollen contamination (including self-pollination), DNA-based methods to confirm the hybrid parentage of seeds produced from controlled crosses are being optimized. Despite the expectation that self-compatibility and pollen contamination would be rare, each cross may result in only a few seeds, so hybrid confirmation is essential. Our initial efforts to confirm several putative hybrids using SSR or AFLP markers uncovered some inconsistencies in the parent trees. Several trees seemed to cluster inappropriately based upon their marker genotype.

We expanded the number of reference samples included for each species of interest and began to carefully document the phenotypes of the exotic ash in our research collection. The ITS region of a subset of individuals was sequenced and these data confirmed that our AFLP-based groupings were consistent with the published phylogenies (Wallander 2008). Based upon both AFLP and ITS sequence results, however, several samples in question were incorrectly identified at the species level.

We were able to trace several incorrectly identified accessions back to the Chinese Academy of Sciences Botanical Garden in Beijing. In one case, the seed was directly distributed from the Chinese Academy to arboreta in the United States in the 1980s, and in another case seed was distributed to a botanical garden in another country in 1963, which then supplied materials to the United States. All accessions derived from this seed source that we have analyzed to date have been determined to be incorrectly identified as *F. chinensis.* In fact, they appear to be *F. pennsylvanica*. Furthermore, several seed lots imported from China by reputable U.S. seed companies have also been shown to be incorrectly identified as either *F. chinensis* or *F. bungeana*. To date, we have found eight specimens and seedlots that were incorrectly identified as either the Asian species *F. chinensis* or *F. bungeana*, all of which are consistent with *F. pennsylvanica*, based on AFLP genotyping. It appears that seed has been routinely collected from *F. pennsylvanica* growing in China and distributed as *F. chinensis* and *F. bungeana*. The seeds from these species are very similar, which has likely contributed to the mistaken identities.

This work accents the importance of confirming the identity of closely related species when initiating a hybrid breeding program or other scientific experiments. For example, comparisons of proteomics, metabolomics, and transcriptomics between EAB-resistant Asian ash species and EAB-susceptible North American ash species are of less value if the species of interest have not been identified correctly. Furthermore, these findings also demonstrate that the number of Asian ash accessions currently in the United States may be even more limited than records show.

It is our long-term goal to overcome the obstacles described here to establish an interspecific breeding program using exotic ash species as sources of EAB resistance that can be introgressed into native North American species. F_1 hybrids with EAB resistance may have immediate value to the nursery industry as street trees. F_1 progeny, as well as subsequent generations, will also be valuable genetic resources for determining the heritability and molecular mechanisms of resistance. Backcross generations created through careful selection of parents for both North American characteristics and EAB resistance may someday provide the resources needed to re-establish EAB-resistant North American ash species in our forests. Gene conservation activities involving North American ash species are a critical component of such a breeding program so that genetic diversity across the native range is preserved for use in advanced generations of breeding.

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POPULATION-LEVEL VARIATION OF *FRAXINUS AMERICANA* L. IS INFLUENCED BY CLIMATE DIFFERENCES ACROSS THE NATIVE RANGE

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Abstract.—This paper reviews population variation in *Fraxinus americana* (white ash) to determine whether intraspecific variation in various physiological traits affects growth and survival in *F. americana* and to correlate any intraspecific variation in *F. americana* with temperature or precipitation differences throughout the species range. Beginning in the 1980s, results from common garden studies indicated that differences in growth and survival in *F. americana* are the result of ecotypic variation in the species. Recently, further insights into the mechanisms controlling these ecotypic differences in growth were made in a common garden at the edge of the species range. This project in northeastern Kansas found significant differences in morphology, phenology, and stomatal regulation among 44 *F. americana* populations. Even after 30 years of growth in the common garden, these population differences were well-correlated with climatic differences (temperature and precipitation) at the location of population origin. Temperature requirements for leaf emergence of *F. americana* populations in the common garden were positively correlated with the latitude of population origin. Differences in water use efficiency among other *F. americana* populations were observed and may be caused by morphological traits, such as variation in leaf mass per area and foliar nitrogen concentration. Thus, the original observations of differences in growth among *F. americana* ecotypes are likely caused by adaptive differentiation among populations.

INTRODUCTION

Interest in the ecology of *Fraxinus* (ash) species has increased due to the recent discovery of an exotic insect pest, the emerald ash borer (*Agrilus planiplennis*), in North America. The wood-boring beetle has caused the death of millions of ash trees (MacFarlane and Meyer 2005) and has seriously threatened the survival of native North American *Fraxinus* species. When tools to conserve and manage ash species are being developed, it is important to consider how ecologically important traits vary among natural populations. High levels of intraspecific variation in morphology, physiology, growth, and phenology are commonly observed in tree species with large ranges that cover different climatic, edaphic, and biotic regions (Donselman and Flint 1982, Geber and Dawson 1993, Abrams 1994, Aspelmeier and Leushner 2004).

Tree physiology often changes along climatic gradients and may be driven by adaptation to temperature differences and/or differences in water availability throughout the species range. In the case of the temperate deciduous tree *F. americana* L. (white ash), the natural species range includes the eastern half of the United States and extends into parts of southern Canada (Fig. 1). Even without accounting for the devastation caused by *A. planiplennis*, models predicting future suitable habitat for the species indicate that its range will decrease in the future as a result of a northward shift in optimum growing conditions (Prasad et al. 2007). An understanding of the natural variation in traits that affect growth, susceptibility to *A. planiplennis*, and survival of *Fraxinus* species is necessary for directing management strategies and conservation efforts.

Figure 1.—Natural range of F. americana in North America (shaded region, USDA Forest Service, www.na.fs.fed.us). Sources (● and o) of the 44 *F. americana* populations grown in the common garden (*) at Nelson Environmental Study Area in northeastern Kansas are also indicated. Ten populations originating from along an east-west precipitation gradient are identified by open circles. From Marchin et al. 2008.

F. americana is a ring-porous angiosperm native to the temperate deciduous forests of the eastern United States. This hardwood is an early successional species that is often one of the first trees to colonize abandoned fields and thus is important for forest regeneration across its range (Schlesinger 1990). The wood of *F. americana* is valued for its strength and is used for furniture and baseball bats (Nesom 2000).

Prior to this century, not much was known about the extent of intraspecific variation in the physiology and growth of *F. americana*. Recent work in a *F. americana* common garden in northeastern Kansas, however, revealed intraspecific variation for several important ecological traits (Marchin 2006, Marchin et al. 2008). Located in a marginal habitat at the western edge of the species range where the required degree of adaptation may exceed the limits of phenotypic plasticity in some genotypes, this study site likely allowed a full expression of range-wide genetic variation in response to abiotic stresses (Paschke et al. 2003, Stanton et al. 2000).

The main focus of this review is to (1) determine whether there are differences in morphology, phenology, gas exchange, and stomatal regulation among *F. americana* populations, (2) determine whether intraspecific variation in these traits affects growth and survival in the species, and (3) correlate intraspecific variation in *F. americana* with a corresponding climate variable to define the causal mechanism driving genetic divergence in the species.

METHODS: THE COMMON GARDEN APPROACH

For many years, it has been known that *F. americana* is genetically variable throughout its range, partly owing to the presence of polyploidy (Wright 1944). These differences in ploidy level vary across the species range, with diploids (46 chromosomes) occurring throughout the species range. Tetraploids (92 chromosomes), however, are restricted to regions south of latitude 35°N, and hexaploids (138 chromosomes) are found between latitudes 35° and 40°N (Black and Beckmann 1983, Schlesinger 1990). Triploid and pentaploid embryos have also been observed, although the prevalence of these individuals in natural populations is unknown (Black and Beckmann 1983).

To better understand how this genetic variation affected growth and to identify the best seed sources for various geographical locations, the U.S. Forest Service, North Central Forest Experiment Station initiated a range-wide provenance test of *F. americana* in 1976 (Clausen 1980, Clausen et al. 1981). Open-pollinated seeds were collected from native parent trees at 59 locations throughout the species' range and planted in a nursery in Illinois. It was observed that under the common environment of the nursery, tetraploids grew taller than other ploidy levels (Clausen et al. 1981). These 1-year-old seedlings were later transplanted to 22 plantations throughout the eastern United States and Canada.

This review primarily focuses on one of the *F. americana* common gardens, located in Kansas at the western edge of the species range (University of Kansas, Nelson Environmental Study Area, 39.0°N, 95.2°W, 299 m asl). At this common garden site, mean annual precipitation is 879 mm, with large interannual variation (± 200 mm SD). Mean annual temperature is 11.9°C, and mean temperature during the growing season (April–September) is 20.4°C (see Jefferson County, Table 1).

Table 1.—Climatic data from the common garden (located in Jefferson County, KS and the locations represented in the common garden. From Marchin et al. (2008 Populations in the latitudinal gradient are indicated by a single cross (†); populations in the east-west precipitation gradient are indicated by a double cross (‡).

Location	Latitude (°N)	Longitude (°W)	Elevation (m)	Annual Precipitation (mm)	Annual Temperature (°C)	Growing Season VPD (kPa)
Jefferson, KS	39.0	95.0	299	879	11.9	0.633
Ontonagon, MI †	46.6	89.5	408	820	4.3	0.400
Forest, WI (2) † †	45.7	89.0	511	777	5.6	0.428
Presque Isle, MI	45.3	83.6	198	749	6.1	0.509
Penobscot, ME (2)	44.8	69.0	85	1083	6.2	0.519
Benzie, MI	44.7	86.0	236	793	6.4	0.530
Onondaga, NY	42.7	76.1	381	940	8.3	0.579
Washtenaw, MI (2)	42.2	83.7	259	799	9.0	0.637
Wayne, OH ‡	40.7	82.0	265	969	9.7	0.592
Otoe, NE ‡	40.6	95.7	259	765	10.8	0.639
Adams, IL ‡	39.8	90.7	213	943	10.9	0.633
Preble, OH ‡	39.6	84.7	305	1035	11.7	0.699
Tucker, WV ‡	39.1	79.5	762	1261	9.3	0.463
Effingham, IL † ‡	39.1	88.4	183	1025	11.9	0.631
Effingham, IL † ‡	39.0	88.4	177	1025	11.9	0.631
Randolph, WV ‡	38.9	79.7	975	1261	9.3	0.463
Jackson, IN ‡	38.9	86.0	191	1142	12.3	0.670
Jackson, IL (2) ‡	37.7	89.4	158	1097	13.3	0.760
Gallatin, IL †	37.6	88.3	152	1125	13.3	0.680
Muhlenberg, KY	37.3	87.2	128	1220	13.9	0.547
Hopkins, KY (2)	37.3	87.6	139	1220	13.9	0.547
Overton, TN (3)	36.5	85.4	357	1408	13.6	0.627
Marion, AR (2)	36.4	92.8	274	1155	14.5	0.672
Boone, AR	36.4	93.0	274	1164	14.2	0.652
Bledsoe, TN (2)	35.5	85.2	396	1408	13.6	0.680
McMinn, TN	35.3	84.5	251	1263	13.9	0.690
Franklin, TN (2)	35.2	85.9	357	1408	13.6	0.680
Pickens, SC	35.0	83.0	229	1295	15.6	0.779
Union, GA	34.8	83.9	914	1378	15.3	0.738
Oktibbeha, MS (3) † † †	33.4	88.8	116	1383	17.2	0.639
East Baton Rouge, LA (2)	31.5	91.0	9	1559	19.3	0.686
George, MS †	30.8	88.8	76	1602	19.4	0.732

Trees in the common garden were 30 years old at the time of the study. The common garden is set up in a block design, with each of the 44 populations (Fig. 1) represented by 25 trees (5 blocks with 5 replicate trees from each population). In 2004, 40 of the 44 populations were still represented by at least 10 individuals, although one population (East Baton Rouge, LA) had no surviving trees in the common garden.

To determine possible mechanisms driving genetic variation in *F. americana*, two subsets of populations were selected *a priori*. One subset of 10 populations formed a north-south latitudinal gradient that ranged from 30.8° to 46.6°N and originated within a narrow longitudinal range (approximately 89°W). Similarly, the second subset of 10 populations formed an east–west precipitation gradient ranging from 79.5° to 95.7°W and originating within a narrow latitudinal range (approximately 39°N). These subsets of populations can indicate the climatic control over known trait variation by maximizing differences caused by one climate parameter (e.g., precipitation) and minimizing differences caused by other factors (e.g., temperature, photoperiod). All physiological measurements were performed on three to five individuals per population, and when possible, all measured trees were located in one block to minimize microsite differences among trees.

RESULTS

Population-level Responses in Growth and Survival

After only 3 years of seedling growth in the originally established 22 *F. americana* common gardens, height varied significantly among populations and plantations, revealing a strong genotype × environment interaction (Clausen 1980). The patterns of variation in height indicated the presence of northern and southern ecotypes divided at approximately 39°N (Roberds et al. 1990). Populations of the southern ecotype had greater growth than the northern ecotype throughout most of the *F. americana* range, excluding the extreme northern latitudes (Clausen et al. 1981, Roberds et al. 1990). Therefore, foresters recommended collecting seed up to 300 km south of a *F. americana* plantation location.

As the *F. americana* trees aged, the height differences among populations became more accentuated at most plantation sites. The mean percentage of total variation attributed to provenance, taken across 10 common gardens, increased from 28.6 percent at age 3 to 38.5 percent at age 13 (Clausen 1984, Rink and Kung 1991). In contrast, the mean variance component attributed to populations-within-provenances decreased from 11.9 percent at age 3 to 7.5 percent at age 13 in the same study sites (Clausen 1984, Rink and Kung 1991), indicating that mean between-provenance variation is more than three times greater than within-provenance variation. Interestingly, the common garden in Kansas was an exception to this trend. Here, the between-provenance variance component for height of 13-year-old trees was 10 percent, while the within-provenance variance component was 31 percent (Rink and Kung 1991.

In the central zone between the northern and southern ecotype, at approximately 39°N, growth and survival are highest among populations that originated from locations closest to the common garden (Schuler 1994, Marchin et al. 2008). This result was confirmed by two common gardens located at opposite edges of the species range. At the common garden in north-central West Virginia, Schuler (1994) examined trees 15 years after establishment and found height and survival were highest in populations originating from the immediate vicinity of the common garden. Among 30-year-old trees in the Kansas common garden, similar results were found: Stem circumference and survival were highest in populations originating closest to the common garden (Fig. 2) (Marchin et al. 2008). In both studies, survival and growth in the common garden were lower in provenances originating either to the north or south of the common garden (Fig. 2).

Taken together, these studies indicate that adaptive differentiation has occurred in *F. americana* in response to range-wide climatic variation. Evidence suggests that the genetically controlled differences in growth and survival among *F. americana* populations are related to differences in temperature and/or precipitation patterns throughout the species' range. There are high degrees of variation in climatic variables throughout the *F. americana* range (Table 1), with mean annual temperature ranging from 4.3 to 19.4 °C and mean annual precipitation ranging from 749 to 1602 mm.

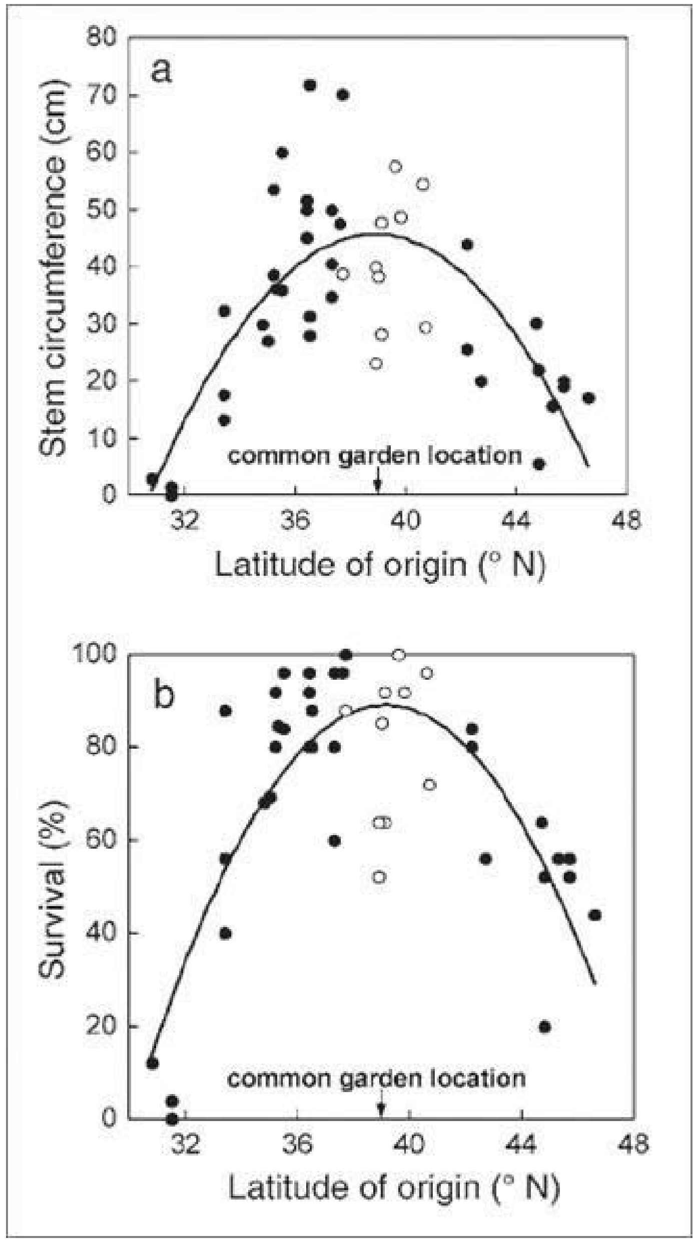

Figure 2.—(a) Stem circumference (n = 1-25) and (b) survival (n = 25) regressed against latitude of origin for 44 *Fraxinus americana* populations grown in a common garden located in Jefferson County, KS. Populations representing an east-west precipitation gradient along latitude 39°N are identified by open symbols. From Marchin et al. 2008.

Temperature-influenced Intraspecific Variation

As indicated by the differences in survival among ecotypes of *F. americana*, there is variation in cold tolerance among *F. americana* populations. Alexander et al. (1984) found that stem tissue of the northernmost populations had lower killing temperatures (–42.6 °C, –41.4 °C) than did the southernmost populations (–33.7 °C, –30.8 °C) in two different years. This result suggests that southern tree populations require lower temperatures or more time to reach maximum potential cold hardening than do northern tree populations. Additionally, it has been shown that southern populations have lower temperature requirements for bud break in the spring in Kansas (Marchin 2006), indicating that these populations may be more susceptible to damaging late-spring frosts. The lower growth and survival of southern *F. americana* populations in the Kansas common garden is likely at least partially controlled by temperature-related traits.

Because killing temperatures of northern *F. americana* populations are low (Alexander et al. 1984), the mechanism controlling differential growth and survival among northern populations is likely different from that of southern populations. The lower stem circumference of northern populations may still be controlled by temperature-related traits, however, because there are phenological differences among populations. The range for mean date of leaf emergence among populations in the Kansas common garden was 26 days, which is comparable to reported time intervals for other deciduous temperate tree species (Scotti-Saintagne et al. 2004, Borchert et al. 2005). A significant linear relationship was found between temperature sum for spring leaf emergence and latitude of origination among *F. americana* populations, where northern populations had higher temperature requirements for bud break than southern populations (Marchin 2006). In forest ecosystems, early greening of canopies is critical for net ecosystem primary production. A difference of a few days in canopy development accounted for more than 20 percent of the interannual change in net photosynthetic production in a northeastern North American forest (Myneni et al. 1997).

Another possible cause of the differential growth and survival observed among *F. americana* populations is differences in photosynthetic capacity or rates of light-saturated photosynthesis (A_{sat}). Leaf gas exchange parameters, such as net photosynthesis (A) and stomatal conductance (g_s), have been found to vary significantly among populations of the closely related species *F. pennsylvanica* (green ash; Abrams et al. 1990). This variation among genotypes in A_{sat} is maintained by simultaneous changes in biochemical and stomatal characteristics during photosynthesis (Geber and Dawson 1997).

Because the amount and intensity of solar radiation in different habitats is a source of population variation in A_{sat} (Fitter and Hay 2002), it is possible that *F. americana* populations along a north-south latitudinal gradient would have differences in A_{sat}. One study comparing 10 *F. americana* populations that originated along such a latitudinal gradient found no significant variation in A_{sat} or g_s (p=0.35, p=0.25, respectively) among *F. americana* populations (Table 2; Marchin 2006). The sample size analyzed in this study was low (n=3-5 individuals), however, and perhaps better sampling would reveal significant differences among populations. Indeed, there does appear to be a trend between mean A_{sat} and latitude, where northern *F. americana* populations have the lowest mean A_{sat} (Table 2). Populations with lower inherent rates of photosynthesis would also have lower potential growth rates (Larcher 2003).

Precipitation-influenced Intraspecific Variation

Changes in water availability across a species range have been found to drive intraspecific differences in drought tolerance in a number of tree species (Schmidtling and Froelich 1993, Roupsard et al. 1998, Adams and Kolb 2004). Water is one of the most important resources for tree growth; up to 90 percent of the variation in the annual width of tree rings can be explained by variations in rainfall (Kozlowski et al. 1991). It is not surprising then to observe population variation in *F. americana* that is related to precipitation differences across the species' range.

Table 2.—Leaf gas exchange (n = 3-5) in *Fraxinus americana* populations originating from locations along a latitudinal gradient and grown in a common garden in Jefferson County, KS

Location	Latitude	A_{sat}(± SD)	g_s (± SD)	Slope of A_{sat}-N Relationship
Ontonagon, MI	46.6	7.94 ± 4.26	0.127 ± 0.136	9.6
Forest, WI	45.7	6.46 ± 1.67	0.062 ± 0.018	2.6
Forest, WI	45.7	8.20 ± 2.09	0.080 ± 0.027	-4.6
Effingham, IL	39.1	9.35 ± 3.30	0.111 ± 0.078	12.5
Effingham, IL	39.0	9.98 ± 2.11	0.118 ± 0.037	3.1
Gallatin, IL	37.6	11. 27 ± 2.68	0.150 ± 0.076	3.2
Oktibbeha, MS	33.4	11. 25 ± 1.83	0.159 ± 0.068	1.3
Oktibbeha, MS	33.4	9.18 ± 3.71	0.118 ± 0.082	-2.7
Oktibbeha, MS	33.4	10.04 ± 2.52	0.104 ± 0.033	2.3
George, MS	30.8	9.85 ± 4.26	0.164 ± 0.101	10.2

Genetic differentiation for water-related traits in *F. americana* can be analyzed in a common garden by choosing a subset of populations where precipitation is known to differ among the locations of origin, but temperature variation is minimized. In the east–west precipitation gradient analyzed here, mean annual precipitation at the source of these populations ranges from 765 mm year^{-1} in the west (Otoe, NE) to 1261 mm year^{-1} in the east (Tucker, WV; Table 1). Among these 10 populations, survival in the common garden was only about 50 percent for the easternmost population (Randolph, WV) but rose to 96 percent for the westernmost population, which originated closest to the common garden (Otoe, NE; Fig. 2). Similarly, stem circumference in the easternmost populations (Tucker, WV; Randolph, WV; Wayne, OH) was only about half that in the westernmost populations (Otoe, NE; Adams, IL).

The stable carbon isotope ratio of leaves (foliar $\delta^{13}C$) provides a time-integrated measure of the ratio of leaf inter-cellular CO_2 concentration to atmospheric CO_2 concentration (c_i/c_a). The c_i/c_a ratio depends on stomatal conductance and photosynthetic demand for CO_2 and thus indicates water-use efficiency (Farquhar et al. 1989, Ehleringer 1991). In the Kansas common garden, foliar $\delta^{13}C$ values were significantly negatively correlated with annual precipitation at the location of origin (Fig. 3; Marchin et al. 2008). Eastern populations from wetter regions exhibited lower $\delta^{13}C$ values and were therefore less conservative in their water use when compared with western populations that originated from drier climates. A more conservative strategy for water use may have contributed to higher survival, particularly when considering that the common garden experienced periods of extreme drought in the past 30 years.

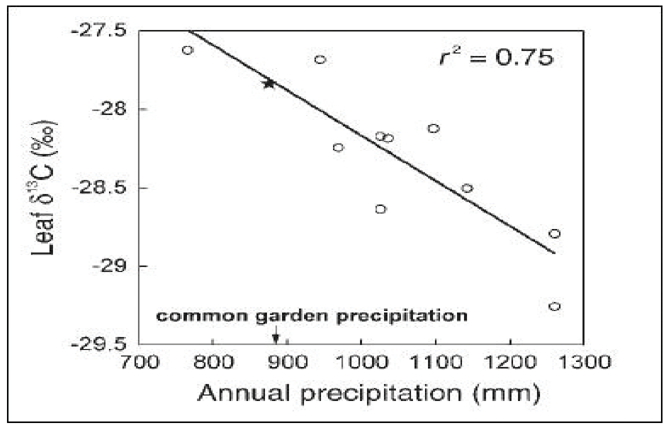

Figure 3.—Foliar carbon isotope ratios of *Fraxinus americana* populations (n = 3-6) from an east-west precipitation gradient when grown in a common garden. The population originating from Jefferson County, KS, near the common garden is indicated by the filled star. (Marchin et al. 2008)

The responses of these *F. americana* populations suggest wide-range adaption of *F. americana* to the local moisture regime. Differentiation in leaf mass per area (LMA) and foliar nitrogen (N) concentration (on an area basis, N_a) was also evident among *F. americana* populations from across the east–west precipitation gradient (Marchin et al. 2008). Increases in LMA and N_a indicate increases in the quantity of photosynthetic apparatus per unit leaf area (Fitter and Hay 2002) and, thus, increases in water-use efficiency (Li et al. 2000, 2004). The superior growth and survival of provenances originating from the western edge of the species range where the climate is driest is thus likely attributable, at least in part, to their high LMA and N_a.

CONCLUSIONS

Genetically based differences in morphology, phenology, and stomatal regulation among populations of *F. americana* are correlated with temperature and/or precipitation differences at the site of population origin. The physiological responses of *F. americana* trees, which are still apparent after 30 years in a different climate, affect the ability of individual populations to grow and survive in novel climates. Populations with the highest growth and survival at the edge of the species range originated from climates of similar annual temperature and precipitation to that of a Kansas common garden. A thorough understanding of population variation can be used to determine the potential for adaptive evolution in *F. americana*, as well as the genotypes that may be most successful in a given region under predicted future climatic and biotic conditions.

The spread of *A. planipennis* to new sites has seriously threatened the continued existence of *Fraxinus* species in North America. The current situation for *Fraxinus* has been compared to the past devastation of *Castanea dentata* (American chestnut) trees by the fungal chestnut blight. Knowledge of the physiological variation among populations can direct seed-collection efforts aimed at conserving diversity in *F. americana*. While we face a difficult challenge in preserving *Fraxinus* species in North American temperate forests, the cultural and ecological importance of these species provides a strong incentive for solving the problem.

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EX SITU CONSERVATION OF ASH SEED IN CANADA

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Abstract.—The National Tree Seed Centre began an ash seed collection program in 2004 in response to the threat imposed on the ash resource in Canada by emerald ash borer (*Agrilus planipennis* Fairmaire). Ash seed stores very well when dried to an 8-percent moisture content and frozen at -20 °C. Collections are made from a minimum of 15 trees per population. Seed is kept separate by tree. Viability tests are conducted to evaluate seed quality before storage. More than 520 collections have been made from five species. At least another 1,200 collections must be made to complete sampling throughout the species' ranges in Canada.

INTRODUCTION

Ash species are an integral component of many of the forest ecosystems in Canada. The range of black ash (*Fraxinus nigra* Marsh.) extends from the Atlantic Provinces, Quebec, and Ontario to the southeastern corner of Manitoba. Green ash (*F. pennsylvanica* Marsh.) is found in parts of Nova Scotia and New Brunswick, and through the southern portions of Quebec and Ontario. Its range extends farther west than black ash, reaching across the lower half of Manitoba and Saskatchewan. White ash (*F. americana* L.) is found throughout the Maritime Provinces and southern Quebec and Ontario. Blue ash (*F. quadrangulata* Michx.) and pumpkin ash (*F. profunda* [Bush] Bush) are not common and are found only in a few locations in the Carolinean Forest of southwestern Ontario (Farrar 1995). Ash wood is strong and durable. White ash is used primarily for tool handles, snow shoes, and baseball bats whereas black ash is prized by First Nations people for weaving baskets. Seeds serve as a food source for wildlife.

With the discovery of emerald ash borer (EAB; *Agrilus planipennis* Fairmaire) in Windsor, ON, in 2002, the future of the ash resource is uncertain. Since that time, the insect has steadily spread into southwestern Ontario but has also leapfrogged to such distant locations as Ottawa and southeast of Montreal (Fig. 1). Mortality of ash trees has had a devastating impact on urban and forest landscapes. With no natural enemies and no inherent resistance within the trees, this exotic insect has the potential to wipe out ash in Canada, resulting in the loss of native species and varieties—with repercussions on the ecosystems where ash occurs. The official position in Canada is that EAB cannot be eradicated (Marchant 2007). Therefore, an effort must be made to collect and preserve the genes, thereby conserving the natural genetic variation that will be critical for the development of resistant planting stock. One means of conservation is collecting and storing seed.

National Tree Seed Centre

The National Tree Seed Centre was established by the Canadian Forest Service in 1967. Its purpose was to collect, store, and provide seed of known origin and quality for research. Since then, its role has been expanded to include the long-term storage of seed for genetic conservation. Seed stored for genetic conservation will be made available only for specific research projects, such as identifying genes that confer resistance, or for hybrid breeding programs, genomics studies, or restoration plantings.

The Seed Centre has excellent storage facilities that consist of three large walk-in freezers maintained at -20 °C. All seed is stored in hermetically sealed glass jars. This method of storage ensures that the moisture content of the seed is maintained during storage, the seed is protected from agents trying to enter the jars, and the local environment is protected from anything trying to escape from the jars.

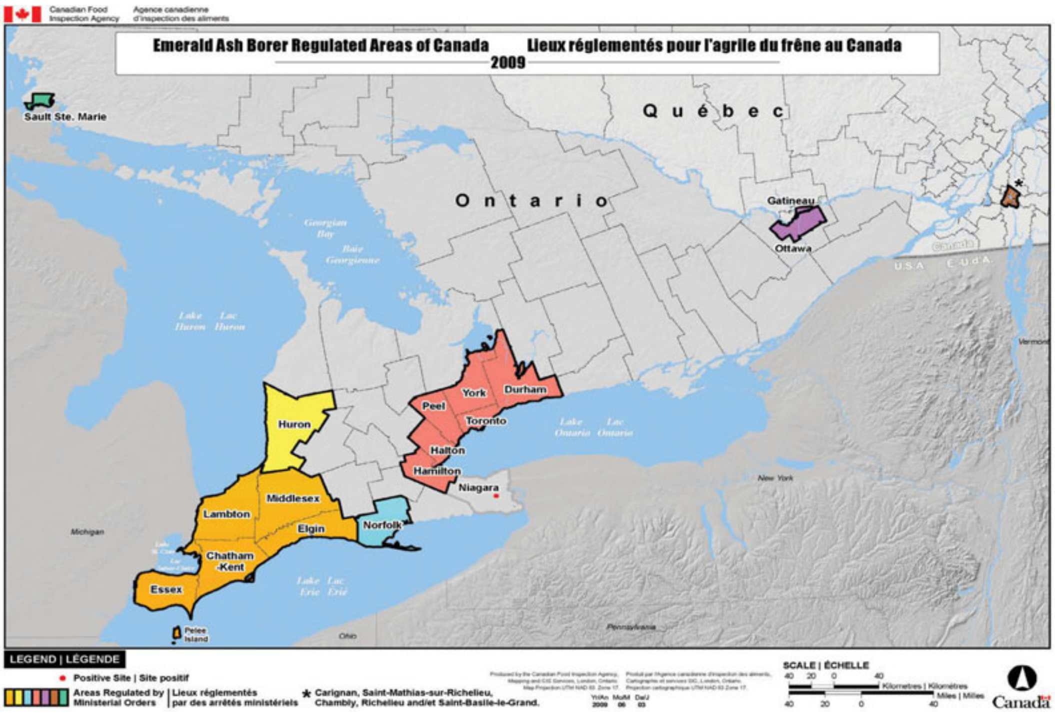

Figure 1.—Locations in Canada where emerald ash borer has been detected. Source: http://www.inspection.gc.ca/english/plaveg/pestrava/agrpla/mc/200907canada.gif

Ash Seed Collection, Processing, Testing, and Storage

Ash seed collections began in 2004 at a time when EAB was emerging as a threat to the ash resource. There was an opportunity for the Seed Centre to become active in conserving the ash genetic resource. Data from ash seedlots already in storage indicated that the seed stored well at -20 °C (Table 1).

Seed collections aim to sample the genetic variation present in a population. A population can comprise a number of stands or a distribution of trees within an environmentally homogeneous area. A minimum of 15 trees are sampled, with the seed kept separate by tree. This protocol serves many purposes (e.g., to evaluate seed quality, assess changes in viability during storage, and evaluate genetic variation among trees within populations). To reduce the chance of collecting seed from related trees, trees are spaced 50–100 m apart. Various techniques are employed for seed collection. The most frequently used method is pole pruning, whereby seed-bearing branches are removed. In some instances, trees are climbed. Generally, 4-5 L of seed and debris are collected.

Good seed collections have been made in the Maritime Provinces, with fewer collections made in other provinces (Fig. 2). The challenge has been to engage seed collectors and agencies in these provinces. There has been more enthusiasm during the last couple of years, so it is hoped that the level of interest will continue to grow and that collectors will be in place to take advantage of

Table 1.—Germination of ash seed from several species stored at -20 °C at the National Tree Seed Centre

Species	Number of seedlots	Years in storage	Germination (%)
F. americana	17	27	76
F. nigra	4	15	78
F. pennsylvanica	3	10	66

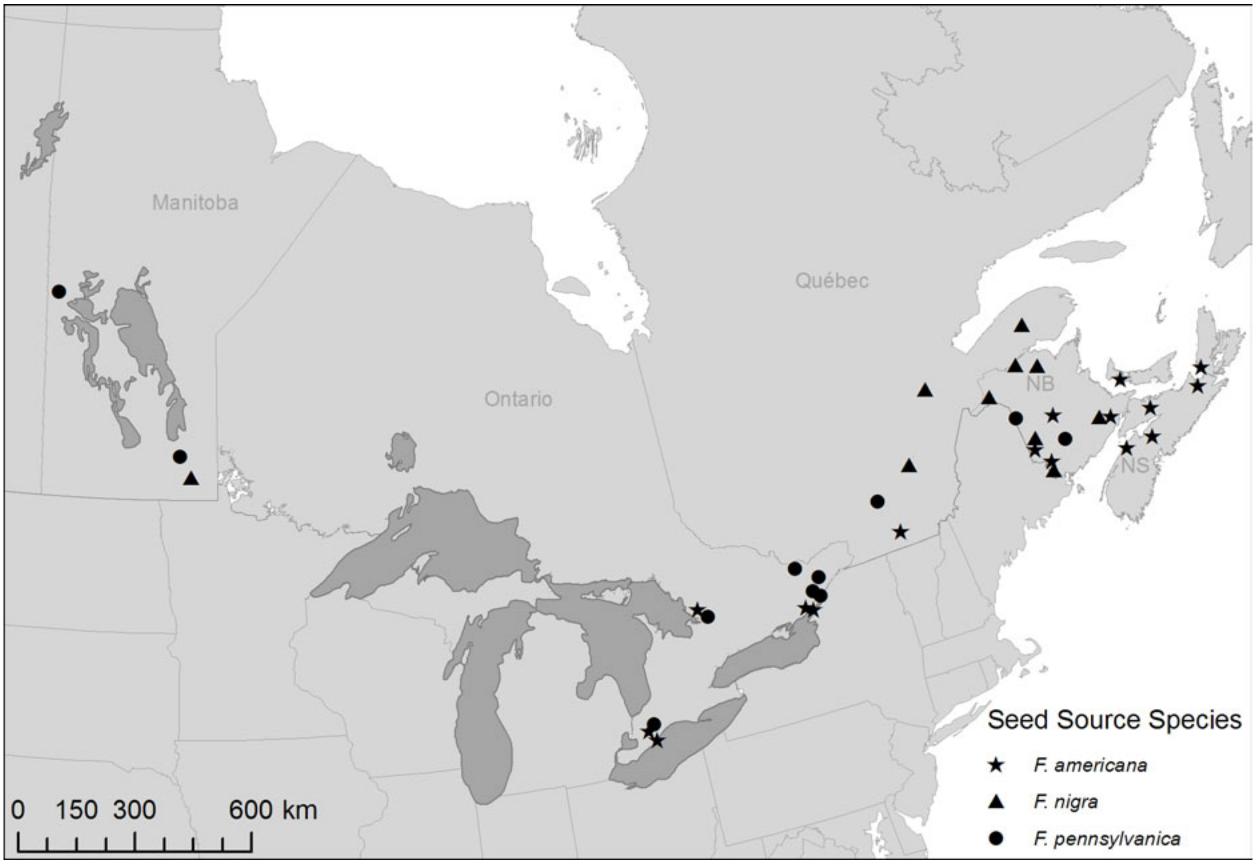

Figure 2.—Locations of ash seed collections stored at the National Tree Seed Centre.

good seed crops. Seed from more than 520 collections is in storage at the Seed Centre (Table 2). About 42 percent of the collections are white ash, 29 percent are green ash, and 28 percent are green ash.

Seed undergo some processing before storage. First, coarse debris (such as leaves, leaf rachises, and stems to which the seeds were attached) is removed. Green and white ash seed are de-winged, whereby about 50 percent of the wing is removed. The seed are then put into an aspirator, which blows away the debris as well as most empty and insect-damaged seed. These processing treatments also reduce the volume of seed to store. However, black ash seed cannot be de-winged in this manner and are stored fully intact. After cleaning and processing, the final volume can be 1-2 L of seed.

Seed quality of each collection is determined by conducting a viability test, which indicates whether an embryo is alive and capable of germinating. Results from viability tests can be obtained within 14 days, whereas germination tests may require 4-12 months to complete, depending on the species, due to treatments required to alleviate seed dormancy. To conduct viability tests,

Table 2. Number of ash seed collections in storage at the National Tree Seed Centre

Species	Number of collections
F. americana	222
F. nigra	149
F. pennsylvanica	154
F. profunda	1
F. quadrangulata	1

the seed is removed from the pericarp and placed in water at 3 °C for 96-120 hours. The purpose of this treatment is to soften the seed coat and allow the seed to absorb water, making it easier to process. After the seed is soaked, a scalpel is used to make a longitudinal incision the length of the seed coat, through which the embryo is removed. The embryo is placed on germination medium (VersaPakJ) in a germination box. Three replications of 25 seed each are prepared. The germination box is placed in a germination cabinet set at 25 °C with a daily light duration of 8 hours. After 14 days, the embryos are assessed. An embryo is viable if it remains the same color as it was when excised, one or both cotyledons turn green, and/or the radicle starts to develop. Of the three principal ash species, green ash seed are least dormant and the embryos often exhibit considerable development during the 14 days, with cotyledons turning green and growing as well as elongation of the radicle. In contrast, black ash embryos are very dormant and exhibit little growth, usually remaining milky white in color with minimal elongation.

An identification card is placed in each sealed jar and the species and seedlot numbers are marked on the lid. Before storage, the moisture content of the seed is determined. The maximum moisture content is 8 percent, and moisture contents are typically 6.5-8.0 percent. Seed at these moisture contents tolerate freezing and store well. All seed are stored at -20 °C.

Seed are stored under two categories: research and conservation. When seed is limiting for a seedlot, the entire quantity is allocated to conservation. Seed stored for research is freely available, upon request, for research. When a seedlot becomes exhausted, a fresh collection is not made. On the other hand, seed stored for conservation will remain there indefinitely. Seed from these collections will be provided only for research that aims to advance the knowledge required for the preservation of the species, such as genomics, breeding, and re-establishment/preservation plantations. Approximately 2,000 seed are stored for conservation; on average, 3,000 seed are stored for research.

THE FUTURE

Seed collecting must continue, particularly in areas where EAB is well established and is killing trees. It is important to sample and store these genes before they are lost forever. Seed must be collected from populations growing on a variety of sites throughout a species' range in order to sample the range of natural genetic variation. As a minimum, the following number of collections must be made: *F. americana* - 230, *F. nigra* - 460, *F. pennsylvanica* - 430, *F. profunda* - 30, and *F. quadrangulata* - 50.

At some point, a genetic analysis should be conducted for each species to evaluate the pattern of genetic variation and possibly identify areas with more or less variation. Additional seed collections can be made in areas exhibiting more genetic variation.

Areas should be revisited after EAB has moved through in case there are trees that are still alive. Such trees may have somehow escaped the ravages of EAB or may be genetically resistant. Such trees (if they exist) should be clonally propagated and their resistance evaluated.

A bank is only as good as its poorest vault. It is prudent to store seed samples at a second location as a back-up in case of a catastrophe at the principal storage facility. The National Tree Seed Centre and the United States National Center for Genetic Resources Preservation have entered into an agreement to store samples of each other's seed.

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ADVENTITIOUS SHOOT REGENERATION OF *FRAXINUS NIGRA* MARSH.

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Fraxinus nigra Marsh. (black ash) is a native ash species occurring in Newfoundland west to Manitoba and south to Iowa, Illinois, West Virginia, and Virginia. Although it is not a commercially important species, it has significant ethnobotanical importance to Native American tribes of the eastern United States. The seeds are important forage for wildlife, the wood is preferred for making splints for basketry, and the wood is also used for flooring and furniture. *F. nigra* have immature embryos at seed set combined with complex stratification requirements, making the species difficult to regenerate naturally from seed. Because of this difficulty, an in-vitro adventitious shoot regeneration system would be beneficial for mass propagation and improvement of this species. The development of such a system will also provide the basis for an *Agrobacterium*-mediated transformation system for emerald ash borer resistance. To date, no in-vitro regeneration protocol for black ash has been developed.

An adventitious shoot regeneration system for *F. nigra* is currently being optimized using hypocotyls extracted from aseptic seeds. Hypocotyls were cultured on a Murashige and Skoog (MS) medium containing 13.3 µM 6-benzylaminopurine (BA) plus 4.5 µM thidiazuron (TDZ) for callus and shoot induction. Shoots were successfully regenerated using a combination of MS medium with Gamborg B5 vitamins (MSB5), 10 µM BA, and 10 µm TDZ for 4 weeks, and then they were transferred to MSB5 medium with 6.7 µM BA, 1 µM indole-3-butyric acid (IBA), and 0.29 µM gibberellic acid (GA_3) for shoot elongation. Once elongated, the shoots were successfully micropropagated using MSB5 medium with 13.3 µM BA, 1 µM IBA, 0.29 µM GA_3, and 0.2 g L^{-1} casein hydrolysate. Rooting of elongated microshoots was successful using woody plant medium containing 4.5 µM IBA plus 5.7 µM indole-3-acetic acid with a 10-day dark incubation, and rooted shoots are undergoing acclimatization.

ESTIMATING OUTSIDE-BARK STEM VOLUME TO ANY TOP DIAMETER FOR ASH IN WISCONSIN

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The future of Wisconsin's estimated 742 million ash trees (5 million of which are in urban settings composing 20 percent of Wisconsin's urban forests) is being considered based on the presence of the emerald ash borer. Part of this discussion includes the stem volumes of these ash trees. An outside-bark volume ratio equation was developed for individual trees, allowing one to estimate outside-bark stem volume (cubic feet) to any top diameter limit for green and white ash trees in Wisconsin. Outside-bark stem diameters were estimated at predetermined heights aboveground on 84 standing trees in central Wisconsin, using two Laser Technology Inc. Criterion RD 1000 optical dendrometers with lines of sight perpendicular to one another. A subset of trees was felled to validate the diameter estimates obtained with the optical dendrometers, and a paired t-test confirmed that no differences exist (p-value = 0.36) between the standing tree diameter estimates and the corresponding felled tree measurements. An outside-bark, total stem volume equation was then developed. The equation explained about 98 percent of the variation present and possessed a mean absolute error of 1.4 cubic feet per tree. A ratio equation was subsequently developed to estimate the proportion of outside-bark stem volume present in a given tree up to a given top diameter. The ratio equation explained about 86 percent of the variation present in the data and possessed a mean absolute error of 0.015 (or 1.5 percent). Applying the ratio equation to volumes derived from the total stem volume equation on validation data resulted in a mean absolute error of 4.5 cubic feet per tree. Use of the outside-bark total stem volume and ratio equations should allow resource managers to make more informed management and utilization decisions about ash trees in Wisconsin.

SURVEY FOR TOLERANCE TO EMERALD ASH BORER WITHIN NORTH AMERICAN ASH SPECIES

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Since the discovery of the emerald ash borer (EAB) near Detroit, MI, in 2002, more than 40 million ash trees have been killed and another 7.5 billion are at risk in the United States. When the EAB outbreak was initially discovered, our native ash species appeared to have no resistance to the pest. Variation in the response of native ash species to EAB may have gone unnoticed in the Detroit area and surrounding suburbs, where ash was a popular street tree. Because the vast majority of street trees are vegetatively propagated horticulture selections, it is likely only a limited number of genotypes were represented. The establishment of "ash-free" zones may have delayed observations of ash trees with any tolerance to EAB in some areas. As the beetle spread away from urban areas into more genetically diverse native stands and woodlots, plots were established to monitor the impact of EAB. The health of more than 3,000 ash trees in infested forests in Michigan and Ohio has been monitored yearly using a canopy health index. In these areas EAB has been present for several years, and almost all of the ash trees are dead. However, our yearly inventory has identified a small number of trees that have persisted. Approximately 1.0 percent of the ash trees have remained alive and 0.1 percent have retained a healthy crown appearance. To date, we have identified and grafted 18 select "lingering ash": 15 *Fraxinus pennsylvanica*, 2 *F. americana,* and 1 *F. nigra.* These trees were selected from the main canopy layer and did not include seedlings or saplings. Initial feeding and landing studies have been performed on a subset of these trees, and preliminary results indicate that some of the lingering ash selections are less preferred by the beetle. We are ramping up propagation of each selection for the establishment of field test plots and further analysis using insect bioassays to distinguish trees that are "escapes" from those that may be tolerant or even resistant to EAB. Those selections that display tolerance/resistance to EAB will be further analyzed using molecular and biochemical methods to identify the underlying mechanisms. Although some of these trees still may succumb to EAB infestation, the mechanisms that allow them to "linger" longer than their counterparts may be further enhanced through breeding. Conceivably, under lower insect pressure, the trees that can survive longer may be capable of co-existing with EAB.

CLIMATIC CONTROLS ON EMERALD ASH BORER DISPERSAL

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We examine whether emerald ash borer (EAB) is limited not only by the presence of ash, but also by climatic factors. Using the WorldClim and CRU TS 2.1 global climate datasets, we delineate the native climatic range of EAB in Asia and the climatically equivalent areas in North America. Using a data-mining approach, we identify which of a suite of bioclimatic variables are important factors in the native range of EAB. A map of locations in North America that satisfy both climatic and host criteria is presented.

A TECHNIQUE FOR MAPPING URBAN ASH TREES USING OBJECT-BASED IMAGE ANALYSIS

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Ash trees are an important resource in the State of Minnesota and a common fixture lining the streets of the Twin Cities metropolitan area. In 2009, the emerald ash borer (EAB), an invasive pest of ash, was discovered in the city of St. Paul. To properly respond to the new-found threat, decisionmakers would benefit from detailed, spatially explicit information on the urban ash resource. Although the Forest Inventory and Analysis (FIA) Program is responsible for inventorying the Nation's forests, it does not currently include tree-covered lands in urban areas unless they meet FIA's definition of "forest." Because this definition includes minimum area, width, and density requirements, shade and boulevard trees are excluded from the inventory. Therefore, we investigate the use of remote- sensing procedures to supplement FIA inventory data. We present a technique for identifying ash tree canopies using eCognition Developer 8 image segmentation software and four-band high-resolution imagery. A fine-scale map of ash trees is produced for a study area in the Twin Cities.

ADVENTITIOUS SHOOT REGENERATION AND ROOTING OF *FRAXINUS AMERICANA*

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White ash (*Fraxinus americana*) trees provide both ecological and economic benefits. Loss of this North American endemic would disturb the environment. The urban ash tree industry in the United States would also suffer, as would manufacturers of baseball bats, furniture, and cabinets. The emerald ash borer (EAB) is an invasive species that is fatal to a tree once the borer infests it. There are no known means of complete eradication or evidence of any innate resistance in *F. americana*. The threat from this pest becomes more urgent with each growing season, making the development of an in-vitro plant regeneration and transformation system a valuable goal. As a first step toward this objective, a protocol for adventitious shoot regeneration and rooting was developed from freshly isolated hypocotyls obtained from mature embryos of white ash. The best regeneration medium for hypocotyls was Murashige and Skoog (MS) medium supplemented with 13.3 µM 6-benzylaminopurine (BA) plus 4.5 µM thidiazuron (TDZ). Sixty-four percent of hypocotyl segments produced adventitious shoots, with a mean of 3.5 adventitious shoots induced per explant. Adventitious shoots from hypocotyls were established as proliferating shoot cultures following transfer to MS medium with Gamborg B5 vitamins (MSB5) supplemented with 10 µM BA plus 10 µM TDZ. For in-vitro rooting trials, woody plant medium with indole-3-acetic acid (IAA) [2.9, 5.7, or 8.6 µM] plus 4.9 µM indole-3-butyric acid (IBA) were tested under 10-day dark treatment followed by culture in the light. Early results show successful (77.1 percent) rooting of in-vitro shoots on medium containing 4.9 µM IBA plus 5.7 µM IAA. Rooted plants were successfully acclimatized to the culture room. This regeneration system from hypocotyls provides a foundation for *Agrobacterium*-mediated genetic transformation of white ash for resistance to the EAB.

RESPONSE OF YOUNG ASH TREES TO CULTURAL TREATMENTS NOT ALL POSITIVE

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Calcium cyanamide (CaN_2), an alkaline nitrogenous fertilizer containing about 22 percent N and 20 percent lime, was evaluated as a fertilizer for young white ash (*Fraxinus americana* L.). Neither height nor diameter of 4-year-old ash trees treated with three annual applications of CaN_2 at rates of 448, 672, 896, and 1,120 kg/ha was significantly different from the unfertilized control trees. Following two applications of CaN_2 at the rates applied to younger trees, 7-year-old white ash were taller and larger in diameter than the unfertilized control trees, but differences were not significant.

The effects of tree shelters on the performance and leaf nutrient concentrations of planted green ash (*F. pennsylvanica)* were investigated at age 3. Mean survival of trees with shelters was 92 percent compared to 62 percent without shelters. Sheltered trees had significantly more stem and root biomass than trees without shelters. Foliar phosphorus, calcium, and magnesium levels were significantly higher and potassium was significantly lower in green ash leaves from trees with shelters than in green ash leaves without shelters.

Annual height measurements taken for green ash in another planting, grown with and without tree shelters, indicated considerable dieback after year 5, and measurements were terminated after year 8. Mean survival was not different between tree shelter treatments, but it declined from 88 percent after 1 year to 55 percent by year 8. Green ash trees with shelters were taller than those without shelters after the first growing season. But by year 5, height differences between treatments disappeared. The exact cause of the dieback and eventual mortality is not known, and there was no difference in the amount of dieback between tree shelter treatments. The author speculated that insects were responsible for the decline.

Michler, Charles H.; Ginzel, Matthew D., eds. 2010. **Proceedings of symposium on ash in North America.** Gen. Tech. Rep. NRS-P-72. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station. 64 p.

Includes 5 papers and 30 abstracts covering topics related to the biology and ecology of the ash species, ash utilization and management, emerald ash borer., and other threats to ash, and genetics and conservation of ash species

KEY WORDS**:** green ash, black ash, white ash, blue ash, emerald ash borer, *Fraxinus.*