# EX SITU CONSERVATION OF ASH SEED IN CANADA

**Dale Simpson**

Natural Resources Canada, Canadian Forest Service—Atlantic Forestry Centre,
PO Box 4000, Fredericton, NB E3B 5P7.
To contact, call (506) 452-3530 or email at Dale.Simpson@nrcan.gc.ca.

**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.

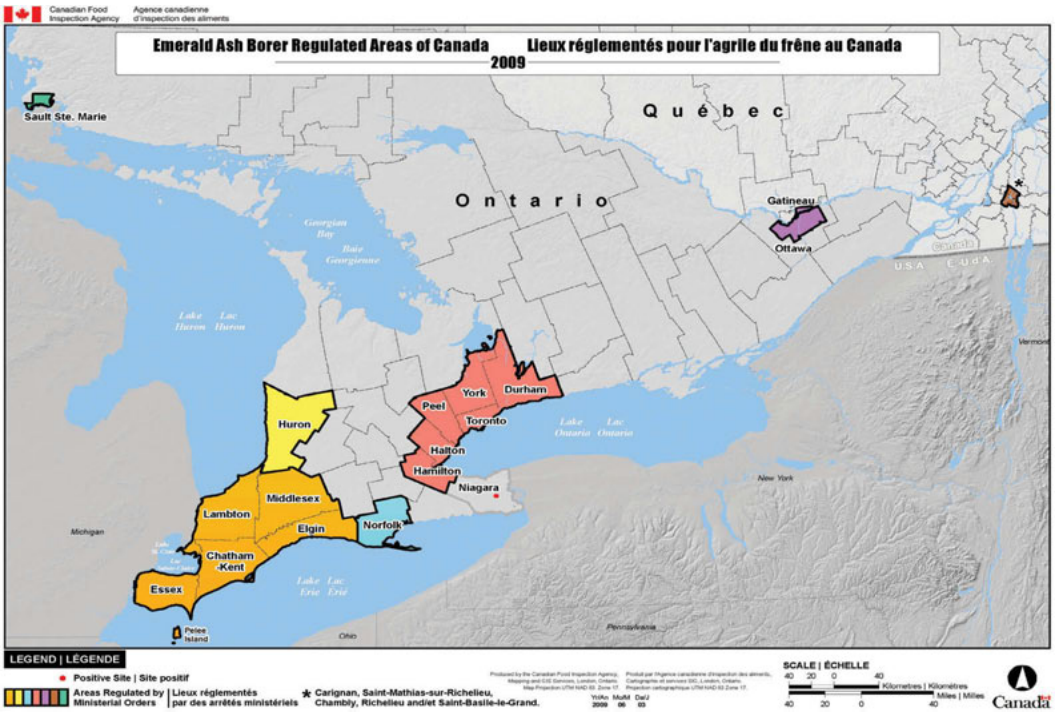


Figure 1.—Locations in Canada where emerald ash borer has been detected. Source: http://www.inspection.gc.ca/english/plaveg/pestrava/agrpla/mc/200907canada.gif

## 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 |

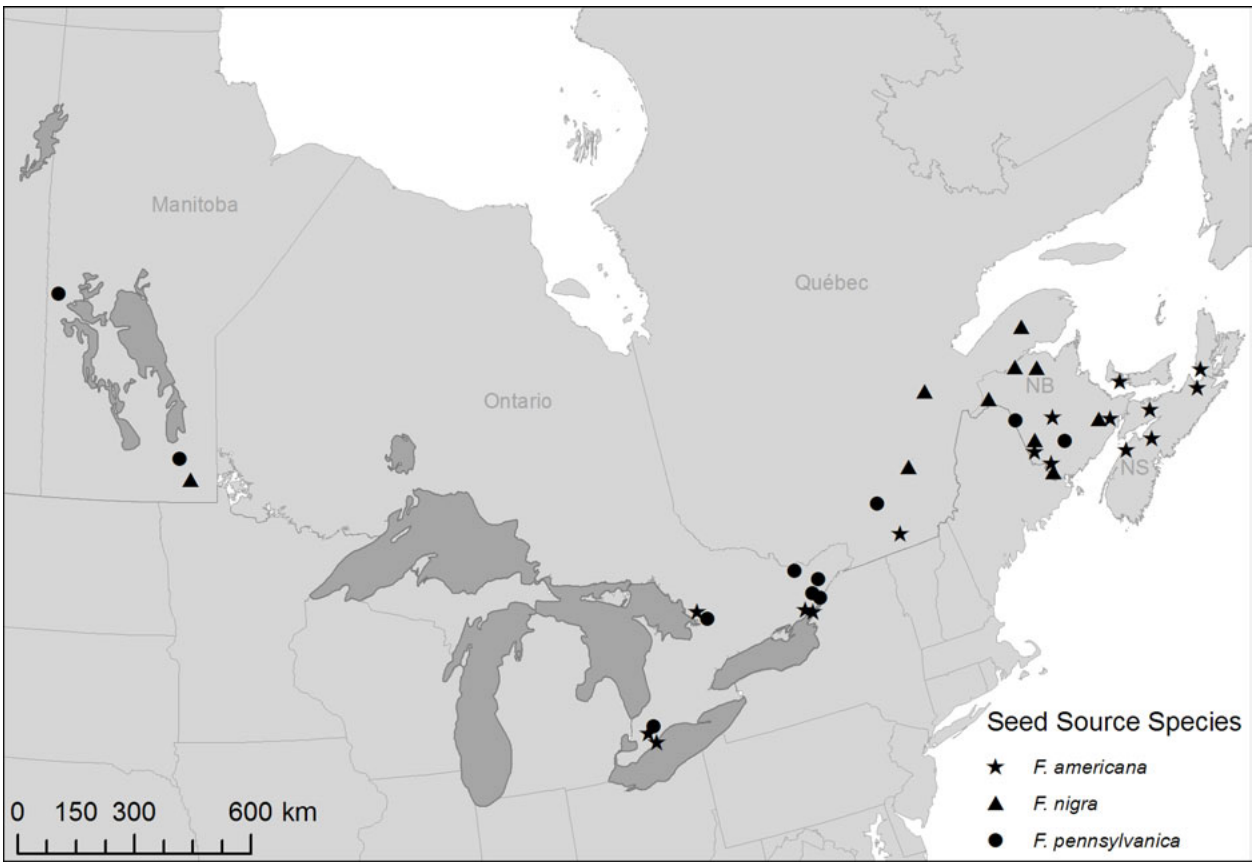


Figure 2.—Locations of ash seed collections stored at the National Tree Seed Centre.

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.

## ACKNOWLEDGMENTS

Appreciation is extended to A. Mosseler for reviewing a draft of this paper and to I. DeMerchant for preparing Figure 2.

## LITERATURE CITED

Farrar, J.L. 1995. **Trees in Canada**. Markham, ON: Fitzhenry and Whiteside Ltd. 502 p.

Marchant, K.R. 2007. **Managing the emerald ash borer in Canada.** In: Gottschalk, Kurt W., ed. Proceedings, 17th U.S. Department of Agriculture interagency research forum on gypsy moth and other invasive species 2006. Gen. Tech. Rep. NRS-P-10. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station: 67-69.