

Seed Collection Success and Failure in *Fraxinus* Gene Conservation Efforts¹

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

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

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

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

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

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