

Recruitment history, current health and conservation genetics of butternut (*Juglans cinerea*) populations in Great Smoky Mountains National Park



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Abstract

In recent decades, butternut populations throughout eastern North America have been decimated by butternut canker, a disease caused by the fungal pathogen *Ophiognomonia clavigignenti-juglandacearum*. Disease mortality, poor regeneration in contemporary forests, and hybridization with Japanese walnut threaten the survival of butternut throughout its historic range. Successful conservation of butternut requires a better understanding of its current survival, conditions under which regeneration occurs, and its genetic integrity where it co-occurs with Japanese walnut. In addition, genetic diversity and gene flow between isolated populations should be assessed to determine best management practices and priorities. We assessed butternut populations within Great Smoky Mountains National Park (GSMNP) to determine recruitment history of populations, post-canker survivorship and health, and degree of hybridization with Japanese walnut. We used National Park Service monitoring records to locate and collect health data for 202 butternut trees across 19 watersheds within GSMNP. Tree core samples were taken from a subset of individuals throughout the Park to assess the pattern of recruitment for the current populations. Leaf samples were collected for genetic analysis to determine the frequency of hybridization. Based upon our results, butternut populations in GSMNP have declined dramatically due to disease mortality and thirty years of minimal regeneration. Tree core samples revealed continuous recruitment since Park establishment (1934) until around 1980, after which regeneration declined drastically across all watersheds. Initial genetic analyses indicate that butternut-Japanese walnut hybrids comprise a small portion of the total trees sampled. The presence of healthy trees and low rates of hybridization offer hope that the trees in GSMNP may contribute to efforts to develop and reestablish disease-resistant populations of this threatened tree species. In the next phase of this study, we will genotype all individuals at 15 microsatellite loci to determine the genetic diversity and population genetic structure to evaluate gene flow patterns among populations that have become increasingly isolated due to disease mortality.

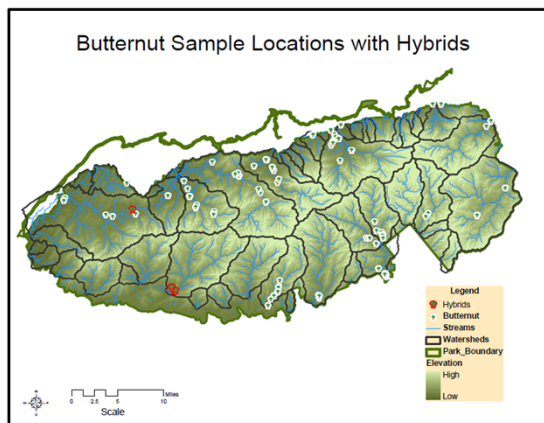


Figure 1: Map of GSMNP with locations of butternut samples. Hybrids shown in red.

Introduction

Within Great Smoky Mountains National Park, after several decades of disease, butternut remain in diffuse populations throughout the Park. In this portion of our study, our objectives were as follows:

1. Assess the survivorship and current health conditions of remaining trees;
2. Determine the degree of hybridization (if any) of *J. cinerea* with *J. ailantifolia*;
3. Determine the age structure of *J. cinerea* populations and recruitment pattern since Park establishment.



Figure 2: Butternut with large basal trunk canker (left) and vigorous butternut (right).

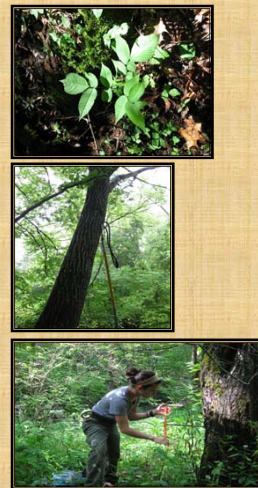


Figure 3: Butternut seedling (top); butternut with arborist slingshot, used for leaf sampling (middle); core sample extraction (bottom)

Methods

Field Collection

- Butternut location database, compiled by NPS during the late 1980's and 1990's
- Our samples: 202 butternut sampled across 19 watersheds
- Survivorship over the past 2 decades estimated from number of trees that were not found or found dead in our survey
- Collected GPS location, dbh, and several health rating measures for each individual
- Leaf samples from 168 trees for genetic analysis

Core Sampling

- 1-2 representatives from each population (defined as all individuals within a watershed)
- Cored all individuals in a subset of 4 watersheds

Genetic Analysis

- DNA extracted from fresh leaves
- All individuals assessed using markers developed to distinguish butternut from butternut-Japanese walnut hybrids (Zhao & Woeste 2010)

- 1 chloroplast marker
- 3 nuclear markers

Results

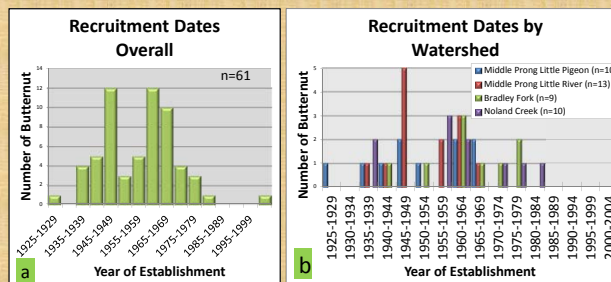


Figure 4: Graph of butternut recruitment pattern, determined by aging tree core samples.

4a: Recruitment dates of combined butternut, in 5 year increments. 61 samples total.

4b: Recruitment dates divided by watershed for the 4 intensively sampled populations. 42 samples, distributed as shown in legend

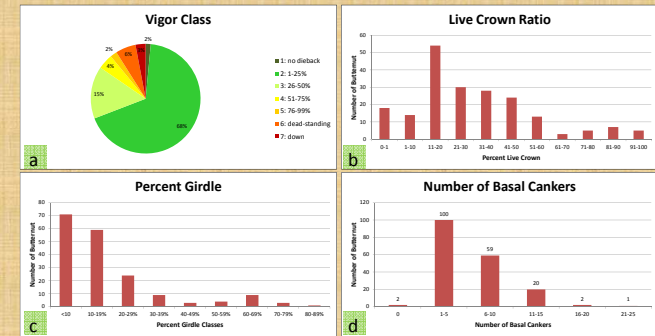


Figure 5: Distribution of ratings of butternut for 4 health measures.

5a: Vigor classes based on percent of crown with dieback or discoloration;

5b: Live crown ratio: an estimated ratio of the total length of the live crown divided by the total length of the tree; values are given in figure as percentages

5c: Percent age of the base of the trunk that has been girdled by coalescing cankers. This measure was included, since variable canker size can confound "number of cankers" as an estimate of health;

5d: Number of trunk cankers below dbh (1.4 meters); cankers tend to join together so counts were of discrete, fusiform cankers; large, hollow cankers were counted as one

Discussion

Results Summary:

- 68% survivorship over the past 2 decades of monitoring
- Most butternut in various stages of decline
- Small percentage of trees are *J. cinerea*/*J. ailantifolia* hybrids (<1%)
- Continuous pattern of recruitment since Park establishment (1934) until about 1980, after which time regeneration declined dramatically across all watersheds
- Individual watersheds had peaks in recruitment between 1945-1965

Future Analysis

- Genotyping at 15 microsatellite loci
- Assessment of population genetic structure and genetic isolation

Although the butternut in GSMNP have experienced high mortality since the arrival of disease, the presence of vigorous trees and low rate of hybridization indicate potential for disease resistance in natural populations, which may be useful in breeding programs and restoration efforts. In addition, the pattern of recruitment, which shows a drop off in regeneration after the succession of agricultural lands and settlement back to forest, indicates that restoration efforts may require site alteration to promote regeneration.

Acknowledgments

We thank the staff at Great Smoky Mountains National Park for their invaluable assistance during field work. We would like to acknowledge Peng Zhao for his extensive assistance in DNA extraction and other genetics lab work.

References

Zhao P, Woeste KE (2010) DNA markers identify hybrids between butternut (*Juglans cinerea* L.) and Japanese walnut (*Juglans ailantifolia* Carr.). Tree Genet Genomes. doi:10.1007/s11295-010-0352-4