RESEARCH ARTICLE

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# Conservation Status of a Threatened Tree Species: Establishing a Baseline for Restoration of *Juglans cinerea* L. in the Southern Appalachian Mountains, USA

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*Natural Areas Journal 33:413–426*

**ABSTRACT**: To mitigate the loss of native tree species threatened by non-native pathogens, managers need to better understand the conservation status of remaining populations and the conditions that favor successful regeneration. Populations of *Juglans cinerea* L. (butternut), a wide-ranging riparian species, have been devastated by butternut canker, a disease caused by a non-native fungal pathogen. We assessed *J. cinerea* within Great Smoky Mountains National Park (GSMNP) to determine post-disease survivorship and health, recruitment history, environmental conditions associated with survival, and the extent of hybridization with a non-native congener. Monitoring records were used to locate and collect data for 207 *J. cinerea* trees in 19 watersheds. Tree cores were collected from a subset of individuals to assess recruitment history. We sampled vegetation plots within areas that contained *J. cinerea* to assess site conditions and overstory species composition of characteristic habitat. We collected leaf samples for genetic analysis to determine the frequency of hybridization. Our reassessment of monitoring records suggests that *J. cinerea* abundance in GSMNP has declined due to butternut canker and thirty years of poor regeneration. Populations displayed continuous recruitment following Park establishment (1934) until around 1980, after which regeneration declined drastically. Ordination analysis revealed that J. cinerea in the contemporary forest was associated with greater distance from homesites and reduced basal area of competing species. Hybrids comprised a small portion of sampled trees. The presence of healthy trees and low rate of hybridization suggest that these trees may contribute to the development of disease-resistant genotypes for future restoration efforts.

*Index terms:* butternut, cohort structure, disturbance regime, forest disease, fungal pathogen, hybridization, mortality, recruitment history

## INTRODUCTION

Exotic diseases are an increasing threat to native biodiversity as global movement of materials introduces non-native pathogens, and changing land use facilitates their spread (Lovett et al. 2006). Fungal pathogens have devastated North American forests in the last century, resulting in fundamental alterations to ecosystems (Ellison et al. 2005; Lovett et al. 2006; Loo 2009; Holzmueller et al. 2010). Baseline (pre-disease) data are necessary to examine the overall effects of species loss, but often extensive mortality occurred before detailed information was collected, making it difficult to determine the true effects of disease on ecosystem processes (Ellison et al. 2005).

Several eastern tree species have been virtually eliminated by introduced pathogens; others have experienced such severe decline that they no longer retain their pre-disease ecological function (Loo 2009). Chestnut blight, caused by *Cryphonectria parasitica* Murrill (Barr) and perhaps the best example of a fungal disease that led to the loss of a keystone species, functionally eliminated *Castanea dentata* [American chestnut, (Marsh.) Borkh] from eastern forests in the early twentieth century. Other fungal diseases, including beech bark disease, Dutch elm disease, and dogwood anthracnose, have caused widespread declines in tree species (Lovett et al. 2006; Loo 2009; Holzmueller et al. 2010). While research has focused on the effects of diseases on common species, less is known about the effects of introduced pathogens on less-common species, for which the consequences of disease may be amplified because of inherently low population density and limited reproduction of the host species.

The four-decade program to produce a blight-resistant hybrid of *C. dentata* is nearing the stage of active restoration (Jacobs 2007). As the program progressed, other researchers have focused on the restoration of other tree species affected by exotic disease (Windham et al. 1998; Griffin 2000; Ostry and Moore 2008). For example, *Juglans cinerea* L. (butternut), a species whose range extends across much of eastern North America, has experienced extensive mortality from butternut canker caused by the non-native fungal pathogen *Ophiognomonia clavigignenti-juglandacearum* (Nair, Kostichka, & Kuntz) Broders & Boland (Woeste et al. 2009). While estimates of mortality caused by butternut canker are as high as 92% (Carlson and Guthmiller 1993), evidence suggests that genetic resistance exists and may potentially be exploited for conservation and restoration efforts (Thompson et al. 2006; Ross-Davis et al. 2008). Efforts are underway to identify resistant individuals

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(Ostry and Moore 2008) and establish a framework for the development of resistant genotypes (Michler et al. 2006).

The persistence of *J. cinerea* in contemporary forests is precarious not only because of the effects of butternut canker, but also due to habitat loss, poor regeneration, and genetic dilution from hybridization with non-native *Juglans ailantifolia* Carr. (Japanese walnut; Hoban et al. 2009). *Juglans ailantifolia* was historically planted within agricultural landscapes (Hoban et al. 2009) and has been present within the range of *J. cinerea* for multiple generations. As with other uncommon species that co-occur with a non-native congener, such as *Celastrus scandens* (American bittersweet) and *C. orbiculatus* (oriental bittersweet), genetic dilution as a result of hybridization presents a serious threat to the persistence of the species (Pooler et al. 2002). When hybridization occurs in conjunction with an invasive pathogen, the threat to a native species is amplified.

Successful restoration of *J. cinerea* not only depends upon mitigating the effects of disease and developing resistant genotypes, but also requires understanding the regeneration dynamics of *J. cinerea* and the community context in which it historically occurred and currently persists (Thompson et al. 2006). In this study, we examined the contemporary health, genetic integrity, canopy associates, and recruitment history of *J. cinerea* populations (defined in this study as all individuals occurring within a watershed; Figure 1) within Great Smoky Mountains National Park (GSMNP), a protected area with numerous well-documented populations of *J. cinerea*. Based upon this examination, we address four primary questions: (1) What is the survivorship and health of *J. cinerea* across an unfragmented landscape represented by GSMNP?; (2) What is the extent of hybridization with *J. ailantifolia*? We hypothesized that the isolation and lack of fragmentation within GSMNP buffers *J. cinerea* populations from invasion by non-native genes; (3) What is the overstory composition in contemporary forests that contain surviving *J. cinerea* trees? We hypothesized that *J. cinerea* occurs with greater abundance in forests with reduced dominance by other early-successional species; and (4) What was the temporal recruitment pattern of surviving *J. cinerea* populations and when did contemporary competitors establish?

## METHODS

### Study Area

Great Smoky Mountains National Park is an internationally renowned center of biological diversity in North America, leading to its designation as an International Biosphere Reserve in 1976 and World Heritage Site in 1983 (Jenkins 2007). Because of its large size (over 210,000 ha), biological diversity, and protected status, GSMNP serves a vital role in conservation within the southeastern United States and observed changes within the biota of GSMNP serve as baselines for comparison to other state and federal lands (Jenkins 2007). GSMNP was once extensively settled and 75% of the Park was logged prior to its establishment in 1934 (Pyle 1985), but forest communities in GSMNP have received minimal direct human disturbance over the last 75 years.

### Focal Species

*Juglans cinerea* typically grows in high-light environments along streams in the moist, fertile soils of riparian forests (Rink 1990), although the species has been observed on drier, rocky sites (Cogliastro et al. 1997). The loss of riparian habitats caused by agriculture, development, alteration to stream courses, and installation of dams has greatly diminished habitat suitable for *J.cinerea* (Clark et al. 2008). When marginal farmland was abandoned in the early-mid twentieth century, *J. cinerea* trees likely established in the resulting early-successional habitat. However, as forest canopies developed, subsequent cohorts of this highly shade-intolerant species were unable to successfully regenerate on sites with less frequent and intense disturbance (Ostry et al. 1994). Although *J. cinerea* has experienced extensive mortality ranging from 77% in the southeastern United States (USDA Forest Service 1995) to 92% in Wisconsin (Carlson and Guthmiller 1993), within GSMNP, numerous scattered individuals and groups of trees remain (Figure 1) within a continuous (non-fragmented) forest that may limit exposure to non-native genes and where high ridges further isolate watersheds.

*Juglans cinerea* has a range of historic and modern uses: Native Americans and early American settlers had several uses for the nuts, bark, and sap, including dyes, foods, and medicines (Ostry and Pijut 2000; Schultz 2003). Historically in areas where *J.cinerea* was common, its wood was nearly equal in value to that of *Juglans nigra* L. (black walnut) (Nicholls 1979). Although *J. cinerea* is largely a riparian species that rarely dominates stands, the loss of *J. cinerea* could have ecological impacts because it is a highly cold-tolerant hard mast species whose nuts are preferred by many species of wildlife (Ostry and Pijut 2000). In addition, *J. cinerea* litter has high concentrations of nitrogen and calcium compared to most other riparian species (Ricklefs and Matthew 1982).

### Survivorship, Health, and Distribution

To assess survivorship and health, we used a National Park Service (NPS) monitoring database to locate *J. cinerea* trees throughout GSMNP, systematically sampling watersheds to represent the full distribution of *J. cinerea*. We used site directions and geographical coordinates to search for individual trees, and those not found at specified locations were assumed to have died since the initial NPS surveys. We searched for 268 trees previously identified in the NPS records, of which we found 178 that were still living. We sampled a total of 207 mature trees in 19 watersheds. The 29 additional mature trees we sampled were newly identified individuals that were discovered in the course of our survey. For each tree, UTM coordinates, diameter at breast height (dbh; 1.4 m), and canopy position (dominant, codominant, intermediate, suppressed) were recorded. Although not all locations in the database were visited, we sampled as many as was logistically possible for a thorough representation of *J. cinerea* habitat.

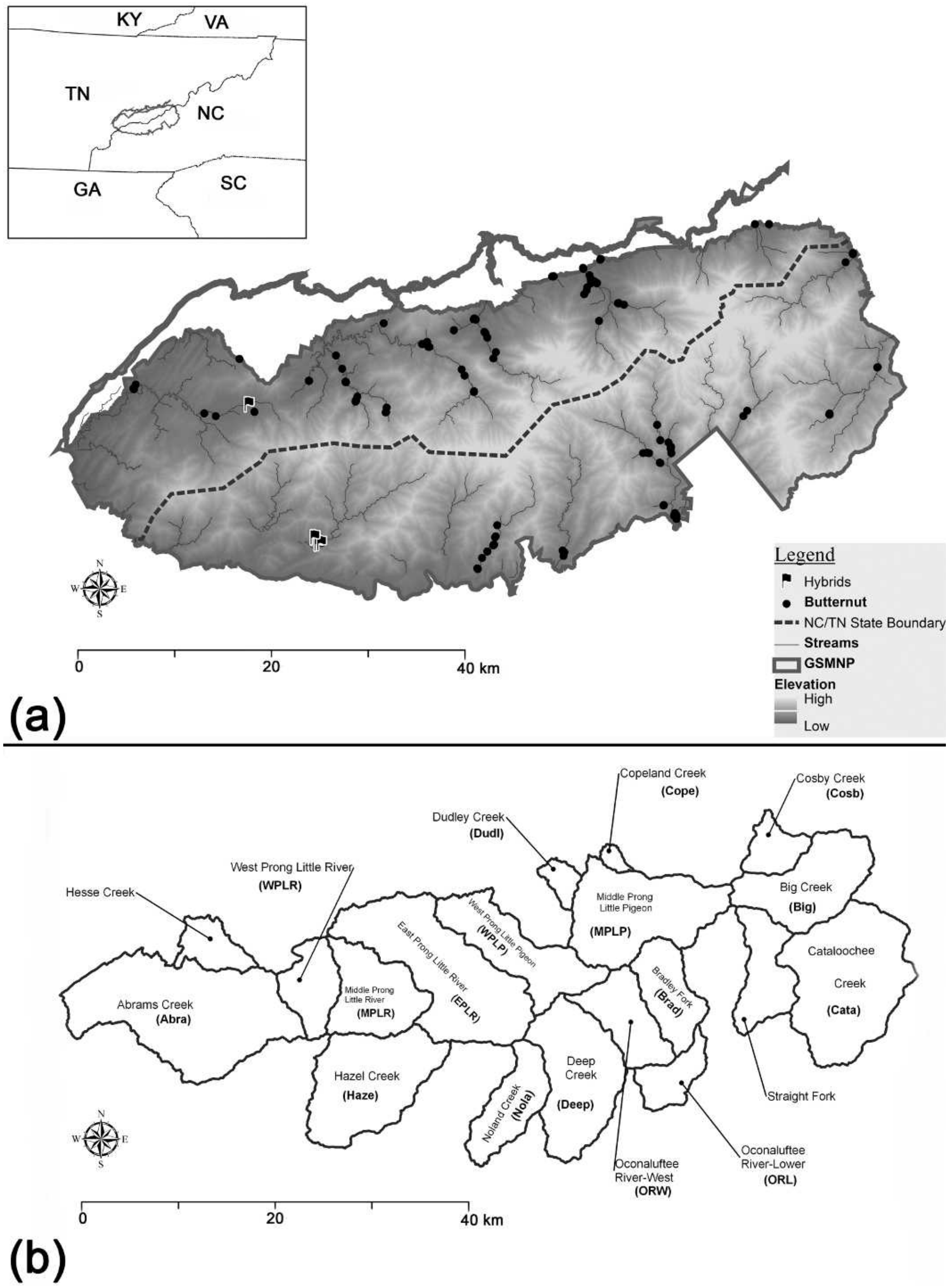

**Figure 1. (a) Locations of 207 *Juglans cinerea* trees surveyed across (b) 19 watersheds in Great Smoky Mountains National Park.**

We used several metrics to characterize disease severity of individual trees: percent live crown (the estimated proportion of the total tree length to the living crown length), vigor (percent of crown with dieback; see Figure 3 for class definitions), presence of epicormic sprouts, number of cankers below dbh, and percent of basal area girdled by cankers. In addition to ease of evaluation, the number of cankers below dbh is a consistent measure of disease because infection typically involves spores from the upper branches washing down the trunk and causing cankers at the tree base (Tisserat and Kuntz 1983).

To assess regeneration, we initiated surveys near adult trees and systematically expanded our search within a circle of approximately 100-m radius around each adult. For each seedling or sapling we found, we recorded UTMs and collected a leaf sample for genetic analysis to distinguish *J. nigra* from *J. cinerea*. Because seedlings of these two species are very similar, morphological identification is difficult.

We compiled spatial summary data using ArcMap (ESRI 2009) and GIS layers acquired from the NPS database (http://www.nps.gov/gis/data_info/) and determined elevation, vegetation association (as defined by White et al. 2003), disturbance history, and historic forest type for each tree location. We derived contemporary vegetation associations from the GSMNP vegetation map, which was developed using aerial photography interpretation, on-the-ground verification, and the national classification system developed by NatureServe (Madden et al. 2004). Disturbance history classes were derived from Pyle (1985), which classified human disturbance based upon ownership records and historic maps into the following categories: corporate logging, diffuse disturbance, diffuse disturbance with large residual trees, concentrated settlement, and undisturbed.

## Leaf Sampling and Genetic Testing

We collected leaf samples from 174 *J. cinerea* trees (including 18 seedlings or saplings) for genetic analysis. Between one and 30 trees were sampled from each watershed using an arborist's slingshot. Samples were refrigerated at the end of each day and shipped weekly to the genetics lab at Purdue University where DNA was extracted using a chloroform-phenol extraction method (Zhao and Woeste 2010). DNA concentration was measured using a NanoDrop-8000 spectrophotometer (Thermo Fisher Scientific, Wilmington, MA) and all samples were diluted to a common working concentration of 10ng/µL prior to PCR reactions.

We assessed hybridization status using one chloroplast marker and three nuclear markers (Zhao and Woeste 2010) to differentiate *J. cinerea* genotypes from *J. ailantifolia* and hybrids of the two species (Table 1). DNA from each individual was examined by performing polymerase chain reaction (PCR) as described by Zhao and Woeste (2010). The resulting product size (cut or uncut, based on the marker) was assessed using agarose gel electrophoresis. For each PCR reaction, control DNA was amplified from individuals of known species origin. In addition, all seedling and sapling samples and several mature trees that were tagged as morphologically cryptic were assessed using the ITS marker, which paired with chloroplast marker *trnT-F*, identifies *J. nigra*: individuals with a *J. cinerea* genotype for the *trnT-F* marker, but a *J. ailantifolia* genotype at the ITS marker were considered black walnut (Zhao and Woeste 2010). Sixteen individuals identified as *J. nigra* were removed from subsequent analyses, but retained as controls for genetic tests.

## Plot Sampling

We installed a total of 20 vegetation plots across 13 watersheds in areas containing *J. cinerea* trees to assess site conditions and overstory composition. Some watersheds were not sampled due to lack of appropriate sites for plot installation (e.g., developed areas and roadsides). Nevertheless, plots were distributed throughout GSMNP and included a range of elevations. We recorded the dbh by species of all stems ≥ 10 cm dbh within a 500-$m^2$ plot. These data were used to calculate relative basal area (species basal area/total basal area of all species), relative density (species density/total density), and importance value (relative density + relative basal area/2) for each species on each plot.

## Vegetation Data Analysis

We used species importance values calculated from our 20 plots to perform nonmetric multidimensional scaling (NMS) based on Sorensen's distance to assess the distribution of tree species and plots along environmental gradients (McCune and Grace 2002). We used random starting configurations and 50 runs with real data with three to five runs performed on each data set. Field and GIS-derived environmental variables (elevation, distance to stream, and distance to former homesite), overstory basal area, and stand age were correlated with each ordination axis and plotted as vectors within ordination space. Distances between plot locations and nearest features in the streams and homesites layers were calculated using Near analysis in ArcMap (ESRI 2009). Stand age was determined from a representative *J. cinerea* tree nearest the plot. Two-way cluster analysis (using Ward's linkage method and Euclidean distance measure) was performed to examine species patterns related to clustering of plots and confirm NMS results.

**Table 1. Markers used in hybrid identification. CAPS: cleaved amplified polymorphic sequence; SCAR: sequence characterized amplified region. See Zhao and Woeste (2010) for additional details.**

| Marker | Type of Marker | *J. ailantifolia* product size (bp) | *J. cinerea* product size (bp) | Hybrid product size (bp) | *J. nigra* product size (bp) |
|---|---|---|---|---|---|
| trnL | CAPS-chloroplast | 471, 380 | 536, 453 | 536, 471, 453, 380 | 536, 453 |
| 22-5 | SCAR-nuclear | 705 | 660 | 705, 660 | --- |
| 16R-2 | SCAR-nuclear | 300 | None | 300 | --- |
| 14R-1 | CAPS-nuclear | 228, 167, 127, 40 | 229, 167 | 229, 228,167, 127, 40 | --- |
| ITS | CAPS-ribosomal | 305, 240, 195 | 430, 305 | 430, 305, 240, 195 | 305, 240, 195 |

### Tree Core Sampling and Analysis

To assess the age of the dominant *J. cinerea* cohort, we collected tree cores from a subset of the 207 trees we located. Trees were selected to represent the spatial distribution of living *J. cinerea* trees across sampled watersheds. All trees were cored at 0.5 m above the ground using a 4.3 mm increment borer. The borer was sterilized with alcohol between sampling individual trees (Clark et al. 2008) to prevent spread of the pathogen. At the request of the NPS, trees showing no sign of disease (< 1% of all trees we encountered) were not cored.

To assess recruitment and cohort structure, we intensively sampled within five watersheds, coring all *J. cinerea* trees within the forest interior (excluding trees along roads and trails), from which an intact sample could be collected. We sampled 8 – 18 individuals per watershed (Table 2). For each of these individuals, we also cored the two nearest overstory competitors to determine the composition of the developing forest at the time of *J. cinerea* regeneration and examine the temporal pattern of recruitment amongst competing species.

Cores were mounted on boards, sanded with a belt sander, and then hand-sanded with increasingly finer grit sand paper (Clark et al. 2008). The annual rings were counted using a dissecting scope. For cores that did not include the pith, we estimated the number of missing years using a graphic with equally-spaced concentric rings equal to the average growth of the innermost rings (Applequist 1958). Because the limited number of surviving trees made cross-dating difficult, trees were placed into five-year recruitment classes to account for possible errors in determining exact year of establishment.

## RESULTS

### Survivorship, Health, and Distribution

Based on our resurvey of *J. cinerea* trees in the NPS database, we estimate survivorship to be 68% over the past two decades. Although *J. cinerea* trees ranged in size from 7 – 74 cm dbh, there was a paucity of individuals in the small diameter classes (Figure 2). Most trees (49%) were classified as codominant, with 9%, 25%, and 13% classified as dominant, intermediate, and suppressed, respectively. Only 14 *J. cinerea* seedlings and saplings were found.

Individual *J. cinerea* trees exhibited a range of disease severity and often trees of similar size within the same area were in different stages of decline. Most trees had a vigor class rating of 2, indicating 1% – 25% crown dieback (Figure 3). Most crowns were small in relation to total tree height, presumably due to disease effects and loss of canopy due to shading. Cankers ranged in size from several centimeters to over 1 meter in length. Epicormic sprouts occurred on 58% of trees. Healthy and severely diseased individuals were evenly distributed across all watersheds.

*Juglans cinerea* occurred across a fairly narrow range of site conditions. Most trees occurred between 400 and 700 m elevation and within 12 of the 37 associations described in the Park vegetation map, with most trees occurring in floodplain (33%) and successional hardwood (28%) forest associations. Fewer trees occurred in cove forests and areas of 'human influence,' which largely consisted of old fields and margins around front-country development. According to the disturbance classes of Pyle (1985), most *J. cinerea* trees (74%) occurred in locations historically dominated by settlement.

### Hybridization

Based on analysis of hybrid identification markers, five out of the 174 samples had hybrid genotypes at all four markers. These hybrids were found in two locations, Cades Cove in the Abrams Creek watershed and the Hazel Creek watershed (Figure 1). Prior to the establishment of GSMNP, Cades Cove was a large agricultural community and the Hazel Creek area once contained Proctor, a town with large logging operations. Only one of five trees sampled in Cades Cove was a hybrid, but all four mature trees sampled in Hazel Creek were hybrids. We cored only one hybrid (from Hazel Creek), which established in 1971. No seedlings or saplings were identified as hybrids.

**Table 2. Number of *J. cinerea* trees surveyed, number of leaf samples, and core samples collected per watershed for the five intensively sampled watersheds. In addition to *J. cinerea* trees, cores were collected from the two nearest canopy competitors in these watersheds. The numbers of samples from the 14 non-intensive watersheds are grouped (Other watersheds; competitors not sampled). Total trees and leaf sample numbers include samples from 14 seedling/saplings that were not used in health assessments of mature trees.**

| Watershed | Number of Trees | Number of Leaf Samples | Number of Core Samples |
|---|---|---|---|
| Middle Prong Little Pigeon | 38 | 30 | 12 |
| Oconaluftee River (Lower) | 31 | 27 | 18 |
| Middle Prong Little River | 25 | 18 | 13 |
| Noland Creek | 13 | 9 | 10 |
| Bradley Fork | 9 | 8 | 8 |
| Other watersheds | 105 | 82 | 20 |
| **Grand Total** | **221** | **174** | **81** |

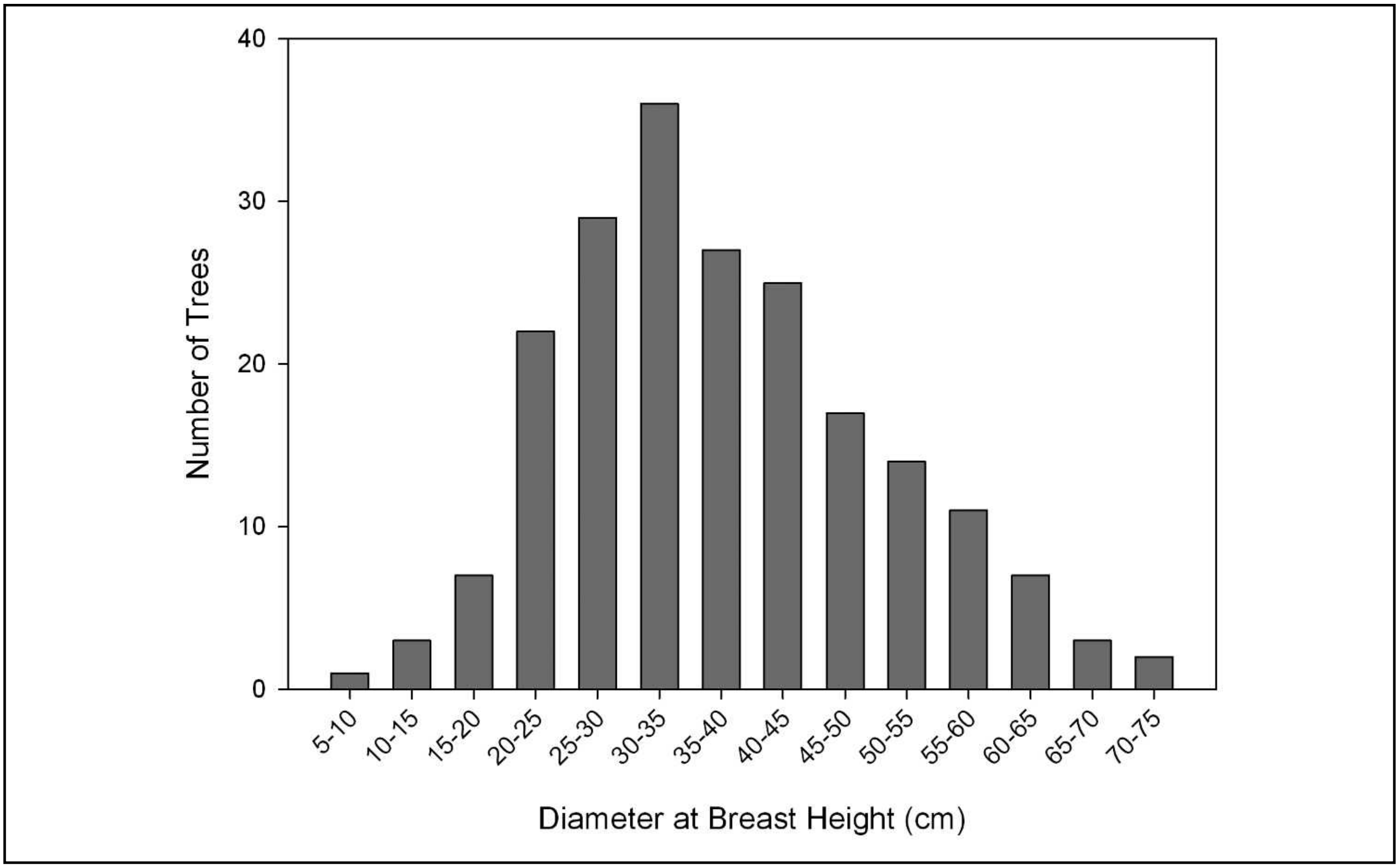

**Figure 2. Diameter distribution in 5 cm DBH classes for 207 *Juglans cinerea* trees surveyed across Great Smoky Mountains National Park.**

### Vegetation Composition

NMS ordination resulted in a two-dimensional solution with a final stress value of 13.86. A Monte Carlo test revealed that the stresses of both Axis 1 ($p = 0.02$) and Axis 2 ($p = 0.05$) were less that than that produced by randomized data. The cumulative variance explained by the ordination was 88% with Axis 1 explaining the most variance ($R^2 = 0.618$) followed by Axis 2 ($R^2 = 0.261$). Distance to homesite and total overstory basal area exhibited the strongest relationships with Axis 1 ($R = -0.478$ and 0.527 respectively), while elevation ($R = -0.609$) exhibited the strongest relationship with Axis 2 (Figure 4, Table 3). Four plot locations clustered away from all other plots and were associated with increased distance from homesites and lower overstory basal area (Figure 4). Two-way cluster analysis clearly separated these four plots at the first division of the dendrogram, supporting the results of the NMS ordination (Figure 5). Although on average *Liriodendron tulipifera* L. (tulip poplar) was the dominant species across our plots, it was absent or had low basal area on all four of the differentiated plots. Another common species, *Carpinus caroliniana* Walter (musclewood), was absent from all but one of these plots. Both of these species were also separated by the first division of the dendrogram (Figure 5). Three out of four of these plots contained large basal area of *J. cinerea*, and two exhibited the greatest *J. cinerea* importance values of any plot. Bubble plots of species importance onto the species ordination plot confirmed these results: *J. cinerea* importance value was inversely related to the importance of *L. tulipifera, Liquidambar styraciflua* L. (sweetgum), and *C. caroliniana* and was associated with greater distance from homesites and lower stand basal area (Figures 4 and 6).

### Recruitment

The oldest *J. cinerea* tree we sampled established in 1925 and the youngest established in 2001 (Figure 7). Data pooled across all watersheds revealed an initial surge of regeneration that continued from the time of Park establishment in the mid-1930s to around 1980, after which regeneration declined dramatically: only one sampled tree established after 1980, and only 14 immature trees were located in our survey. Generally, individual watersheds exhibited trends similar to the pooled watersheds, with recruitment spanning the same time frame but with some variability in initial *J. cinerea* establishment or peak year (Figure 8). The establishment of competitors peaked around the same time as *J. cinerea*, but competitor recruitment was generally more continuous through time (Figure 8).

## DISCUSSION

### Survivorship, Health, and Distribution

Our survivorship estimate of 68% since the late 1980s was considerably greater than

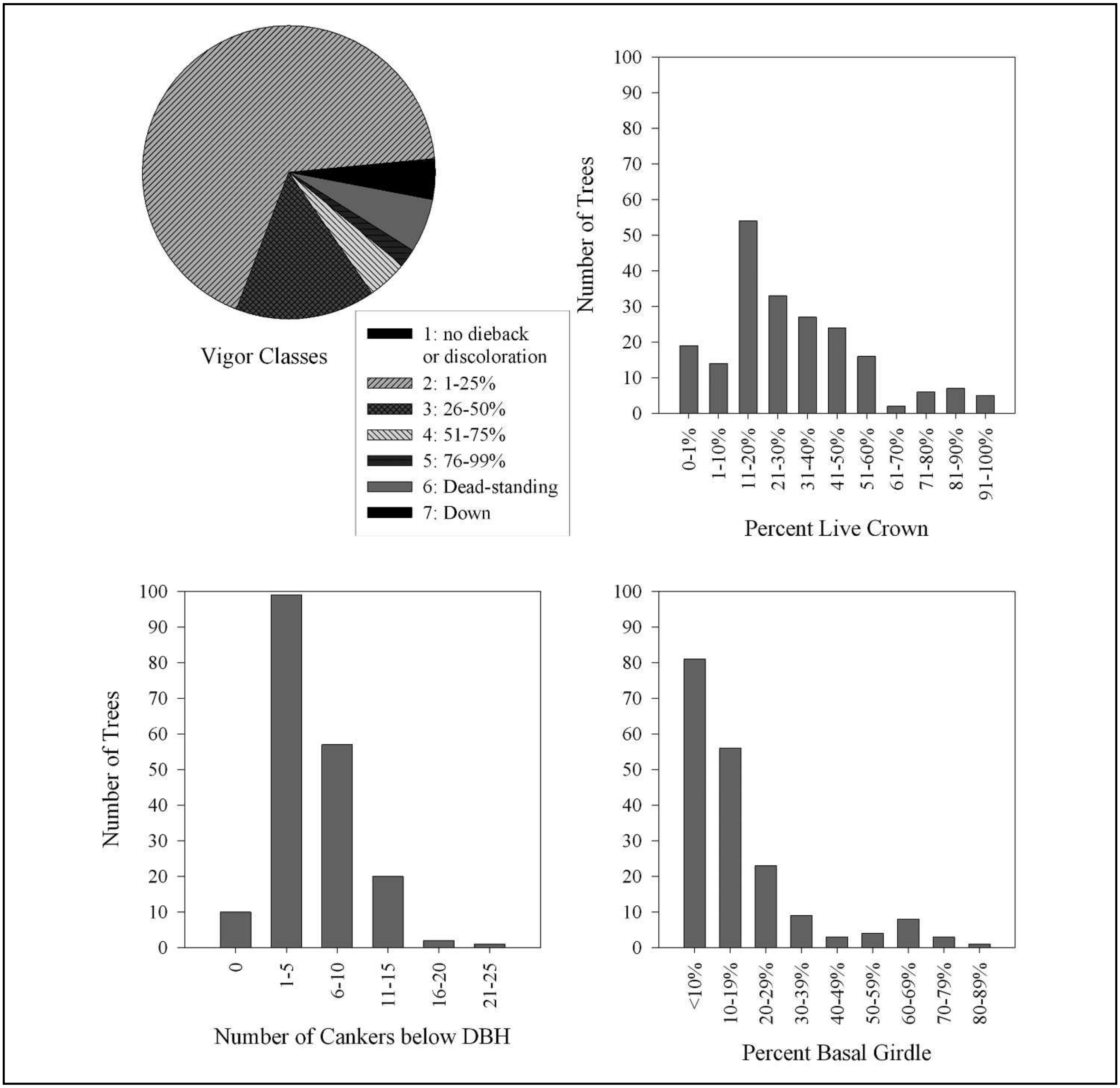

**Figure 3. Health data summary for 207** *Juglans cinerea* **trees surveyed across 19 watersheds in Great Smoky Mountains National Park. Vigor class represents the percent of the crown with dieback or discoloration. Percent live crown represents the proportion of the total tree length to the living crown length.**

the 23% survivorship observed in North Carolina and Virginia between 1966 and 1986 (USDA Forest Service 1995). This earlier (USDA Forest Service) estimate may reflect mortality since the onset of disease; but the disease had presumably been active in the southeastern United States for at least two to three decades before the NPS surveys (Ostry et al. 2003), and many individuals likely died prior to the initiation of monitoring. Consequently, the Park database may not capture the full extent of mortality and the individuals we surveyed likely represent a group that survived the initial wave of disease. While cankers were present on most trees we sampled, most had < 20% girdling, suggesting that they have been able to limit canker growth and avoid girdling. Since we know the pathogen produces a prolific number of spores that can be spread by precipitation, wind, and living vectors (Kuntz et al. 1979; Tisserat and Kuntz 1983), we assume all individuals clustered closely in a watershed had

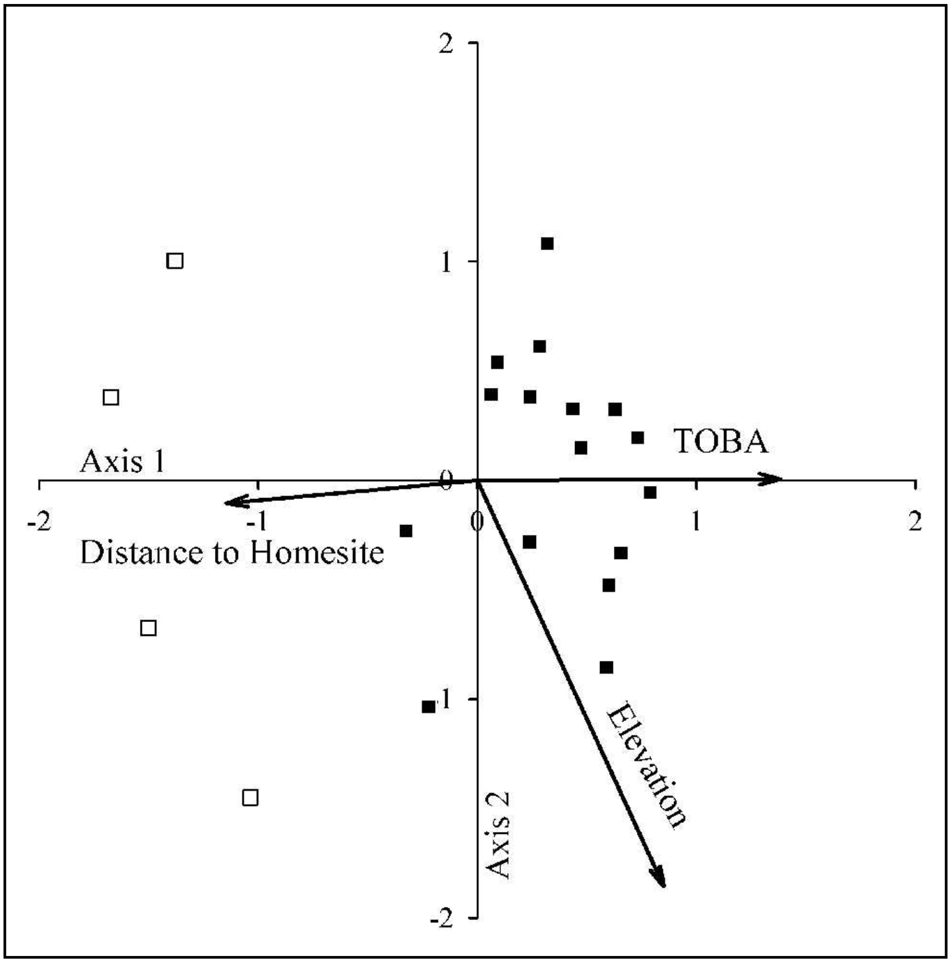

**Figure 4. Non-metric multidimensional scaling ordination of overstory vegetation data from 20 plots distributed across 13 watersheds in Great Smoky Mountains National Park. Dominant environmental variables are represented as vectors [distance to homesite, elevation, total overstory basal area (TOBA)]. Hollow squares represent the four plots that clustered away from other plots, and are characterized by high *Juglans cinerea* basal area and low *Liriodendron tulipifera* (LIRITUL) basal area relative to all other plots.**

about the same time interval of exposure to the pathogen. If true, the variation in health among living trees in our study may reflect varying levels of disease resistance. Individuals that remained healthy in close proximity to dead or declining trees may be the best sources for resistance genes since it is unlikely that they escaped infection and may have qualities that enabled them to better tolerate the canker disease.

Our results indicate that *J. cinerea* occurs with greatest frequency under a fairly limited range of environmental conditions (400 – 700 m elevation, low stand basal area, floodplain forests). Other studies have described *J. cinerea* as occurring in low elevation alluvial floodplains, but also in upland areas and cove forests with sufficient light and well-drained soils (Ostry et al. 2003). When assessing the distribution of *J. cinerea*, land-use history must be considered because human-mediated dispersal of this species and its congeners may be reflected in the current distribution and hybridization status of populations. In addition, anthropogenic disturbance likely created the site conditions under which *J. cinerea* historically regenerated and persisted. Although *J. cinerea* seeds are dispersed naturally by rodents, streams, or gravity (Schultz 2003), human activity also likely contributed to the movement of *J. cinerea* in GSMNP because trees historically were planted at homesites and farms (Ross-Davis et al. 2008). While we cannot determine whether the origin of *J. cinerea* in a given watershed was through

**Table 3. Environmental variables (Mean ± 1 standard error) and associated correlations with nonmetric multidimensional scaling (NMS) ordination axis scores for 20 overstory vegetation sampling plots sampled across 13 watersheds.**

| | | | | Axis 1 | | Axis 2 | |
|---|---|---|---|---|---|---|---|
| Variable | Mean±SE | Min | Max | r | P-value | r | p-value |
| Elevation (m) | 623.2 ± 35.4 | 347 | 907 | 0.131 | 0.581 | -0.609 | 0.004 |
| Distance to Stream (m) | 41.2 ± 6.2 | 5.6 | 93.8 | 0.269 | 0.252 | 0.099 | 0.678 |
| Distance to Homesite (m) | 289.1 ± 108.8 | 14.3 | 1888.3 | -0.478 | 0.033 | -0.145 | 0.541 |
| Total Plot Basal Area (m2/ha) | 36.5 ± 3.3 | 10.1 | 78.5 | 0.527 | 0.017 | 0.025 | 0.917 |
| Stand Age (years) | 54.3 ± 3.8 | 23 | 85 | -0.042 | 0.86 | -0.313 | 0.179 |

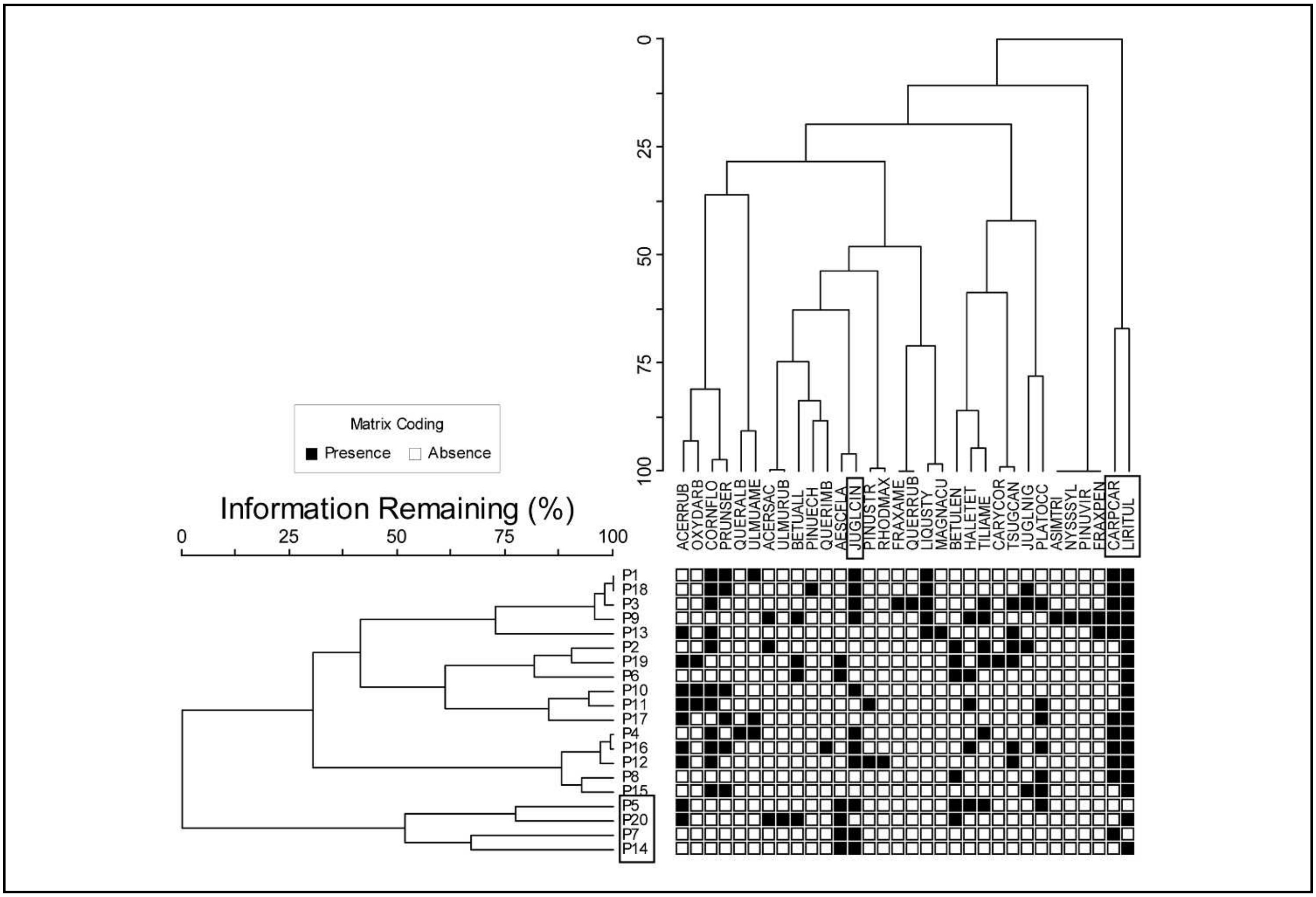

**Figure 5. Two-way dendrogram of plots and overstory species in 20 vegetation plots distributed across 13 watersheds in Great Smoky Mountains National Park. The four plots described in Figure 4 are highlighted in the plot/sample column. *Liriodendron tulipifera* (LIRITUL), *Carpinus caroliniana* (CARPCAR), and *Juglans cinerea* (JUGLCIN) are highlighted in the species row. Other species: *Acer rubrum* (ACERRUB), *Acer saccharum* (ACERSAC), *Aesculus flava* (AESCFLA), *Asimina triloba* (ASIMTRI), *Betula alleghaniensis* (BETUALL), *Betula lenta* (BETULEN), *Carya cordiformis* (CARYCOR), *Cornus florida* (CORNFLO), *Fraxinus americana* (FRAXAME), *Fraxinus pennsylvanica* (FRAXPEN), *Halesia tetraptera* (HALETET), *Juglans nigra* (JUGLNIG), *Liquidambar styraciflua* (LIQUSTY), *Magnolia acuminata* (MAGNACU), *Nyssa sylvatica* (NYSSSYL), *Oxydendrum arboreum* (OXYDARB), *Pinus echinata* (PINUECH), *Pinus strobus* (PINUSTR), *Pinus virginiana* (PINUVIR), *Platanus occidentalis* (PLATOCC), *Prunus serotina* (PRUNSER), *Quercus alba* (QUERALB), *Quercus imbricaria* (QUERIMB), *Quercus rubra* (QUERRUB), *Rhododendron maximum* (RHODMAX), *Tilia americana* (TILIAME), *Tsuga canadensis* (TSUGCAN), *Ulmus americana* (ULMUAME), *Ulmus rubra* (ULMURUB).**

human planting or natural reproduction, most of the contemporary trees we sampled established after original landowners were removed from GSMNP.

## Hybridization

Although widespread settlement in the southern Appalachians may have promoted the introduction of *J. ailantifolia*, we hypothesized that the isolation, topographic complexity, and lack of fragmentation in GSMNP favored low occurrence of hybrids compared to more-fragmented landscapes. Hybrids comprised < 3% of our sampled trees, but hybridization rates over 90% have been documented in more fragmented landscapes (Hoban et al. 2009). Within GSMNP, we theorized that increased isolation from developed areas provided a buffer from hybridization due to increased barriers to invasion by non-native pollen. Also, because *J. ailantifolia* was planted, areas with higher historic human populations should have higher proportions of hybrids. Anecdotally, this appears to be the case. The few hybrids we observed were in historically settled areas near the present-day Park boundary. However, other sampled watersheds were formerly settled and near the Park boundary, but exhibited no evidence of hybridization. Existing hybrids may also be the progeny of *J. ailantifolia* trees that were planted within GSMNP.

## Vegetation Composition

NMS analysis revealed an association between greater abundance of *J. cinerea* and lower importance of other early-successional species. *Liriodendron tulipifera* was the dominant species across our study sites and sustained disturbance associated with land use at homesites (soil disturbance, lack of litter accumulation) may have created seedbed conditions that favored germination and establishment of this and other small-seeded and widely-dispersed species (Hosner and Graney 1970; Jenkins and Parker 2001). Reduced dominance of *L. tulipifera* and other species farther away from homesites may have allowed *J. cinerea* to survive because of reduced competition. Plantation studies have shown that controlling competing vegetation is critical for the successful survival and growth of *J. cinerea* seedlings (Lambert et al. 1994).

## Recruitment

Based on existing knowledge of butternut silvics, we predicted that contemporary *J. cinerea* trees regenerated following the abandonment of agricultural land and that subsequent recruitment in maturing forests would be rare. The overall pattern of recruitment we observed across all watersheds exhibited an initial peak in the late 1940s, approximately 10 – 15 years after Park establishment. A similar lag time has been observed for the establishment of other early successional species in abandoned agricultural fields (Peroni 1994; Jenkins and Parker 2000). Clark et al. (2008) found most *J. cinerea* regeneration occurred within nine years; but, unlike the abandoned fields in GSMNP, their data were collected following clearcutting. Secondary succession following harvest is typically the result of stump sprouting or seeds from the intact seedbank (Oliver 1981), but advanced regeneration and seeds of tree species are typically reduced or eliminated following decades of agricultural use (Wijdeven and Kuzee 2000). Since *J. cinerea* seeds are not stored in the seed bank, dispersal from nearby sources would be necessary for re-establishment. Although *J. cinerea* seedlings may perform well in the open conditions of abandoned fields, its large seeds may make re-establishment difficult due to limited dispersal (Battaglia et al. 2008).

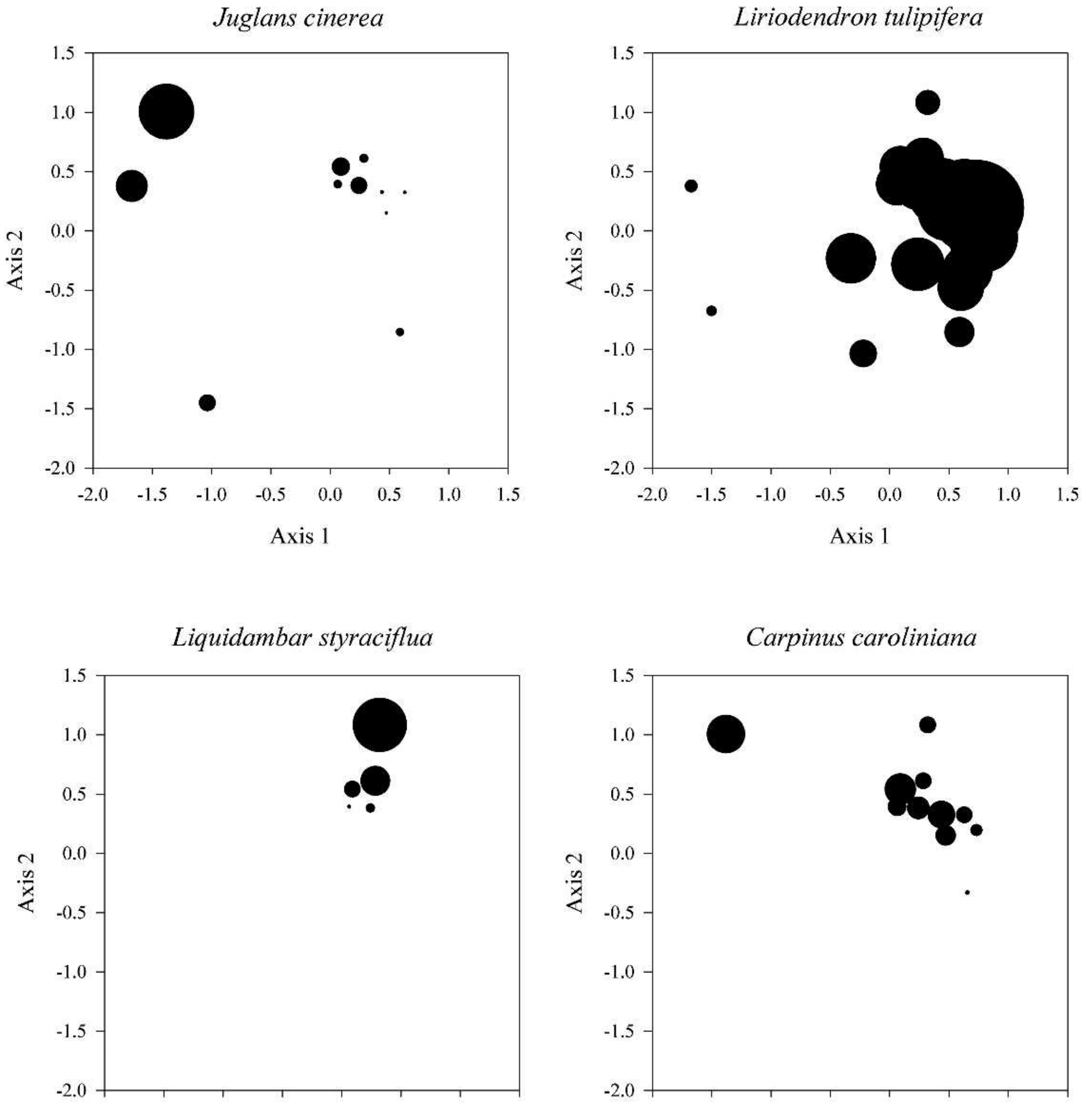

**Figure 6. Bubble plots of overstory species importance value overlaid onto the NMS overstory ordination. Symbol size is proportional to the importance value (relative density + relative basal area/2) of a species on a given plot.**

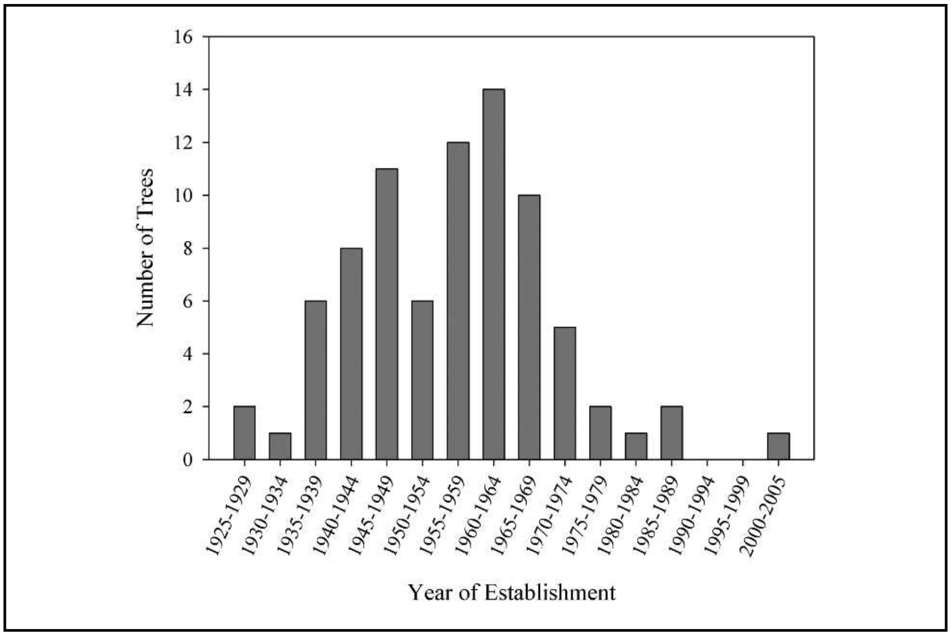

**Figure 7. *Juglans cinerea* recruitment within five-year intervals between 1925 and 2005 pooled across all sampled watersheds in Great Smoky Mountains National Park. Tree cores were collected in 2010-11.**

We identified continuous recruitment of competing species concurrent with recruitment peaks of *J. cinerea*, indicating that *J. cinerea* was able to compete, to some degree, with other trees in developing stands. As the forest matured, competition intensified and light became less available for subsequent regeneration. The precipitous drop in regeneration after 1980 likely resulted from continued forest development and the effects of disease. Regeneration that did occur likely had low survival due to shading and disease (Kuntz et al. 1979).

The lack of regeneration over the past several decades has serious implications for future *J. cinerea* conservation, regardless of disease impacts. While *J. cinerea* can establish in silvicultural openings, overstory competition in post-harvest stands may accelerate decline (Clark et al. 2008). In our study, the oldest cohort of *J. cinerea* trees established on abandoned agricultural land where substrate disturbance and a lack of vegetation legacies resulted in a reduced rate of successional development and, consequently, reduced interspecific competition. Site alterations that reduce competition may be needed to promote the establishment and persistence of the species, even if disease-resistant lineages are developed in the future (Thompson et al. 2006). Although *J. cinerea* often successfully establishes in natural forest openings, such as along stream banks, habitat destruction in riparian areas and reduced population densities may necessitate active management and the creation of forest openings to supplement natural reproduction (Ostry et al. 1994). In addition to managing disease effects, different management prescriptions may be necessary for different life stages of *J. cinerea* to promote regeneration, establish new cohorts, and facilitate continued recruitment (Woeste et al. 2009).

## Conclusions and Management Implications

Other studies have reported frequent hybridization of *J. cinerea* with its non-native congener *J. ailantifolia*. The frequency of hybridization in our study was quite low, however. Plant populations in unfragmented landscapes, such as GSMNP, may be buffered from gene swamping by invasive species. As such, these landscapes may serve as refugia for genetically-uncontaminated populations and may play a critical role in maintaining the diversity of the species. Future restoration efforts should focus on unfragmented landscapes where newly-established populations are protected from genetic contamination. Although butternut canker was present in all areas, we observed many vigorous trees that displayed symptoms of low disease severity. Natural selection may have increased the frequency of these relatively healthy-appearing trees; butternut canker is thought to have been present in the study area for over 50 years and many highly susceptible trees may have already died, leaving trees that have relatively greater resistance (Ingwell and Preisser 2010). Genetically pure and diverse populations, such as those found in GSMNP, may prove critical to future efforts to breed resistant genotypes of this species by serving as sources of propagation material for restoration efforts centered on intraspecific (non-hybrid) disease resistance.

Breeding efforts designed to enhance disease resistance in *J. cinerea* may require the introduction of genes from *J. ailantifolia* in a manner similar to the breeding programs using non-native genes for blight resistance in *C. dentata* (Griffin 2000; Jacobs 2007). However, additional studies are necessary to determine the ecological implications of this strategy. Although it remains to be seen if *J. cinerea* restoration will require the incorporation of non-native genes from *J. ailantifolia*, we recommend that any introduction of non-native genes into the gene pool of *J. cinerea* or other native species should be minimal. Hybrids used for restoration need to be carefully evaluated for altered traits such as morphology, environmental tolerance, and competitive ability that will affect their ability to fill the ecological niche of *J. cinerea*.

Most *J. cinerea* trees in the watersheds we sampled were recruited following agricultural land abandonment. Contemporary populations persist in areas where prior disturbance created an environment of reduced interspecific competition. Over the past 30 years, *J. cinerea* recruitment has declined severely due, in part, to a less-intensive contemporary disturbance regime. Following the development of resistant genotypes, active restoration of this species will require control of competing vegetation to allow planted trees to successfully reach the forest canopy and substrate disturbance combined with

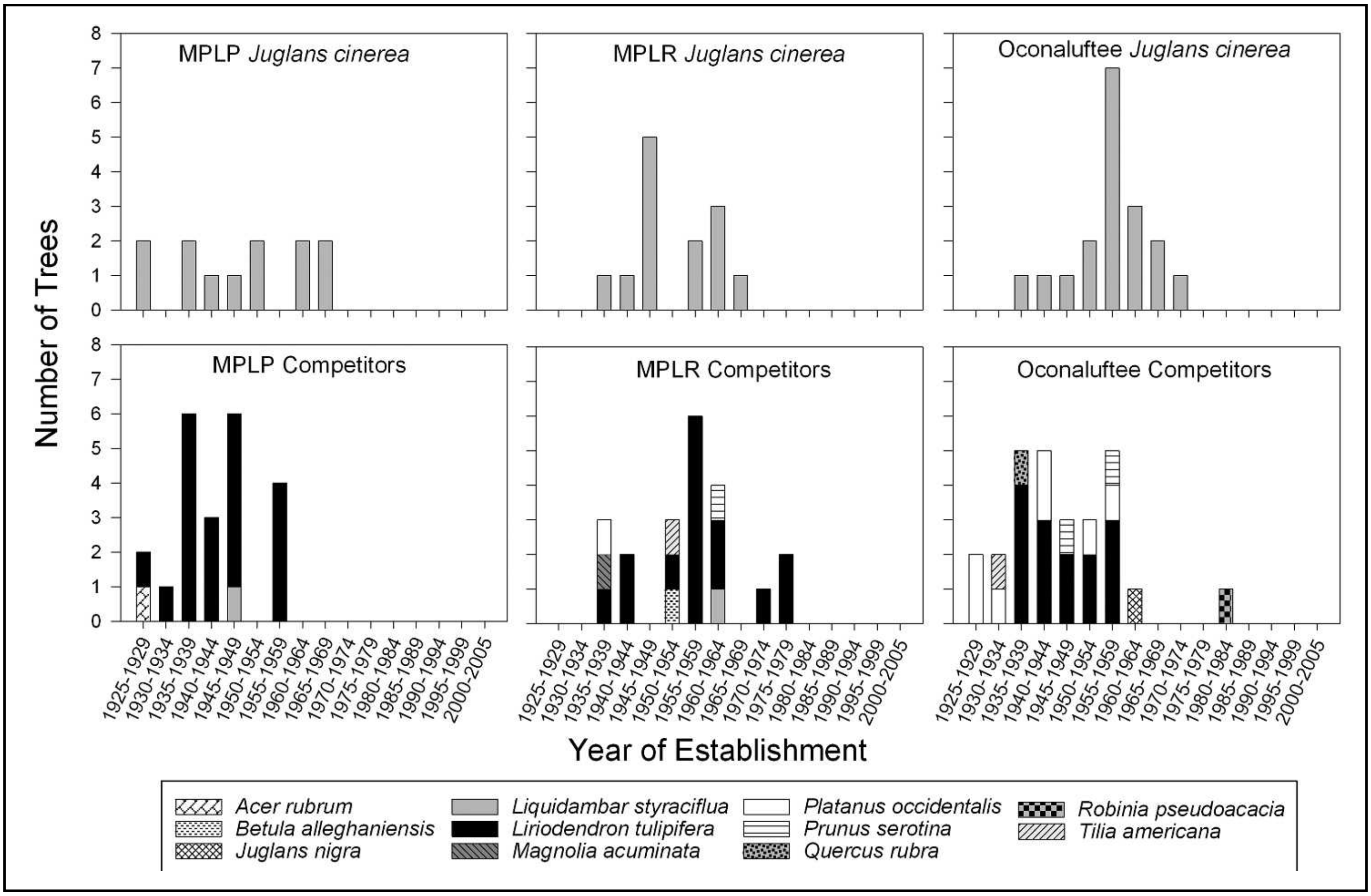

**Figure 8. Recruitment of *Juglans cinerea* and competitors within five-year intervals in three watersheds with greater than 12 tree core samples: MPLP (Middle Prong Little Pigeon), MPLR (Middle Prong Little River) and Oconaluftee River.**

canopy thinning or removal to allow successful germination and establishment of seedlings.

## ACKNOWLEDGMENTS

We thank the staff of GSMNP for their support, especially Tom Remaley, Janet Rock, Keith Langdon, Kristine Johnson, Glenn Taylor, Emily Darling, Emily Guss, and Paul Super. We thank Peng Zhao, Michael Saunders, and Robert Morrissey for their assistance. Kim Steiner, Eric Holzmueller, and three anonymous reviewers provided helpful comments on earlier versions of the manuscript. This work was supported by the Hardwood Tree Improvement and Regeneration Center (USDA FS NRS) and the Department of Forestry and Natural Resources at Purdue University. The use of trade names is for the information and convenience of the reader and does not imply official endorsement or approval by the USDA or the Forest Service of any product to the exclusion of others that may be suitable.

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# Conservation Status of a Threatened Tree Species: Establishing a Baseline for Restoration of *Juglans cinerea* L. in the Southern Appalachian Mountains, USA

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Source: Natural Areas Journal, 33(4):413-426. 2013.
Published By: Natural Areas Association
DOI: http://dx.doi.org/10.3375/043.033.0404
URL: http://www.bioone.org/doi/full/10.3375/043.033.0404