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ARTICLE

Butternut (*Juglans cinerea*) health, hybridization, and recruitment in the northeastern United States

A. Boraks and K.D. Broders

Abstract: Butternut (*Juglans cinerea* L.) trees are being extirpated from their natural range by an epidemic caused by a fungal pathogen. Widespread mortality is reminiscent of past epidemics on American chestnut (*Castanea dentata* (Marsh.) Borkh.) and American elm (*Ulmus americana* L.). Butternut has remained relatively understudied, resulting in unsampled areas and gaps in our understanding of this forest epidemic and the future outlook of this species in North America. The previously unsampled area consisting of the northeastern United States was surveyed for the presence of *J. cinerea*, and several population health metrics were recorded, including recruitment, disease pressure, and hybridization. A total of 252 butternut trees were sampled. Analysis indicates that there is insufficient *J. cinerea* recruitment to maintain population sizes. Further compounding low recruitment, butternut saplings demonstrate elevated levels of disease impact from the fungal pathogen *Ophiognomonia clavigignenti-juglandacearum* Broders & Boland. Natural hybridization of butternut with introduced congenics such as *Juglans ailantifolia* Carrière is strongly associated with lower disease impact. Hybrid trees displayed an average of 2.4 cankers per tree compared with 4.5 cankers for nonhybrid butternut. Further niche and resistance studies are required to assess whether butternut hybrids can replace butternut in a natural setting. It still remains uncertain whether tree size or habitat affect disease impact; however, smaller trees, often residing in riparian habitats, were found to have a greater number of cankers. The data presented here, combined with past studies, provide critical information for use in butternut management strategy plans.

Key words: *Juglans cinerea*, butternut canker, hybrid, demographic, endangered species.

Résumé : Une épidémie causée par un champignon pathogène est en voie de faire disparaître le noyer cendré (*Juglans cinerea* L.) de son aire naturelle. L'ampleur de la mortalité rappelle les épidémies passées sur le châtaignier d'Amérique (*Castanea dentata* (Marshall) Borkh.) et l'orme d'Amérique (*Ulmus americana* L.). Le noyer cendré a été relativement peu étudié, d'où les régions non échantillonnées et les lacunes dans notre compréhension de cette épidémie et des perspectives d'avenir de cette espèce en Amérique du Nord. La région qui n'a pas encore été échantillonnée, soit le nord-est des États-Unis, a été inventoriée pour la présence du noyer cendré et plusieurs métriques associées à l'état de santé de la population ont été notées incluant le recrutement, la pression de la maladie et l'hybridation. Au total, 252 tiges de noyer cendré ont été échantillonnées. L'analyse révèle que le recrutement du noyer cendré est insuffisant pour maintenir la taille des populations. Aggravant davantage le faible recrutement, les gaules de noyer cendré subissent un impact sévère de la maladie causée par le champignon pathogène *Ophiognomonia clavigignenti-juglandacearum* Broders & Boland. L'hybridation naturelle du noyer cendré avec des espèces co-isogéniques introduites telles que *Juglans ailantifolia* Carrière est étroitement associée à un plus faible impact de la maladie. Les arbres hybrides avaient en moyenne 2,4 chancres par arbre comparativement à 4,5 chancres sur les noyers non hybrides. D'autres études de niche et de résistance sont nécessaires pour déterminer si les noyers hybrides peuvent remplacer le noyer cendré dans un environnement naturel. On ne sait toujours pas avec certitude si la taille des arbres ou l'habitat influencent l'impact de la maladie. Cependant, les arbres plus petits, souvent présents dans les habitats riverains, avaient un plus grand nombre de chancres. Les résultats présentés ici combinés aux études antérieures fournissent une information cruciale pour planifier la stratégie d'aménagement du noyer cendré. [Traduit par la Rédaction]

Mots-clés : *Juglans cinerea*, chancre du noyer cendré, hybride, population, espèce menacée.

1. Introduction

Juglans cinerea L., known as the butternut tree or white walnut, is an economically and ecologically important member of the eastern deciduous forest. Native to eastern North America, the range of butternut stretches from southern New Brunswick and Quebec west to Minnesota and as far south and southwest as Georgia and Missouri. This medium-sized tree is not abundant in forests (Delcourt 1979) and is usually found as small stands associated with other mixed hardwoods (Schultz 2003). Butternut is a relatively short-lived species, reaching maturity at 15–20 years and rarely exceeding 75 years in age (Ostry et al. 2003). A wind-pollinated species, butternut produce significant seed crops every 2–3 years, with lighter yields during interim years (Rink 1990). The heavy seed requires dispersal by gravity, water, or scavengers and may not survive adjacent to the parent tree due to allelopathy and resource competition (Hartman et al. 2000). Maternally produced naphthoquinone combined with shade intolerance, seed predation by animals, and narrow range of habitable growing sites make sapling recruitment infrequent.

Further compounding the effects of low recruitment, butternut is under threat of extinction by an exotic fungus. A notable disease on butternut was first reported in Wisconsin in 1967 (Renlund 1971) and is widely known as butternut canker caused by the ascomycete fungus *Ophiognomonia clavigignenti-juglandacearum*

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(*Oc-j*) Broders & Boland (Broders and Boland 2011). Today *Oc-j* threatens butternut throughout the natural range and is responsible for mortality rates approaching 80% (Ostry et al. 2003). Estimations of mortality rates appear low, as trees infected with *Oc-j* normally perish within several years from canker girdling (personal observation). *Oc-j* can infect butternut trees of all ages, providing an additional stressor on low recruitment. The devastating effect of this disease has led to the butternut being designated as an Endangered Species in Canada in 2003 (Neilson et al. 2003), and a recovery strategy has been outlined based on previous survey data (Poisson and Ursic 2013). The United States (US) has yet to list butternut as an endangered species, likely due to an incomplete assessment of the trees' status in the northeastern US where suitable habitat is present but surveys have not been conducted to determine population health.

Initially discovered in Wisconsin in 1967, butternut canker was not present in New England and New York (Anderson and LaMadelaine 1978). First reports of butternut canker in the northeastern US began in 1982 (Kostichka 1982). More recent surveys of the northeastern US reveal a disease incidence rate of nearly 100% and a mortality rate of 25% (Bergdahl and Bergdahl 2009). It still remains unclear how *Oc-j* spread throughout butternut's range so rapidly, but coleopteran vectors (Halik and Bergdahl 2002) combined with anthropogenic mediated jump and dispersal via infected seed (Innes and Rainville 1996) and multiple introductions (Broders et al. 2012) may have all contributed to the rapid dispersal.

Resistance to *Oc-j* is essential for the long-term survival of butternut (Michler et al. 2006). While some butternut trees appear to be affected by butternut canker to a lesser degree than others, no specific mechanism of resistance has been identified. Further complicating observations of resistance, butternut has the ability to naturally hybridize with at least two other exotic congeners: Japanese walnut (*Juglans ailantifolia* Carrière) and English walnut (*Juglans regia* L.), each of which appears to be only mildly affected by butternut canker (Hoban et al. 2009, 2012). Likewise, hybrid and backcrossed butternut trees display greater resistance to *Oc-j* than do true butternuts (Mckenna et al. 2011). Understanding the parameters of butternut canker in a natural setting can help guide conservation policy and perhaps elucidate factors that contribute to *Oc-j* resistance. Furthermore, monitoring butternut recruitment and frequency of hybridization with *J. ailantifolia* will provide further insight on demographic status of the species and its potential future in the landscape.

New Hampshire, Maine, and New York have a large number of acres in mixed hardwood forests that are ideal habitat for butternut; however, there are little or no data on the number of trees or the health of trees present in these areas. Furthermore, these states may contain previously untapped sources of butternut diversity and resistance that will be important to conserve. With reportedly low recruitment frequency, understanding characteristics that allow butternut to grow until seed-bearing maturity is important for future restoration projects. The overall objective of this project was to aid butternut restoration by providing statistical information on the health, ecology, and recruitment of butternuts. To accomplish this objective, we surveyed 20 butternut populations across the northeastern US to assess overall butternut health and determine if there were specific habitats or phenotypes that persisted in the landscape. Observational results from previous research has suggested that butternut with deep-fissured bark appear to be more resistant to *Oc-j* than butternut with shallow-fissured bark (Ostry et al. 2003). We therefore evaluated susceptibility to infection for butternut of varying bark phenotype. Additionally, we investigated variation in disease severity among butternut growing in upland versus riparian habitats. Finally, we evaluated the rate of hybridization (*J. cinerea* × *J. ailantifolia*) in natural populations of butternut and determined if these hybrids displayed greater resistance to *Oc-j* than do true *J. cinerea*.

2. Materials and methods

2.1. Study location

Field sampling occurred during the months of August 2011 and June 2012. Fieldwork entailed locating, observing, and sampling butternut trees. Location and population data were available from researchers and foresters (courtesy of D. Bergdahl and K. Lombard) and census data collected from public and private landowners. Areas of suspected butternut growth were also explored. The selection of sampling sites was more dependent on the number of reported trees in a location rather than the geographic location. Sampling sites were chosen based on a minimum number of reported individuals. Any location containing greater than 10 individuals was deemed sufficient to represent a sampling site and was further investigated.

Sampling in August 2011 was limited to New Hampshire and focused around the Connecticut River basin and adjacent properties. Butternut stands were identified with the help of state foresters. In total, four locations along the Connecticut River were sampled: sites 1, 2, 11, and 17 (Fig. 1). In addition to these Connecticut river sampling locations, one site (site 12) was located in central New Hampshire (Fig. 1).

Sampling efforts in 2012 totaled 12 locations across Maine, Vermont, and New York, encompassing an area of approximately 100 000 km² (Fig. 1). The majority of sampling locations were located on privately owned land. Owners were contacted with the help of Cornell Cooperative extension and the New York State Forest Owners Association. All butternut sampling for the 2012 field season occurred over the span of two weeks in June.
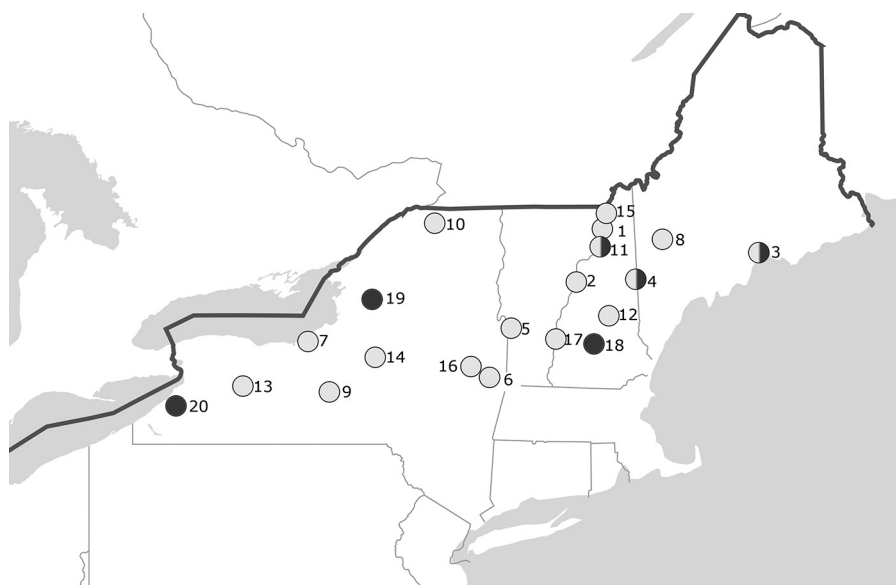
Within a butternut stand, dead butternut trees would often be encountered as downed trees or snags. Without live tissue, it would not be possible to test the hybrid status of a dead tree. To avoid confusion in our dataset, dead trees were not included in the dataset because our experimental design did not compensate for factors such as time since death or cause of death.

2.2. Survivorship and health

The following statistical parameters were recorded for each tree: GPS coordinate and elevation, diameter at breast height (DBH), a rating of tree vigor, habitat type, number of cankers, level of crown dieback, and the number of epicormic growths. These parameters are similar to the parameters chosen by Ostry et al. (1994) designed to designate healthy butternut trees. Ostry et al. suggest a 70–20–50 rule in which trees with more than 70% live crown and less than 20% of the circumference affected by cankers should be considered healthy. Trees with 50% live crown and no cankers are also considered healthy. These guidelines have been used by other butternut researchers (Parks et al. 2011) and were used in the present study to establish a baseline of general health, age, and geographic location. Other measures of health were recorded and are outlined below.

An ordinal rating scale for tree vigor was used as an estimation of tree health. Parameters for vigor have been classified into four general categories: (1) nearly dead, the tree has clearly not experienced any growth within the last season; (2) dying, some life remains though the tree is clearly desiccating; (3) fairly vigorous, while there is some evidence of desiccation, the tree is predominately thriving; and (4) vigorous, the tree is clearly thriving with little or no evidence of desiccation. While estimation of vigor is subjective, the broad nature of these parameters increased the likelihood of consistent assignment. Other parameters measuring general tree health, number of cankers, crown dieback, and epicormic growth were used to reinforce estimations of vigor. Canker number was categorized by activity and location on the trunk, which was used to complement estimations of vigor. Cankers were counted on trunk segments between 0.3 and 2.7 m, and both active and healed cankers were counted. Crown dieback was estimated by visual observation and categorized according to foliage

Fig. 1. Distribution of sample sites across the northeastern US included in this study. Locations were composed of only *Juglans cinerea* (light circles), only *Juglans ailantifolia × cinerea* hybrids (dark circles), or both *J. cinerea* and *J. ailantifolia × cinerea* hybrids (light–dark circles).

abundance on a scale of 1 to 4. Crown dieback in canopies was assessed with an ordinal rating scale as follows: (1) minimal dieback (<10%); (2) moderate dieback (10%–50%); (3) heavy dieback (50%–80%); and (4) severe dieback (>80%). The number of epicormics was recorded and used as a supplemental estimator of canopy health and ratings of tree vigor.

Butternuts were categorized as growing in either upland or riparian habitats. Determining whether a butternut is categorized as upland or riparian depended on the vicinity to, and the relative elevation from, a water source. Trees that were located within 50 m of a permanent body of water or within a floodplain were designated as riparian. All other butternuts that did not meet these criteria were categorized as upland. This manner of habitat differentiation is similar to that used in other studies on butternut and habitat assessment (Hoban et al. 2014).

2.3. Vegetation sampling

A small amount of tissue was removed from each tree. For the majority of trees, an arborist slingshot was used to retrieve canopy leaf tissue. For trees with unreachable canopies, a small section of cambium was excised from under the bark. Leaf tissue was preferred because of larger quantities of quality DNA compared with cambium tissue. Plant sample tissue was organized into individual plastic bags, which were placed on ice in a cooler until they could be lyophilized for DNA extraction.

2.4. DNA extraction

Total genomic DNA was isolated from 10 mg of lyophilized plant tissue using the CTAB method (Doyle 1987). DNA was suspended in 200 μL of 10 mmol·L^{-1} Tris–HCL buffer, and the concentrations were estimated using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Wilmington, Massachusetts, USA). The DNA was aliquoted for standardizing to a concentration of 10 ng·μL^{-1} and preserved at −20 °C for short-term storage. Remaining DNA was frozen at −80 °C for archiving.

2.5. Hybrid analysis

In selecting sampling sites, reports of butternuts located in or near towns were generally avoided. Established towns, and even long-established farmsteads, are prone to having nursery trees planted nearby, increasing the likelihood of *J. ailantifolia* or butternut hybrids, as proximity to human-modified landscapes was shown to be a predictor of butternut hybrids (Hoban et al. 2012).

To ensure that trees were true *J. cinerea* and not hybrid butternut, a hybrid diagnostic assay developed by McCleary et al. (2009) was used. Briefly, aliquots of 10 μL of cleaved amplified polymorphic sequence (CPS02) amplicons were subsequently digested using enzyme MSP I (New England BioLabs) and bovine serum albumin (BSA) (New England BioLabs) at 37 °C for 1 h. Digested CPS02 amplicons were assessed on a 1.5% agarose gel stained with ethidium bromide. Trees containing true *J. cinerea* chloroplast DNA display a single amplicon band of 332 bp. In comparison, trees containing *J. ailantifolia* chloroplast DNA have a cleaved amplicon of 235 bp and 97 bp in length. Caution should be heeded as this assay is able to detect only hybrids originating from a maternal *J. ailantifolia*, leading to type II error. This assay detects nearly 90% of all butternut hybrids based on asymmetrical hybridization. Samples confirmed to contain *J. ailantifolia* DNA were set aside, and samples that could not be resolved in this hybrid test were removed from *J. cinerea* only analyses.

2.6. Data analysis

Individuals that were missing more than 15% of data metrics were removed from the sample set. Variables were tested for statistical normality by evaluating skewness (asymmetry) and kurtosis (peakiness) using PC-ORD v6.08 (MjM Software, Gleneden Beach, Oregon, U.S.A.).

To evaluate the distribution of health, ecological, and growth parameters, subpopulations were tested for between and among diversity of DBH, canker number (0.3–2.7 m), percent canker girdling, and number of epicormics using ANOVA. Crown class and vigor (ordinal data) were analyzed using contingency tests. These analyses were performed in JMP v10 (SAS Institute Inc., Cary, North Carolina, U.S.A.). ANOVA was used to test variation between continuous data, and Fisher exact tests were used for the comparison of ordinal data. To evaluate butternut demographics and recruitment, mean DBH was regressed with a rating of overall vigor using a logistic regression.

To test the hypothesis that deep-fissured bark is more resistant to butternut canker, a Student's *t* test compared bark type with vigor, number of epicormics, number of trunk cankers, and percent girdled. A multivariate discriminant analysis separated bark phenotype and discriminated among canker number, epicormic number, DBH, and healed cankers. To test the hypothesis that upland and riparian trees vary in disease severity, an ANOVA

Table 1. Sampling locations and growth characteristics.

	Latitude (°N)	Longitude (°W)	N	Hybrid (%)	Bark	DBH (cm) Mean*	SD	Habitat	No. of cankers per tree (mean)*	No. of epicormics per tree (mean)*
Sample site										
1	44.655	−71.566	20	0	S	27.2cd	21.2	R	4.8bc	0.5c
2	44.047	−72.064	22	0	S	21.5d	15.9	R	8.0a	4.7ab
3	44.425	−69.006	7	22	S	37.6bc	16.2	U	3.2cde	0.5c
4	44.136	−71.009	2	50	D	42.4abcd	5.1	U	2.5abcde	0.0abc
5	43.507	−72.930	2	0	S	29.5bcd	8.5	U	7.5abcd	0.0abc
6	42.670	−73.650	10	0	I	57.4a	16.9	U	2.6cde	0.0c
7	43.223	−76.608	16	0	S	35.3bc	22.7	U	1.6de	0.4c
8	44.511	−70.520	5	0	I	41.1abc	17.0	U	3.2bcde	1.0bc
9	42.534	−76.696	12	0	I	30.5bcd	13.0	U	1.2e	0.4c
10	44.924	−73.780	13	0	I	43.5ab	29.1	U	5.1bc	0.8c
11	44.475	−71.622	26	7	S	23.2d	9.9	R	3.3cde	6.2a
12	43.657	−71.503	15	0	S	35.7bc	22.9	U	3.7bcde	0.0c
13	42.515	−77.891	13	0	S	26.6cd	24.0	R	4.6bc	0.0c
14	42.904	−75.633	11	0	D	41.3b	5.8	U	5.8abc	2.4bc
15	44.756	−71.621	6	0	I	28.1bcd	19.1	R	6.6abc	0.0c
16	42.719	−74.111	14	0	I	39.1bc	19.7	U	5.7abc	0.5c
17	43.520	−72.297	27	0	I	34.1bc	8.7	U	6.6ab	1.5c
Total			221							
Hybrid Site										
3	44.425	−69.006	2	22	S	62.2	25.1	U	0.0	2.0
4	44.136	−71.009	2	50	D	40.4	13.9	U	3.0	0.0
11	44.475	−71.622	2	7	S	21.4	14.2	R	1.0	0.0
18	43.178	−71.460	12	100	I	36.1	12.2	U	3.3	0.6
19	43.808	−75.886	1	100	I	43.6	0.0	U	1.0	0.5
20	42.247	−79.314	12	100	D	75.9	32.7	U	1.8	0.0
Total			31							

Note: *N*, sample number; DBH, diameter at breast height; SD, standard deviation. Bark phenotype: S, shallow; I, intermediate; D, deep. Habitat: R, riparian; U, upland.

*Means for a particular variable with no letter in common are significantly different.

contrasted vigor, number of epicormics, number of trunk cankers, and percent girdled to trees grouped as upland or riparian. A multivariate discriminant analysis performed in JMP v10 separated upland from riparian trees and discriminated between total epicormic, DBH, and number of cankers.

Hybrid trees were excluded from all data analysis unless otherwise explicitly mentioned. Analyses that included hybrid data were used in a comparative manner between butternut and hybrid trees. Hybrid butternut trees are reported to be more resistant to *Oc-j* than true butternut trees. This hypothesis was tested by ANOVA using measures of vigor (number of epicormics, crown class, canker number, DBH, crown dieback, and habitat) to compare between both hybrid and true butternut trees. A discriminant analysis categorized butternut from hybrid trees and ordinated epicormic count, DBH, and canker count.

3. Results

3.1. Subpopulation summary

Combining the two sampling seasons, pairwise Euclidean distances between 20 sampling locations averaged 343 km, and locations varied in growth habitat ranging from flood basin to mature upland forest. In total, 252 trees were sampled from 20 sites across the states of New York, Vermont, New Hampshire, and Maine (Table 1). When sample unit outliers were detected by skewness and kurtosis, only epicormic count had skewness > 1. Overall skewness for epicormic count was remedied by the removal of an epicormic outlier datum. Hybrids were identified using molecular methods. Thirty-one trees (13%) were verified to contain *J. ailantifolia* chloroplast DNA and were separated from the *J. cinerea* dataset. Significance of all regressions was tested using ANOVA and Fisher exact tests to interpret the significance for contingency tables.

Diameter at breast height (DBH) and the number of epicormics per tree differed significantly between subpopulations following a Bonferroni correction ($P < 0.001$). The total number of cankers per tree was nearly significantly different between subpopulations following a Bonferroni correction ($P = 0.054$). Contingency tests revealed significant variation between subpopulations for crown class ($P < 0.001$) and vigor rating ($P < 0.001$) but not for percent girdled ($P = 0.921$). The rating of tree vigor had no significant relation with the number of cankers found on a tree ($P = 0.412$). The Bonferroni correction for the subpopulation significance tests was based on 271 pairwise comparisons and was applied to all pairwise comparisons in an effort to reduce the frequency of type I error.

3.2. *Juglans cinerea* compared with butternut hybrids

Even though sites containing putative hybrid trees were avoided, of the 252 trees sampled, 31 trees that were phenotypically indistinguishable from true butternut were determined to contain *J. ailantifolia* chloroplast DNA by molecular methods. Two sampling locations contained only hybrid trees (24 in total); the remaining seven hybrids were detected growing among naturalized butternut stands (Table 1; Fig. 1). A comparison of butternut and hybrid trees revealed a significant difference in DBH in that hybrid trees had greater DBH ($P < 0.001$; Table 2). Hybrid trees were found to have significantly deeper fissured bark ($P = 0.008$; Fig. 2), had a higher likelihood of growing in upland habitats ($P = 0.003$; Table 1), increased vigor rating ($P < 0.001$; Fig. 3), fewer cankers ($P = 0.008$), and less crown dieback ($P < 0.001$) when compared with true butternuts (Table 2). Hybrid trees did not vary from true butternuts in the number of epicormics ($P = 0.277$) or crown class ($P = 0.253$). A discriminant canonical analysis of hybrid and true butternut trees confirmed univariate results: hybrid
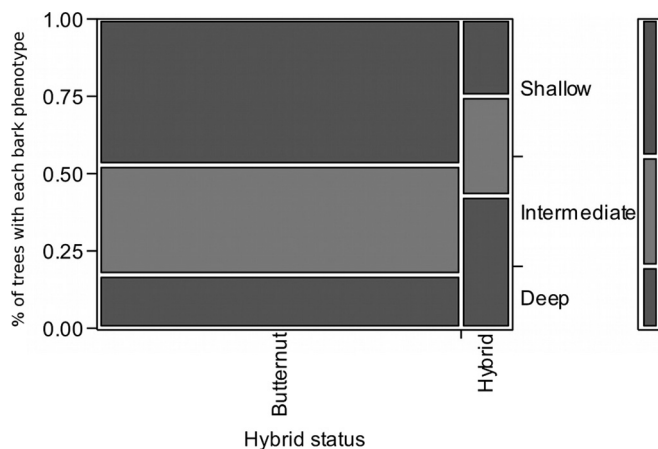
Table 2. Association between growth characteristic and phenotype.

	N	DBH (cm) Mean	DBH (cm) SE	Canker number (mean)	Epicormic count (median)	Crown dieback*
Hybrid status						
Butternut	221	**32.9**	1.4	**4.5**	1.7	**2**
Hybrid	31	**54.7**	4.0	**2.5**	0.5	**1**
Bark phenotype						
Shallow	114	**24.3**	19.1	**5.3**	1.0	1
Intermediate	76	**38.3**	14.4	**4.3**	1.5	1
Deep	37	**49.4**	18.4	**3.2**	2.1	1
Habitat						
Riparian	90	**26.9**	18.9	4.9	**2.7**	1
Upland	137	**37.2**	18.7	4.3	**0.8**	2

Note: N, sample number; DBH, diameter at breast height; SE, standard error. Significance ($P < 0.05$) indicated in bold.

*Crown dieback scale: (1) minimal dieback (<10%); (2) moderate dieback (10%–50%); (3) heavy dieback (50%–80%); and (4) severe dieback (>80%).

Fig. 2. Contingency test comparing butternut and hybrid bark phenotype. Distribution along x axis displays sampling disproportion. Hybrid trees tended to have significantly deeper fissured bark compared with true butternut.

Fig. 3. Contingency table of vigor rating for butternut and butternut hybrids. Distribution along x axis displays sampling disproportion. Hybrid trees are significantly more vigorous than true *Juglans cinerea* ($P < 0.001$).

trees were positively ordinated with DBH, total number of cankers was positively ordinated with butternut trees, and neither hybrid nor butternut were strongly associated with epicormic number (Fig. 4).

3.3. Recruitment

Analyses on the magnitude of butternut recruitment revealed an average DBH of 35.2 cm. A box plot for the logistic regression of DBH and vigor was nonsignificant ($P = 0.161$) but indicated a trend of positive association between DBH and vigor. DBH and total canker number were found to be negatively associated: trees of smaller DBH had significantly more cankers per tree ($P = 0.005$; Fig. 5).

3.4. Bark phenotype

Vigor rating and bark phenotype were nonrelated as evaluated by contingency test ($R^2 = 0.02$) and Fisher two-sided exact test ($P = 0.174$). Total epicormic count and bark phenotype were also not related ($P = 0.366$; Table 2). Canker number was not significantly correlated to bark phenotype following a Bonferroni correction ($P = 0.104$) but indicated a general trend that trees with shallow-fissured bark had more cankers than trees with deep-fissured bark. The percent canker girdling was significantly related to bark phenotype ($P = 0.008$): the highest level of canker girdling occurred on trees with shallow-fissured bark.
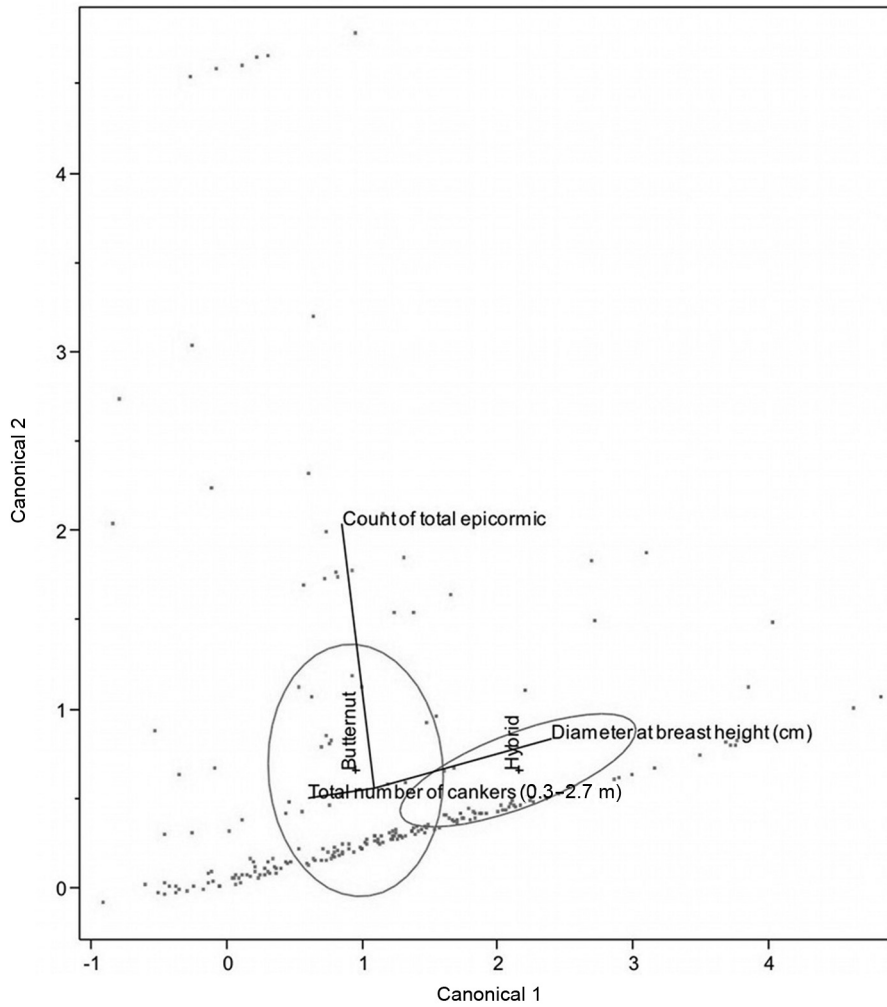
3.5. Habitat

Upland and riparian habitats varied considerably in resource distribution. No significant difference was found in the vigor between trees growing in upland versus riparian zones ($P = 0.2058$), nor did habitat type significantly affect the total number of cankers ($P = 0.1521$) or the percent girdled ($P = 0.1176$). There was, however, a difference between upland and riparian sites for the number of epicormics per tree. Riparian trees had significantly more epicormics than upland trees ($P < 0.001$; Table 2). A canonical discriminant analysis separating habitats found that epicormics and canker number positively correlating with riparian trees; furthermore, there was a positive correlation between DBH and upland trees (Fig. 6).
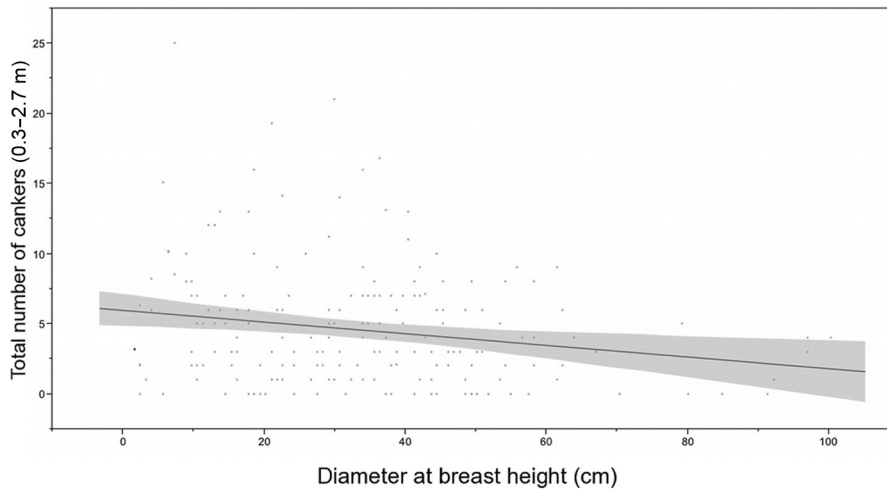
4. Discussion

Although convenience sampling can bias the outcome of a study, by virtue of landowners recommending sampling sites, sampled individuals are likely to experience some level of anthropogenic disturbance. This may have a tendency to bias samples by including genetics of selected or planted trees. Butternuts occurring in highly modified landscapes were avoided, potentially acting as a source of bias for the present study. Although this study may have been subject to slight bias, the greater number of trees sampled by product of convenience sampling likely provides a more realistic estimation of butternut populations compared with serendipitous sampling. The subjective nature of convenience sampling reduces the ability to calculate ecological inferences on relative abundance and distribution of butternut. We have therefore reduced the scope of our discussion related to relative abundance and range distribution of butternut. The variables recorded from each tree were grouped and typified by a normalized curve. Split into sample locations, measurements of vigor, DBH, and crown class varied significantly. This variation between subpopulations allowed for hypothesis testing between subpopulations, while the dataset as a whole remained normal. The number of cankers per tree differed significantly between sampling locations, which may be the result of several factors, including a difference in site demographics or time since initial exposure. The percent girdled did not vary between sample locations, indicating an even spread of butternut canker across the northeastern US. This corroborates with the nearly 100% canker incidence rate observed by Bergdahl and Bergdahl (2009). Despite the uniform presence of butternut canker, Bergdahl and Bergdahl (2009) note a variation in disease severity. This corresponds with the significant variation in vigor and crown class observed in the present study. Speculation of resistant butternut trees is not a

Fig. 4. Discriminant canonical analyses of butternut and hybrid trees. Ellipses each contain 50% data points for butternut and hybrid trees. Butternut trees are ordinated more closely with the total number of cankers, whereas hybrid trees are positively correlated with DBH.
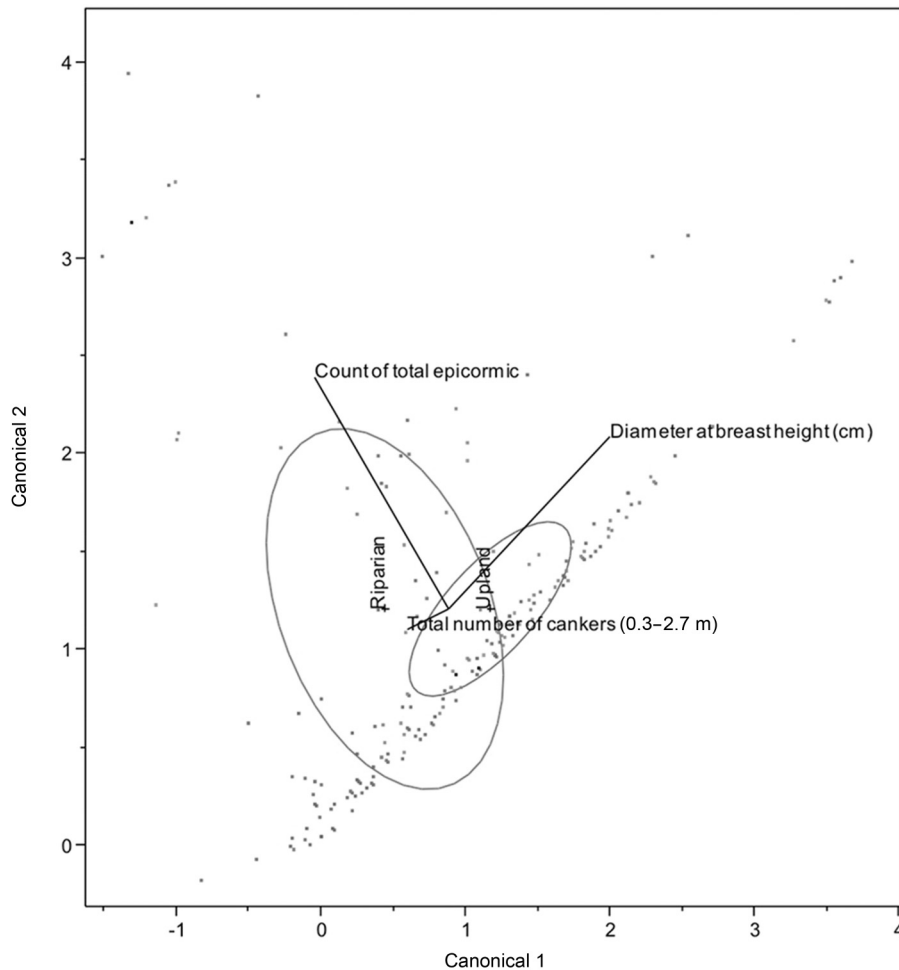

Fig. 5. Linear regression of diameter at breast height and the number of trunk cankers. Line of best fit indicated ($r^2 = 0.04$) surrounded by the standard error (shaded area). Tree size is inversely correlated with canker number, with smaller trees having more cankers than larger trees ($P < 0.005$).

novel concept (McKenna et al. 2011; Orchard et al. 1982; Ostry and Woeste 2004; Ostry and Moore 2008), although the mechanism of resistance remains uncertain. The present study was not designed to test the mechanism of resistance; however, our approach allowed for empirical testing of disease impact and vigor between sampling locations, demonstrating that butternut canker is uniformly present across the northeastern US, although disease impact remains variable (Table 1).

Fig. 6. Discriminant canonical plots separated by habitat type. Normal ellipse regions contain 50% of each habitat type with an error of 36% misclassified data.

Butternut age distribution from the present study indicated that there is a lack of butternut recruitment. The relative abundance and distribution of butternuts across the northeastern US were not explicitly tested for in the present study, although some inferences may be drawn from the available data. The most frequently sampled DBH cohort occurs between 20 and 40 cm (Table 1). The average DBH from this study is 38.2 cm. A demographic plot of butternut DBH from this study would exemplify a severely undersampled young cohort. One explanation to the absentee young cohort can be explained somewhat by the sampling method; older butternut trees are easier to locate and identify than younger trees. Alternate hypotheses are that *Oc-j* kills young butternut trees more rapidly than older trees. Younger trees were found to have more cankers per tree than older trees (Fig. 5). *Oc-j* affects butternut trees of all ages, but the youngest are most susceptible. An emerging hypothesis from this scenario relates to genetic resistance. Previous reports and population genetic analysis indicate that the canker-causing strain of *Oc-j* was likely recently introduced into the northeastern US (approximately 24 years ago) (Anderson and LaMadelaine 1978) when compared with the average life-span of butternut (around 70 years). It is possible that those susceptible allele combinations that remain in the butternut gene pool are being recruited in the younger cohort. If this scenario were true, plant breeders would have reason to look for genetic resistance. An alternative hypothesis to this scenario is that canker resistance is the product of both genetic and preformed resistance, as older trees generally have thicker bark.

A variety of bark phenotypes, from light grey with shallow fissures to darker grey with deeper fissuring, has been observed. Initially suggested in 2003, dark-grey deep-fissured trees appeared to be less affected by butternut canker than nearby light-grey shallow-fissured types (Ostry et al. 2003). This hypothesis was further tested, and it was found that the dark phenotype had significantly fewer canker disease symptoms than the corresponding light phenotype (Ross-Davis et al. 2008). The present study used fissure depth to phenotype trees. Trees with a shallow-fissure phenotype were more likely ($P = 0.008$) to have extensive canker girdling than trees with a deep-fissure phenotype. No other significant associations were found between bark phenotype and canker number, epicormic number, or vigor rating. Diameter at breast height was the best predictor of bark phenotype in that trees of a larger DBH also had deeper fissuring. Because shallow-fissure bark phenotype is correlated with more extensive girdling and a smaller DBH, it appears that bark phenotype has little or no relation to canker resistance.

Butternut trees were equally affected by butternut canker whether they were growing in an upland or riparian habitat, as they did not differ in canker number, vigor, or percent girdled. Interestingly, the number of epicormics per tree was significantly greater in riparian habitats than in upland habitats. This can likely be attributed to the closed canopy generally observed in upland habitats. Butternut trees are shade-intolerant, and epicormics initiated under a closed canopy would likely not survive. Riparian zones generally have a less developed canopy, allowing

butternut epicormics enough light resource to persist. Conservation policy should consider the preservation of both upland and riparian trees as it appears that both riparian and upland butternut trees are equally affected by butternut canker. From personal observation, butternut growing among riparian habitat had relatively less irradiance resource competition than those growing in upland habitat. Encouraging favorable growing conditions is recommended to reduce growth stress and increase potential butternut canker tolerance. Guidelines for individual habitat preservation may be found within the Recovery Strategy for Butternut in Canada (Poisson and Ursic 2013).

Despite an attempt to avoid the sampling of hybrid trees, 31 trees were confirmed to contain *J. ailantifolia* chloroplast DNA. Because we attempted to sample pure butternut exclusively, one should heed caution when comparing the ecological and phenotypic differences with hybrid trees as our sampling scheme may affect results. In addition, only a single maternally inherited chloroplast gene was used to identify hybrids. This molecular approach was able to identify hybrids with a paternal *J. cinerea* and maternal *J. ailantifolia*. This approach is likely to be sufficient for our analytical needs as 90% of butternut hybrids contain a maternally inherited *J. ailantifolia* chloroplast (Hoban et al. 2009). Complex backcrosses and hybrids not detected by our assay would likely have some but minimal influence on the large sample size used in the present study. Three sampling sites contained only hybrid trees. Site 19 contained only a single tree, from which it is difficult to draw conclusions. Sites 18 and 20 each had reasonably sized populations (12 individuals per site), so general characteristics from each may be examined. Both locations were classified as upland, each was located on private property near a house, and both sites had what appeared to be selectively planted trees near the house and younger trees in the surrounding forests. Anthropogenically modified landscapes can be used as a predictor of hybrid trees (Hoban et al. 2012). The aforementioned factors allude to the hybrid trees of sites 18 and 20 being the result of selective *J. ailantifolia* planting. Other sites containing hybrid trees (sites 3, 4, and 11) are likely the result of genetic drift via seed dispersal, as only maternal *J. ailantifolia* hybrids were detected by our assay. Considering the somewhat limited dispersal distance of heavy butternut seed, natural populations containing introgressed hybrids are likely geographically proximal to a *J. ailantifolia* seed source.

Butternut bark is light grey with flat, closely furrowed ridges. Exceptions to this phenotype have been noted, and although the present study did not explicitly test for phenotypic differences between hybrids and true butternut, it should be noted that hybrid trees are more likely to have a darker grey and deeply fissured bark (Fig. 2). This is likely attributed to the larger average DBH observed in hybrid trees. Hybrid trees were observed to have a higher average vigor, less crown dieback, and fewer cankers. Butternut trees and hybrids are indistinguishable by the number of epicormics grown or the relative crown class.

The current state of demographic normality among butternut trees of the northeastern US allows for a range-wide approach to management. Butternut is being eliminated from mature forest stands due to their relatively short life-span, intolerance to shade, and the *Oc-j* disease epidemic. Natural disturbances such as flood and fire will aid in the recruitment of new butternut stands. The potential for breeding resistance seems limited due to lack of resistance in true butternut and the magnitude of disease impact. A more effective approach to developing resistant trees may involve including resistant, naturally occurring, hybrid trees that are already phenotypically indistinguishable from true butternut into breeding programs. These hybrid trees could be quickly integrated into naturalized populations, based on current naturalization of hybrid butternut trees. The Recovery Strategy for Butternut in Ontario considers hybridization as a potential threat to the preservation of butternut (Poisson and Ursic 2013). How-

ever, introgression of genetic resistance from conspecifics may be the most viable method of preserving butternut diversity. The chestnut foundation has been working towards this goal for a number of year by actively crossing American chestnut with Chinese chestnut and then backcrossing resistant progeny to American chestnut until they obtained a tree with all of the phenotypic characteristics of American chestnut while retaining resistance from the Chinese chestnuts (Hebard 2006). In the case of butternut, these types of advanced backcross hybrid butternut trees already exist in natural populations in North America and represent a fortuitous head start in the development of resistant trees for use in restoration projects. Future research into niche modeling would provide additional insight into whether hybrids can fulfill a similar ecological role as butternut has in the past.

Acknowledgements

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