

Inter-population Response of White Ash to the Emerald Ash Borer: Is Vegetative Regeneration Possible?

By

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Abstract

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Emerald ash borer (EAB) (*Agilus plannipennis* Fairmaire) (Coleoptera: Buprestidae) is the costliest invasive pest in North American history (Aukema et al., 2011; McCullough, 2019) due to its extensive killing of ash trees (*Fraxinus* spp.). Ash species account for 7.5% of all eastern hardwood forests, with an undiscounted compensatory stumpage value at \$282.3 billion (Federal Register, 2003). The impacts of EAB invasion are expected to be more ecologically destructive than the loss of American chestnut from eastern hardwood forests in the early 1900s, increasing invasive species in forest understories (Hausman et al., 2010), contributing to forest mesophication (Dolan and Kilgore, 2018), and reducing the carbon sequestration abilities of forests (Fei et al., 2019). EAB-induced biomass losses are 1.813 Tg C per year above background levels, second only to Dutch elm disease (2.386 Tg C/year lost) (Fei et al., 2019). Because of this extreme threat, EAB has become an emerging model organism for understanding how invasive pests can alter ecosystems and reduce natural resources that are economically and socially valuable.

It remains unclear whether white ash (*F. americana*) will persist or what form it will take following long-term EAB effects. White ash will likely lose its role as a timber species (Stewart and Krajicek, 1973) and will be diminished in its role as an ecologically valuable species. Previously documented host-insect dynamics provide some insight about potential paths forward for white ash. It is unlikely that white ash will persist similarly to American beech as mortality rates across North America have already exceeded beech mortality (Knight et al., 2013;

Wieferich, 2013). Unfortunately, it is likely that white ash will follow a similar path as American chestnut in North American forests. American chestnut was functionally eliminated from the landscape (Anagnostakis, 1987) and exists only as root collar sprouts (Paillet, 2002; Jacobs et al., 2013). I examined whether two forms of vegetative regeneration, root collar and epicormic sprouts, were a viable persistence mechanism for white ash and what predicts their occurrence. I found that root collar sprouts, but not epicormic sprouts, have the potential to persist beyond primary trunk mortality. Presence of collar sprouts was not predicted by ploidy level or sex, but tree canopy status (TCS) and diameter at breast height (cm) (DBH) were significant predictors. Several populations also did not produce one or both forms. Count of both sprout forms was predicted by sex, and the overdispersion of zeroes was predicted by both tree canopy and diameter at breast height. My findings suggest that tree size and health during EAB-infestation will determine an individual's chance of persistence via vegetative regeneration, but that tree sex will ultimately determine number of collar sprouts.

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Chapter I

Introduction: The rising prevalence of invasive pests and implications for white ash

Nonnative insects and pathogens pose significant financial and ecological risks to United States trees. More than 450 tree-feeding insects and pathogens have invaded the United States, with approximately 2.5 established nonnative insects discovered per year from 1860 to 2006 in the United States alone (Aukema et al., 2010). The majority of these invaders entered the continent accidentally, a consequence of international trade and hitchhiking on shipments (Brockerhoff and Liebhold, 2017). Nearly every native woody plant genus in North America is associated with at least one nonnative species, most of which have negative effects on their host (Mattson et al., 2007). In particular, phloem- and wood-boring insects are the most damaging subset of these invaders, despite other groups such as sap and foliage feeders being more prevalent. Phloem- and wood-boring insects are financially costly due to the cost of quarantine regulations and the impacts on communities, homeowners, and industries (Aukema et al., 2011). Financially, the largest burden falls on homeowners and local governments. Predicted annual costs to these groups is \$1.7 billion/year for local governments, \$830 million/year in residential property value losses, and \$760 million/year in household costs (Aukema et al., 2011).

The ecological impacts of nonnative insects and pathogens are also significant. Forest community composition is changing due to tree species losses, often resulting in “mesophication” or increased prevalence of shade-tolerant species (Dolan and Kilgore, 2018). Recent evidence suggests that riparian areas may not suffer from increased invasive species, but instead elevated sedge diversity (Engelken et al., 2020). Water tables in non-riparian sites are also likely to change because of increased herbaceous species diversity (Slesak et al., 2014).

Similarly, many biological control programs call for the removal of infected trees, but eradication of canopy species increases duration and intensity of light. The result is often increases in understory species diversity via expansions in invasive plant diversity (Hausman et al., 2010). Further, for only the 15 most damaging, nonnative insects, there was an additional (above background levels) 5.53 Tg Carbon (C) lost per year in forest biomass, likely reducing C sequestration of United States forests (Fei et al., 2019). Even more concerning is the prediction that greater than 40% of total live biomass in United States forests are still at risk to these invaders, suggesting annual biomass losses will only increase. The two largest contributors to biomass loss were Dutch elm disease (2.386 Tg C lost annually) and the emerald ash borer (1.813 Tg C lost annually between 2002 and 2015) (Fei et al., 2019).

Despite these major financial and ecological effects, tree species vary in their responses to insect and pathogen invaders. Some species may persist following outbreaks and establishment of nonnative insects and pathogens. For example, beech bark disease (*Neonectria* spp.) is an exotic canker fungus spread by an invasive sap-feeding beech scale insect (*Cryptococcus fagisuga* Lind.) which infects American beech (*Fagus grandifolia*). In upper Michigan, approximately 55.6% of beech stems and 92.4% of beech basal area were killed by beech bark disease, while in lower Michigan only 38.9% of stems and 25.6% of beech basal area were killed (Wieferich, 2013). While beech bark disease thus leaves some beech trees on the landscape, many other tree species do not survive pests. One of the major pests for species that caused massive mortality of trees, resulting in extirpation (sensu Valiente-Banuet et al., 2016), is chestnut blight (*Cryphonectria parasitica*). American chestnut (*Castanea dentata*) was functionally eliminated from the landscape when the chestnut blight killed over 3.5 million trees in the southeastern United States between 1900 and 1940 (Anagnostakis, 1987). Today,

American chestnut exists on the landscape solely as root collar sprouts. These collar sprouts never reach reproductive maturity (Jacobs et al., 2013) and rarely grow out of the understory (Paillet, 2002). Thus, these two tree species represent extremes along the continuum of tree responses to invasive pests: American chestnut fared poorly and was functionally eliminated from landscapes while American beech is still present, albeit less prominent and with reduced roles in the ecosystem.

A third and final case study is that of Dutch elm disease (DED), the single largest contributor to annual lost biomass to pests at 2.386 Tg C (Fei et al., 2019). The American elm (*Ulmus americana*) was planted across the United States because of its aesthetic value (Hubbes, 1999) but many of these trees were killed by DED, which is a fungus (*Ophiostoma ulmi*) transmitted by bark beetles (*Scolytus multistriatus*; *Hylurgopinus rufipes*) (Richardson and Cares, 1976). Of the 77 million elms in the United States prior to DED, only about 34 million survived to 1976 (Hubbes, 1999). While American elm was removed from most parks and is no longer as prevalent in forest canopies, elm remains an important species in hardwood-dominated lowland ecosystems (Richardson and Cares, 1976). American elm is therefore highly similar to American beech, both representing possible species persistence despite pest infestation.

Ironically, many dying elm in cities and parks were replaced by ash (*Fraxinus* spp.) (MacFarlane and Meyer, 2005), which are now threatened by the invasive emerald ash borer (EAB; *Agriulus planipennis*). Ash species account for 7.5% of all eastern hardwood forests, with an undiscounted compensatory at \$282.3 billion (Federal Register, 2003). First sightings of EAB in North America were on trees in Detroit, Michigan, and Windsor, Ontario in 2001 (Herms and McCullough, 2014). By 2003, approximately 5-7 million ash trees were dead or dying in southeastern Michigan alone (Cappaert et al., 2005; Poland and McCullough, 2006). Due to

rapidly spreading infestation, EAB is currently found in 35 states and three Canadian provinces (Emerald Ash Borer Information Network, 2020) and EAB is the most costly invasive pest in North America (Aukema et al., 2011; McCullough, 2019). Approximately 99% of infested trees die within four years of EAB signs appearing, and 100% mortality is predicted within six years (Knight et al., 2013).

Notably, there is significant interspecific variation in response to EAB amongst ash species. Manchurian ash (*F. mandshurica*), an Asian ash species, is highly resistant to EAB, while North American species and cultivars are all highly susceptible (Rebek et al., 2008) with possible exception to blue ash (*F. quadrangulata*) (Anulewicz et al., 2007; Tanis and McCullough, 2012). These trends may be explained by evolutionary history because EAB is a native pest for Manchurian ash, while North American ash are naïve to EAB. Manchurian ash may have evolved defenses via phloem phenolic compounds which North American ash largely lack (Whitehill et al., 2011; Whitehill et al., 2012). Asian ash species also rarely experience significant disturbance from EAB (Wei et al., 2004; Baranchikov et al., 2008), further highlighting their potential resistance and interspecific differences in response to EAB.

Within only North American ash species, there is also significant interspecific variation. Time to death and injury levels due to EAB vary widely across ash spp. (Steiner et al., 2019), but mortality still reaches similarly high levels of >75% for most North American species (Rebek et al., 2008) and all North American ash species are infected (Subbarayalu and Sydnor, 2018). Despite similar infection, EAB larval density varies widely by ash species: Tanis and McCullough (2015) found that both black (*F. nigra*) and green (*F. pennsylvanica*) ash have EAB larval densities approximately 5x higher than white ash (*F. americana*) and 130x higher than blue and Manchurian ash (see also Anulewicz et al., 2008). More evidence points to interspecific

differences in nutritional and defensive chemistry between black, green, and white ash (Chen and Poland, 2010; Hill et al., 2012) and responses to EAB treatment programs among white, green, black, and Manchurian ash (Whitehill et al., 2014).

There is far less evidence of intraspecific differences in ash responses due to a lack of research in this area. Rebek et al. (2008) suggested that intraspecific differences are likely nonsignificant because North American ash cultivars vary little in responses to EAB and resistance to EAB did not transfer to a black ash x Manchurian ash hybrid. However, recent work in green ash suggests there may be significant intraspecific differences resulting in species persistence. While mortality still approaches 97-99%, some green ash survive EAB infestation, termed “lingering” ash (Knight et al., 2013; Koch et al., 2015). Lingering ash are individuals with rare phenotypic combinations of elevated tree defenses resulting in increased EAB larvae mortality and/or reduced preferences by EAB for oviposition. Notably many of these lingering ash have since died (Knight et al., 2014). Even more recently, Steiner et al. (2019) found significant intraspecific differences in 57 green ash for mortality, cumulative density of EAB signs, and crown condition. It remains to be seen whether such intraspecific variation exists in other ash species.

Even less evidence of intraspecific variation exists in white ash specifically. While five white ash populations in a common garden in Pennsylvania were found to differ significantly in mortality, cumulative density of EAB signs, and crown conditions (Steiner et al., 2019), others have found no intraspecific differences among white ash cultivars (Rebek et al., 2008). Few to no other studies question intraspecific differences in white ash response to EAB. To help fill this knowledge gap, we studied a diverse array of white ash populations grown in a common garden

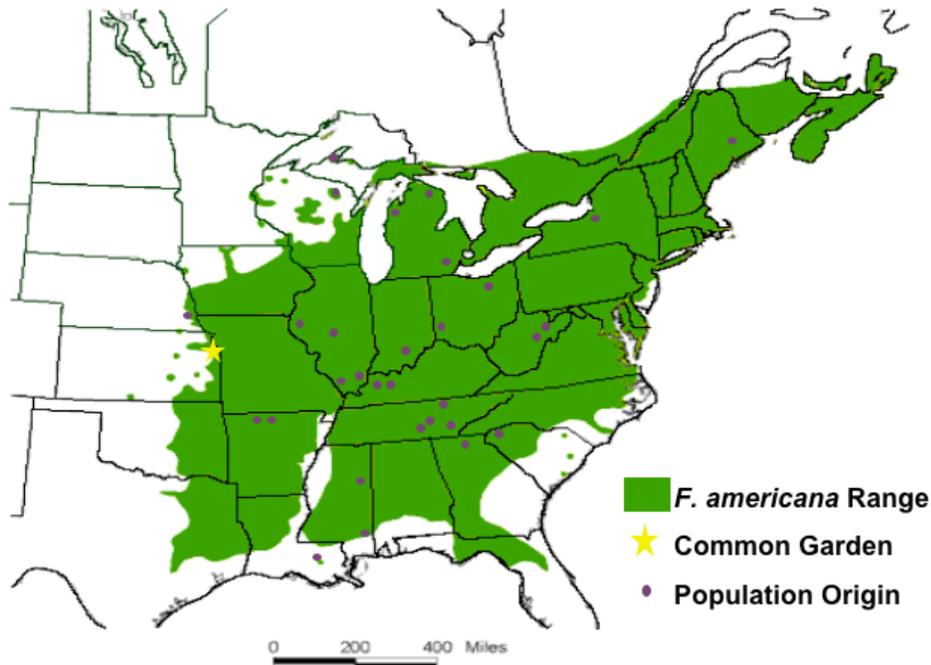


Figure 1-1. The native range (green) of *F. americana* (white ash) includes much of the eastern United States and extends into southern Canada. Purple dots indicate the origin of populations from which seeds were collected. The yellow star indicates the location of the KUFS common garden.

at the University of Kansas Field Station. This common garden is located at the western edge of the species range (Fig. 1-1) and represents a relatively dry location relative to the vast majority of the full range. Initially, 1,100 trees were planted there from 30 distinct locations, representing 43 different populations. The Ward lab began examining population-level differences in response to water limitation over 15 years ago. This work found that populations differ in their diameter growth, survival, and several morphological and physiological measures (Marchin et al., 2008). More specifically, populations located closer to the same latitude as our common garden survived more frequently and grew more quickly than those from northern or southern locations (see also Schuler, 1994; Knight et al., 2013). Further, in a subset of populations across a narrow latitudinal band, we found that populations from the wetter, eastern end of the gradient had lower survival and growth rates than the populations native to drier locations closer to the common garden. Work in the Ward lab has also examined intraspecific differences in phenology and physiology of white ash during climactic extremes. Leaf out at the site was accelerated by 22 d

during the extreme heat year of 2012 (Carter et al., 2017). Further, white ash populations respond similarly to extreme heat for both leaf out (Carter et al., 2017) and stable carbon isotopes (Carter et al., 2020), maintaining rank order (i.e., no significant interaction of population and extreme heat) of population-level responses across moderate to the most extreme years with respect to precipitation and temperature (see also Dawson et al., 2002 for a review of similar findings across species).

Recently, the common garden has faced attack from EAB, providing an excellent opportunity to study intraspecific variation in white ash response to EAB. EAB likely arrived at our site in the early 2010s, because in 2016 signs of infestation were only found on 50% of trees (N = 41) that were nearly dead or had recently died (L.J. Haavik, unpublished data). Beginning in 2016, we annually quantified tree canopy status data and have periodically collected physiological data on water relations, wound healing, carbon isotopes, and phloem chemistry. Our results from these studies indicate that carbon isotope discrimination is reduced under EAB attack (Fig 1.2), but rank order between populations was still maintained as previously found (Carter et al., 2020). We suggest that the reduction in carbon isotope discrimination is a stomatal effect triggered by EAB attack. When stomatal conductance is reduced (i.e., more stomata closure), fractionation is limited, and plants thus discriminate less between ^{13}C and ^{12}C . There is also significant intraspecific variability in white ash mortality by latitude of origin (Fig. 1-2). Trees from the southern U.S. were more likely to be healthy (lower tree canopy status values) whereas several populations from northern locations were approaching complete mortality (tree canopy status = 5). Four populations had tree canopy status means greater than three, suggesting average canopy dieback in these populations was greater than 50%. Specifically, these populations represent locations closest to the initial site of EAB outbreak in North America

because all are from Michigan or Wisconsin. Moreover, recent research supports our findings of intraspecific latitudinal gradients: Steiner et al. (2019) found less EAB exit holes on green ash populations native to the south and east, but significantly higher levels in southern Canada and northern central U.S., including Michigan. Altogether, these novel results suggest there may be intraspecific variation in EAB-induced white ash mortality and thus southern trees may be a restoration source for northern locations post-EAB.

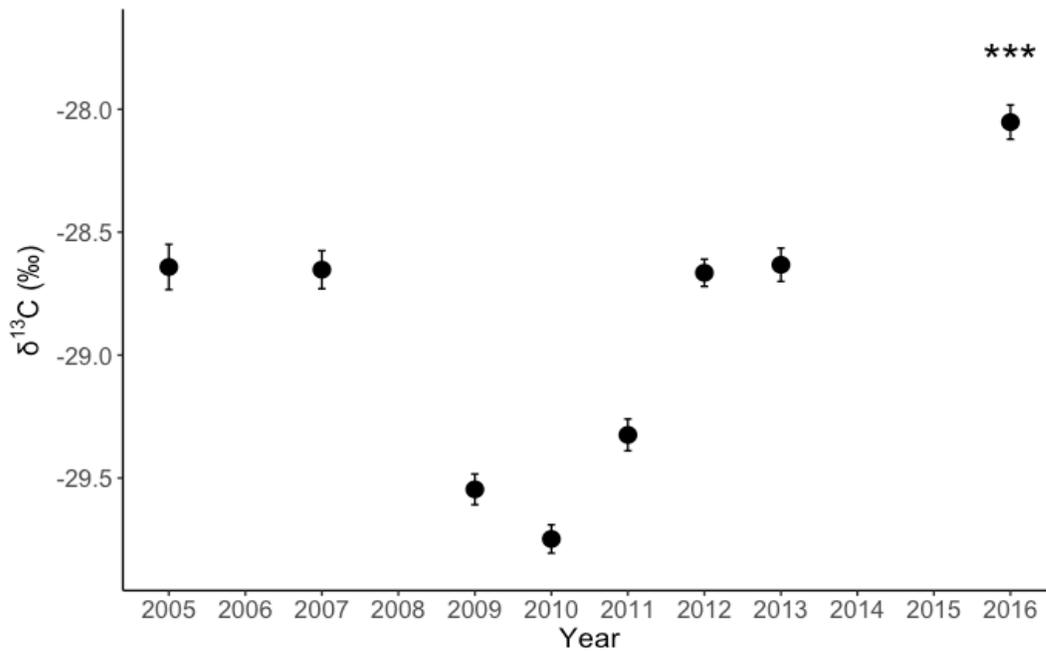


Figure 1-2. Interval plot of mean carbon isotope discrimination by year (Haavik et al., unpub.). Carbon isotopes were collected from at the University of Kansas Field Station in the years shown for all 44 populations. Population differences were not removed (i.e., not estimated marginal means). Our two-way ANOVA found that both population (data not shown; $F_{34, 1050} = 9.55$, $P < 2.2 \times 10^{-16}$) and year ($F_{7, 1050} = 95.8406$, $P < 2.2 \times 10^{-16}$) were significant. There was no interaction effect (data not shown; $F_{1, 1088} = 1.0113$, $P = 0.4499$) suggesting maintenance of rank order between populations across these years. Bars represent +/- 1 SE. We did not collect isotopes in 2006, 2008, 2014, or 2015.

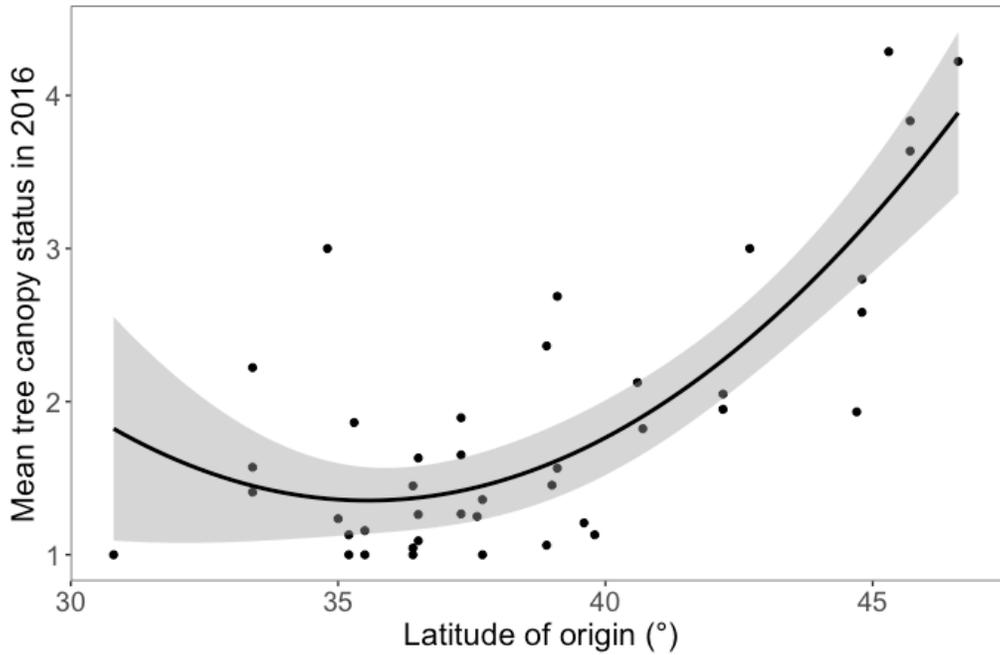


Figure 1-3. Scatterplot of mean tree canopy status in July 2016 by populations plotted against latitude of origin (°) (Haavik et al., unpub). Tree canopy status (Smith, 2006) was collected for every individual at the common garden at the University of Kansas Field Station and averaged by population ($n = 44$). One population, East Baton Rouge, LA, was removed from analysis because there was only one individual. Grey cloud represents 95% confidence interval. Quadratic regression was significant ($F_{2,39} = 37.05$, $P = 9.632 \times 10^{-10}$, $R^2 = 0.66$) for both terms.

Chapter II

White ash trees may survive emerald ash borer attack through collar but not epicormic sprouting.

Abstract

The emerald ash borer (EAB) has killed millions of ash trees since initial infestation in North America. Mortality rates regularly exceed 99%, functionally eliminating ash species from the landscape and causing immense ecological disturbance. It is unclear whether white ash may persist, and if so, in what form. Seeds are not a feasible mechanism for species persistence because viable seed count is negatively correlated with mortality. An alternative is vegetative regeneration because it would allow ash persistence on the landscape, but it has been ignored or rejected as a viable option in white ash. To fill this gap, I questioned whether populations varied in their vegetative regeneration, to what extent individual-level characteristics (tree canopy status, ploidy level, sex, diameter at breast height [DBH]) determine presence and count of two forms of vegetative regeneration (epicormic and collar sprouts), and explored the persistence of both sprout forms beyond primary trunk mortality. In summer 2019, I counted both types of sprouts, measured DBH, and identified sex and ploidy level where possible on 732 trees in a common garden at the University of Kansas Field Station. Both collar and epicormic sprouts were uncommon at the site, occurring approximately in one out of every five or six trees, respectively. I found minimal variation in both forms of sprouting among populations, concluding that populations do not differ in vegetative regeneration. However, individual-level traits predicted both forms of sprouts. Both count and probability of presence for both types of sprouts were predicted by DBH, and tree canopy status also predicted collar sprouts presence and count. Sex and ploidy level were not strong predictors for any analysis. Collar sprouts persist

beyond full canopy dieback, but epicormic sprouts do not. Long-term studies are necessary to fully understand the vigor of collar sprouts.

Introduction

Emerald ash borer (EAB) (*Agilus plannipennis* Fairmaire) (Coleoptera: Buprestidae) is the most ecologically destructive (Herms and McCullough, 2014) and financially costly (Aukema et al., 2011; McCullough, 2019) invasive pest in North American history. Ash species account for 7.5% of all eastern hardwood forests, with an undiscounted compensatory stumpage value at \$282.3 billion (Federal Register, 2003). EAB-induced biomass losses are 1.813 Tg C per year above background levels, second only to Dutch elm disease (2.386 Tg C/year lost) (Fei et al., 2019). First sight of EAB in North America was in Detroit, Michigan, and Windsor, Ontario in 2001 (Herms and McCullough, 2014). By 2003, approximately 5-7 million ash trees were dead or dying in southeastern Michigan alone (Cappaert et al., 2005; Poland and McCullough, 2006). Infestation quickly spread, and EAB is currently found in 35 states and three Canadian provinces (Emerald Ash Borer Information Network, 2020). While there is some heterogeneity in attack severity and frequency within stands leading to variation in mortality (Cappaert et al., 2005), and some “lingering” ash exist longer than most individuals (Knight et al., 2013; Koch et al., 2015), mortality rates exceed initial predictions and even most lingering ash die (Knight et al., 2014). Approximately 99% of trees die within four years after signs of EAB appear, and 100% mortality is predicted within 6 to 15 years (Knight et al., 2013).

Ash species may recover from seed, but that is unlikely. In Michigan, the epicenter of the EAB attack in North America, viable seed count decreases with increasing mortality, and once mortality exceeds 99%, viable ash seeds are not found in seedbanks within 5 years (Klooster et al., 2014). Two factors contribute to seed absence: the lack of adult reproduction due to their

mortality and the limited persistence of ash in seedbanks to 3-4 years (Clark, 1962). All that remains in these sites is an “orphaned cohort” of previously established seedlings (Klooster et al., 2014), but their survival is also unlikely. Mortality in the smallest group of ash plants is high (Kashian and Witter, 2011) likely because many of these are infected with EAB (Aubin et al., 2015). Ash can be infected by EAB at sizes as small as 2.5 cm in diameter at breast height (DBH) (McCullough et al., 2008). Young ash plants also face significant browsing pressure (Hausman et al., 2010). Notably, this orphaned cohort has not grown into the overstory in recent studies (Kashian and Witter, 2011; Burr and McCullough, 2014; Aubin et al., 2015), likely because of slow growth rates (Bond and Midgley, 2001) and outshading by dominant canopy species (Burr and McCullough, 2014). In this way, the “regeneration niche” (reseeding ability) (Grubb, 1977) and its significance for species continuation is nearly eliminated (but see Granger et al., 2017).

Another possible route for ash species survival is via the “persistence niche” (vegetative regeneration or sprouting) (Bond and Midgley, 2001; Nzunda et al., 2007). Vegetative regeneration can take many forms. The most significant form is collar sprouts (Fig. 2-1A) because these have the highest likelihood of forming a new trunk in most tree species (Del Tredici, 2001). The collar is a morphologically vague location except in mature trees, where the collar exists at or below the ground level. This a crucial detail: collar sprouts are thus in contact with soil and may form adventitious roots, allowing them to become autonomous of the parent trunk (Sakai et al., 1995; Del Tredici, 2001). Many species produce opportunistic sprouts (Del Tredici, 2001) and/or stem epicormic sprouts (“epicormic sprouts”) (Fig. 2-1B) (Bellingham and Sparrow, 2000) from primary trunks. Very few North American species may also produce specialized stems, such as lignotubers or rhizomes, which also develop sprouts (Fig. 2-1C) (Del

Tredici, 2001). Similarly, many angiosperms produce sprouts from roots (root suckers) far away from the primary trunk (Fig. 2-1D) (Del Tredici, 2001). Formation of these sprouts may come from preventitious buds, those created by apical meristems and/or associated tissues, or adventitious buds, those formed due to injury (Paciorek et al., 2000).

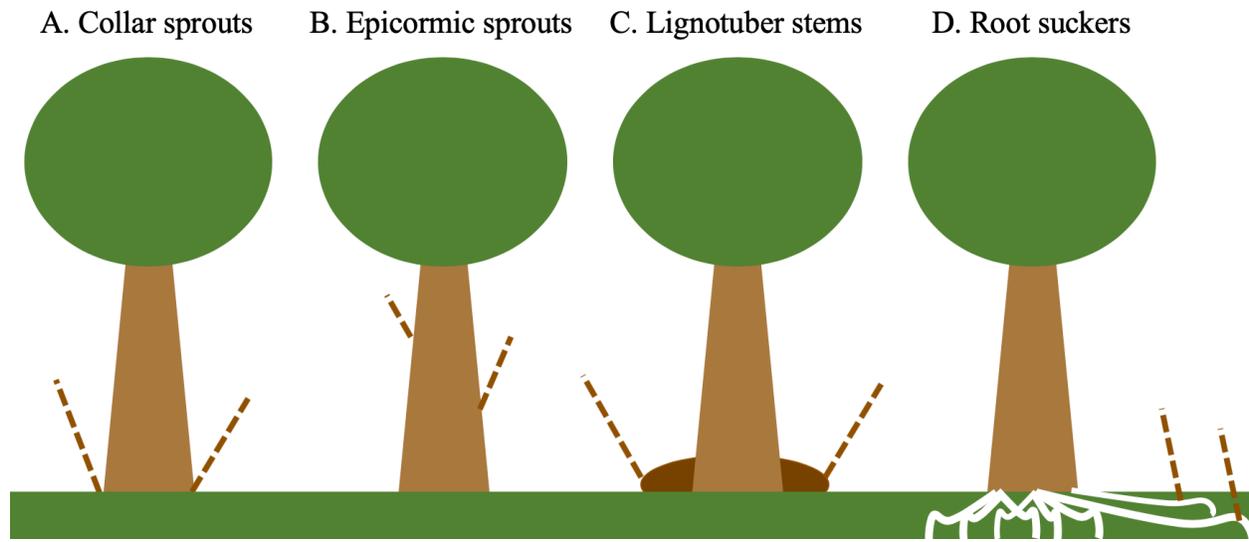


Figure 2-1. Four dominant modes of vegetative regeneration in trees. Primary trunk (light brown trapezoid) and canopy (green ovals) are the original tree. Dotted lines represent vegetative regeneration. In C, dark brown component is the lignotuber, a storage organ and extension of the primary trunk. In D, white lines are roots (not shown for A, B, or C). Ground is represented as green to limit confusion with trunk and lignotuber.

Vegetative regeneration may allow species to persist on the landscape past primary trunk death, although functional roles may change (Aubin et al., 2015). Persistence is key to maintaining ecosystem integrity, reducing species turnover (Bond and Midgley, 2001), limiting disturbances (e.g., Hausman et al., 2010; Slesak et al., 2014; Dolan and Kilgore, 2018; Fei et al., 2019; Engelken et al., 2020), and diminishing species reliance on seeds (Bellingham and Sparrow, 2000). The persistence niche is especially important when there is low reproductive output, establishment, and/or recruitment from seeds (Ibrahim, 1991; Nzunda et al., 2007; Lasso et al., 2009), senescence (Schier and Campbell, 1980), and/or when species occur at range edges

(Koop, 1987; Peterson and Jones, 1997). Nearly all species can vegetatively regenerate, even obligate re-seeders (Bellingham and Sparrow, 2000), but it is rare in gymnosperms (with exception to genera *Pinus*, *Juniperus*, and *Abies*) (Zeppel et al., 2015). Notably, Nzunda et al. (2007) found no evidence of phylogenetic constraint for vegetative regeneration ability in the subtropics, and other trends suggest a similar lack of constraint (Burns and Honkala, 1990; Del Tredici, 2001).

The mechanisms underlying vegetative regeneration are not fully elucidated, leading to a weak understanding of interspecific variation. Recent work by Moreira et al. (2012) provides a three step model for sprouting: (1) ability to sprout and initiation of sprouts, (2) resprouting vigor, and (3) post-resprouting survival. Species vary in which of these steps limit vegetative regeneration. However, there are key similarities at initiation. Firstly, sprouting is triggered by disturbance. Disturbances like root wounding (Fraser et al., 2004), hurricanes and typhoons (Bellingham et al., 1994; Miura and Yamamoto, 2003), browsing pressure (Morris et al., 2004), fire (Schier and Campbell, 1978; Kauffman, 1991; Pausas and Keeley, 2017; Warrix and Marshall, 2018), girdling (Chupp and Battaglia, 2017), drought (Bond and Midgley, 2001), and pathogens and pests (e.g., Ibrahim, 1991; Mallett, 2002; Morris et al., 2004; Cappaert et al., 2005; Nyland et al., 2006; Wagner et al., 2010; Jacobs et al., 2013; Hughes et al., 2018) have been found to induce one or more forms of sprouts. Disturbance severity, measured as proportion of above-ground biomass lost, may determine which type of sprouts form. For example, epicormic sprouts form under intermediate severity, while collar sprouts form under severe disturbance (Bellingham and Sparrow, 2000). This is likely because aboveground biomass losses reduce apical dominance (Schier, 1981). Secondly, trees are constrained by bud bank strength, resource allocation patterns, and carbohydrate reserves (Clarke et al., 2015). Carbohydrate

reserves are particularly important because they provide the initial energy supply for sprouts (Walters et al., 2005; Nzunda et al., 2008; Clarke et al., 2010; Moreira et al., 2012) and limit which sprout form is produced (Smith et al., 2018). Lastly, health pre-disturbance underlies sprouting ability in species like trembling aspen (Frey et al., 2003) but it is unclear whether this effect is mediated by or independent of carbohydrate reserves. The two remaining stages of the model (resprouting vigor, post-resprouting survival) are not related to carbohydrate reserves and both have been suggested to vary widely between species (Moreira et al., 2012).

Intraspecific differences in sprouting are far less understood. Population-level differences are generally not found (e.g., Żywiec and Holeksa, 2012). This is likely because sprouting is a highly individualized behavior and dependent on environmental variation and disturbance (see also Schier, 1981). Individual traits like age and size may be key. In aspen, sprouts are generally most frequent during the first year post-disturbance (Frey et al., 2003), but they thin down to one stem over time (Sandberg, 1951; Sandberg and Schneider, 1953; Schier and Campbell, 1980). Relationships of size with both number of sprouts and ability to sprout have been studied extensively among species and there are examples of positive (e.g., Bellingham et al., 1994), negative (e.g., Ward and Williams, 2018), or no association (e.g., Negrelle, 1995). Del Tredici (2001) notes that sprouts are rare in temperate deciduous trees larger than 30 cm DBH, while trees 5-15 cm DBH often sprout abundantly. Intraspecific variation in size and sprout number relationships has received less attention, but some evidence exists for positive (Miura and Yamamoto, 2003; Moreira et al., 2012) and negative (Mallett, 2002) associations.

Other individual traits like sex and ploidy level and their relationships with vegetative regeneration also largely lack examination. In particular, little to no research examines these traits and their associations with sprouting in trees. Much research has theorized that

polyploidization may be a general response to abiotic and biotic stress (Scholes and Paige, 2015) and that polyploids have an advantage in host/pest dynamics (King et al., 2012), but there is little experimental evidence. More evidence exists for differences between sexes, but it is solely in shrubs or herbaceous species, and studies vary widely in their conclusions. No differences in sprouting (non-reproductive shoots) were found between male and female stinging nettle individuals (Oñate and Munné-Bosch, 2009), while Mizuki et al. (2005) found that females of a clonal yam invested significantly less in vegetative regeneration than males. Moreover, observational evidence also demonstrates that *Myrsine* spp. males produce more vigorous and frequent sprouts post-fires (Hoffman, 2004).

These gaps in knowledge about intraspecific variation in sprouting and the nearly complete mortality of white ash in North America motivated my project. Specifically, I questioned if and to what extent 43 white ash populations differed in their abilities to sprout from trunks (epicormic sprouts) or collars (collar sprouts). I also questioned whether epicormic and collar sprout counts or probability of presence could be predicted by individual traits (size, health status due to EAB, ploidy level, sex). Previous research suggests sprouting is a highly individualized process with minimal population effects (e.g., Żywiec and Holeksa, 2012), but most of these studies have fewer populations than my work ($n = 43$). I collected my data at the white ash common garden at the University of Kansas Field Station, located at the dry edge of the species range. The latter detail is essential because under relatively high levels of water limitation (compared with other parts of the species range), ash species often see higher levels of attack as noted in other studies (Wei et al., 2004; Cappaert et al., 2005) and elevated performance of EAB larvae (Chakraborty et al., 2014). In this way, I examined familial differences in sprouting of white ash trees that had experienced several years of physiological stress from high

temperatures (Carter et al., 2017) and water-limitation (Marchin et al., 2008; Carter, 2015). Moreover, drier conditions are believed to be more frequent and intense across the globe (Sheffield and Wood, 2008; Taylor et al., 2012; Keellings and Engström, 2019; Koutroulis et al., 2019) and thus white ash must be able to respond to it and EAB infestation simultaneously as climate change continues.

Materials and methods

Study species and common garden

Fraxinus americana is a dioecies, ring-porous angiosperm native to the eastern United States and southern Canada (Fig. 1-1) (Schlesinger, 1990). Within *F. americana*, there is significant ploidy variation whereby the diploid (2n) occurs across a wider geographic range than the polyploids. *F. smallii* is a tetraploid (4n) occurring from eastern Texas to Florida, and north to Missouri, Ohio, and Pennsylvania. *F. biltimoreana* is a hexaploid (6n) with similar distribution to the tetraploid but it is rare in Arkansas, Louisiana, and Missouri and does not occur in Texas. Here, I treat all individuals as *F. americana* but note ploidy level as intraspecific variation because previous work in the lab has found few differences between ploidy levels (Carter, 2015).

I completed my work at a common garden with initially 1,100 trees from 43 populations of *F. americana*. Populations originated from locations across the species range (Fig. 1-1). Seeds were collected from open-pollinated, native parent trees by the North Central Forest Experiment Station (USDA Forest Service). Before planting at the University of Kansas Field Station (KUFS) in Lawrence, KS (39.0°N, 94°W, 299 m a.s.l.), the North Central Forest Experiment Station planted the seeds in a nursery in Illinois and transplanted them as one-year old seedlings

in common gardens across the species range to examine provenance effects (Clausen, 1984b, a; Rink and Kung, 1991). Each population had 25 individuals at transplant in 1976 and they were planted in replicates of five across five blocks (Fig. 2-2).

The KUFS common garden is located at the xeric edge of the species range, where annual precipitation averages 879 ± 200 mm. Mean annual temperature is 13 °C and temperatures may range from -7 °C in January to 32 °C in July. Precipitation is relatively low compared to the rest of the species range ($749 - 1602$ mm) while temperature is warmer than over half of the species range ($4.3 - 19.4$ °C) (National Oceanic and Atmospheric Administration National

Climatic Data Center, 2004; Marchin et al., 2008).

Mortality at the site was high prior to the arrival of the emerald ash borer (EAB): only 689 of the original 1,100 trees had any canopy in 2016 and 51

had no canopy. EAB likely arrived between 2010

and 2015 as evidenced by signs of attack on 50% of dead or nearly dead trees ($n = 41$) in 2016 (Haavik et al., in prep).

I report data on 42 of the 44 original populations (Appendix A, Table 1). Two populations saw significant mortality pre-EAB and were not included in my analysis. For example, only one tree remained of the population from East Baton Rouge, LA (96% mortality).

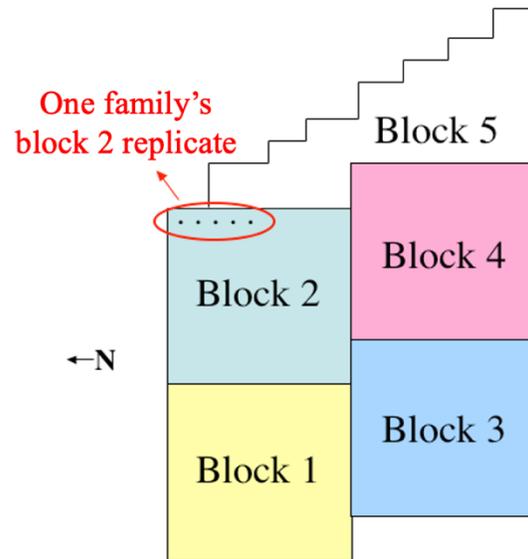


Figure 2-2. The experimental design of the *F. Americana* common garden at KUFS. Each population had 25 trees with five individuals per block. Reproduced with modification from Marchin (2006).

These 42 populations represent 29 distinct locations. Populations from the same location are distinct physiologically (Carter, 2015) and thus analyzed separately here.

Tree health and size

In July and August of 2019, I collected data on tree health and size of all standing trees in the common garden. Tree canopy status (TCS) was measured on an ash canopy health scale of 1-5 using protocols from Smith (2006) which were adapted from bronze birch borer (Ball and Simmons, 1980). Briefly, trees ranked as 1 had full, healthy canopies. Minimal thinning of canopies ranked 2. Trees showing moderate dieback, specifically dead branches at canopy tops, were ranked as 3. Trees with greater than 50% dieback were ranked as 4. If a tree had no foliage in the canopy, it was ranked as 5. This last ranking included previously dead trees (TCS = 5 in 2017 or 2018) if the primary trunk still stood ($n = 39$). Further, vegetative sprouts did not influence these health ratings. For example, trees with vegetative regenerations but no foliage in the canopy were still ranked as 5 (Smith, 2006).

For all standing trees at the common garden, I measured circumference (cm) at breast height (1.3 m) and converted to diameter at breast height (DBH). For multi-stemmed trees, the larger stem was counted in full for DBH and half of the diameter of smaller stem(s) was added. Collar and epicormic sprouts (see below) were not included in DBH measures.

Tree ploidy level and sex

In July 2019, I established ploidy of individuals based on morphology (Nesom 2010)

from at least two samples per tree and merged findings with preexisting data (Carter, 2015) ($n_{\text{diploid}} = 345$, $n_{\text{tetraploid}} = 39$, $n_{\text{hexaploid}} = 37$). Diploid individuals (2N) lacked hairs and had V- to U- shaped petiole bases and leaf scars. Tetraploid individuals (4N) were also hairless but had oblong to widely obovate petiole bases and leaf scars. Hexaploid individuals (6N) had similarly oblong to widely obovate petiole bases and leaf scars, but also had small hairs on twigs, petioles, and leaf rachises.

Limited data was available for sex ($n_{\text{female}} = 226$; $n_{\text{male}} = 62$). Preexisting data (Carter, 2015) was collected during springs of 2010-2013 based on flower anatomy. *F. americana* is a dioecious species, so individuals either have male (stamen) or female (pistils) reproductive organs but not both. I contributed to this dataset by noting individuals bearing seeds in July 2019. These individuals are female, but do not represent all females because 2019 was not a masting year in white ash.

Vegetative reproduction

In August 2019, I quantified two forms of vegetative regeneration on all standing trees at the common garden. I counted stems from the trunk (epicormic sprouts) below breast height (1.37 m). I also counted the number of stems from the root collar (collar sprouts) within 0.1 m of the tree trunk. All collar sprouts originated from below soil level. For both types of sprouts, I did not count stems with zero, dead, or browning foliage.

Data analysis

I completed all statistical analyses using R version 3.6.1 (R Development Core Team, 2010). Both population and individual-level factors could not be assessed in the same model due to singularity. Further, not all trees have been sexed ($n_{\text{female}} = 226$; $n_{\text{male}} = 62$) because of masting behavior in white ash. Ploidy levels are also only identified for trees with low-hanging branches ($n_{\text{diploid}} = 345$, $n_{\text{tetraploid}} = 39$, $n_{\text{hexaploid}} = 37$). Additionally, ploidy levels are not distributed across populations equally because tetraploids and hexaploids do not occur in parts of the species range as aforementioned.

Thus, I first assessed population-level differences in associations with tree canopy status for epicormic and collar sprouting abilities with a Fisher's exact test with a simulated p-value based on 2000 replicates. All individuals of a given population were removed from further analysis if the population may lack the ability to sprout. I interpreted populations that had one or more trees exhibiting dieback ($\text{TCS} > 1$) but not showing one of the sprout forms as lacking the ability to produce that form of sprouts based on the assumption that vegetative regeneration is triggered by biomass loss (Bellingham and Sparrow, 2000). Thus, two data sets were created, with populations that did not produce epicormic sprouts removed from one and populations that did not produce collar sprouts removed from the other.

Before predicting presence and count of both sprout forms, I assessed normality for both sprout forms with a Shapiro-Wilk normality test. Both epicormic and collar sprouts exhibited zero-inflated distributions (Figs. 2-3a, 2-3b). Therefore, I predicted count of both sprout forms with zero-inflated Poisson regression via maximum likelihood using the *pscl* package (Zeileis et al., 2008; Jackman, 2017). I report the count model results here (Poisson with log link) (e.g., Ward and Williams, 2018). For each vegetative regeneration form, I used ploidy level, sex, DBH, and TCS as predictors in the regression. When singularity was not an issue, I also

examined interaction effects. I compared zero-inflated Poisson regressions with standard Poisson regressions using a Vuong test (Zeileis et al., 2008; Jackman, 2017). I dropped non-significant predictors from models in a second iteration of each regression. To examine differences among levels of a predictor, I ran a Wald test using the aod package (Lesnoff and Lancelot, 2012). For predicting presence of collar and epicormic sprouts, I used a binomial error distribution in a generalized linear model. All graphs were made using base graphics and ggplot2 (Wickham, 2016).

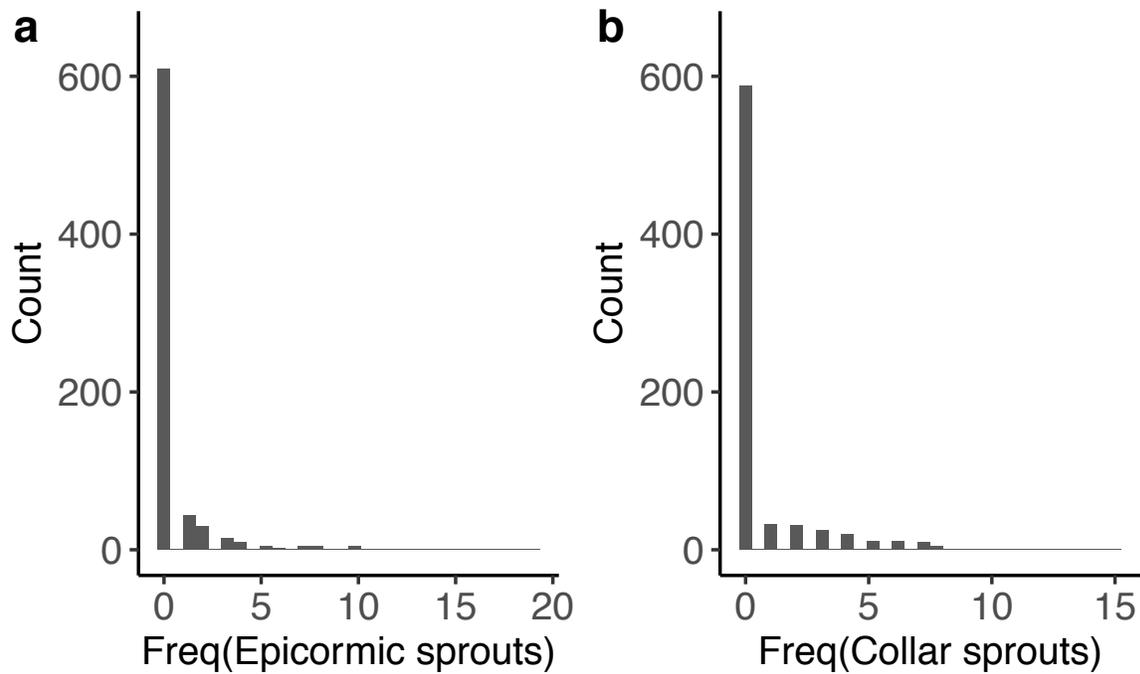


Figure 2-3. Distributions of (a) epicormic sprout frequencies and (b) collar sprout frequencies.

Results

Differences among populations in regeneration ability

To start, I examined whether populations differed in their presence of both types of sprouts by tree canopy status level (TCS) through Fisher's exact tests. Overall, I found minimal effects of population. I found that seven populations only exhibited collar sprouts, four only exhibited epicormic sprouts, 30 exhibited both, and one population exhibited neither sprout form. I found no differences for any populations and TCS for epicormic sprouts ($P > 0.05$) and population was removed as a factor in all future sprout prediction models. However, there were five populations that did not produce epicormic sprouts despite having dying trees. Muhlenberg, KY had 13 full-canopied (TCS = 1) and two dying trees (TCS = 3). Gallatin, IL had 22 full-canopied trees, two dying trees (TCS = 2), and one individual with no canopy (TCS = 5). Penobscot, ME had one full-canopied individual and four dying trees (TCS = 3-4). Lastly, two of three populations from Overton, TN lacked epicormic sprouts: Overton, TN (1) had 20 full-canopied trees and two dying trees (TCS = 2), while Overton, TN (3) had 17 full-canopied trees and one dying tree (TCS = 2). Notably, the remaining population from Overton, TN (2) showed epicormic sprouts on two trees. I removed the five populations without epicormic sprouts from further analysis because they may lack the capacity to epicormically sprout. All other populations ($n=37$) had sprouts on at least one individual.

Contrarily, I found that presence of collar sprouts varied with tree canopy status for nine populations. Fisher's exact test confirmed that eight populations were more likely to have collar sprouts if they exhibited any dieback (TCS = 2-5): Jackson, IL ($P = 0.0055$), Wayne, OH ($P = 0.0085$), Tucker, WV ($P = 0.0099$), both Hopkins, KY populations ($P = 0.0005$; $P = 0.001$), Onondaga, NY ($P = 0.0215$), Benzi, MI ($P = 0.0165$), McMinn, TN ($P = 0.036$). On the other

hand, Presque Isle, MI only had collar sprouts on individuals with moderate dieback (TCS = 3), but not on trees with no or heavy dieback and dead trees (TCS = 4-5) ($P = 0.045$). Despite an association of collar sprouts with heavier canopy dieback in those eight populations, there were always trees exhibiting canopy dieback that did not have collar sprouts in these populations. In all other populations ($n = 33$), collar sprouts were not significantly associated with TCS levels. Despite moderate population differences in collar sprouting, I did not include population as a factor in all future collar sprout prediction models. I kept all of these above populations in the analysis of collar sprouts because I used TCS in the regression models, therefore accounting for these differences between populations. However, two populations had no collar sprouts despite having trees with some dieback. Adams, IL had 21 full-canopied (TCS = 1) and two trees with low to moderate dieback (TCS = 2, 3). Muhlenberg, KY, which also lacked the ability to epicormically sprout, lacked did not produce collar sprouts. Both of these populations were removed from further analysis because they may lack the ability to sprout.

Predicting vegetative regeneration

I aimed to predict probability of presence and count of both epicormic and collar sprouts. To better understand epicormic sprouting, I examined whether TCS, sex, ploidy, and/or size predict sprout presence and/or number of epicormic sprouts. Overall, approximately 16.7%

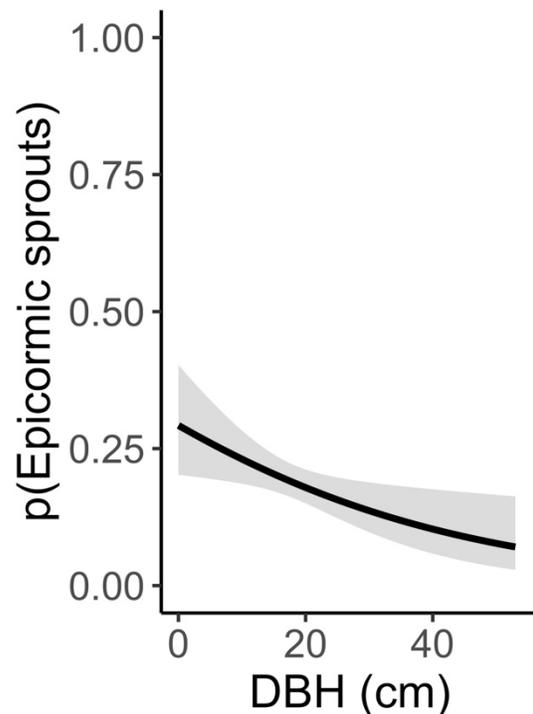


Figure 2-4. Probability of epicormic sprout presence by DBH (cm). Grey cloud represents 95% confidence interval.

of all trees had epicormic sprouts (122 of 731 trees). Presence of epicormic sprouts was not predicted by ploidy ($\chi^2 = 1.3$, $df = 2$, $P = 0.51$), sex ($z = 1.007$, $P = 0.3139$), or TCS ($\chi^2 = 4.1$, $df = 4$, $P = 0.4$). These factors were removed and a model with solely DBH was significant ($\chi^2 = 6.099$, $df = 1$, $P = 0.0135$). Probability of epicormic sprouting was always less than 0.3. For every unit increase in DBH, the probability of sprouting dropped approximately 0.0024 (Fig. 2-4; $z = -2.428$, $P = 0.0152$).

Across the site, I found that the average number of sprouts was 0.521. Sprout number was significantly predicted by a zero-inflated Poisson regression ($P = 0.00168$) and this model performed better than a standard Poisson regression ($z_{\text{vuong}} = 5.983$, $P = 1.094 \times 10^{-9}$). Neither ploidy ($\chi^2 = 2.2$, $df = 2$, $P = 0.33$), sex ($z = -1.487$, $P = 0.137$), or TCS ($\chi^2 = 4.7$, $df = 4$, $P = 0.31$; Appendix B Fig. 1) were significant predictors of sprout count. The only significant predictor of sprout count was DBH ($z = 2.415$, $P = 0.0157$), whereby larger trees were predicted to have less sprouts (Fig. 2-5). Notably, the predicted number of sprouts was always less than one.

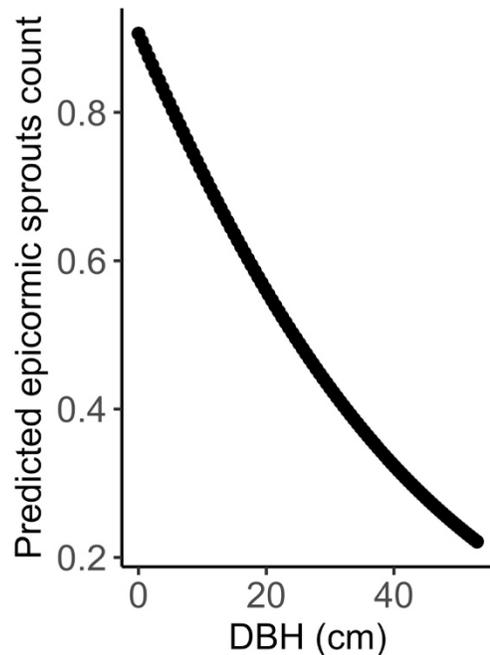


Figure 2-5. Predicted frequency of epicormic sprouts by DBH (cm).

Similarly, I examined whether TCS, sex, ploidy, and/or size predicted collar sprout probability of presence and/or number of collar sprouts. Overall, approximately 19.6% of trees had collar sprouts (143 of 731). Presence of collar sprouts was not predicted by ploidy ($\chi^2 = 1.3$, $df = 2$, $P = 0.51$) or sex ($\chi^2 = 0.91$, $df = 1$, $P = 0.34$) and these variables were dropped from the subsequent model. The reduced model was significant ($\chi^2 = 221.1904$, $df = 9$, $P = 1.16 \times 10^{-42}$). I

found that DBH ($z = -5.436$, $P = 5.45 \times 10^{-8}$), TCS ($\chi^2 = 13.8$, $df = 2$, $P = 0.0079$), and their interaction ($\chi^2 = 10.8$, $df = 4$, $P = 0.029$) were all significant predictors of collar sprout presence. Trees with no to moderate canopy dieback (TCS = 1-3) show decreasing likelihoods of collar sprouting as they get larger, predicting no collar sprouts if DBH was larger than 20 cm (Figs. 2-6.1, 2-6.2, 2-6.3). On the other hand, trees with heavy dieback (TCS = 4) had a stable ~75% chance of having collar sprouts (Fig. 2-6.4). Trees with no canopy (TCS = 5) are more likely to have collar sprouts as they get larger, increasing their chances from 0.50 at small DBH to nearly 0.70 at larger DBH (Fig. 2-6.5).

I found that the average number of collar sprouts across the site was

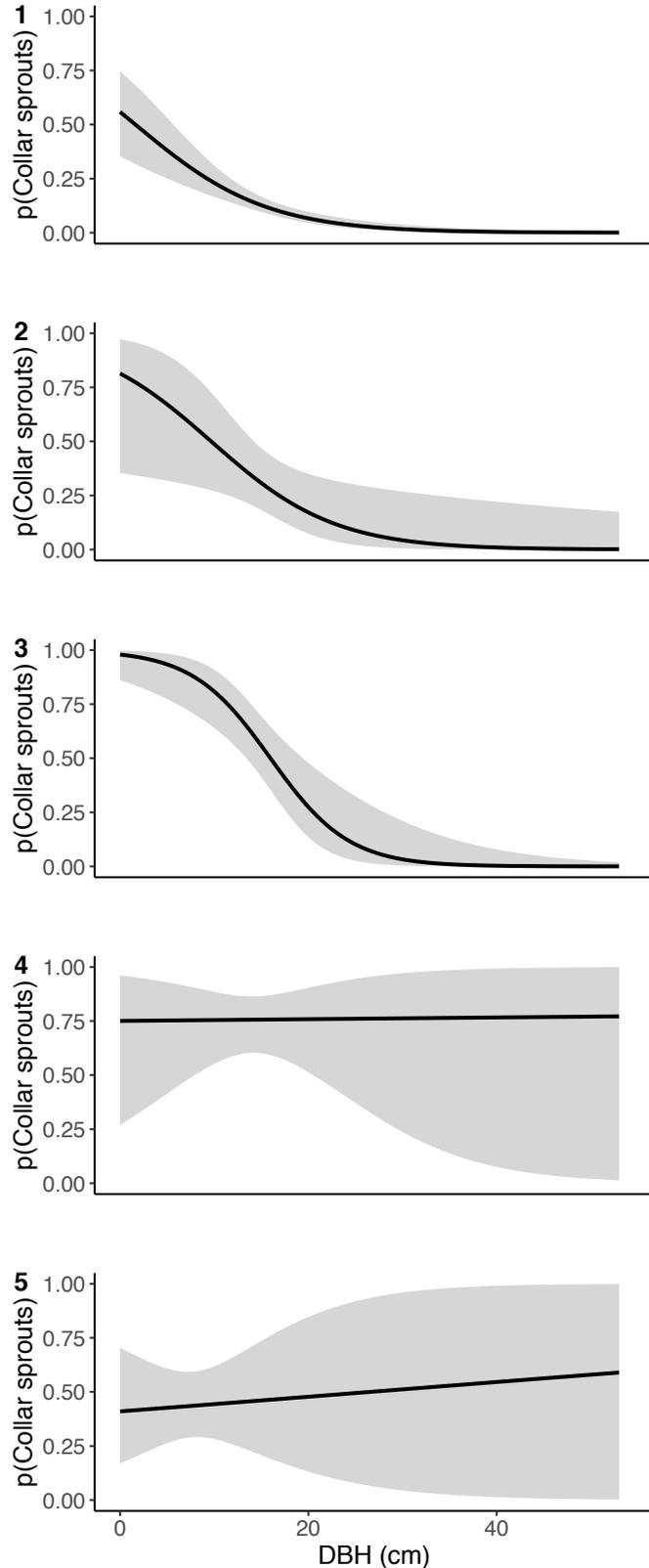


Figure 2-6. Probability of collar sprout presence by DBH (cm). Panel labels (1-5) represent TCS levels 1-5. Grey clouds represent 95% confidence intervals.

0.659. Count of collar sprouts was significantly predicted by a zero-inflated model ($P = 3.988 \times 10^{-53}$) and this model was better than a standard Poisson regression ($z_{\text{Vuong}} = 2.557$, $P = 0.005$). Collar sprout count was not predicted by ploidy level ($\chi^2 = 3.2$ $df = 2$, $P = 0.2$) or sex ($z = 1.816$, $P = 0.0694$). However, males tended to have 0.5 more collar sprouts and hexaploid individuals generally had 0.25 less collar sprouts than diploid or tetraploid individuals. I removed sex and ploidy level from the subsequent model and found that DBH ($z = 4.724$, $P = 2.31 \times 10^{-6}$) was a significant predictor of collar sprout count, but TCS ($\chi^2 = 2.3$, $df = 4$, $P = 0.69$) and the interaction of DBH and TCS ($\chi^2 = 6.3$, $df = 4$, $P = 0.17$) were not. Overall, larger trees were predicted to have less collar sprouts. No collar sprouts were expected for trees larger than 30cm in DBH if trees exhibited no to moderate dieback (TCS = 1-3). However, trees near death (TCS = 4) were marginally significant ($P = 0.51$) and predicted to have more collar sprouts as they get larger, nearly tripling in count (Fig. 2-7). This trend may be driven by individual trees with many collar sprouts (Appendix B Fig. 2).

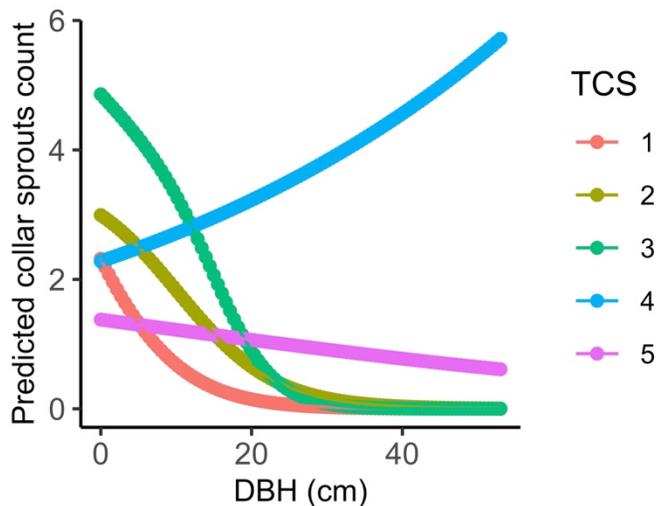


Figure 2-7. Predicted frequencies of collar sprouts by tree health status (TCS) and size (DBH). Differences between TCS level 4 and others was marginally significant ($P = 0.51$).

Discussion

My work sought to fill gaps in knowledge about intraspecific variation in sprouting and how it may contribute to persistence of white ash despite nearly complete mortality of the species in North America. Specifically, I questioned if and to what extent 42 white ash populations differed in their abilities to sprout from trunks (epicormic sprouts) or collars (collar sprouts). I found minimal variation in sprouting between populations (see also Żywiec and Holeksa, 2012), suggesting that sprouting is an individual-level process. I also questioned whether epicormic and collar sprout counts or probability of presence could be predicted by individual traits (DBH, TCS, ploidy level, sex). Both types of sprouts, and both count and probability of presence for both types of sprouts, were predicted by tree size (DBH), and TCS was a significant predictor of both count and probability of presence of collar sprouts. Sex and ploidy were not significant predictors in any analysis.

Differences among populations in sprouting

Minimal variation in both types of sprouting was due to population. Neither epicormic or collar sprouts showed clear trends for why some populations did not sprout, but it could be that these populations lack the ability to develop epicormic and/or collar sprouts, that apical dominance was especially strong in these populations (Schier, 1981), that bud banks are limited (Clarke et al., 2015), and/or that these populations stored too little carbohydrates to initiate sprouting (Walters et al., 2005; Clarke et al., 2010; Moreira et al., 2012; Clarke et al., 2015). I suggest that cause of no sprouting likely varies by sprout form. Sprout form is important because epicormic sprouts are more costly to nonstructural carbohydrate reserves than collar sprouts in *Eucalyptus* (Smith et al., 2018) and this trend may hold in other species. If it does, the population from

Adams, IL must have significant reserves because they exhibited epicormic, but lacked collar, sprouts. It is possible that because epicormic sprouts form first (Bellingham and Sparrow, 2000), carbohydrate reserves were depleted and too low when disturbance was severe enough to trigger collar sprouts. Of the five populations that lacked epicormic sprouts, four had collar sprouts, suggesting that the lower carbohydrate cost might be significant in white ash. Finally, the population from Muhlenberg, KY lacked both types of sprouts and merits further investigation.

Predicting vegetative regeneration

Some individual-level traits were predictive of sprouting. Sex and ploidy level had no significant effect on probability of presence of either sprout form. The lack of significant differences in sprouting between male and female trees suggests tradeoffs related to dioecious resource allocation may not impact vegetative regeneration (Bellingham and Sparrow, 2000; Obeso, 2002; Oñate and Munné-Bosch, 2009; McKown et al., 2017). However, there was a trend that males had more collar sprouts, and this trend may be exaggerated in masting years (2019 was not a masting year; T. E. Burnette, *pers. obs.*). Ploidy level also lacked significant differences, but hexaploids tended to have 0.25 fewer collar sprouts than diploids or tetraploids. If collar sprouts are considered a stress response as previously noted by Bellingham and Sparrow (2000) and Bond and Midgley (2001), this trend contradicts theory that higher ploidy levels provide advantages to plants in host/pest dynamics (King et al., 2012) and stress (Scholes and Paige, 2015).

Size (DBH) was the predominant factor for determining count and probability of presence for both sprout types, but tree canopy health altered the associations for collar sprouts. In almost every case, larger white ash trees are less likely to sprout and had fewer sprouts of

either form, supporting other findings that sprouting decreases with increasing size (Del Tredici, 2001; Mallett, 2002). Epicormic sprouts decreased in probability of presence and count as DBH increased, but epicormic sprouts are likely unimportant to white ash persistence given their low probability of presence across all sizes (~ 0.25) and their low predicted counts across all sizes (< 1). Three cases do not conform to this negative association of size and DBH, instead supporting intraspecific variation in sprouting found in *Castanopsis cuspidate* (Miura and Yamamoto, 2003). Trees with severe canopy dieback have nearly constant probability of collar sprout presence (Fig. 2-6.4) and trees with no canopy see a 0.2 increase in probability of collar sprout presence as DBH increased (Fig. 2-6.5). Count of collar sprouts for trees with severe canopy dieback is also positively associated with DBH, tripling in count (Fig. 2-7). It is important to note that these differences in size relationships for collar sprouts are tied to tree canopy status. Trees close to death are likely to have more collar sprouts, and because collar sprouts are the most likely form to become independent of the original trunk (Sakai et al., 1995; Del Tredici, 2001), this may be evidence for persistence.

Persistence

Evidence of collar sprouts on white ash trees with no canopy suggests that these sprouts may have the ability to persist. Only one epicormic sprout was found on a tree originating from Penobscot, ME with no canopy, suggesting epicormic sprouts are unlikely to persist beyond primary trunk mortality (but see Kappler et al., 2018). Similarly, Kappler et al. (2018) found collar sprouts on green ash trees in an Ohioan floodplain with no canopies, and collar sprouts were much more common than in my study: 75% of trees had collar sprouts. Lower collar sprout frequency in my study is possibly due to drought stress associated with the location of the common garden at the xeric edge of the white ash species range. It is also noteworthy that in

mixed hardwood forests, sprouts of either form in ash species do not last beyond canopy death (Klooster et al., 2014). On the other hand, *Castanopsis cuspidate* var. *sieboldii* individuals with dead primary trunks can continue to support sprouts and even develop new ones (Miura and Yamamoto, 2003). Further, there is some evidence for persistence of collar sprouts across years at our common garden because approximately 7.1% (35 of 496) of trees that died prior to 2019 had root collar sprouts in 2019, representing 20 of the 44 families (Burnette & Ward, unpublished). This data requires further investigation to ensure connection of these sprouts to collars but is a promising preliminary finding.

Several concerns about the long-term survival of vegetative regeneration merit discussion. Primarily, there is conflicting evidence about sprout growth into the canopy. While reviews suggest that sprouts fill canopy gaps quicker than seedlings (e.g., Bond and Midgley, 2001), others have found that sprouts grow more slowly, arguing it is because they are rebuilding crowns and maintaining large root systems (Paciorek et al., 2000). Further, in green ash, sprouts do not grow into the canopy (Burr and McCullough, 2014; Aubin et al., 2015). These findings in green ash may be because EAB attacks sprouts larger than 2.5 cm DBH (McCullough et al., 2008), which will likely be a challenge for white ash sprouts as well. Further, while persistence of these individuals may limit ecological disturbance, it is only a temporary solution if collar sprouts do not produce seeds. Collar sprout life span may be short (Bond and Midgley, 2001) and thus not reach the typical reproductive age of 20 years and size of 20-25 cm DBH in white ash (Schlesinger, 1990). However, the most recent evidence in green ash suggests sprouts younger than seven years old and greater than 4 cm DBH can produce seeds (Kashian, 2016). This is a promising finding for green ash, but it is unclear whether other ash species such as white ash will behave similarly.

Implications and conclusions

Both collar and epicormic sprouts were uncommon at the site, occurring approximately on one out of every five or six trees, respectively. I found minimal variation in sprouting between populations (see also Żywiec and Holeksa, 2012). Both types of sprouts, and both count and probability of presence for both types of sprouts, were predicted by tree size (DBH) but other traits (sex, ploidy level) were less important. There is some evidence for persistence of white ash past primary trunk mortality via collar sprouts, but this merits further long-term examination. While white ash persistence would reduce the effects of disturbance on ecosystems, it is merely an elongation of an individual's lifespan unless collar sprouts can produce seeds. In other words, white ash will not persist in the long-term without seed production.

The other primary gap is carbohydrate storage in white ash. Many studies have demonstrated carbohydrate storage is likely a key variable in determining whether an individual tree can sprout (Walters et al., 2005; Nzunda et al., 2008; Clarke et al., 2010; Moreira et al., 2012). If populations differ in their storage abilities, this may lead to population level differences in ash sprouting, despite claims that populations do not differ in sprouting (e.g., Żywiec and Holeksa, 2012) and may explain my small differences between populations. Storage may also impact sprout form because of differing carbohydrate costs as in *Eucalyptus* (Smith et al., 2018). It is unclear if this trend holds in other species, and even less clear in species facing EAB infestation and drought stress like white ash.

Chapter III

Conclusions

The emerald ash borer (EAB) is the costliest tree-killing insect to invade North America (Aukema et al., 2011). The results of this invasion are expected to be more ecologically destructive than the loss of American chestnut from eastern hardwood forests in the early 1900s, possibly elevating invasive species in forest understories (Hausman et al., 2010), contributing to mesophication (Dolan and Kilgore, 2018), and reducing forests' carbon sequestration (Fei et al., 2019). Ash is one of the most widely distributed tree genera in North America and may be functionally or completely eliminated from many forest and urban landscapes in the near future due to EAB (Aubin et al., 2015). Because of this extreme threat, EAB has become an emerging model organism for understanding how invasive pests can alter ecosystems and decrease natural resources that are economically and socially valuable.

It remains unclear whether white ash will persist and in what form following EAB infestation and future elevated drought stress (Sheffield and Wood, 2008; Taylor et al., 2012; Keellings and Engström, 2019; Koutroulis et al., 2019). White ash will lose its role as a timber species (Stewart and Krajicek, 1973) and will no longer be a dominant species in North American forests. Previous outbreaks of nonnative insects and pests provide some potential paths forward for white ash. It is unlikely that white ash will persist similarly to American beech as mortality rates across North America have already exceeded beech mortality (Knight et al., 2013; Wieferich, 2013), and this is evident even at our common garden (Haavik et al., in prep). It is more likely that white ash will be functionally eliminated from the landscape like American chestnut (Anagnostakis, 1987), whereby American chestnut only exists only as root collar sprouts (Paillet, 2002; Jacobs et al., 2013).

My findings support the possibility of white ash losing its functional roles and persisting on the landscape like American chestnut given high mortality at the common garden (Haavik et al. in prep) and because collar sprouts seem to live past primary trunk mortality. I found that both presence and count of collar sprouts were predicted by tree size (DBH) and tree health (TCS), whereby larger trees closer to death were most likely to have collar sprouts. Epicormic sprouts were predicted solely by DBH, but they do not persist beyond primary trunk mortality and they occur infrequently and at low counts. While there is still a need for long-term persistence studies of collar sprouts in white ash, specifically examining their growth into canopies and their ability to produce seeds, I am the first to document potential persistence of white ash via vegetative regeneration, and my findings contradict previous research that sprouts are not a viable persistence mechanisms (e.g., Klooster et al., 2014).

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Appendices

Appendix A.

Table 1. White ash tree populations at the common garden and their latitude (°) and longitude (°) of origin.

Population	Latitude (°)	Longitude (°)
Adams, IL	39.8	90.7
Benzie, MI	44.7	86
Bledsoe, TN (1)	35.5	85.2
Bledsoe, TN (2)	35.5	85.2
Boone, AR	36.4	93
Effingham, IL (1)	39	88.4
Effingham, IL (2)	39.1	88.4
Forest, WI (1)	45.7	89
Forest, WI (2)	45.7	89
Franklin, TN (1)	35.2	85.9
Franklin, TN (2)	35.2	85.9
Gallatin, IL	37.6	88.3
George, MS	30.8	88.8
Hopkins, KY (1)	37.3	87.6
Hopkins, KY (2)	37.3	87.6
Jackson, IL (1)	37.7	89.4
Jackson, IL (2)	37.7	89.4

Jackson, IN	38.9	86
Marion, AR (1)	36.4	92.8
Marion, AR (2)	36.4	92.8
McMinn, TN	35.3	84.5
Muhlenberg, KY	37.3	87.2
Oktibbeha, MS (1)	33.4	88.8
Oktibbeha, MS (2)	33.4	88.8
Oktibbeha, MS (3)	33.4	88.8
Onondaga, NY	42.7	76.1
Ontonagon, MI	46.6	89.5
Otoe, NE	40.6	95.7
Overton, TN (1)	36.5	85.4
Overton, TN (2)	36.5	85.4
Overton, TN (3)	36.5	85.4
Penobscot, ME (1)	44.8	69
Penobscot, ME (2)	44.8	69
Pickens, SC	35	83
Preble, OH	39.6	84.7
Presque Isle, MI	45.3	83.6
Randolph, WV	38.9	79.7
Tucker, WV	39.1	79.5

Union, GA	34.8	83.9
Washtenaw, MI (1)	42.2	83.7
Washtenaw, MI (2)	42.2	83.7
Wayne, OH	40.7	82

Appendix B.

Figure 1. Scatterplots of observed epicormic sprout frequencies versus DBH (cm). Blue line represents least-squares regression for each TCS level. Panels (1-5) represent tree health status levels (TCS levels 1-5).

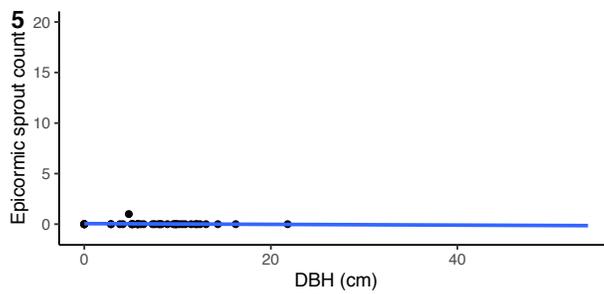
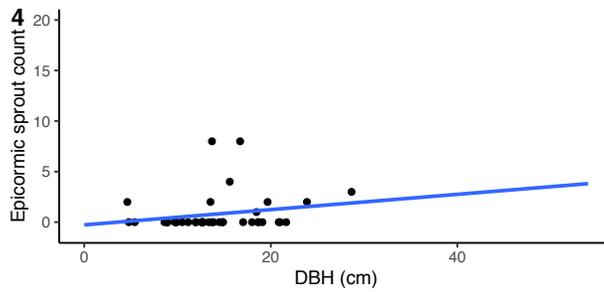
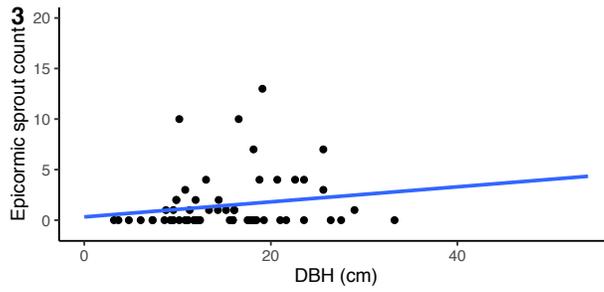
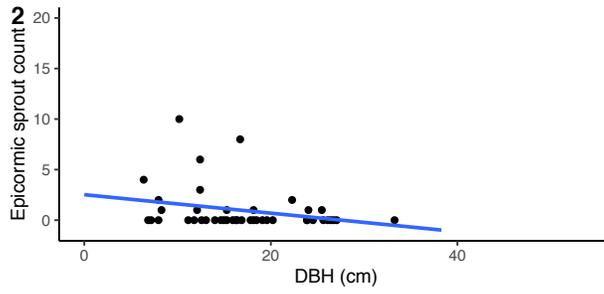
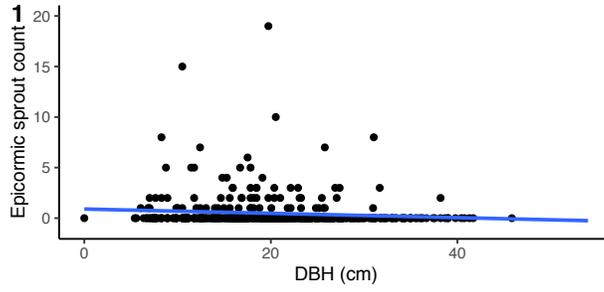


Figure 2. Scatterplots of observed collar sprout frequencies versus DBH (cm). Panels (1-5) represent tree health status levels (TCS levels 1-5). Blue line represents least-squares regression for each TCS level.

