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Distribution and Spread of an Invasive Shrub (Pyrus calleryana Decne) Across Environmental Gradients in Southern Indiana

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DISTRIBUTION AND SPREAD OF AN INVASIVE SHRUB (*PYRUS CALLERYANA*, DECNE.) ACROSS ENVIRONMENTAL GRADIENTS IN SOUTHERN INDIANA

by

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This thesis is dedicated to my children, Lane Dunn and Griffin Dunn, who I hope will share in their parent’s passion for the forest and nature.

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Invasive species represent one of the greatest challenges to ecological management today. With new species introductions occurring every year, understanding the impacts, mechanisms of spread, and characteristics of invaded habitats is vital in developing appropriate control methods. One newly escaped invasive species, *Pyrus calleryana*, is rapidly expanding its invasive range and potentially altering forest structure and composition in southern Indiana. This research investigated characteristics of invaded environments and patterns of spread in *P. calleryana*. I found an association between *P. calleryana* and areas with high levels of light and high stocking of shade intolerant species. Data further revealed an association between *P. calleryana* and dryer aspects. These results indicate *P. calleryana* is associated with more xeric environments with high light availability and will likely be found in early successional environments. The genetic structure of the study population indicated it was likely composed of two populations. This population structure indicated the importance of density to the spread of *P. calleryana* as one population corresponded most closely with areas composed of high densities of *P. calleryana* and the second population was more associated with the expanding edge of the invasion. These results indicate that bird driven dispersal, introduction of new individuals via horticulture, and population density are the factors with the greatest influence on *P. calleryana* overcoming its self-incompatibility. Overall, *P. calleryana* has the potential to rapidly expand into disturbed environments and successfully invade, particularly when population densities are high.

CHAPTER 1. INVASIVE HISTORY OF *PYRUS CALLERYANA* IN THE UNITED STATES

Non-native plant invasions are among the greatest issues facing natural resource management today, with economic impacts associated with control efforts and ecological impacts due to loss of habitat and biodiversity. These impacts increase with time and, the longer an invasive species is present, the greater the likelihood it will expand across the landscape and produce shifts in species composition and forest structure (Arim et. al, 2006; Williamson and Fitter, 1996). Further, more advanced invasions are more difficult to control with effective means of eradication and management coming at a high economic costs estimated at more than $120 billion (USD) annually (Pimental et. al, 2005; Burt et. al, 2007).

Theories of Invasion

An invasive species is defined as one that has or is likely to spread into new habitats, develop self-sustaining populations, and become a disruptive or dominant species (Reichard et. al, 2001). While invasive species may be defined as native or non-native, this study focused on non-native invasive species. The likelihood of invasive plants spreading is a function of mode of introduction. When a non-native species is introduced intentionally, often for horticulture, its likelihood of spreading to new areas is increased. This is because these species are more likely to undergo multiple introductions over a wide area. They are also likely to have been selected to have a high likelihood of success in their new environment (Arim et. al, 2006; Burt et. al, 2007; Culley et. al, 2011). When a species is introduced accidentally (via contaminated seed mix), its likelihood of spreading is variable and dependent on the application of the contaminated material and extent of contamination (Reichard et. al, 2001). For example, if there is a large degree of contamination in

a seed mix to be distributed in city parks across a state there is a higher likelihood of spread. Alternatively, where there is a low level contamination and the material will only be applied in the yard of a single home, the likelihood of spread is reduced. Species introduced widely via horticultural plantings have greater likelihood of developing into an invasive (Culley et. al, 2011). This advantage stems from the increased probability that a species or horticultural variety will be introduced to a suitable habitat alongside other individuals of the species or genus aiding in reproductive success and recruitment (Drenovsky et. al, 2012).

Following introduction to a new area, many non-native species enter a lag phase where the population either survives and reproduces within the native community, or is outcompeted, and eradicated (Culley and Hardiman, 2007). The length of the lag phase varies due to a poorly understood suite of biological and environmental factors inferring a general lack of predictability (Reichard et. al, 2001). One particularly important biological factor which can be determinant of successful (or unsuccessful) establishment is biotic resistance. Biotic resistance is the culmination of species interactions that makes an area resistant or susceptible to invasion. Biotic resistance postulates that communities with higher diversity are more resistant to invasion because the interactions among species are more tightly linked and there is less available niche space (Terhorst and Lau, 2015). However, analysis of species interactions and the likelihood of invasion in plant communities indicate biotic resistance has weak influence on invasion by non-native species (Levine et. al, 2004; Maron and Vila, 2001).

Successful establishment and expansion of introduced plants, as predicted by the evolution of increased competitive ability (EICA) hypothesis, are dependent upon phenotypic changes during the lag phase (Blossey and Notzold, 1995). These phenotypic changes lead to a greater investment by introduced species in biomass production and reproduction as opposed to

investment in defense. The lack of investment in defense mechanisms and structures is largely due to the escape of these non-native species from the enemies present in their native range (Blossey and Notzold, 1995). An introduced population is usually not recognized as problematic until it exits the lag phase and has undergone some adaptation to its new environment (Jaric and Cvijanovic, 2012; Keane and Crawley, 2002). As the invasion moves from the lag phase to the expansion and saturation phase, the increased success of introduced plant species over native members of the community may result in a great deal of ecological damage. At this point, cost of control can be quite high because the population has grown in spatial extent and density, both of which can pose a significant hindrance to control efforts (Pimentel et. al, 2005).

Another explanation of invasive species is offered by the enemy release hypothesis (ERH) which states that individuals introduced outside their natural range are released from inhibition by natural enemies enhancing their ability to compete with native populations (Keane and Crawley, 2002). This hypothesis is related to EICA in that it sets the stage for the shift in resource allocation from defense to biomass accumulation following introduction (Blossey and Notzold, 1995). An example supporting this hypothesis exists in *Alliaria petiolata* (garlic mustard); this species experiences lower levels of herbivory in its introduced range in the United States. The reductions in herbivory on the plant appear to be connected to differential biomass accumulation and chemical defense production among individuals in *A. petiolata*'s introduced and native ranges (Lewis, 2006). Overall, support for EICA is relatively limited in the literature whereas there is far more support for ERH (Bossdorf et. al, 2005; Keane and Crawley, 2002; Maron and Vila, 2001). This difference in support, despite the tight interaction between these two hypotheses, can be attributed to two major factors: first, the often generalist behavior of many herbivores and second, the

difficulty in providing a full analysis of EICA which requires a full assessment of growth and defense in a species in both its native and introduced ranges (Bossdorf et. al, 2005).

Innate characteristics of a species may also contribute to its invasiveness. For example, many of the most problematic invasive plant species in the United States originate from East Asia. The enhanced invasiveness of these species likely results from climatic similarity between native and introduced ranges and the extended leaf phenology of East Asian species (Fridley, 2012; Heberling et. al, 2017). This extended phenology is a preadaptation that provides a competitive edge for introduced species in the form of an extended growing season relative to native species. An example of a species demonstrating this trait is *Lonicera maackii* (Amur honeysuckle), a Eurasian shrub species introduced to the United States in the late 19th century. *Lonicera maackii* is documented producing leaves earlier in spring than its native competitors and retaining these leaves much later into the fall (Fridely, 2012; Luken and Thieret, 1996).

Modes of Introduction

Introduction of non-native woody species is primarily anthropogenic and intentional (Burt et. al, 2007; Heberling et. al, 2017). Woody species are introduced for a variety of reasons including erosion control, aesthetics, and/or to confer disease resistance to a native species (Sakai et. al, 2001). Some of the most problematic invasive species such as *Eleaegnus umbellata* (autumn olive), *Rosa multiflora* (multifora rose), and *Pueraria montana* (kudzu) were originally introduced to reduce soil erosion from poor farming practices. For example, 85 million cuttings of *P. montana* were offered to farmers in the southern United States with a planting incentive of approximately $3 per hectare. Promotional efforts were successful and *P. montana* is now one of the most serious invaders of the southern United States (Reichard et. al, 2001). In many cases, introduced species

are also “bred for success.” *Lonicera maackii*, underwent selective breeding both before and after introduction to enhance horticultural success (Luken and Thieret, 1996).

In the past, intentionally introduced plants were more likely to be widely planted, thus enhancing the likelihood they would be placed near suitable environments (Culley et. al, 2011). Ideally, non-native species should be monitored for opportunistic behavior, though instances of this monitoring are relatively rare. Instead most monitoring focuses on prediction of invasions following introduction (Reichard et. al, 2001; Thuiller et. al, 2005). Further, this observation period may coincide with the lag phase of establishment, which can mask traits associated with invasiveness. For example, the longer juvenile period of woody species and the poor correlation of controlled greenhouse settings with field conditions may inhibit the identification of invasiveness.

The horticulture industry is responsible for the introduction of many non-native ornamental species. These introductions are in response to demand for plants that are easy to care for, aesthetically pleasing, or improve existing species through breeding. Of 235 woody species identified as naturalized, 85% were introduced primarily or secondarily for horticultural use (Culley et. al, 2011).

Despite the overwhelming contribution of the horticulture industry to the pool of invasive plants, the majority of introduced species do not become invasive (Reichard et. al, 2001). This is reflected in the Rule of Tens which describes the variable success of non-native species at becoming invasive. This rule hypothesizes that out of 1000 introduced species, 10% will escape cultivation, and of those that escape, 10% will establish, and of those that establish (i.e. reproduce and disperse to other habitats), 10% will successfully become invasive (Williamson and Fitter, 1996). The Rule of Tens suggests that not every species is able to successfully establish and

develop increased competitive ability and even those that do, still may not develop into an invasive pest. However, the Rule of Tens was developed purely on the basis of statistical models for introduced species, regardless of mode of introduction (Williamson and Fitter, 1996). Because this theory focuses purely on proportions and fails to take into account traits or modes of introduction of non-native species, it is likely that this rule will fail to accurately predict invasion success of species introduced via horticulture. This is due to the fact that species introduced via horticulture are often selected upon post-introduction in order to improve the environmental tolerance of the introduced species. Additionally, this rule further fails to account for the source of introduced populations. For example, East Asian invasive species tend to be highly successful invaders in North America due to extended leaf phenology. Extended leaf phenology confers an advantage to invasive species from East Asia because they are able to leaf out earlier in the spring and hold their leaves later into the fall allowing them to have a longer growing season than their native competitors (Fridley, 2012). This is an important short-coming of the theory because there are great variations in the regions from which introduced species stem and these differences result in a range of innate species characteristics that may increase invasiveness. Further, species traits (both natural and enhanced via cross-breeding) can have profound impact on introduction success (Jaric and Cvijanovic, 2012). For example, as mentioned above, horticultural breeding programs associated with *L. maackii* are thought to be an important factor in its successful invasion across the United States (Luken and Thieret, 1996).

Another essential factor in the spread of a successful invasive species is post establishment dispersion and recruitment. Here, I define recruitment as occurring when new individuals are added to a population through seed dispersal or vegetative reproduction. Seed and pollen dispersal are critical to recruitment and offer population level mechanisms by which an invasive species

expands its introduced range through the establishment of new individuals. Because seeds represent the dominant mobile stage for plants, seed dispersal patterns determine the spatial extent of recruitment where other ecological interactions such as predation, competition, and mating occur (Nathan and Muller-Landau, 2000). Dispersal of pollen by insects, birds, mammals, or wind can greatly affect the long-term success of introduced species. The disjunct range over which pollen and fruits are dispersed poses a specific challenge to introduced species as pollen dispersal is vital to the fecundity and successful spread of introduced species.

Focal Species

Pyrus calleryana was introduced to the United States from Asia following expeditions conducted from 1916 to 1918 by Frank Meyer and Frank Reimer of the United States Department of Agriculture (USDA). Meyer collected *P. calleryana* seed from five locations in China, and Reimer collected seed from Korea and Japan (Culley and Hardiman, 2009). Multiple shipments of seed were transported to the United States (Vincent, 2005). The resulting *P. calleryana* seed was planted at research stations in Oregon and Maine. These seeds were used in breeding programs with *Pyrus communis* (common pear) which was being decimated by the fungus, *Erwinia amylovora* (fire blight). To accomplish this, *P. communis* was grafted onto rootstock of *P. calleryana* seedlings exhibiting fire blight resistance (Culley and Hardiman, 2007).

*Pyrus calleryan*a became established as a viable ornamental species in the 1950s when its aesthetic potential was recognized and it continues to be cultivated as an ornamental species to this day (Vincent, 2005). To maintain uniformity across cultivars, *P. calleryana* trees are propagated by grafting the desired cultivar onto the rootstock. This process produces progeny which have genetically identical scions that are incapable of selfing (Culley and Hardiman, 2009). Several cultivars originated from the original Asian seed. Several others resulted from crossing of

propagated individuals over successive generations such as the Whitehouse cultivar, originating from an unintended cross between the Bradford cultivar and an unknown *P. calleryana* cultivar (Culley et. al, 2011). The unknown parentage of the Whitehouse cultivar suggests the species was capable of escape early in propagation. Now, more than 25 cultivars of *P. calleryana* exist, many of which are genetically distinct and capable of crossing with other cultivars. *Pyrus calleryana* remains a popular ornamental species due to its abundant and early flowering; compact, rounded crown shape; and tolerance of a variety of environmental conditions (Culley and Hardiman, 2009). As of 2005, the species has naturalized populations established and spreading in more than 26 states and as of 2007, the species was classified as invasive or on a watch list in 10 states (Culley and Hardiman, 2007; Vincent, 2005).

Pyrus calleryana was initially thought to be incapable of sexually reproducing and becoming naturalized due to its self-incompatible nature (Gilman and Watson, 1994). The most determinant factor in the escape of *P. calleryana* was its escape from infertility. This escape occurred as flaws in the ornamental character of the original cultivar, Bradford, were recognized. Improvements to ornamental characteristics led to the development of many genetically distinct cultivars capable of sexually reproducing with one another. The development of ornamental *P. calleryana* created an abundance of genetic diversity across cities and suburbs (Culley and Hardiman, 2009). The proximity of genetically distinct mates across plantings, paired with the abundant fruit production of *P. calleryana* led to an enhanced ability of the species to spread into natural areas (Cuizhi and Spongberg, 2003; Culley et. al, 2011). In early spring, *P. calleryana* is among the first species to flower and does so before leaf production; it also demonstrates extended leaf phenology relative to native species, similar to other East Asian invasive woody species (Fridley, 2012).

Pyrus calleryana trees produce 6-12 flowers per inflorescence (Cuizhi and Spongberg, 2003). These flowers have approximately 20 stamens with 25 fused carpels and 2 ovules per flower. This produces a maximum of 10 seeds per fruit, averaging 4-6 seeds. There is the potential for production of seeds that are not viable, though actual viability rates of seeds have yet to be assessed (Jackson, 2003). While the range of seed production of individuals, is not known, they are capable of producing viable seed as early as three years of age (Cuizhi and Spongberg, 2003). This early age of first reproduction is thought to contribute to the potential of the species to rapidly establish and recruit into new areas. The potential limiting factor for this self-incompatible species is pollen dispersal. Fruits of *P. calleryana* are consumed and dispersed by a variety of bird species, which creates the potential for seed to be transported great distances. However, insect pollination occurs over a shorter distance limiting recruitment of individuals far outside the established range (Burd, 1994; Nathan and Muller-Landau, 2000).

An extensive population of *P. calleryana* exists on Naval Support Activity (NSA) - Crane in Crane, Indiana. Initially, *P. calleryana* was likely introduced into the area that became Crane through a nursery present in the 1930s. During the Great Depression, several thousand hectares of abandoned farmland surrounding the nursery were purchased by the United States Department of Agriculture. As World War II escalated, the Navy sought an in-land base to store munitions. Therefore, the USDA transferred the initial purchase to the Navy, which later purchased several thousand additional hectares including the now defunct nursery. In the 1970s, a golf course was constructed and additional *P. calleryana* trees were planted on the base near where the nursery had once been. A few decades later, *P. calleryana* was recognized as spreading into other areas of the base and this expansion continues today. The current invasion is likely a product of rootstock remaining from the nursery crossing with cultivars planted on the golf course. Other horticultural

planting on NSA Crane (including planting associated with a beautification project) likely also contributed to the current extent and abundance of the invasive population.

The *P. calleryana* population on Crane is distributed across most of the base, and suggests that birds have distributed seeds to new areas, as observed in other invasive plants (Merow et. al, 2011). However, because this species is self-incompatible, pollen dispersal is likely the limiting factor for subsequent recruitment and genetic diversity across the population (Merow et. al, 2011). This idea is supported by the existence of isolated mature *P. calleryana* stems far from the invasive edge waiting for a distinct pollen source to reach them and allow production of viable fruits and successful reproduction. Once fertilized, seeds from these isolated individuals have the potential to expand the population further into new areas. In an urban setting, long-distance dispersal events may be more successful due to the increased availability of *P. calleryana* trees originating from different cultivars. Long distance dispersal events are a particularly important factor in the spread of invasive species (Hastings et. al, 2005). The remoteness and size of the base suggests that off-base source populations are not within a distance that allows significant crossing. Spread of the existing population is dependent upon dispersal of genetically distinct seed and pollen to new areas allowing establishment and recruitment (Merow et. al, 2011). Aside from cross-compatibility of the many cultivars of *P. calleryana*, little is currently known about the effects and mechanisms of invasion in *P. calleryana* (Culley and Hardiman, 2009). As this species continues to invade into new environments, understanding mechanisms driving success and effects of invasion represent important lines of research.

In Chapter 2 of this thesis, I investigated how the density of *P. calleryana* varies across environmental gradients. Understanding influential environmental gradients controlling distribution of this species is a vital step in identifying environments vulnerable to invasion. The

advanced age of the invasion on Crane presented a unique opportunity to examine environmental conditions that favored invasion through time.

In Chapter 3, I examined genetic similarity as a proxy for relatedness of individuals to assess patterns of dispersal of *P. calleryana*. By analyzing allele frequencies of 202 individuals across nine loci, I was able to assess genetic relatedness of individuals at a spatial scale that elucidated patterns of seed and pollen dispersal. Understanding these patterns allowed for improved understanding of potential mechanisms for the successful spread and recruitment of *P. calleryana* (Barribal et. al, 2015). By determining relatedness of individuals across the landscape, the different distances over which pollen and seed dispersal occurs and how the invasive population is spreading is better understood. Determination of spread in *P. calleryana* is a key step in understanding the process of successful invasion; an essential piece in determining appropriate control mechanisms (Arim et. al, 2006).

CHAPTER 2. ENVIRONMENTAL GRADIENTS ASSOCIATED WITH INVASIVE *PYRUS CALLERYANA* IN A SOUTHERN INDIANA FOREST

Abstract

Understanding the ecosystem characteristics that affect the likelihood of invasion is essential to predicting the vulnerability of uninvaded areas to invasion. This understanding is of critical importance during the expansion stage of an emerging invasion of a relatively understudied exotic species. This research focuses on a population of one such invasive species, *Pyrus calleryana,* located in southern Indiana. I sought to identify characteristics of invaded forest communities through measurements of the biotic environment (i.e, overstory, sapling, and regeneration layer), abiotic environment (i.e, canopy cover, slope position, and aspect), associated species tolerances to shade, drought, and water-logging represented by Niinemets derived community score. Sample plots had *P. calleryana* densities ranging from 198 to 18,911 stems ha^{-1}. I found that increased *P. calleryana* density was associated with shade intolerant species in the sapling and regeneration layer, and with drought tolerant species in the regeneration layer. *Pyrus calleryana* also demonstrated a relationship to aspect. Unsurprisingly, I found that older stems of *P. calleryana* were associated with higher abundances of *P. calleryana* stems, demonstrating a relationship between residence time and density of invasion. Overall, my results indicated that *P. calleryana* is expected to more commonly invade recently disturbed or early successional habitats with high light availability relative to more mature forests. There is also an apparent preference of *P. calleryana* for aspects on south and southwestern-facing slopes. This information is valuable to management of *P. calleryana,* as it identified vulnerable environments and can help focus eradication efforts as the invasion continues to expand into new areas.

Introduction

In invasive plant ecology, most research efforts have focused on well-established species that are already impairing ecosystem function and where control is difficult and eradication is practically impossible (Richardson and Pysek, 2012; Hobbs and Mooney, 2005; Levine et. al, 2003; Lee et. al, 2004; Barribal et. al, 2005). While these efforts are noble, it is arguably more important that we understand what makes an environment vulnerable to invasion, dispersal mechanisms of invasive species, and the autecology of species in active expansion that may be just beginning to affect ecosystem function and become a management problem. Understanding the ecological behavior of these more recently introduced species will allow early identification of aggressive invaders and better prediction of their rate and pattern of dispersal and establishment.

Invasive trees and shrubs pose particular challenges to forest management (Webster et. al, 2006). In addition to altering species composition and stand structure, invasive woody plants may create legacy effects that shift ecosystem function (Kulmatiski and Beard, 2011). For example, invasive shrubs such as *Lonicera maackii* (Amur honeysuckle) have been shown to reduce the seedling abundance and diversity of native tree species and altered nutrient cycling (Gorchov and Trisel, 2003; Shields et. al, 2015). Often, these changes in composition, structure, and function create negative legacy effects that do not necessarily end with removal or control of the invasive, and thus further exacerbate existing management issues. For example, successional trends and lack of forest manipulation and disturbance in the Central Hardwoods Region have resulted in regeneration failure of *Quercus* spp. and a reduction in the overall diversity of the regeneration layer (Brose and Stout, 2014; Fei and Steiner, 2009; McEwan et. al, 2011). Negative effects of long-established invasive species and introduction and expansion of new species further exacerbate this issue (Hastings et. al, 2005; Shifley et. al, 2014). While not all newly introduced

species become invasive, managers must first identify and then mitigate their impacts early during establishment. This task can be difficult, but one of the key steps is determining which forest stands are most vulnerable to invasion. Vulnerability may be predicted from a range of biotic and abiotic factors such as structure, species composition, light availability, and moisture availability, but the relative importance of these factors vary with the physiological characteristics and environmental tolerances of a given invasive species (Drenovsky et al, 2012).

Invasion by non-native trees and shrubs can greatly influence light availability in the understory layers of forest communities, reducing the establishment of native competitors and reinforcing the dominance of the invasive (Hedja et. al, 2009). Throughout much of the eastern United States, *Pyrus calleryana* (Callery pear) is an emerging invasive species. *Pyrus calleryana* was introduced to the United States from China, Korea, and Japan in the early 1900s for horticultural purposes and has developed into a wildly popular ornamental tree species (Culley et. al, 2011; Culley and Hardiman, 2007). The species has become widespread across its introduced range in the eastern United States (Vincent, 2005). Since its naturalization, *P. calleryana* has established in a variety of forest types aided by its tolerance of a broad range of environmental conditions (Cuizhi and Spongberg, 2003).

Pyrus calleryana has only recently been identified as an aggressive species expanding into forested habitats. However, the characteristics of vulnerable forest stands have not yet been identified. Based upon observations of other woody invasive species, managers fear that *P. calleryana* invasion may lead to further simplification of forest structure and composition by outcompeting native species in the understory layer (Luken and Thieret, 1996; Cuizhi and Spongberg, 2003).

This research focused on a population of *P. calleryana* located in southern Indiana that established in the 1970s and spread across a large area of forest. Today, this forest is a part of Naval Support Activity – Crane, a naval base. The forests on the base are semi-continuous and composed of multi-age stands intermixed with roads and mowed edges. I characterized forest communities where the *P. calleryana* occurred and investigated biotic and abiotic factors that were correlated with greater abundance of the species. To this end, I examined species composition (including *P. calleryana*) of the under- and overstory layers across a range of environmental gradients (i.e. elevation, aspect, percent canopy cover, basal area, etc) to determine how environmental and community characteristics favored the establishment and persistence of *P. calleryana.* The age, density and spatial distribution of abundance of *P. calleryana* across the base provided an opportunity to investigate how environmental factors and community characteristics correlate with the abundance of *P. calleryana*.

Existing literature on *P. calleryana* indicates a tolerance to a variety of light and moisture conditions however, these characterizations have not been formally assessed (Culley and Hardiman, 2007). This research sought to understand how the distribution of *P. calleryana* is associated with native woody species across environmental gradients. This research further sought to understand how the distribution of *P. calleryana* is related to the physiological characteristics of native woody species in the forest understory. Invasion by *P. calleryana* may result in increased shade and suppressed growth of more shade intolerant species leading to a compositional shift towards more shade tolerant species (Culley and Hardiman, 2007; Vincent, 2005). I further sought to understand the relationship between density (stems ha^{-1}) of *P. calleryana* and residence time of invasion (as represented by age of large individuals).

Methods

Study Area

This research focused on a population of *P. calleryana* located on Naval Support Activity – Crane (38.870003 N, -86.831494 E), hereafter referred to as Crane, a 254 km^2 base located in southern Indiana (Fig. 2.1). Soils across the base are predominantly shallow silt loams derived from shale and limestone parent materials of the Crawford Upland Section of the Shawnee Hills Natural Region (Homoya et. al, 1985). The base was established in the 1940s to primarily function as a munitions storage facility. Prior to becoming a naval base, the area was largely composed of degraded farmland. Since establishment, these lands have returned to forest and are now dominated by *Quercus-Carya* (oak-hickory) and *Acer-Fagus* (maple-beech) forests with varying densities of invasive *P. calleryana* in the understory. The population of *P. calleryana* pre-dates establishment of the base and is thought to have primarily originated from two sources: 1) a nursery for ornamental plants existed in the 1930s before the base was established but was abandoned along with hundreds of acres of farmland during the Great Depression; and 2) a golf course, established in the 1970s but now converted to other uses, that contained several planted *P. calleryana* trees. *Pyrus calleryana* also occurs in several other, more recent ornamental plantings throughout Crane.

Field Sampling

In March of 2015, I conducted a reconnaissance survey of the *P. calleryana* invasion on Crane using timed field searches of locations distributed along roadways throughout the base. Areas were classified as “severely invaded” where stems were located within 50 m of the roadway and found within five minutes of searching. Areas were classified as “diffusely invaded” where stems were located within 100 m of roadway and found within 10 minutes of searching. Lastly, areas were

classified as “absent” where stems were not located within 200 m and after 10 minutes of searching.

I used this classification (severe, diffuse, and absent) to create a stratified sample design for the location of vegetation plots which were used to assess the abundance and age of *P. calleryana*, plant community structure and composition, and canopy cover (Fig. 2.2). Plots were randomly placed within each strata at a density of 2.5 plots per km^2 using ArcGIS 10.3. Because Crane is an active military base, some plot locations were rejected due to safety or security concerns. Of the 324 plots sampled; 104 were in the severe category, 126 were in the diffuse category, and 94 were in the absent category (Fig. 2.2).

I sampled vegetation using a combination of variable and fixed radius plots. The overstory in each plot was sampled using a 2.3 m^2 ha^{-1} (10 ft^2 ac^{-1}) basal area factor (BAF) variable radius prism (Avery and Burkhart, 2002). Overstory trees were defined as those exceeding 11 cm in diameter at breast height (DBH; 1.38 m). Saplings, here defined as woody stems between 1.5 and 11.0 cm DBH, were inventoried using a 0.01 ha concentric circular subplot. Species and DBH were recorded for all measured overstory trees and saplings. Woody regeneration (i.e. DBH <1.5cm) was measured within four separate 4.52 m^2 subplots placed 6.87 m from plot center at 45º, 135º, 225º, and 315º azimuths. Abundance was recorded by species and height classes, defined as: 1: <10 cm height; 2: 10-30 cm in height; 3: 30.1-60 cm in height; and 4: >60 cm in height.

Percent canopy cover at the plot center was estimated using the average of four spherical densiometer readings, each taken at breast height facing in one of the four cardinal directions at plot center. The average of the four readings was multiplied by 1.04 and subtracted from 100% to obtain a percent canopy cover reading.

All *P. calleryana*, regardless of diameter or height, were tallied within in the 0.01 ha sapling subplot to estimate density of invasion. To estimate age of invasion, the largest stem of *P. calleryana* greater than 1.5 cm DBH and within 50 meters of plot center was cut with a pruning saw at breast height. A cookie, defined as a cross-section of the tree trunk, was then collected from this cut stump. For stems smaller than 1.5 cm, a cookie was not collected due to the distortion of rings that occurred by the cutting process barring the accurate counting of rings. In plots where a single *P. calleryana* stem was present but a cookie was not collected due to size, age was assumed as one year. Similarly, where a cookie was not collected, plots containing one to five stems were assigned an age of three years and plots containing five or more stems were assigned an age of five years. These assignments were based on the assumption that increased age results in increasing density. Where a cookie from the stem was collected, age was assessed by counting rings using a hand lens. Diameter of each cookie was also recorded.

Data Preparation

Species dominance at each plot was relativized using basal area of overstory and saplings, and the stem density of regeneration. Relative basal area of overstory or saplings was calculated by taking basal area of a given species divided by the total basal area at that plot. Relative density of regeneration was calculated by taking density of a given species divided by the total density at that plot. These relativizations were assigned to each species in each of the three strata: overstory, sapling, and regeneration. The main matrix consisted of species relative dominance (RD) from the overstory (basal area in m^2 ha^{-1}), sapling (basal area in m^2 ha^{-1}), and regeneration (stems ha^{-1}) strata.

In order to prepare my data for analysis using Non-metric multi-dimensional scaling, an environmental matrix consisting of several classes of variables was created. This matrix consisted

of three categories of variables: location and edaphic variables, invasion variables, and community variables. Location and edaphic variables included elevation (m), transformed aspect (Beers et. al, 1966), slope position (represented as a value from 0-1 with zero representing the lowest elevation within 200 m of the sample point and one representing the highest), and distance to nearest road (m). Invasion variables included *Pyrus calleryana* density (stems ha^{-1}) as a proxy of intensity of the invasion; and age of oldest *Pyrus calleryana* at each plot as a proxy for duration of invasion. Lastly, community variables included percent canopy cover, and Niinemets derived community scores for shade tolerance, drought tolerance, and water-logging tolerance of all species in the plot (Niinemets and Valladares, 2006). Niinemets derived community scores are represented as a value between one and five where one represents very intolerant and 5 represents highly tolerant. The Niinemets score for shade tolerance (ST) was originally calculated by performing a linear regression across different shade tolerance classifications of North American and European species ranked according to their shade tolerances to create a numerical index for each species. These classifications include both measurements on minimal light requirements and more subjective assessments of species shade tolerances (Niinemets and Valladares, 2006). The Niinemets score for water-logging tolerance (WT), defined as tolerance to reduced soil oxygen availability in the root-zone, was originally derived by a cross-calibration across several existing datasets on the water-logging of species. The WT measure is highly qualitative and defines the 5 scores as follows: 1) incapable of tolerating water-saturated soils for more than a few days during the growing season; 2) tolerant of water-saturated soils during the growing season for one to two weeks; 3) capable of surviving water-logged soils for 30 consecutive days during the growing season; 4) will survive deep water-logging for an entire growing season; and 5) tolerant of deep and prolonged water-logging lasting more than a year (NIinemets and Valladares, 2006) The Niinemets score for

drought tolerance (DT) was originally derived based on physiological tolerance to three major species characteristics: physiological tolerance to water-stress, morphological adaptations to cope with scarce water, and water availability typical of sites where the species frequently occurs. The scores are defined as follows: 1) more than 600 mm precipitation and low variation over the growing season; 2) 500-600 mm precipitation over the course of the growing season with variation in that precipitation characterized by a coefficient of variation less than 10% and no drought period; 3) 400-500 mm of precipitation during the growing season with a coefficient of variation of 10-15% and up to one month of drought; 4) 300-400 mm of precipitation with a coefficient of variation of 20-25% with two to three months of drought; and 5) less than 300 mm of precipitation with a coefficient of variation greater than 25% and more than three months of drought (Niinemets and Valladares, 2006). For this research, I created a single index value for community-level environmental tolerance each strata within the plot by multiplying the relative basal area or relative density of each species by the Niinemets score for that species and summing according to strata.

Niinemets scores across all the species in a stratum provided an assessment of the physiological tolerance of each strata and suggested how overstory composition may change through time. Differences in scores between the overstory, sapling, and regenerating strata allowed me to assess the competitive environment in which *P. calleryana* established. Niinemets scores utilized were overstory shade tolerance (ST_O), sapling shade tolerance (ST_S), regeneration shade tolerance (ST_R), overstory drought tolerance (DT_O), sapling drought tolerance (DT_S), regeneration drought tolerance (DT_R), overstory water-logging tolerance (WT_O), sapling water-logging tolerance (WT_S), and regeneration water-logging tolerance (WT_R). However, because the scores are ordinal in scale and represent relative rankings, changes between scores do not necessarily represent equal (cardinal) changes in physiological tolerance.

Statistical Analysis

Non-metric multidimensional scaling

Woody species composition along environmental gradients was assessed using non-metric multidimensional scaling (NMS; Kruskal, 1964; Mather, 1976) in PC-ORD Version 5.31 (McCune and Mefford, 2006). NMS ordinations examined the distribution of *P. calleryana* within forest communities across measured environmental and site variables. The main matrix consisted of species represented by their relative basal area or relative density according to strata. The environmental matrix consisted of *P. calleryana* stem density (stems ha^{-1}), *P. calleryana* age of oldest individual, percent canopy cover, distance to roads (m), total basal area by plot, transformed aspect (Beers et. al, 1966), elevation (m), drainage (as represented by a value between 0 and 1 with 0 being poorly drained and 1 being well drained), ST_O, DT_O, WT_O, ST_S, DT_S, WT_S, ST_R, DT_R, WT_R, and slope position (as represented as a value between 0 and one calculated as the relative position of the plot to the lowest and highest points located within 200 m). The initial iteration included all 324 plots and basal area in m^2 ha^{-1} (overstory and saplings) and stem density in stems ha^{-1} (regeneration). On later iterations, I reduced the main matrix by eliminating plots which did not contain *P. calleryana*, removed rare species which occurred on less than five plots in any of the strata, and used relative basal area and relative density as data inputs. These manipulations were made to reduce the stress of the final ordination solution. The final ordination included 73 plots and 83 species. I utilized the autopilot mode in PC-ORD 5.31 to conduct my NMS analysis on the slow and thorough setting. The autopilot mode used a random starting configuration and starting dimensionality of 6, instability criterion of 0.00001 with 40 and 50 randomized runs each of 500 iterations using Sorenson's dissimilarity coefficient (McCune and Mefford, 2006).

Regression Trees in randomForest

Environmental and *P. calleryana* density data were further analyzed with random forest regression trees using the randomForest package (Liaw and Wiender, 2002) in R version 3.3.2 (R Core Team, 2016). This analysis specifically examined factors related to *P. calleryana* density whereas my previous NMS analysis looked at factors driving overall species composition. The independent variables included in randomForest analysis for each plot were: genera, basal area by genera, age of *P. calleryana* stems, percent canopy cover, distance to roads (m), total basal area, transformed aspect, ST_O, ST_S, ST_R, DT_O, DT_S, DT_R, WT_O, WT_S, WT_R (Niinemets and Valladares, 2006). Two additional independent factors included in this analysis were *Quercus* spp. (oak) RD of the overstory and sapling layers at each plot and oak basal area. My response variable was *P. calleryana* density (stems ha^{-1}).

Linear Regression

Linear regression was performed in R version 3.3.2 (R Core Team, 2016) to investigate the relationship between *P. calleryana* density (stems ha^{-1}) and variables identified as important in the NMS and random forest analyses. These variables were age of oldest *P. calleryana* stem found at each plot, Niinemets score for shade tolerance of regenerating species (ST_R), Niinemets score for shade tolerance of sapling species. A regression was also performed to evaluate the relationship between total basal area and ST_R where basal area acts as a proxy for light availability. Residual plots and quarter quantile plots were used to examine normality of data. Due to these checks, a log transformation was performed on *P. calleryana* stem density to ensure the data better conformed to the assumptions necessary for regression (Neter et. al, 1985).

Results

Diameter at breast height of the oldest *P. calleryana* in plots as determined by cookie collected ranged from 0.5 cm to 50 cm (Fig. 2.3). Stem density of *P. calleryana* in invaded plots ranged from 198 stems ha^{-1} up to 18,900 stems ha^{-1} with a mean of 2306 ± 50 stems ha^{-1}. The average age of oldest *P. calleryana* stem found in invaded plots was 11.5 ± 0.1 years, but the oldest stem sampled was 50 years old. Average percent canopy cover was 88.8% ± 0.23, ranging from 0 to 100% cover. Basal area of overstory trees and saplings averaged 14.0 m^2 ha^{-1} ± 0.1 with a range of 0.0 to 39.1 m^2 ha^{-1}. Density of regenerating stems averaged 29,843 ± 1762 stems ha^{-1} with a range of 0 to 451,000 stems ha^{-1}. Plots were, on average, 124.5 ± 1.2 m from a road ranging from 5 m to 341 m. Finally, plots sampled were at an average elevation of 649.3 ± 4.5 m above sea level with a range of 481 to 842 m above sea level.

NMS ordination determined a three-dimensional solution as the most stable with a final stress of 15.40. The ordination explained a cumulative variance of 81.9%, with axis 1 explaining the most variance (R^2 = 0.429) followed by axis 3 (R^2 = 0.220). WT_O showed the strongest relationship with axis 1 (R = -0.283) and DT_S showed the second strongest relationship with axis 1 (R = -0.239; Table 2.1). The relationship of WT_O to axis 3 was R = 0.192, the relationship of DT_S to axis 3 was R = -0.052 (Table 2.1). Density of *P. calleryana* stems per hectare showed the strongest relationship with axis 3 (R = 0.551). ST_R showed the second strongest relationship with axis 3 (R = -0.479). The relationship of *P. calleryana* density to axis 1 was R = 0.071; the relationship of ST_R to axis 1 was R = 0.122 (Table 2.1). The strongest variables associated with axis 2 were WT_O (R = 0.343) and ST_R (R = -0.290).

Species most closely associated with the *P. calleryana* gradient along axis 3 were *Ulmus americana* (elm) saplings and *Fagus grandifolia* (American beech) saplings. Species which

distinctly separated from *P. calleryana* were *Quercus alba* (white oak) saplings, *Carya ovata* (mockernut hickory) saplings, *Quercus velutina* (black oak) saplings, *Cercis canadnesis* (eastern redbud) saplings, *Carpinus caroliniana* (ironwood) saplings, *Acer rubrum* (red maple) overstory, and *Sassafras albidum* (sassafras) saplings. Overall, there was not strong separation of species relative to shade tolerance with most species concentrated near the origin of axis 1 and axis 3 (Fig. 2.5). The ST_R vector was associated most with my shade intolerant species, specifically *Quercus* spp. (oak), *Pinus resinosa* (red pine), *Juniperus virginiana* (eastern red cedar), *Pinus banksiana* (Jack pine), and *Liriodendron tulipifera* (yellow-poplar). WT_O and DT_S vectors showed a potential association with *Fraxinus* spp. (ash), *Quercus alba* (white oak), and *Prunus serotina* (black cherry) (Fig. 2.4, 2.5). The environmental vectors identified as most important by the ordination revealed a positive association between higher densities of *P. calleryana* and DT_S. They also revealed a negative association between ST_R and *P. calleryana* density (Fig. 2.4).

RandomForest analysis determined that *P. calleryana* stem density was most closely associated with transformed aspect (MSE = 26.30), *P. calleryana* age (MSE = 22.29) and ST_R (MSE =19.06; Fig. 2.6). It showed the least association with total basal area by species and species groups. The association between ST_R and *P. calleryana* stem density (MSE = 21.79) was similar to correlations observed in NMS analysis (Fig. 2.4, 2.5).

Linear regression revealed significant relationships between log transformed *P. calleryana* stem density and age of oldest *P. calleryana* stems found at each plot, regeneration and sapling layer shade tolerances, and combined basal area of overstory trees and saplings (m^2 ha^{-1}; Table 2.2, 2.3). The relationship between *P. calleryana* stem density and age of oldest stem displayed a positive association ($p < 0.001$*). Pyrus calleryana* stem density was negatively associated with ST_R ($p < 0.001$). *Pyrus calleryana* stem density was negatively related to ST_S ($p < 0.001$). *Pyrus*

calleryana stem density was not associated with WT_O ($p = 0.754$). *Pyrus calleryana* density displayed a weak ($R^2 = 0.100$) but significant ($p<0.001$) relationship with DT_S (Fig. 2.7, Table 2.2). While significant, ($p < 0.001$), total basal area displayed a weak positive relationship with ST_R ($R^2 = 0.049$; Fig. 2.8, Table 2.2).

Discussion

As predicted, where older stems of *P. calleryana* were present, I found greater abundance of *P. calleryana* stems (Fig. 2.7). This relationship between age and density of *P. calleryana* is not necessarily true for all invasive populations of *P. calleryana*. While not demonstrated by these data, the inability of *P. calleryana* to self-pollinate means older stems may exist where density of *P. calleryana* is very low or even limited to a single stem resulting in an area with a single old stem (high residence time) and low overall density (Culley and Hardiman, 2007). During population expansion, invasion may be occurring discreetly via individuals who are isolated from mates. Therefore, invaded habitats may be sparsely populated by nonbreeding, undetected individuals but later experience rapid increases in density as genetically distinct mates become increasingly available (Chapter 3). However, these populations are primed for expansion into new areas as soon as new individuals or mates are close enough to allow cross-pollination, reproduction, and successive recruitment to occur highlighting the economic importance of early detection in plant invasions (Wilson et. al, 2011; Pimental et. al, 2005; Hobbs and Mooney, 2005). This relationship also highlighted the increased potential for recruitment and invasive spread with older established populations (Arim et. al, 2006).

The ability of *P. calleryana* to stump sprout and regenerate readily under a variety of environmental conditions means management efforts must be persistent to prevent invasive populations from expanding and overcoming a threshold beyond which expansion accelerates

(White et. al, 2005; Levine et. al, 2003; Theoharides and Dukes, 2007; Vincent, 2005). There are several traits of *P. calleryana* which suggest an increased ability to establish, compete, and suppress native species in new environments including rapid reproduction, compatibility with native seed dispersers, and tolerance to broad spectrum of environmental conditions (Drenovsky, et. al, 2012).

My data demonstrated a negative relationship between ST_R and ST_S and *P. calleryana* density. I found that plots with high *P. calleryana* density were more closely associated with plots that contain more shade intolerant regenerating species. This result suggests that, despite its Niinemets score (1.35), *P. calleryana* is not a very shade tolerant species. Alternatively, this result may be a function of the opportunistic behavior of invasive species which lead them to invade early seral environments with higher light availability (Drenovsky et. al, 2012). The observed relationship could be a commonality of environmental tolerances among shade intolerant species and *P. calleryana* as opposed to a causative relationship between shade tolerance and *P. calleryana* density. Another important consideration here is the age of the invasion. *Pyrus calleryana* has been actively establishing across the base since the 1970s meaning it has had time to outcompete native species within vegetation communities. This result may also indicate that *P. calleryana* is incapable of competing with shade tolerant species such as *Acer* spp. in the regenerating layer of mature forests in a fashion similar to *Quercus* spp. seedlings. In either case, my data supported that competition for light in the understory is a potentially important factor in determining vulnerability to invasion by *P. calleryana*.

Another important factor that influences the distribution of plant species is soil moisture conditions. NMS ordination and linear regression supported a positive, significant relationship between *P. calleryana* and DT_S (Table 2.1, 2.2, Fig. 2.4). *Pyrus calleryana* may prefer more xeric

environments since the species was associated with communities dominated by species with higher drought tolerances (Fig. 2.7). This finding was supported by randomForest analysis which determined that *P. calleryana* density was most associated with aspect, specifically south facing slopes. This may indicate a response to increased light on south facing slopes or it may be a function of site preference regarding moisture. Aspect can have a strong impact on the environmental gradients that influence species composition meaning the relationships between *P. calleryana* and aspect, and *P. calleryana* and DT_S may be confounding one another (Stage and Salas, 2007). Further the way I generated the index for Niinemets derived community scores for plots may have resulted in a loss of overall resolution due to the ordinal nature of the original index.

My research found that *P. calleryana* appears to be associated with habitats that are more xeric and characterized by higher available light. The association between *P. calleryana* and saplings with higher drought tolerance and overstory trees with lower water-logging tolerance suggests that drier sites may be more vulnerable to invasion. This site preference is contrary to initial observations recorded in collections of seed from China, Korea, and Japan by the USDA in 1916 to 1918. While *P. calleryana* was found across a wide variety of moisture conditions in my study and in the literature (Culley and Hardiman, 2009), my data did show an association between *P. calleryana* and drought tolerant species, shade intolerant species, and aspect. This site preference has the potential to impact regeneration and success of other species that show an association with drier site conditions and south-facing aspects such as *Quercus* spp. (Fekedulegn, 2004). Evidence for this in my data included the separation of *P. calleryana* saplings and regeneration from *Q. alba* saplings, *Q. velutina* saplings, and *C. tomentosa* saplings (Fig. 2.5).

Future research should focus on identifying the effects of *P. calleryana* invasion intensity on the composition and function of forest ecosystems. Specifically, there should be investigation into potential effects of *P. calleryana* abundance on the herbaceous community in forests as well as effects on soil chemistry and nutrient cycling. My findings identified potentially vulnerable habitats and the environmental factors associated with *P. calleryana* invasion. Understanding the full suite of effects, and how they vary with intensity and duration of invasion, are important for restoration efforts in invaded regions (Drenovsky et. al, 2012).

Tables and Figures

Table 2.1 Axis correlations of three axes for non-metric multi-dimensional scaling ordination across 73 plots and 83 species. Axis 1 was the most dominant axis ($R^2 = 0.429$) followed by axis 3 ($R^2 = 0.220$) and axis 2 ($R^2 = 0.170$). Combined, the three axes explained a cumulative variance of 81.9%.

	Axis 1	Axis 2	Axis 3
Pyrus calleryana Density	0.071	-0.096	0.551
Pyrus calleryana Age	-0.160	-0.078	0.284
Percent Canopy Cover	-0.069	0.075	-0.280
Distance to Roads (m)	0.050	-0.214	-0.138
Basal Area (m^2 ha^{-1})	0.023	-0.178	0.055
Transformed Aspect	-0.139	-0.161	-0.117
Elevation (m)	0.235	-0.135	-0.089
Overstory Shade Tolerance	-0.108	0.199	-0.168
Overstory Drought Tolerance	0.138	-0.11	-0.333
Overstory Water-logging Tolerance	-0.283	0.343	-0.192
Sapling Shade Tolerance	0.090	0.082	-0.220
Sapling Drought Tolerance	-0.239	0.239	-0.052
Sapling Water-logging Tolerance	-0.086	0.058	0.087
Regeneration Shade Tolerance	0.122	-0.290	-0.479
Regeneration Drought Tolerance	-0.222	0.264	0.534
Regeneration Water-logging Tolerance	0.151	0.281	-0.112
Slope Position	0.034	-0.086	-0.178

Table 2.2 Estimate, standard error, t value and p value for linear regressions against log transformed *Pyrus calleryana* stem density across 73 plots included in non-metric multidimensional scaling and random forest analysis where *Pyrus calleryana* was present.

	Estimate	Standard Error	t value	p value
Intercept Age of Oldest *Pyrus calleryana* stem (years)	5.414	0.072	75.08	<0.001
Age of Oldest *Pyrus calleryana* stem (years)	0.081	0.006	14.16	<0.001
Intercept Regeneration Shade Tolerance	8.581	0.265	32.33	<0.001
Regeneration Shade Tolerance	-0.853	0.093	-9.22	<0.001
Intercept Sapling Shade Tolerance	7.546	0.163	46.21	<0.001
Sapling Shade Tolerance	-0.407	0.046	-8.80	<0.001
Intercept Water-logging Tolerance of Overstory	6.224	0.149	41.87	<0.001
Water-logging Tolerance of Overstory	-0.029	0.093	-0.31	0.754
Intercept Drought Tolerance of Sapling Layer	4.741	0.189	25.03	<0.001
Drought Tolerance of Sapling Layer	0.514	0.065	7.91	<0.001

Table 2.3 Estimate, standard error, t value and p value for linear regressions with total basal area (m^2 ha^{-1}) from 73 plots included in non-metric multidimensional scaling and random forest analysis where *Pyrus calleryana* was present.

	Estimate	Standard Error	t value	p value
Intercept Total Basal Area (m^2 ha^{-1})	2.581	0.064	40.61	<0.001
Total Basal Area (m^2 ha^{-1})	0.011	0.003	3.91	<0.001

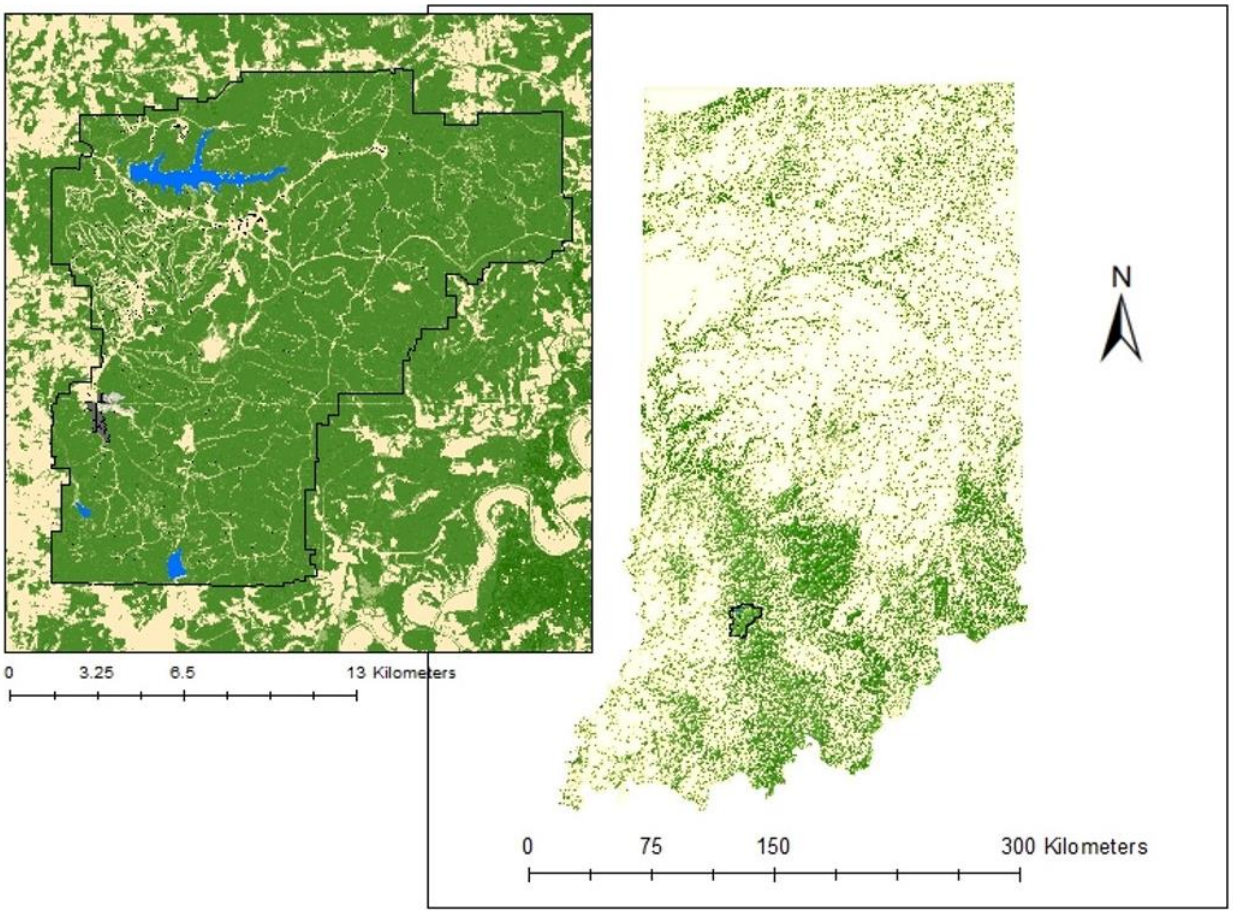

Figure 2.1. Naval Support Activity – Crane located in Martin County, Indiana. Insert map displays boundaries of the installation and area covered by forest. The state map shows forested land.

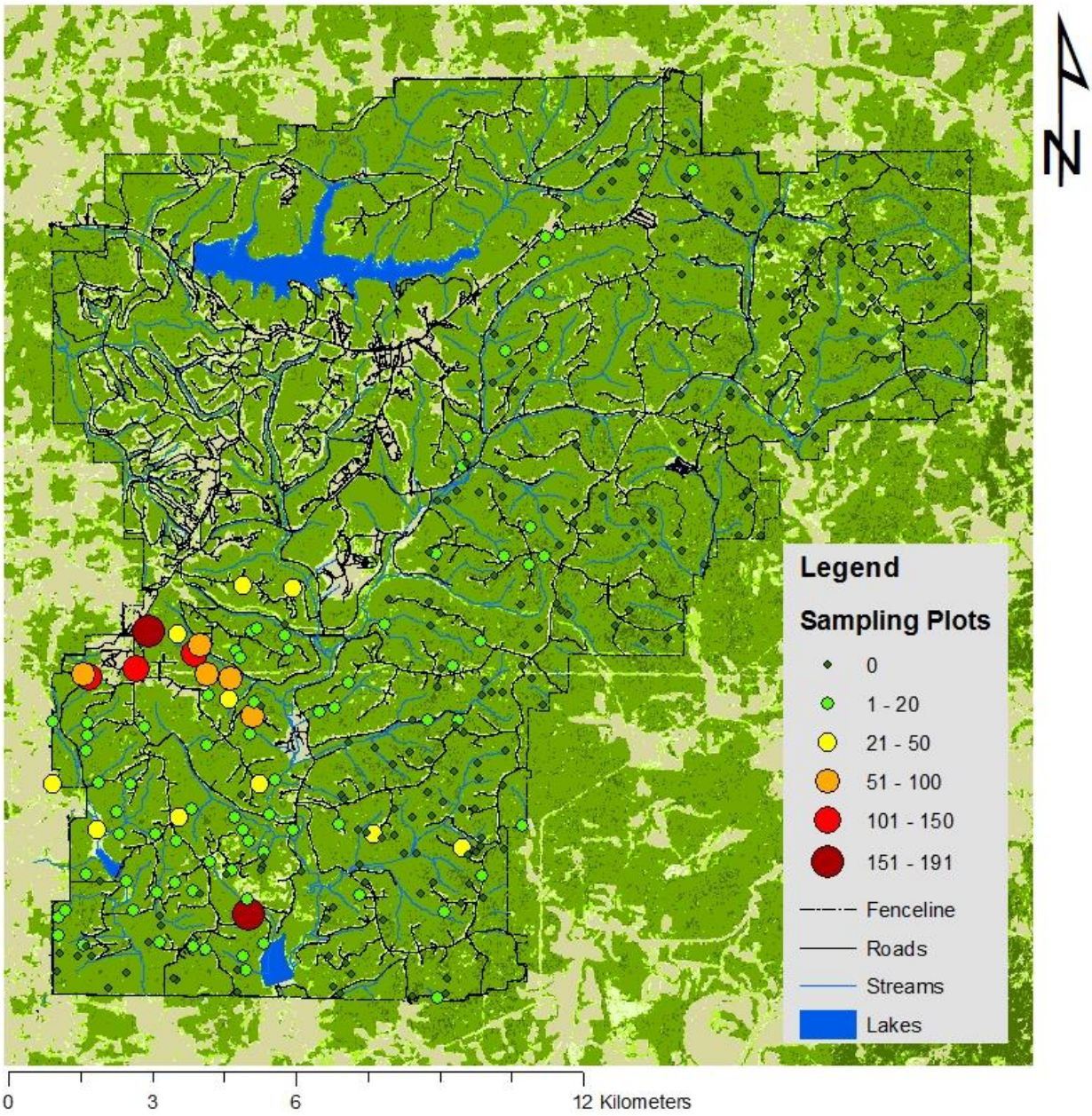

Figure 2.2 Randomly distributed sampling plots on Naval Support Activity – Crane located in Martin County, Indiana. Larger red dot symbols indicate greater density of *Pyrus calleryana* (stems ha^{-1}) on a given plot.

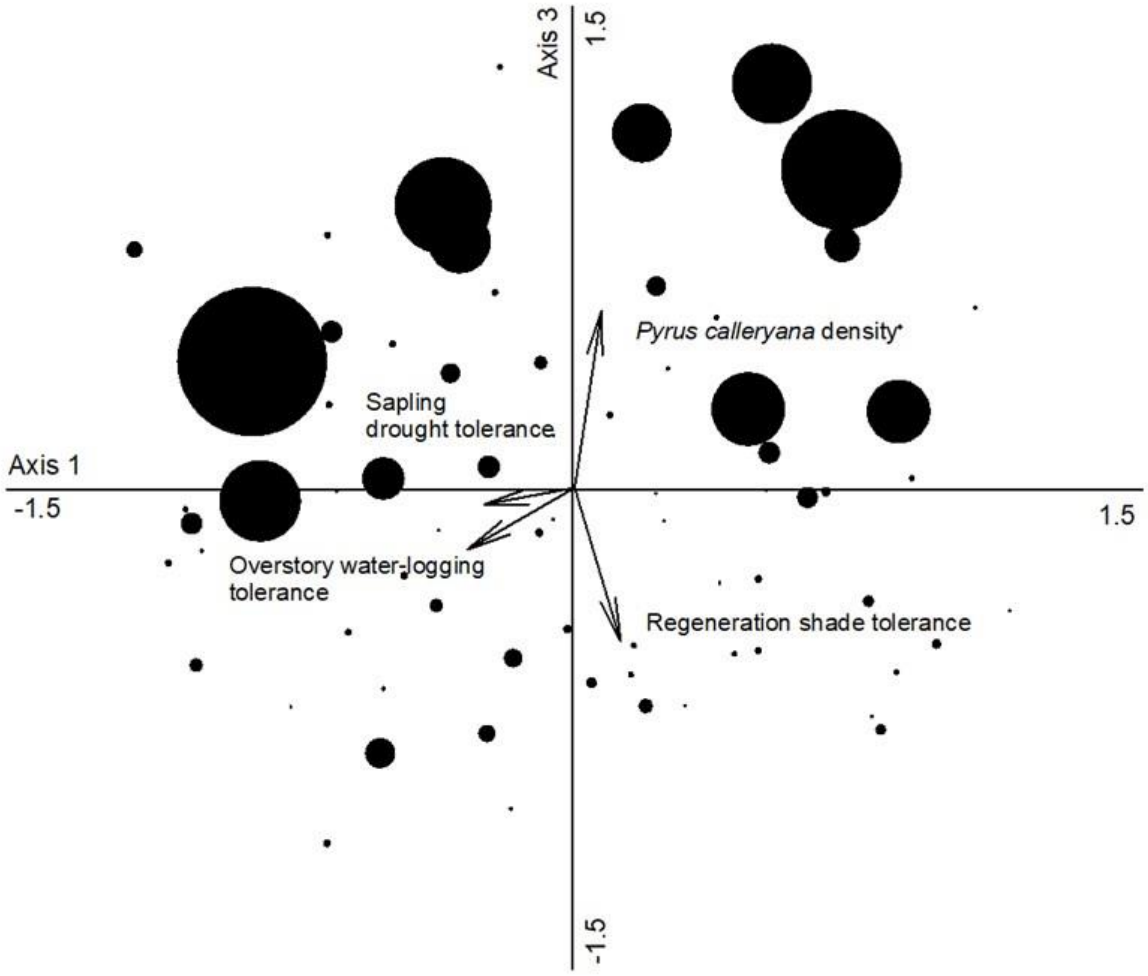

Figure 2.4 Non-metric multidimensional scaling ordination of overstory, sapling, and regeneration layer data from 73 plots and 83 species across sampling area on Naval Support Activity – Crane. Points are sized according to relative density of *Pyrus calleryana* present at plot. Dominant environmental variables are represented as vectors: *Pyrus calleryana* stem density (stems ha^{-1}), Niinemets score for regenerating layer shade tolerance, Niinemets score for overstory layer water-logging tolerance, and Niinemets score for sapling drought tolerance.

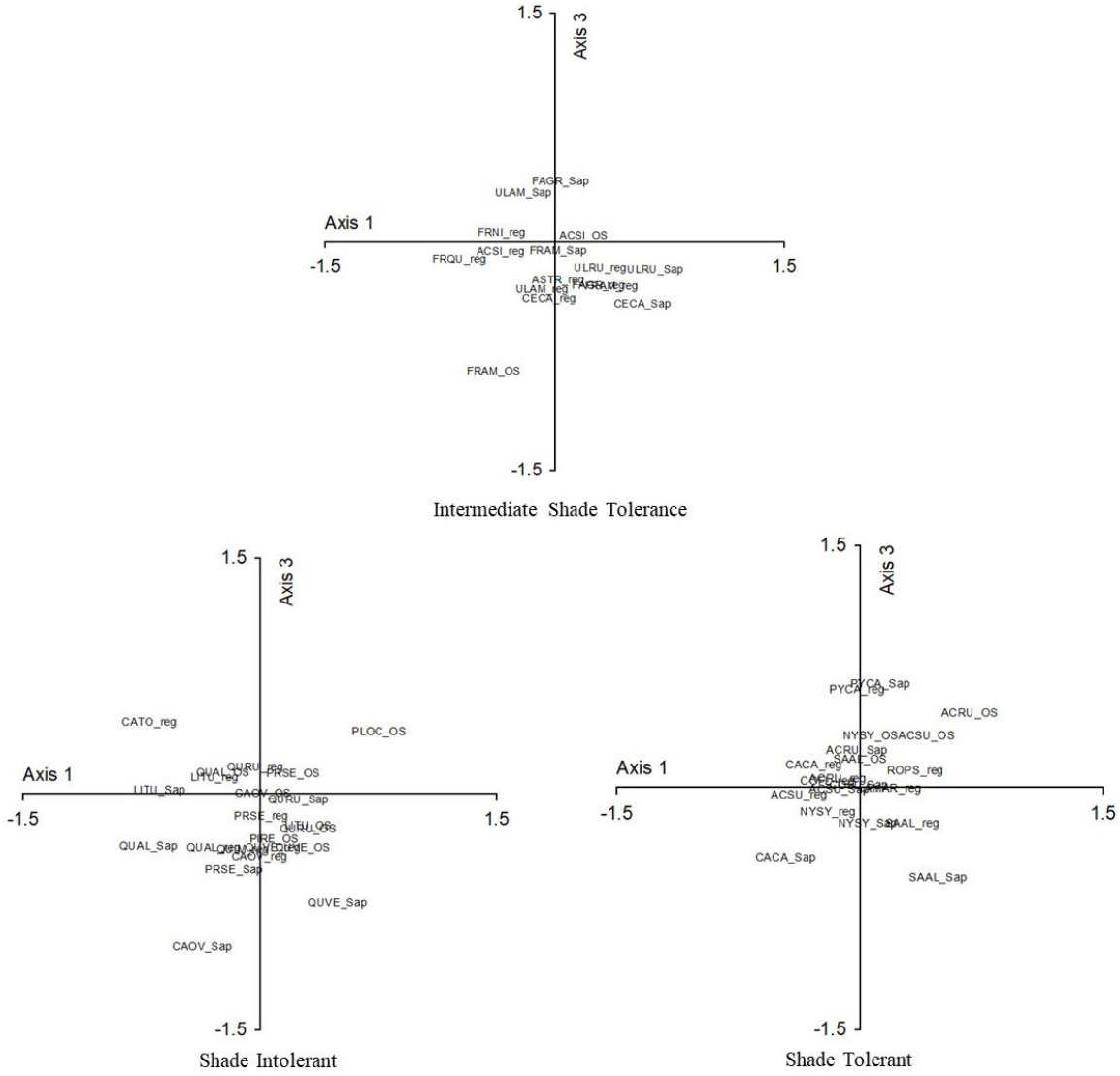

Figure 2.5 Species coordinates for axes one and three as determined in non-metric multidimensional scaling across 73 plots and 83 species. Species are organized by shade tolerance and labeled as: intermediate shade tolerance, shade intolerant, and shade tolerant. Species are coded as the first two letters of the genus and the first two letters of the species (Appendix A) and, following the underscore, abbreviation of the layer of which the species was a member (OS - overstory, Sap - sapling, and reg –regeneration).

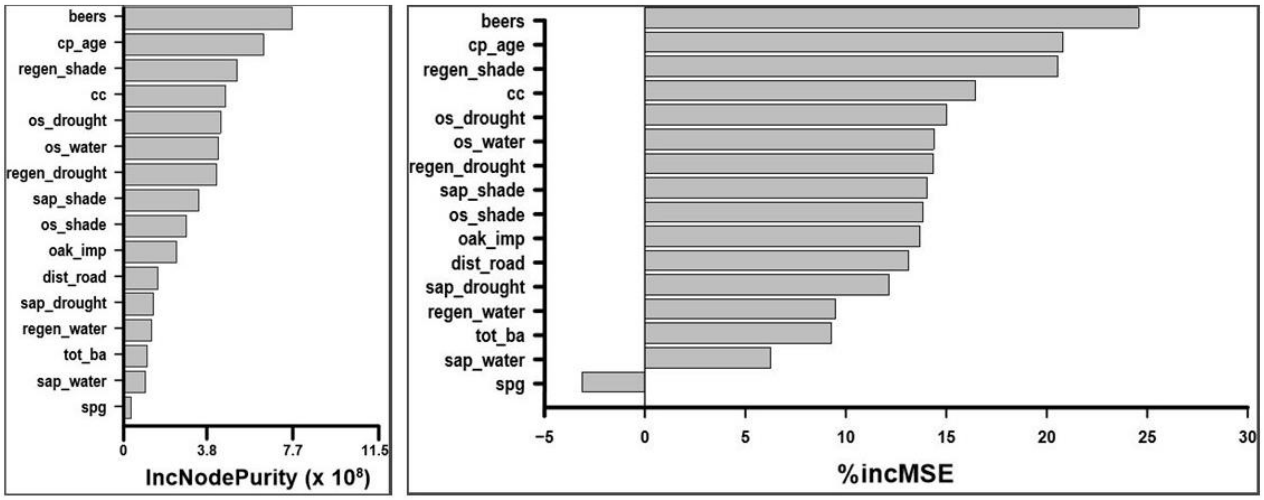

Figure 2.6 Increment node purity (x 10^8) and percent Increment mean squared error of variance in *Pyrus calleryana* stem density explained by each of the environmental variables on the y axis across 73 plots and 16 variables used in random forest analysis. Abbreviations for 16 variables are defined in Appendix B.

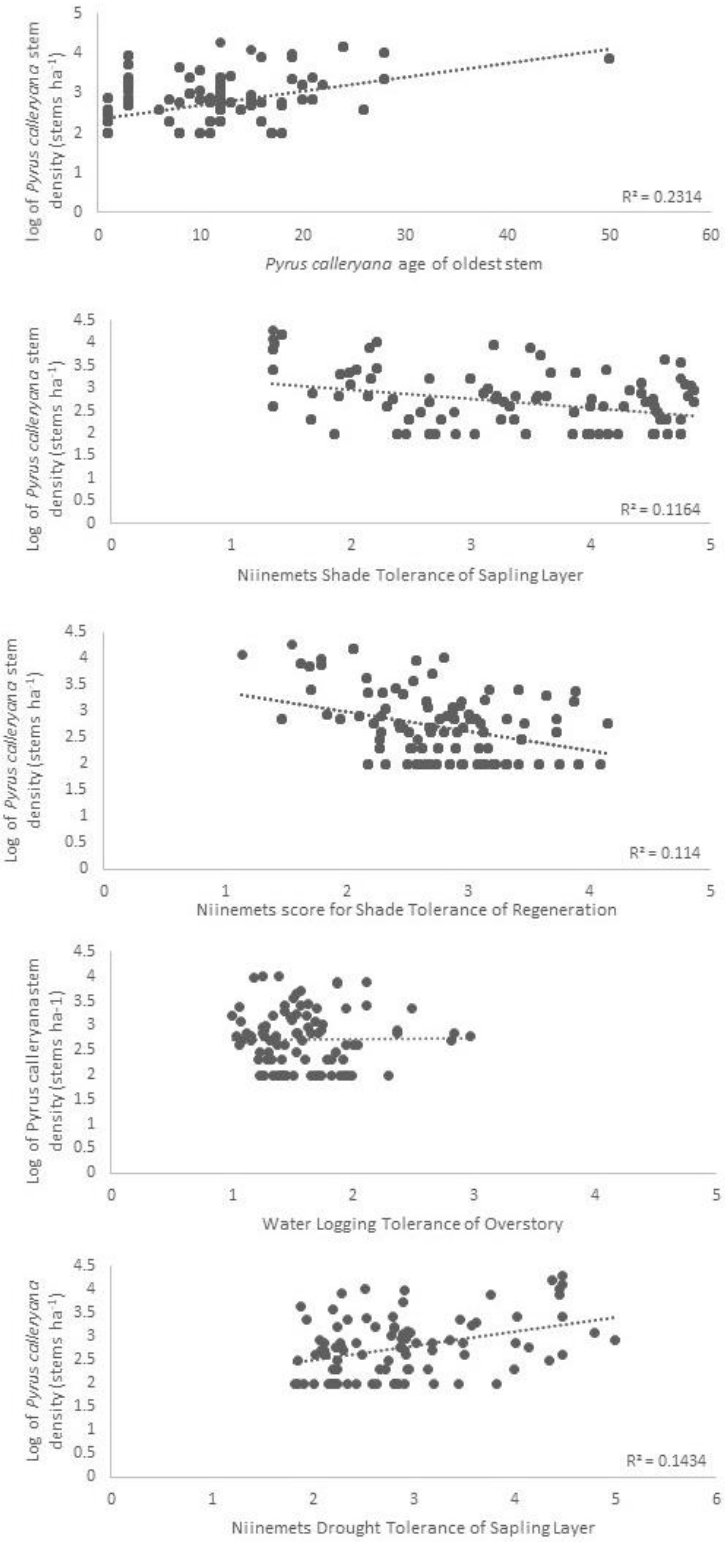

Figure 2.7 Linear regressions of log transformed *Pyrus calleryana* stem density (stems ha^{-1}) vs. environmental variables identified in NMS and randomForest analyses. Regressions reflect only those plots included in non-metric multidimensional scaling and randomForest analysis where *Pyrus calleryana* was present.

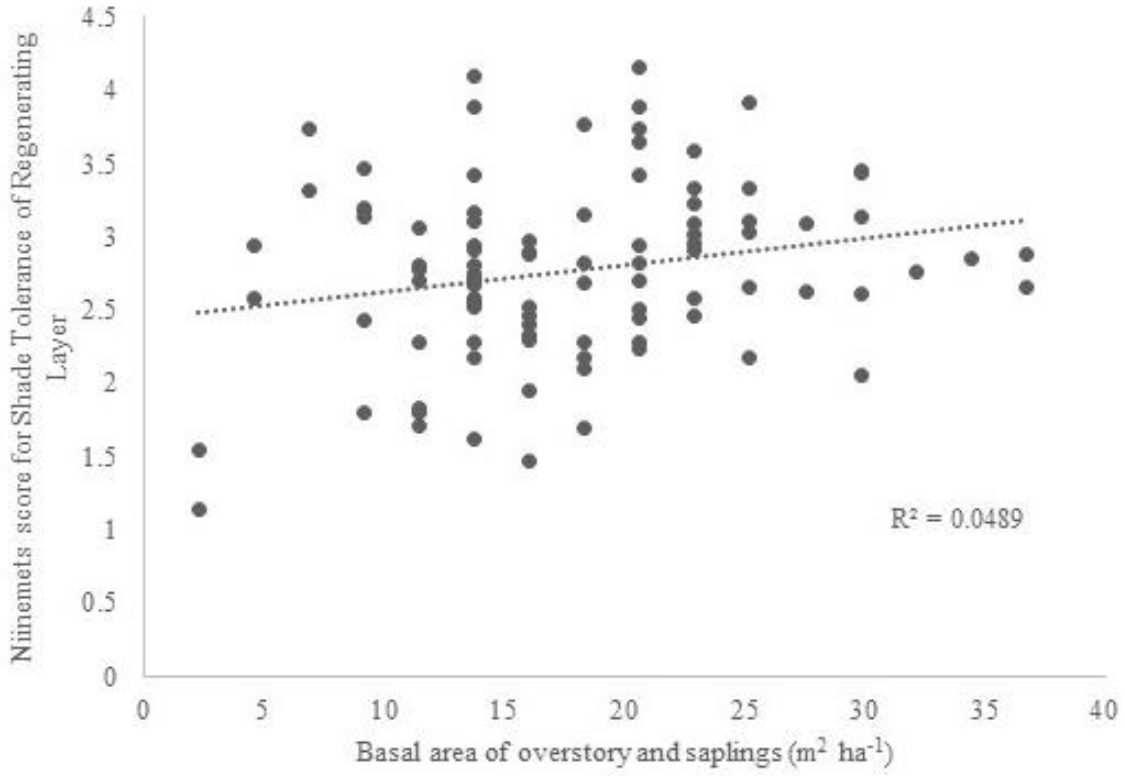

Figure 2.8 Linear regression of basal area of overstory and sapling layer against Niinemets score for shade tolerance of species in regenerating layer across 73 plots. Data included in regression reflects only those plots included in non-metric multidimensional scaling and randomForest analyses.

Appendix A

Table 2A Definitions for species codes included in non-metric multi-dimensional scaling as shown in figure 2.5.

Code	Species
ACRU	*Acer rubrum*
ACSI	*Acer saccharinum*
ACSU	*Acer saccharum*
AIAL	*Ailanthus altissima*
AMAR	*Amelanchier arborea*
ASTR	*Asimina triloba*
CACA	*Carpinus caroliniana*
CACO	*Carya cordiformis*
CAGL	*Carya glabra*
CAOV	*Carya ovata*
CATO	*Carya tomentosa*
CECA	*Cercis canadensis*
COFL	*Cornus florida*
FAGR	*Fagus grandifolia*
FRAM	*Fraxinus americana*
FRNI	*Fraxinus nigra*
FRQU	*Fraxinus quadrangulata*
LITU	*Liriondendron tulipifera*
NYSY	*Nyssa sylvatica*
PIRE	*Pinus resinosa*
PLOC	*Platanus occidentalis*
PRSE	*Prunus serotina*
PYCA	*Pyrus calleryana*
QUAL	*Quercus alba*
QUIM	*Quercus imbricaria*
QURU	*Quercus rubra*
QUVE	*Quercus velutina*
ROPS	*Robinia pseudoacacia*
SAAL	*Sassafras albidum*
ULAM	*Ulmus americana*
ULRU	*Ulmus rubra*

Appendix B

Table 2B Definitions of codes for variables included in randomForest regression tree analysis as shown in figure 2.6.

Code	Definition
beers	Transformed aspect
cp_age	Age of oldest *Pyrus calleryana* stem
regen_shade	Niinemets shade tolerance of regeneration layer
cc	Percent canopy cover
os_drought	Niinemets drought tolerance of overstory layer
os_water	Niinemets water-logging tolerance of overstory layer
regen_drought	Niinemets drought tolerance of regeneration layer
sap_shade	Niinemets shade tolerance of sapling layer
os_shade	Niinemets shade tolerance of overstory layer
oak_imp	Oak importance value for individual plots
dist_road	Distance to roads (m)
sap_drought	Niinemets drought tolerance of sapling layer
regen_water	Niinemets water-logging tolerance of regeneration layer
tot_ba	Total basal area (m^2 ha^{-1})
sap_water	Niinemets water-logging tolerance of sapling layer
spg	Species group

CHAPTER 3. DISPERSAL AS A FACTOR IN THE INVASION SUCCESS OF *PYRUS CALLERYANA*

Abstract

The success of invasive species is determined by a variety of environmental and biological factors. One major factor in establishment and recruitment is dispersal, which can be reflected in the genetic structure of invading populations. Differential dispersal of seed and pollen can have a great influence on genetic structure and impose limits on recruitment rates of invasive species. The population structure and relatedness among individuals of invasive species, such as *Pyrus calleryana*, can identify the potential mechanisms of dispersal. This research sought to understand *P. calleryana* dispersal by determining the genetic structure of an invasive population of *P. calleryana* located in southern Indiana. I found a weak influence of local spatial structure that indicated long-distance dispersal events may be an important factor of spread of fruits and the overall population dynamics of this self-incompatible, insect pollinated species. Historical land uses and horticultural use of this species also may have been important contributors to the spread and maintenance of this population of *P. calleryana*. This research suggested that population density, bird driven fruit dispersal, and other factors are important in spread of *P. calleryana*. Due to the apparent importance of density to invasion success, management of *P. calleryana* should focus on control within densely populated plant patches.

Introduction

Recruitment and establishment are two of the most important components of invasion success. These components depend both upon successful pollen dispersal to receptive mates, and fruit dispersal to environments with appropriate conditions for germination to occur (Drenovsky et. al, 2012; Hastings et. al, 2005; Nathan and Muller-Landau, 2000; Rejmanek and Richardson, 1996). Understanding the rate and spatial range of invasive species dispersal across a landscape is an important factor in designing appropriate management strategies. For example, species capable of long-range dispersal events present a greater challenge to landscape-scale planning than species with locally concentrated dispersal (Hastings et. al, 2005). Species capable of long-range dispersal are capable of spreading across a landscape at a greater rate than those dependent on shorter-distance dispersal methods.

Dispersal and recruitment rates may be reflected in the genetic relatedness within and among groups of individuals across spatial scales. The pairwise relatedness among individuals is an important indicator of seed and pollen dispersal within a population (Vekemans and Hardy, 2004). This relatedness reflects genetic differentiation across populations. Differentiation among individuals in populations reflects the activity of gene dispersal so even in large, continuous populations, differentiation that results in population structure will occur where gene exchange (dispersal of pollen and fruit) is restricted. Such restriction leads to isolation by distance (Wright, 1943; Wright, 1946; Wright, 1978). Isolation by distance is a direct function of pollinator flight behavior and seed dispersal which are often limited by the tendency of dispersers to travel frequently between neighboring plants (Turner et. al, 1981).

In addition to activity of dispersers, population structure can be an indicator of pre-zygotic and post-zygotic barriers to gene flow. For example, overall pollen performance, as determined by

successful germination, in insect pollinated, self-compatible *Alstroemeria aurea* (Peruvian-lily) was found to be influenced by distance between parents. In self-compatible species such as this, inbreeding can lead to overall low levels of genetic diversity across space (Pleasants and Wendel, 1989). However, *A. aurea* has relatively high levels of diversity across both local and distant scales. This diversity was found to be indicative of a selective barrier in the pollen tube which favored more genetically distinct pollen sources (Souto et. al, 2002). Another class of species are those which are self-incompatible, or obligate out-crossers. Despite typically high rates of heterozygosity, these species are at a disadvantage when it comes to colonizing new areas as available mates will be severely restricted (Baker, 1955). The success of self-incompatible species in terms of reproduction and development of genetic diversity are likely influenced by human-mediated migration processes, extended reproductive periods, non-specific pollinator requirements, high seed set, and temporary breakdown of self-incompatibility (Barrett, 1988; Sun and Ritland, 1998; Pandey, 1980).

Examples of dispersal limitations of self-incompatible species are abound in the literature. For example, pollen of the self-incompatible *Ascelpias exaltata* (poke milkweed) can be dispersed over great distances due to the behavior of its large butterfly pollinators. Successful pollination in this species was, however, limited by the genetic relatedness of plants involved in the exchange; more genetically similar pairings resulted in reduced seed-set and viability (Broyles and Wyatt, 1991). Similarly, reproductive success in self-incompatible, insect-pollinated *Diplotaxis erucoides* (white wallrocket) was shown to be density dependent. Distantly spaced plants produced lower levels of fruit set and seed production when compared to those with nearby neighbors (Kunin, 1992). *Primula vulgaris* (primrose) is another self-incompatible plant species that shows

correlation between reproductive success and population size, with smaller populations showing reduced success (Brys et. al, 2004).

Pyrus calleryana is an invasive, self-incompatible tree species that is rapidly spreading across the eastern United States. This species was introduced in the early 1900s by the United States Department of Agriculture (USDA) with sources from China, Japan, and Korea. *Pyrus calleryana* was used in breeding programs for *Pyrus communis* (common pear) to confer disease resistance to *Erwinia amylovora* (fire blight). During cultivation, desirable characteristics were identified in the species and outplanting shifted to primarily ornamental and horticultural uses. To date, more than 25 cultivars are available (Culley and Hardiman, 2007; Vincent, 2005). Since its widespread introduction, *P. calleryana* has spread into field edges, open fields, and the understory of intact forest stands altering species composition (Vincent, 2005). The wide environmental tolerance of the species allows it to potentially invade a variety of site conditions (Culley and Hardiman, 2007; Liu et. al, 2012; Vincent, 2005; Chapter 2). In addition, *P. calleryana*'s invasiveness is increased by its ability to flower and produce fruit as early as three years of age (Cuizhi and Spongberg, 2003). The fruits are produced early in spring and retained through the summer and into fall when they are dispersed by birds. Abundant fruit production, while not officially quantified, is seen in urban populations of *P. calleryana* and is also likely present in escaped populations. However, not all fruits produce viable seeds (Culley et. al, 2011).

As is true of many *Prunus* species, and other members of the family Rosaceae, *P. calleryana* is self-incompatible (Entani et. al, 2003, Culley et. al 2011; Culley and Hardiman, 2007). This character prevents self-pollination and may limit the ability of the species to spread. According to Baker (1955), species capable of selfing are expected to be more successful in colonization, dispersal, and recruitment than obligate out-crossers (Hao et. al, 2011). There are

exceptions to Baker's Law, however, a self-incompatible, invasive vine in Florida, *Paederia foetida* (skunkvine) was found to overcome the limits of dispersal imposed by its self-incompatible character through the visitation of native and non-native pollinators which allowed the species to successfully increase its invasive range (Liu et. al, 2006). While some insect pollinators are specialists, research has shown that many insect pollinators are generalists (Waser et. al, 1996). So the spread of *P. calleryana*, similar to *P. foetida*, *D. erucoides*, and *P. vulgaris*, may not be limited by pollinator availability and visitation but rather by its self-incompatibility and the proximity of viable mates. Self-incompatibility may be regulated by multiple self-incompatibility alleles. The presence of multiple alleles of this type increases the probability of disassortative mating and reproductive success between individuals. The number of self-incompatibility alleles present in *P. calleryana* is undocumented, so the potential effect of this factor on the invasiveness of *P. calleryana* is unknown.

Previous research on the genetic structure of wild *P. calleryana* in its native range revealed that geographic distance influences genetic differentiation among subpopulations and populations (Liu et. al, 2012). It also showed that in wild populations of *P. calleryana*, the greatest levels of genetic diversity was within populations rather than among populations (Liu et. al, 2012). This supports research showing density dependent reproductive success for other asexual species and further indicates that restrictions on breeding between geographically separated individuals may drive genetic differentiation and diversity in populations (Baker, 1955; Brys et. al, 2004; Hao et. al, 2011; Kunin, 1992). This is important relative to *P. calleryana* as patterns of diversity can be used to evaluate rates of reproductive success within and among populations.

Studies of *P. calleryana* invasion have indicated that hybridization in the species may lead to increased invasiveness. Increased genetic variation resulting from hybridization further

increases the likelihood of development of compatible phenotypes such as those associated with increased fecundity and size (Hovick and Whitney, 2014; Stebbins, 1959). This idea is supported by studies of early generation hybrids of *P. calleryana* which documented increased root mass in hybrid individuals relative to parental genotypes (Culley and Hardiman, 2010). Greater root mass may mean escaped hybrids of *P. calleryana* will exhibit increased hardiness. Other studies on hybrid *P. calleryana* cultivars have documented increased photosynthetic rates and stomatal conductance in later generations (Merritt et. al, 2014). These results indicated hybrid vigor in *P. calleryana*, so mating between phenotypes selected for cultivation may lead to a phenotype adapted for invasion.

Birds are expected to be the main mediators of long distance dispersal (as well as dispersal over shorter distances) for *P. calleryana*, as birds are observed most often predating upon the fruits of *P. calleryana*. Pollen dispersal in *P. calleryana* is completed primarily by insects in this species. Pollen dispersal limitations can strong strongly impact invasive expansion (Arim et. al, 2006; Liu et. al, 2012; Nathan and Muller-Landau, 2000; Souto et. al, 2002). Insect pollination events are also likely what maintains the higher levels of genetic diversity noted within populations of *P. calleryana*, highlighting the importance of population density to the reproduction of self-incompatible species (Brys et. al, 2004; Kunin, 1992; Liu et. al, 2012).

I studied an invasive population of *P. calleryana* located on Naval Support Activity (NSA) – Crane (hereafter referred to as Crane) located in southern Indiana. This population was selected because of knowledge of its major establishment events, its long residence time on the base, and the large area encompassed by the invasion. This population was initially established in the 1930s, began expanding in the 1970s and has continued to spread in the understory of the forests and early successional habitats of Crane (Chapter 2). This research was designed to understand the spread

of *P. calleryana*, a self-incompatible species, through analysis of genetic diversity in populations surrounding mother trees (demes) sampled across the base.

Because pollen dispersal distance is expected to be at least an order magnitude less than the distances over which seeds are dispersed by birds, I hypothesized a relatively uniform level of relatedness across increasing distances among trees surrounding a central large mother tree. This is based on the behavior insect pollinators which typically operate within plant patches rather than between (even where capable of travelling greater distances; Brys et. al, 2004). In other words, offspring located near their assumed mother (10-40 m) will be no more related than those located far away (80-100 m). I also hypothesized that each mother tree would be the source of a genetically-identifiable population on the base. I suspected this pattern because insect pollinators are often noted travelling shorter ranges than the total capable flight distance and these pollinators, such as bees, most often move within plant patches rather than between them (Brys et al, 2004; Pasquet et. al, 2008). Based on the study by Liu et. al (2012) and reproductive success patterns in other self-incompatible species, finally I hypothesized that there will be higher levels of genetic diversity within demes than among them (Brys et. al, 2004; Kunin, 1992).

Methods

Study Area

Genetic samples were gathered from a population of *P. calleryana* in southern Indiana located on Naval Support Activity (NSA) - Crane in southern, Indiana (38.870003 N, -86.831494 E; Chapter 2, Fig. 2.1). The base encompasses 254 km^2 located on the Crawford Upland section of the Shawnee Hills landscape (Homoya et. al, 1985). Soils across the base are shallow and poor in quality relative to other soils found in Indiana. The soils at Crane are predominantly silt loams

with shale and limestone parent materials. The naval base was established in the 1940s to serve as a munitions storage facility. Prior to becoming a naval base, the site was primarily composed of degraded farmland but since its establishment, the base has re-forested and is now dominated by *Quercus-Carya* (oak-hickory) and *Acer-Fagus* (maple-beech) forests with varying densities of invasive *P. calleryana* in the understory (Chapter 2). The population of *P. calleryana* pre-dates establishment of the base and likely resulted from a combination of events. First, a nursery for ornamental plants established in the 1930s and was abandoned on what is now part of the base. Second, a golf course established on the site in the 1970s was planted with *P. calleryana*. The area most severely invaded on the base was adjacent to both the golf course and the nursery. Third, *P. calleryana* continues to be planted and maintained around the base for ornamental purposes.

Plant Materials

Genetic data were collected on the basis of a neighborhood model that utilized sample sites with known distances between individuals to estimate dispersal and relatedness across those set distances (Adams and Birkes, 1991). Ten mother trees were selected with the goal of sampling large-diameter individuals in areas most affected by the invasion (Chapter 2). I selected sample sites based upon locations known to contain large individuals that were easy to access. Each large individual selected, or mother tree, was deemed plot center. The mother tree was assumed to be the oldest tree in a given plot due to size (Fig. 3.1). In each plot, five to 20 fresh leaves of each mother and up to two other adult *P. calleryana* trees located within 50 m of plot center were collected. Diameter at breast height (DBH) of the mother tree and any other large adults within the plot was recorded. Five to 20 leaves were also sampled from trees in the youngest cohort (>1.5 cm DBH) growing at 10, 20, 40, 80, and 100 meters in each of the four cardinal directions from the mother tree. This sampling method was designed to capture the relationship between dispersal

distance from mother trees and relatedness of both younger trees and other nearby older trees in the plot. Examining large diameter individuals was intended to provide insight into the establishment and genetic relatedness among older trees that established earlier in the invasion.

DNA Extraction

Genomic DNA was extracted from leaves with a Qiagen DNeasy plant mini extraction kit (Qiagen, United States of America) with the following modifications. First, leaves were disrupted using liquid nitrogen and immediately frozen. Frozen ground tissues were then placed in a screw-capped tube with 800 µL of Buffer AP1 (instead of the designated 400 µL), 4 µL of RNase A, and a ceramic bead (Qiagen, 2015). This additional buffer allowed the sample to be further disrupted by the ceramic bead when placed in the fastprep tissue disruptor machine (VWR 2017). The samples were ground using the fast prep for five cycles of 20 seconds each and were then incubated for 2 h at 50°C. Following extraction, DNA samples were stored at -80ºC until used in Polymerase Chain Reactions (PCRs). Gel electrophoresis was performed on 20 random DNA samples to verify DNA quality. DNA concentration (ng/µL) was measured and recorded for each sample using a Qbit (Promega Corporation, 2016).

Microsatellite Analysis

Genetic comparisons among plots were performed using microsatellite markers (Simple Sequence Repeats or SSRs) and microsatellite genotyping. I used SSRs because of their abundance in the genome, polymorphism, and co-dominant inheritance (Yamamoto et. al, 2002). Nine microsatellite primer pairs developed from species closely related to *P. calleryana* and shown to have success in amplifying DNA of *P. calleryana* were selected for use in this study. KA14, KA16 and KU10 were originally designed for *P. communis, Pyrus pyrifolia,* and *Pyrus ussuriensis* and

primers CH01F02, CH01H01, CH01H10, CH02D11, CH02B10, and CH02D12 were initially designed for use with *Malus domestica* (Yamamoto et. al. 2002; Guilford et. al 1997; Gianfranceschi et al, 1998). DNA samples were run in polymerase chain reactions (PCR) in 22 µL reaction volumes with 6-60 ng of genomic DNA, 10 µL of MyTaq 2X mix, 5 µL of ddH_2O, and 1.5 µL each of the forward and reverse primers. Forward primers were fluorescently labeled and reverse primers were unlabeled. Primers were suspended in Tris EDTA (TE) buffer at a concentration of 0.1 nmol/µL.

PCR reactions were run under the following thermocycler conditions on an Eppendorf Mastercycler (Eppendorf North America, Hauppauge, NY): 95ºC for 4 min, followed by a cycle of 94ºC for 45 s, 63ºC for 45 s, and 72ºC for 45 s, then a cycle of 94ºC for 45 s, 59ºC for 45 s, and 72ºC for 45 s, then 5 cycles of the same conditions with a step down of 1ºC from 58ºC to 54ºC. This was followed by 30 cycles of 94ºC for 45 s, 53ºC for 1 min, and 72ºC for 1 min, and then a final extension of 72ºC for 10 min and 4ºC for 10 min. PCR products were multiplexed post PCR reaction in the following primer group combinations: group 1 with KU10, CH02D12, and CH02B10; group 2 with KA16, CH01H10, and CH01F02; and group 3 with CH01H01, CH02D11, and KA14. Samples were genotyped by the Purdue Genomics Core Facility on an illumina sequencer. Genotyping results were analyzed and binned using Genemapper 3.0 software (Applied Biosystems, 2012).

Data Analysis

Each sample was genotyped at nine microsatellite loci and the genotypes were used for subsequent genetic analyses. Identity of samples was hidden and samples were genotyped randomly to prevent bias. Three randomly selected DNA samples were also repeated on each plate to serve as a control for variation in PCR reactions and allele binning.

Population structure and substructure was assessed using the Bayesian software STRUCTURE 2.3.4 (Pritchard et. al, 2000). Population membership assignment was initially based on the most proximal mother tree. The analysis used a 20,000 repetition burnin period followed by 200,000 Markov Chain Monte Carlo (MCMC) reps after burnin. Ten iterations were run at each of K = 1 to 10 populations. Most likely number of populations was calculated using the Evanno method which identifies the most likely number of populations as the result with the highest delta K value determined from the log likelihood of the true number of populations (K; Evanno et. al, 2005) Results were uploaded to Structure Harvester to confirm these calculations (Earl and VonHoldt, 2012).

STRUCTURE has a documented error associated with falsely returning K = 2 subpopulations (Janes et. al, 2017). To prevent this error, individuals were separated into their identified populations and re-analyzed using STRUCTURE. To further ensure accuracy of the number of populations identified, a dummy population with alleles distinct from those which occur naturally was created to determine if STRUCTURE could detect the outliers and assign them to a population other than those identified for the non-dummy populations.

Analysis of Molecular Variance (AMOVA) was performed in GenAlEx 6.503 on all individuals sampled on Crane with individuals sorted into their populations as determined in STRUCTURE. F statistics (F_{ST}) were calculated using GenAlEx software to understand relationship between population structure detected by STRUCTURE. GenAlEx was also used to calculate linear genetic and Nei's genetic distance among the populations (Peakall and Smouse, 2012). Nei's genetic distance (GD) was calculated for mother tree subpopulations which included mother trees, other large trees, and offspring. Nei's GD was also calculated between mother tree adult groups (composed of mother trees and associated large trees) and for populations identified

by Structure analysis (Nei, 1972). These metrics identify the degree of relatedness among demes and among individuals in the two populations.

Maternity analysis was conducted using Cervus (Marshall et. al, 1998). This software uses genetic markers to assign parents and offspring using likelihood analysis. The simulation of maternity analysis function was used to assign a mother to individuals in my data set. The threshold was set an 80% confidence for assignment. Maternity was based on comparison of offspring genotypes with presumed maternal genotypes and other large adults sampled. Candidate mothers were assumed to be all mother trees and large reproducing adults. Offspring were assumed to be the youngest individuals from which leaves were collected in each deme. GenAlEx was used to calculate Nei's genetic distance between offspring and their assigned parent.

Calculations for estimation of Hardy-Weinberg Equilibrium (HWE) were completed in GenAlEx. HWE assumes a population is experiencing no genetic drift, immigration or emigration (i.e. it is a closed population), not undergoing mutations, experiencing random mating patterns, and not subject to natural selection (Hardy, 1908). A chi-square analysis was conducted to determine if STRUCTURE population membership differed between mother tree populations.

Results

According to GenAlEx analysis, my population deviated from HWE. This indicates there is some change or selection occurring in my study population. This is important as my analysis software, (STRUCTURE, Cervus, and GenAlEx analyses) assumed populations are in HWE meaning my results should be interpreted with caution. Heterozygosity was calculated and shown to be higher than expected in all loci indicating an excess of heterozygotes in my population and explaining

deviation of the population from HWE (Table 3.3). When all samples were grouped in a single population, all nine loci were polymorphic averaging 9.33 alleles per locus.

Using STRUCTURE software, I determined that my samples were drawn from two populations (Fig. 3.2). When I included a dummy population, STRUCTURE determined three subpopulations as the most likely solution, the previously identified population, (one and two), and a third population composed entirely of the dummy samples. The spatial distribution of the populations did not reflect any obvious underlying edaphic or environmental factors. Members of each population were not necessarily located near each other (i.e. members of both population were found across the entire sampled space on the base). Further, in some cases I found members of population one and two located adjacent to one another (Fig. 3.4). Chi-square analysis on mother tree populations determined that membership in STRUCTURE populations was significantly more different among sampled sites than was expected ($p < 0.001$), based on proportion of total individuals belonging to each STRUCTURE population (Table 3.4).

Nei's genetic distance (GD) between the two populations was 0.870 with an average GD of 0.566 between all individuals sampled and GD of 0.721 between mothers. AMOVA determined an F_{ST} of 0.033 between populations. Nei's genetic distance revealed that that mother trees, adults, and offspring were more closely related than the mothers and adults alone (Table 3.4, Table 3.5). Parentage assignments as determined by Cervus demonstrated a great deal of mixing of assigned parentage across the assumed offspring in Crane, with parentage assignments showing no clear relationship to biological or geographical barriers (Fig. 3.6).

Discussion

In this research, I sought to understand the relationship between spatial distance and genetic relatedness by sampling 10 random demes across the invaded space. Understanding this relationship is vital in understanding drivers of spread in this species, factors contributing to success, and why, despite its self-incompatible character, *P. calleryana* invaded across Crane. I hypothesized uniform relatedness across space in *P. calleryana* between mother and offspring. This hypothesis was supported by my data with little variation in GD across distances from their mother (Table 3.4). I further hypothesized 10 subpopulations would be present in the invasive range corresponding to the mother trees sampled. This hypothesis was negated by my STRUCTURE analysis (Fig. 3.2, 3.4). Finally, due to activity of bird fruit dispersers, I hypothesized that I would find greater levels of genetic diversity (as represented by GD) within mother tree populations than between. This hypothesis was supported by my data with an F_{ST} of 0.033 (Fig. 3.5). It is important to note that my population deviated from HWE indicating some level of change in genetic structure was occurring over time. Further, HWE is a central assumption in all software used for analyzing my data. The higher than expected levels of heterozygosity identified by GenAlEx are likely contributing to this deviation.

Contrary to predictions of Baker (1950), *P. calleryana* seems to be successfully colonizing and recruiting in many parts of its range despite the self-incompatibility of the species. The lack of population inhibition may be a function of pollinator and seed dispersal activity. That is, generalist insect pollinators on Crane readily visit *P. calleryana* and transmit pollen between trees leading to production of viable fruits (Pasquet et. al, 2008). Further, fruit consumption by unspecified bird species led to abundant dispersal activity over a variety of distances. Although it is possible that the population of *P. calleryana* on Crane may have escaped self-incompatibility,

my data showed high heterozygosity and no progeny genotypes consistent with self-pollination. This suggests no breakdown of self-incompatibility. However, my research did not specifically identify self-incompatibility alleles. Further, *P. calleryana* is closely related to the *Malus* genus with many microsatellite markers being cross-compatible between species. This research utilized PCR primers originally designed for *Malus domestica* (orchard apple) but proven effective in *P. calleryana* as well. One means by which *P. calleryana* could escape self-incompatibility is through hybridization with a species belonging to the *Malus* genus such as *Malus angustifolia* (flowering crabapple) or other member of the Rosaceae family.

The lack of relationship between distance and GD at each sampled site indicated that progeny were the result of matings that were independent of distances up to 100 m (Table 3.4, 3.6). Both pollen dispersers and fruit dispersers are active at these distances. In some mother tree demes, there was increasing Nei's GD with spatial distance from mothers and adults (M1, M2, M3); however, these increases were not statistically significant (Table 3.5).

The excess of heterozygotes noted in my population explains its departure from HWE. The observed excess heterozygosity may indicate disassortative mating caused by the requirement that parents express complementary (different) self-incompatibility alleles. Disassortative mating in my population favored crosses between individuals which were less than average heterozygosity.

The hypothesis that the invasive population as a whole would be composed of 10 subpopulations, according to deme, was contradicted by my STRUCTURE which showed *P. calleryana* at Crane comprised two populations. The genetic differentiation between the populations was detectable, but low (F_{ST}= 0.033; Fig 3.3). I observed less GD between younger trees of each deme than between mature trees in the same deme (Table 3.6, Table 3.7). This result indicated that the adult trees at the sampled sites belonged to cohorts that were more similar than

the similarity of offspring at the same site. I found that many of the offspring were not derived from local parents but were drawn from a different gene pool than the one from which the parents were derived, i.e., the offspring were the result of crosses within a cohort distinct from the one that produced the adults. The genetic difference between adults and offspring at each of the demes may reflect a second wave of invasion, or at least the influx of additional genetic variance. It may also reflect inputs from horticultural plantings on the base which were not captured in my sampling design as I sampled from only escaped *P. calleryana*. Finally, my hypothesis that I would find greater levels of genetic diversity (measured by GD) within than between populations was supported. I found that mother tree populations had lower GD relative to one another than GD found within that population between mother trees and offspring (Table 3.4, Table 3.6).

Differences in phenology may contribute to the population differentiation I observed. The timing of fruit and pollen production in different *P. calleryana* cultivars has not been established, so it is possible that phenology differences produced a phenotypic barrier to crossing between individuals. Any difference in fruiting time may also affect the behavior of birds. If certain trees are fruiting earlier, this phenotypic difference can determine where birds forage; dispersers may visit certain trees and not others at a given time. The consumption of seeds of *P. calleryana* individuals fruiting at the same time can lead to their joint dispersal, maintaining spatial covariance of genotypes and increasing the relatedness of offspring where the seeds are deposited. I did not record phenology data at Crane, but phenotypic differences could result in non-random establishment and ultimately non-random mating, and tend to increase differentiation between populations while decreasing differentiation within populations. It is possible this effect was revealed by my parentage analysis which showed that mother trees tended to be surrounded by seedlings by which they were not the parents but to which they were closely related. For example,

demes M3 and M5 (GD = 0.370; Table 3.5) shared offspring, as did M7 and M3 (GD = 0.534), and M6 and M7 (GD = 0.522), whereas there were no offspring of deme M9 in M10 (GD = 0.816), and no sharing between M9 and M1 (GD = 0.997), demes which the parents were more distantly related (Table 3.5, Fig. 3.4). This result may reflect bird behavior if mothers M3 and M5, M7 and M3, and M6 and M7 fruited at the same time. Another factor that could have produced the observed pattern of relatedness among mother tree demes is behavior of insect pollen dispersers. Trees in patches with the same bloom time would be more likely to exchange pollen and produce offspring that are related. While insect pollinators are capable of pollen transfer over large distances, they more commonly forage locally in small vegetation patches (Pasquet et. al, 2008).

A possible explanation for the presence of two populations of *P. calleryana* at Crane is effects related to density. By comparing the frequency of membership in each population at each mother tree with the densities of *P. calleryana* at plots identified in Chapter 2 of this thesis, I observed that population one appears to coincide with regions with the greatest density of *P. calleryana* (Chapter 2, Fig. 2.2; Fig. 3.4). A possible reason for density-based population separation is self-incompatibility. Long residence time in an area can contribute to higher stem density of *P. calleryana* as dispersers introduce regeneration. In areas with old trees and new regeneration, inter-generational mating and reproduction can result in more local increases in genetic diversity and increased heterozygosity. If new cultivars of *P. calleryana* are introduced into an area, then regeneration in areas of high population density may also increase opportunities for hybridization and hybrid vigor in the offspring (Gaskin, 2017). Where there is higher density, there is also an increased probability of the presence of viable mates. If new genotypes of *P. calleryana* are introduced into an area, local increases in the number of hybrid and introgressed genotypes is expected. In highly genetically diverse local demes there is more genetic variance on

which selection can act increasing the potential for adaptions and increased invasiveness (Gaskin, 2017; Hardiman and Culley, 2010).

It is possible that the introduction of multiple cultivars of *P. calleryana* by the nursery at Crane (in the 1940s), followed by subsequent introductions of new cultivars (in the 1970s) were the basis of the patterns observed today. Accordingly, population one may represent a gene pool of more recently introduced cultivars and their hybrids and population two may represent the oldest gene pool at Crane and hybrids of the members of this pool. This model is supported by the parentage assignments that showed more mixing among demes dominated by population two versus population one. M1, M2, and M5 were strong examples of this effect; relatively few mothers contributed to the offspring at these sites (Fig. 3.4, Fig. 3.6). This kind of density dependence is important to invasive species management indicating the value of treating areas of high population density necessitating intense levels of management in densely populated areas to achieve control or the lofty goal of eradication. It also indicated that detecting densely populated plant patches early in invasion is vitally important to preventing greater impact on native species.

The findings in this research demonstrated that the spread of *P. calleryana*, and other self-incompatible species may not be limited as Baker (1950) suggests; particularly where high densities and abundant available mates are present. The self-incompatibility in *P. calleryana* was among the main selling points of the species as an ornamental because it was hypothesized to be incapable of escape. Over time, horticultural flaws led to breeding programs to introduce additional genetic variance, increasing the opportunities for release from the constraint of self-incompatibility (Culley et. al, 2011; Culley and Hardiman, 2007; Vincent, 2005). *Pyrus calleryana* is now one of the most popular species for ornamental plantings in urban centers and in the landscaping industry (Vincent, 2005). The large number and genetic diversity of *P. calleryana* used in the horticultural

industry encourages the species invasiveness and the range over which the invasion is likely to occur. Large, invasive populations similar to the one at Crane are likely to become more common.

Overall, the environmental preferences of *P. calleryana* for drier sites and association with shade intolerant species indicate a preference for early seral environments (Chapter 2). Desirable native tree species, such as *Quercus* and *Carya* spp., can expect increased competition over time with *P. calleryana* (McEwan et. al, 2011). The high levels of heterozygosity present in the invasive study population are an indication that it will continue to spread, hybridize, and increase its density across its invaded range. More generally, the potential density-dependent reproductive success in *P. calleryana* coupled with the horticultural popularity of the species suggests *P. calleryana* will continue to escape and recruit into new areas, increasing its invasive range. The probability of shifts in forest structure, composition, and community species diversity will undoubtedly follow.

Tables & Figures

Table 3.1 Primers utilized in Polymerase Chain Reactions and genotyping of *Pyrus calleryana*.

Primer	Sequence (5'-3')
KU10	AGTATGTGACCACCCCGATGTT AGAGTCGGTTGGGAAATGATTG
CH02D12	AACCAGATTTGCTTGCCATC GCTGGTGGTAAACGTGGTG
CH02B10	CAAGGAAATCATCAAAGATTCAAG CAAGTGGCTTCGGATAGTTG
KA16	GCCAGCGAACTAAATCT AACGAGAACGACGAGCG
CH01H10	TGCAAAGATAGGTAGATATAGCCA AGGAGGGATTGTTTGTGCAC
CH01F02	ACCACATTAGAGCAGTTGAGG CTGGTTTGTTTTCCTCCAGC
CH01H01	GAAAGACTTGCAGTGGGAGC GGAGTGGGTTTGAGAAGGTT
CH02D11	AGCGTCCAGAGCAACAGC AACAAAAGCAGATCCGTTGC
KA14	TCATTGTAGCATTTTTATTTTT ATGGCAAGGGAGATTATTAG

Table 3.2 Data used in the Evanno method for determining true number of populations (K) as determined from STRUCTURE analysis.

K	Reps	Mean LnP(K)	Standard Deviation LnP(K)	Ln'(K)	[Ln''(K)]	Delta K
1	10	-65077.96	0.27	-	-	-
2	10	-6381.84	1.68	126.14	303.56	181.11
3	10	-6559.26	46.63	-177.42	128.72	2.76
4	10	-6607.96	52.85	-48.70	32.47	0.61
5	10	-6624.19	70.92	-16.23	165.72	2.34
6	10	-6806.14	384.36	-181.95	230.81	0.60
7	10	-6757.28	138.17	48.86	92.87	0.67
8	10	-6615.55	117.47	141.73	696.33	5.93
9	10	-7170.15	86.52	-554.60	587.08	0.65
10	10	-7137.67	693.72	32.48	-	-

Table 3.3 Sample size, number of alleles, observed heterozygosity, and expected heterozygosity for each mother tree population which included mother, large adults, and any offspring.

	Locus	CH02B10	CH02D12	KU10	CH01F02	CH01H10	KA16	CH01H01	KA14	CH02D11
M1	Sample size	13	13	13	13	12	13	13	13	13
	no. alleles	5	5	9	5	6	7	3	6	6
	Observed Heterozygosity	1.000	1.000	1.000	1.000	1.000	0.923	1.000	1.000	0.846
	Expected Heterozygosity	0.675	0.604	0.805	0.725	0.757	0.734	0.624	0.802	0.675
M2	Sample size	21	21	21	21	20	21	19	21	21
	no. alleles	3	6	9	6	5	9	6	8	8
	Observed Heterozygosity	1.000	1.000	1.000	0.810	0.850	0.810	1.000	1.000	0.952
	Expected Heterozygosity	0.561	0.622	0.820	0.680	0.734	0.749	0.695	0.802	0.834
M3	Sample size	23	23	22	23	23	23	23	22	23
	no. alleles	4	6	12	5	5	9	7	7	9
	Observed Heterozygosity	1.000	1.000	1.000	0.826	0.870	0.957	1.000	1.000	0.957
	Expected Heterozygosity	0.595	0.647	0.856	0.667	0.760	0.856	0.704	0.822	0.836
M4	Sample size	18	18	18	18	18	18	16	18	18
	no. alleles	4	6	11	6	6	9	6	7	8
	Observed Heterozygosity	1.000	1.000	1.000	1.000	0.833	0.889	0.938	1.000	0.889
	Expected Heterozygosity	0.573	0.637	0.877	0.781	0.739	0.818	0.719	0.827	0.781
M5	Sample size	18	18	18	18	18	18	18	18	18
	no. alleles	5	5	7	6	5	8	6	7	9
	Observed Heterozygosity	0.944	0.944	0.944	1.000	0.833	0.944	0.944	0.778	1.000
	Expected Heterozygosity	0.713	0.617	0.769	0.765	0.736	0.782	0.691	0.730	0.826
M6	Sample size	20	20	20	20	19	20	20	20	20
	no. alleles	4	7	11	9	6	8	7	8	8
	Observed Heterozygosity	0.900	0.950	1.000	0.950	1.000	1.000	1.000	1.000	0.950
	Expected Heterozygosity	0.536	0.628	0.871	0.843	0.765	0.739	0.708	0.758	0.818
M7	Sample size	22	23	23	23	23	23	23	23	23
	no. alleles	4	7	9	9	6	10	5	8	10
	Observed Heterozygosity	0.955	1.000	0.913	1.000	0.957	1.000	0.957	1.000	0.913
	Expected Heterozygosity	0.681	0.667	0.853	0.844	0.740	0.771	0.721	0.805	0.861
M8	Sample size	23	23	23	23	23	23	23	23	23
	no. alleles	3	6	8	7	6	9	7	8	10
	Observed Heterozygosity	0.957	1.000	0.652	0.957	1.000	1.000	1.000	1.000	1.000
	Expected Heterozygosity	0.651	0.677	0.832	0.783	0.711	0.771	0.748	0.822	0.887
M9	Sample size	20	21	21	21	21	21	21	21	21
	no. alleles	4	8	7	5	6	8	6	8	7
	Observed Heterozygosity	1.000	0.905	0.667	1.000	0.905	0.952	1.000	1.000	0.952
	Expected Heterozygosity	0.634	0.661	0.782	0.763	0.712	0.745	0.737	0.765	0.813
M10	Sample size	22	22	22	22	22	22	22	20	22
	no. alleles	4	8	10	7	5	8	7	6	9
	Observed Heterozygosity	1.000	1.000	0.955	0.955	0.909	1.000	1.000	0.950	1.000
	Expected Heterozygosity	0.581	0.713	0.798	0.780	0.701	0.796	0.738	0.776	0.856

Table 3.4 Nei's genetic distance of mothers and large adults to offspring cohorts at distances 10, 20, 40, 80, and 100 meters.

	M1	**M2**	**M3**	**M3A1**	**M3A2**	**M4**	**M5**	**M5A1**	**M6**
10	0.274	0.393	0.358	0.523	0.439	0.453	0.427	0.508	0.730
20	0.437	0.388	0.333	0.431	0.545	0.631	0.650	0.488	0.758
40	0.336	0.391	0.335	0.328	0.793	0.661	0.622	0.380	0.713
80	0.584	0.442	0.519	0.573	0.508	0.896	0.665	0.416	0.774
100	0.583	0.507	0.581	0.361	0.559	0.380	0.664	0.365	0.732
	M7	**M7A1**	**M7A2**	**M8**	**M8A1**	**M8A2**	**M9**	**M10**	**M10A1**
10	0.563	0.711	0.511	0.565	0.534	0.587	0.393	0.720	0.497
20	0.446	0.421	0.480	0.565	0.504	0.787	0.492	0.593	0.478
40	0.575	0.585	0.687	0.852	0.534	0.617	0.500	0.539	0.478
80	0.592	0.740	0.481	0.624	0.729	0.643	0.762	0.474	0.638
100	0.718	0.823	0.380	0.629	0.698	0.617	0.592	0.708	0.643

Table 3.5 Result of chi-square analysis of mother tree population membership to STRUCTURE populations (one and two).

Mother Tree	Population	Observed	Expected	$(O-E)^2/E$	X^2
M1	One	1	5.835	4.007	8.534
	Two	10	5.165	4.527	
M2	One	0	11.140	11.140	23.727
	Two	21	9.860	12.587	
M3	One	0	10.079	10.079	21.468
	Two	19	8.921	11.388	
M4	One	1	9.018	7.129	15.184
	Two	16	7.982	8.055	
M5	One	1	4.774	2.984	6.355
	Two	8	4.226	3.371	
M6	One	15	8.488	4.996	10.642
	Two	1	7.512	5.645	
M7	One	17	9.018	7.064	15.046
	Two	0	7.982	7.982	
M8	One	21	11.140	8.726	18.586
	Two	0	9.860	9.860	
M9	One	21	11.140	8.726	18.586
	Two	0	9.860	9.860	
M10	One	10	6.366	2.075	4.419
	Two	2	5.634	2.344	
				Chi-square value:	142.547
				degrees of freedom:	9
				p-value:	<0.001

Table 3.6 Nei's genetic distance between sample sites including all trees found at each sample site as calculated by GenAlEx.

	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
M1	0.000									
M2	0.144	0.000								
M3	0.158	0.088	0.000							
M4	0.158	0.117	0.081	0.000						
M5	0.199	0.156	0.118	0.171	0.000					
M6	0.247	0.317	0.253	0.301	0.219	0.000				
M7	0.242	0.283	0.203	0.251	0.184	0.099	0.000			
M8	0.293	0.364	0.250	0.303	0.243	0.122	0.057	0.000		
M9	0.296	0.408	0.275	0.319	0.266	0.117	0.074	0.054	0.000	
M10	0.169	0.227	0.147	0.192	0.157	0.120	0.082	0.093	0.098	0.000

Table 3.7 Nei's genetic distance between mother trees and associated large adults compared to other mother trees and associated large adults at sample sites (no offspring) as calculated by GenAlEx.

	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
M1	0.000									
M2	0.558	0.000								
M3	0.566	0.725	0.000							
M4	0.864	0.793	0.625	0.000						
M5	0.602	0.650	0.370	0.732	0.000					
M6	0.889	0.937	0.385	1.003	0.618	0.000				
M7	0.804	0.900	0.534	1.087	0.584	0.522	0.000			
M8	0.958	1.257	0.649	1.023	0.681	0.617	0.640	0.000		
M9	0.997	0.911	0.567	1.281	0.667	0.357	0.577	0.751	0.000	
M10	0.725	0.842	0.452	0.816	0.598	0.596	0.354	0.572	0.816	0.000

Table 3.8 Sample size, number of alleles, observed heterozygosity, and expected heterozygosity for the two subpopulations identified by Structure analysis.

	Locus	CH02B10	CH02D12	KU10	CH01F02	CH01H10	KA16	CH01H01	KA14	CH02D11
Subpopulation one	Sample size	105	105	104	105	102	105	101	104	105
	no. alleles	5	10	14	9	6	10	10	8	10
	Observed Heterozygosity	0.990	0.990	0.990	0.914	0.882	0.914	0.980	0.962	0.943
	Expected Heterozygosity	0.662	0.635	0.893	0.758	0.780	0.833	0.705	0.810	0.840
Subpopulation two	Sample size	95	97	97	97	97	97	97	95	97
	no. alleles	4	11	13	10	6	10	8	8	10
	Observed Heterozygosity	0.958	0.969	0.814	0.979	0.948	0.990	0.990	0.989	0.959
	Expected Heterozygosity	0.641	0.684	0.846	0.826	0.733	0.783	0.743	0.800	0.873

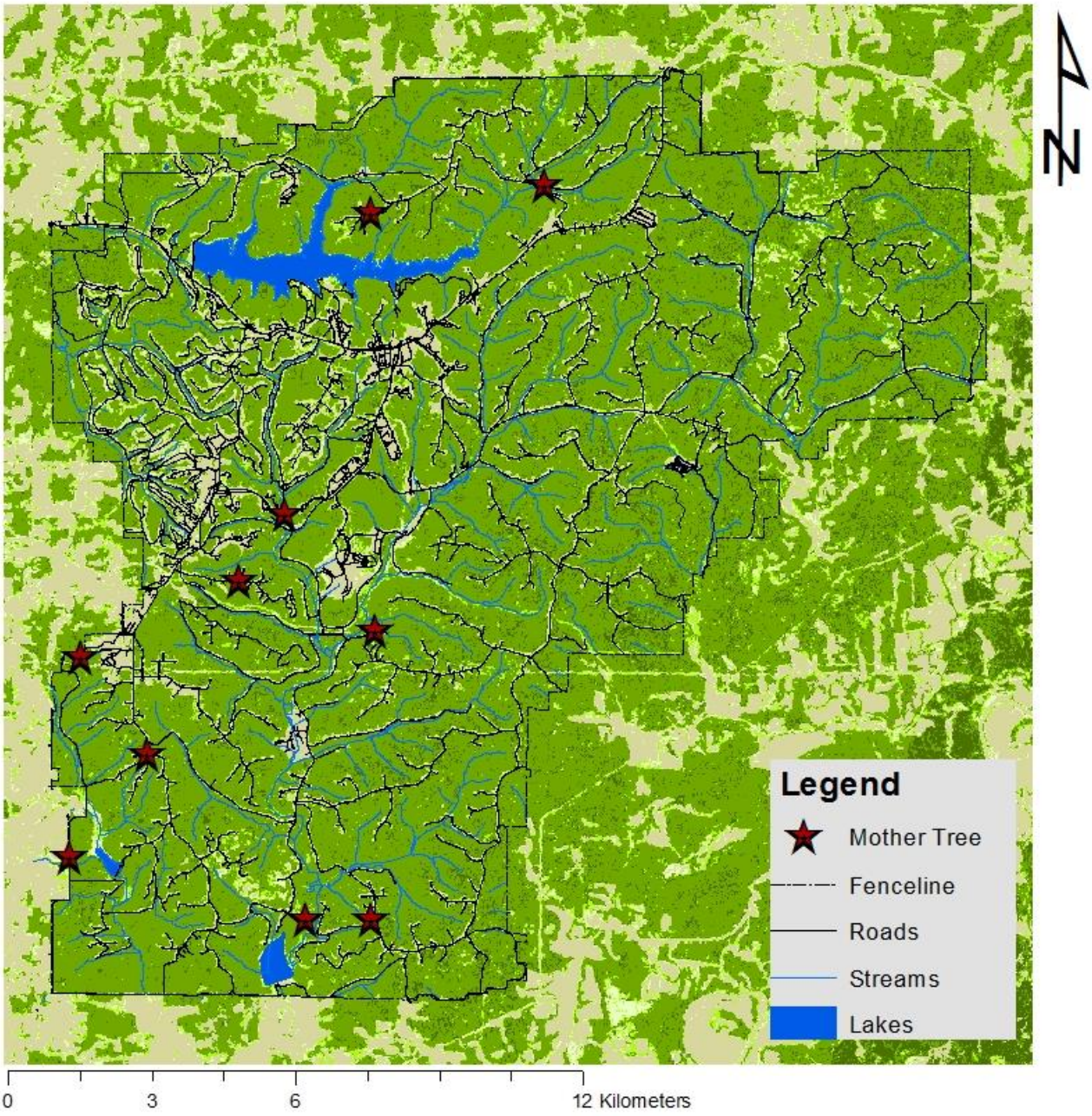

Figure 3.1. Location of mother trees (large, reproducing adult *Pyrus calleryana* trees) on Naval Support Activity – Crane located in Martin County, shown with forest cover. Mother trees were identified and selected on the basis of knowledge of existing large adults within the invasive range of *P. calleryana* on the base.

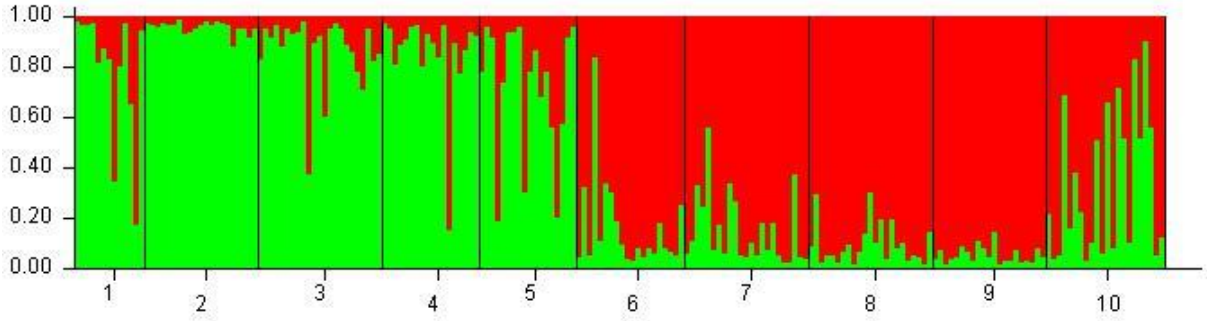

Figure 3.2 Population assignments of offspring and Mother Trees on Naval Support Activity – Crane with Mother Tree number shown on the x-axis according to STRUCTURE. Population one is shown in red and population two is shown in green.

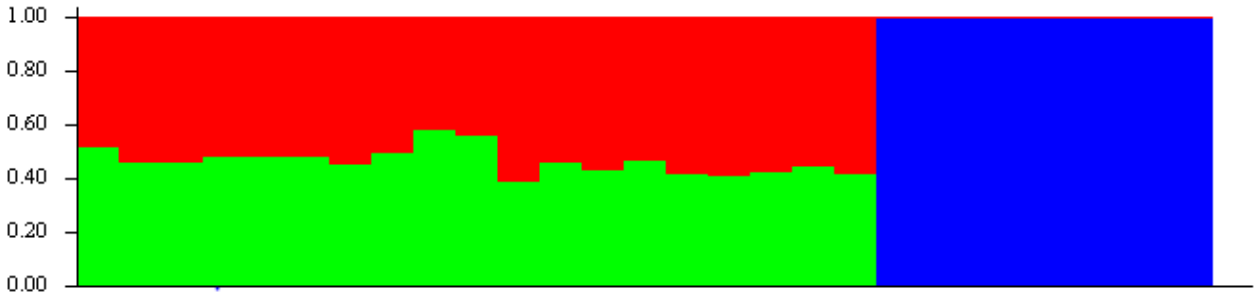

Figure 3.3 Result of STRUCTURE analysis verification test which included the dummy population with population one shown in red, population two shown in green, and the dummy population shown in blue.

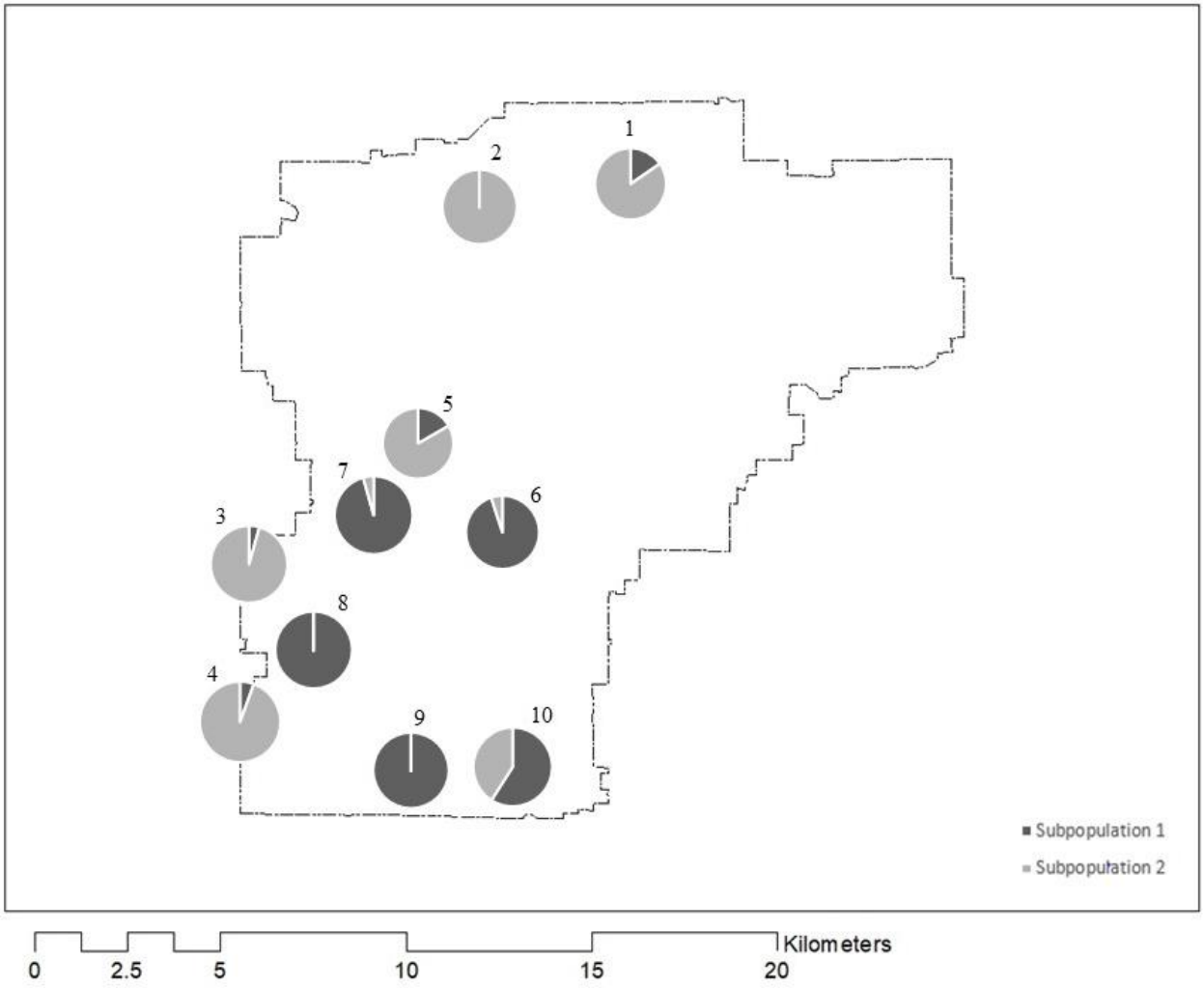

Figure 3.4 Proportion of population assignments (subpopulation one or two) determined by structure at each mother tree located on Naval Supporty Activity – Crane.

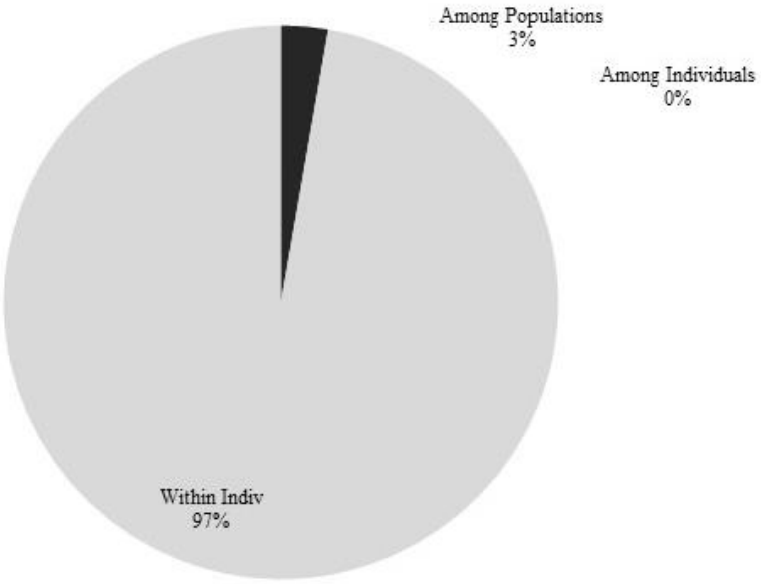

Figure 3.5 Variation among populations and within and among individuals as determined from F_{ST} calculated in GenAlEx.

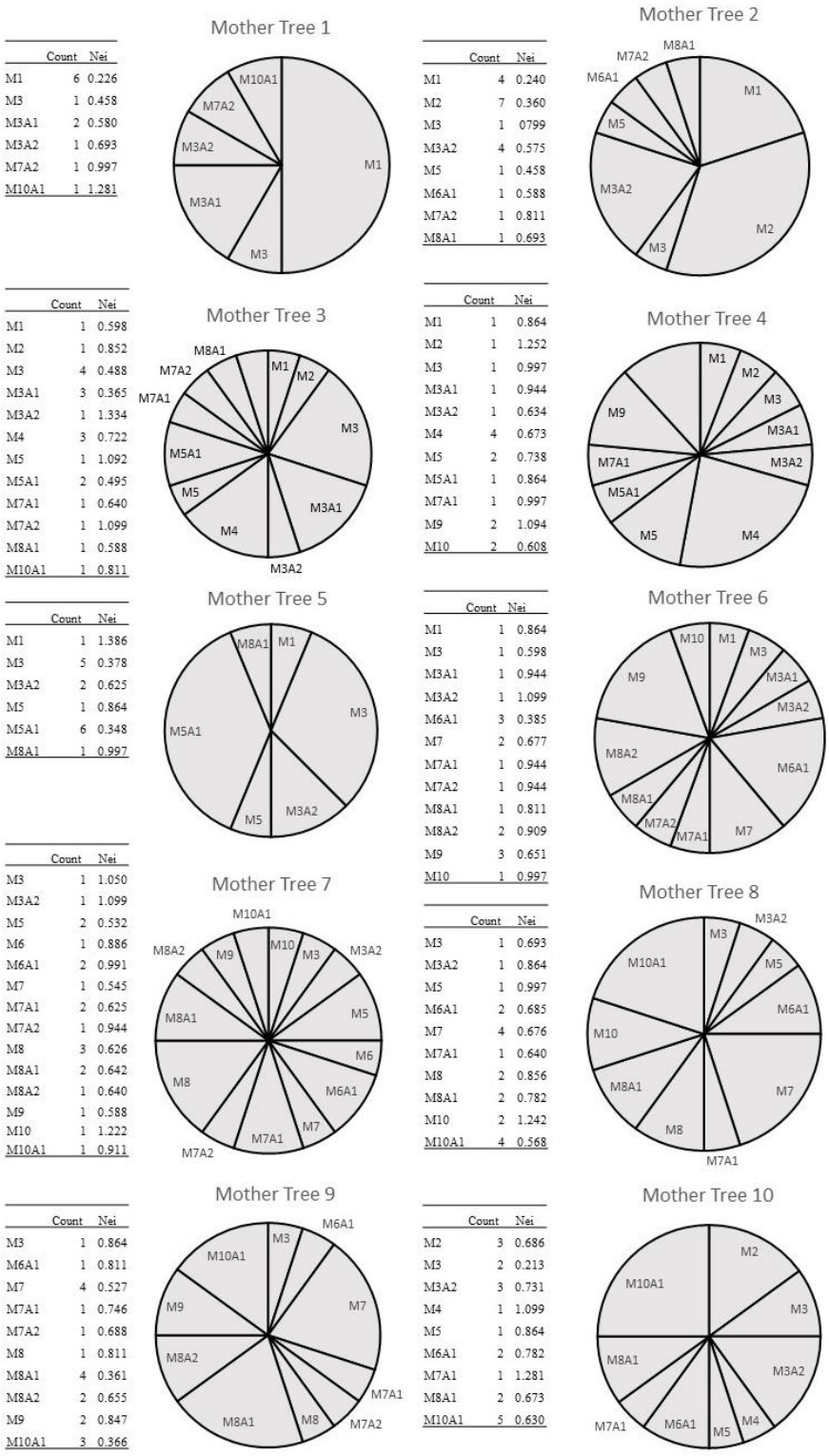

Figure 3.6 Parentage assignments as determined by Cervus across the 10 demes. Number of offspring assigned to each mother as well as Nei's genetic distance between those offspring and that mother is given in the table displayed on the left of each pie chart. Mother tree codes are defined in Appendix A.

Appendix A

Table 3A1 Abbreviation definitions for mother trees sampled in study.

Abbreviation	Definition
M1	Mother tree one
M2	Mother tree two
M3	Mother tree three
M3A1	Additional adult at mother tree three
M3A2	Additional adult at mother tree three
M4	Mother tree four
M5	Mother tree five
M5A1	Additional adult at mother tree five
M6	Mother tree six
M6A1	Additional adult at mother tree six
M7	Mother tree seven
M7A1	Additional adult at mother tree seven
M8	Mother tree eight
M8A1	Additional adult at mother tree eight
M8A2	Additional adult at mother tree eight
M9	Mother tree nine
M10	Mother tree 10
M10A1	Additional adult at mother tree plot 10

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