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STUDY OF MORPHOLOGICAL AND PHYSIOLOGICAL PROPERTIES AND POLLINATORS OF THE INVASIVE CALLERY PEAR

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STUDY OF MORPHOLOGICAL AND PHYSIOLOGICAL PROPERTIES AND POLLINATORS OF THE INVASIVE CALLERY PEAR

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ABSTRACT

Callery pear is an invasive tree in 15 states of United States and is currently present in 37 states. Its management has challenged landowners and land managers. Despite being listed as a noxious weed in four states, its study is limited. The accurate estimation of Callery pear biomass will give a clearer picture of the level of invasion and help land managers develop different strategies to control its population. Similarly, identifying possible pollinators of Callery pear is essential to have some insights on pollinators associated with this tree. Hence, the objectives of this study are 1) to calculate total above-ground biomass and prepare allometric equation, and 2) identify the pollinator taxa and calculate diversity metrics and community composition of pollinators using Callery pear.

Forty trees from six different sites were destructively harvested. We measured diameter at breast height (DBH), total tree height, fresh weight, oven-dry weight, C:N ratio and moisture content of different tree components (i.e., leaves, fruits, stem, and branches) and examine their relationship with each other. Biomass of each tissue component was significantly related to DBH. Total wet biomass ranged from 326.75 g (1.27 cm DBH) to 160 kg (17.52 cm DBH), and total dry biomass ranged from 193.25 g to 97 kg for the same DBH. The average moisture content ranged from 39% to 42 % and the average wood density was found to be 0.88 gm/cm^3. The model/equation determined for the biomass is Log linear model with 91% variation explained by the model.

Five sites in South Carolina were surveyed using yellow, blue, white colored plastic cups and sweep net methods. A total of 756 insects were collected, belonging to 15 families in three orders; Diptera (611), Hymenoptera (135), and Coleoptera (10). The most common families of insects visiting Callery pear were Syrphidae, Sarcophagidae, Anthomyiidae, and Andrenidae with common species being *Toxomerus*, *Andrena*, *Apis mellifera*, *Osmia*, and *Lasioglossum.* Because of highest number in all sites, we propose that *Toxomerus* spp. from Syrphidae family are the major visitors of Callery pear's flower whereas sweep net and yellow bowl trap are most suitable methods of collecting them.

Keywords: allometric equations, biomass, diversity, invasive, pollinators

DEDICATION

I want to dedicate this thesis to my grandparents (Mohan and Saraswoti Devkota), parents (Ek Raj and Bimala Devkota), siblings (Priti and Prakrit), brother-in-law (Jerry Nettles), in-laws (Chandrakant and Ganga Paudel), my dear husband (Abinash Paudel) and my whole family for their continuous support and motivation. Thank God for always being there for me.

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CHAPTER ONE

LITERATURE REVIEW

1.1 INVASIVE SPECIES

The term "invasive species" was coined by Charles Elton in his book (Elton, 1958), but was legally defined in the United States in 1999 by President Bill Clinton as "a species that is non-native to the ecosystem under consideration and whose introduction causes or is likely to cause economic or environmental harm or harm to human health" (Clinton, 1999). While invasive species can be introduced intentionally or unintentionally, deliberate introduction, such as horticultural and ornamental plants (64% of plants that were introduced deliberately), is the primary vector of invasive angiosperms in the United States (Lehan et al., 2013; Mack & Erneberg, 2002; Reichard & White, 2001). Among the 1,285 identified invasive plant species in the United States, 61% are available through plant trade, such as commercial nurseries and e-commerce (Beaury et al., 2021). Of the 688 species (54% of 1,285) regulated by states, 50% are available for purchase. Of the 98 federal noxious weed species, 20 (20%) are available for purchase (Beaury et al., 2021). Global studies show that only 0.5% to 0.7% of woody plants (trees and shrubs) are invasive outside their native range (Richardson & Rejmánek, 2011). However, this number is rapidly increasing, with North America being the third highest in terms of the number (163) of woody invasive alien species in the world after Australia (183) and South Africa (170) (Richardson & Rejmánek, 2011).

After introduction to a novel ecosystem, invasive plant species may take months or years to become invasive (i.e., lag time) (Sakai et al., 2001) depending on site variables, such as climate, and plant characteristics, such as longevity and reproduction. Some extinctions of introduced species occur in the lag phase; however, once the species overcome the barriers of invasion (i.e., introduction, acclimatization, establishment, and landscape escape) (Theoharides & Dukes, 2007), it can be nearly impossible to eradicate them (Mack et al., 2000). Therefore, prevention, early detection, and rapid response to invasives is the best way to reduce biological invasion (Reaser et al., 2020). Early detection minimizes the impact of invasives and reduces their long-term costs and damages (Epanchin-Niell, 2017).

Invasive species pose severe threats to biodiversity and community structure (Early et al., 2016). Invasive plants may alter ecosystems by affecting biodiversity, nutrient cycling, loss of groundwater, and forest dynamics, resulting in harm to native species (Zedler & Kercher, 2004). For example, tree-of-heaven (*Ailanthus altissima*) and Chinese tallow (*Triadica sebifera*) produce thousands of seeds and displace native species once established (Webster et al., 2006; Wickert et al., 2017). Melaleuca or Paper-Bark Tree (*Melaleuca quinquenervia*) causes loss of groundwater because of increased evapotranspiration (Webster et al., 2006). Chinese privet (*Ligustrum sinense*) has caused a reduction in pollinator diversity (Ulyshen et al., 2020), native plants (Wilcox & Beck, 2007), native earthworms (Lobe et al., 2014), and beetles (Ulyshen et al., 2010).

Besides negatively impacting biodiversity and community structure, invasive species cause severe economic impacts requiring a large investment of money in their management, control, and eradication (Larson et al., 2011). Management costs of invasive species in the United States total $1.22 trillion, with the second major economic impact ($190.45 billion) coming from the plant invaders (Fantle-Lepczyk et al., 2022).

This cost has risen from ~$10.30 billion annually between 1960 and 1969 (adjusted for inflation) to $21.08 billion annually between 2010 and 2020 (Fantle-Lepczyk et al., 2022). Therefore, several agencies have prioritized research on quantifying the biology, impacts, and interactions of invasive species, their management, and altered ecosystems due to invasion (Poland et al., 2021). Due to the financial investments to prevent and manage invasive species, it is important to make rational decisions based on data. Quantifying the amount of biomass of an invasive species in the landscape and understanding its ecological impacts will help decision-makers make sound management decisions.

1.2 CALLERY PEAR (PYRUS CALLERYANA DECNE.)

Callery pear (*Pyrus calleryana* Decne., Rosaceae) is native to East and Southeast Asian countries such as China, Taiwan, Korea, Vietnam, and Japan inhabiting a broad ecological range including steep slopes, plains, and mixed valley forests at elevation of 100-1800 meters (Culley & Hardiman, 2007). Some studies report that first Earnest H. Wilson accessioned and grew several seed lots at the Arnold Arboretum in 1908 (Culley & Hardiman, 2007). Others report that it was introduced by George Compere with the California State Commission of Horticulture (Compere, 1913; Lalk et al., 2021). Later, it was collected in large quantities by Frank Meyer of the USDA Foreign Plant and Seed Introduction Division to graft the European pear (*P. communis*) to *Pyrus calleryana* rootstocks to save the former from fire blight disease, caused by *Erwinia amylovora* (Culley, 2017). While there are inconsistent reports of its introduction into the United

States, the primary reason behind its original importation was to save the commercial pear industry from fire blight-caused losses.

In 1952, John Creech from the USDA identified features (i.e., glossy leaves, globular form, and lack of sharp spurs) of one of the remaining 33-year-old *P. calleryana* trees from Meyer's Chinese seeds, which would make the species an attractive ornamental tree. He then grafted scions of this tree to another *P. calleryana* rootstock to produce a new cultivar that is genetically identical to its original mother tree leading to the development of the first ornamental cultivar of *P. calleryana*, the 'Bradford' pear (Culley, 2017). Since then, 25 additional P. *calleryana* cultivars have been developed which were planted as ornamentals until the late 1900s, at which point the presence of these cultivars allowed the self-sterile plant to begin cross-pollinating each other, producing viable seeds readily spread by birds and wildlife (Dirr, 1990; Griffiths & Huxley, 1992; Vincent, 2005). Callery pear is now present in 37 states in the U.S. and is listed as 'invasive' in 13 of those (Georgia - center for invasive species and ecosystem health, 2023), growing on roadsides, old fields, vacant urban/suburban lots, and, more recently, encroaching into forested areas where it forms dense thickets with branches covered in sharp thorns (Coyle et al., 2021). Where it has become invasive, fallen fruit creates a slippery mess on sidewalks, branches with sharp thorns cause damage to equipment, spring flowers have a very foul smell, and, most crucial, stems and branches tend to split and break in winds and storms causing damage and injury (Culley, 2017).

Callery pear is a perennial, deciduous (Sedgley, 1990), medium-sized, fast-growing tree, reaching about 35 ft. in height, with clusters of white flowers that bloom in early spring (Niemiera, 2010). These white flowers can cross-pollinate with other *Pyrus*, including other cultivars of *P. calleryana*, to form dark brown seeds in the fall, which are then dispersed primarily by birds (Miller et al., 2011). Seeds can germinate in almost any soil condition and young saplings then compete for resources with natives (Culley & Hardiman, 2007). Callery pear has simple, alternate, glossy leaves that turn red-orange in the fall and an asymmetrical, oval to round-shaped crown that reaches up to 25 ft. in width (Niemiera, 2010). Wild Callery pear tends to have a more open branching pattern than horticultural cultivars and is often much more heavily armed with thorny spur shoots than those grown in the nursery (White et al., 2005).

Many potential reasons behind the spread and successful establishment of Callery pear into new habitats exist including the development and widespread planting of various ornamental cultivars, such as the ‘Aristocrat’ cultivar, allowing this self-incompatible tree to produce viable seeds (Culley et al., 2011) as well as its release from natural enemies upon entering novel habitats of North America [i.e., the enemy release hypothesis (Hartshorn et al., 2022)]. Callery pear also has high rates of gene flow, genetic diversity, and indications of a high mutation rate and, therefore, an ability to adapt to new environments rapidly (Sapkota et al., 2022). Seed and bud dormancy in Callery pear also allows it to produce seeds that can withstand extreme temperatures and form a seed bank that poses a risk of invasion for up to 11 years with 45-87 % seed germination (Serota & Culley, 2019; Zhang et al., 2022). Additionally, their roots also exhibit secondary dormancy if exposed to warm temperatures in late winter creating favorable conditions for new stems to grow from rootstocks (Culley & Hardiman, 2007; Swearingen et al., 2002).

Callery pear spread is likely to increase as it alters soil conditions, promoting reduced availability of labile Carbon (C) and reductions in soil pH, directly affecting native species in infested areas (Woods et al., 2021). Untreated single-stem Callery pear trees cause more soil alteration than treated sprouted seedlings because of their leaves' higher ratio of Carbon to Nitrogen (N) (Woods et al., 2021). Because of its rapid spread and severe impacts, Callery pear has been increasingly identified as a noxious weed in multiple states including Ohio (Lalk et al., 2021), South Carolina (Clemson University Regulatory Services, 2021), Delaware (Sue Barton, 2022) and Pennsylvania (Pennsylvania Department of Agriculture, 2023) making its sale and distribution illegal in those states.

1.3 BIOMASS

Biomass is defined "the weight of vegetative matter produced by trees, shrubs, herbs and other vegetative matter per unit area above the ground" (Khanna & Chaturvedi, 1982). For woody plants, equations to calculate biomass can be done at either the tree-level or the stand-level. Stand level equations are used to estimate biomass within the sample plots established in the target areas using other variables such as diameter at breast height (DBH), height, and volume. Tree-level equations are frequently used to estimate the biomass of individual trees and typically connect the biomass of sampled trees to characteristics that are simple to measure for a large number of trees, such as DBH and tree height (Temesgen et al., 2015). Since Callery pear trees are typically scattered and do not commonly form a whole stand, tree-level equations might provide a better estimate of their biomass. While DBH is a reliable variable for quantifying the biomass of an individual tree, it can be affected by other variables such as age, height, site quality, and crown size (Li & Zhao, 2013). Biomass estimation can be done via direct (i.e., destructive sampling) or indirect methods (i.e., using basic equations or converting tree volume using basic density (the ratio of dry mass over green volume) (Njana, 2017). Estimating tree biomass by destructive sampling (i.e., harvesting) is considered the most accurate estimate of biomass measurement (Daryaei & Sohrabi, 2016).

Biomass and primary production of trees and shrubs is commonly studied using non-destructive methods, such as establishing regression equations that allow readily measured parameters, such as tree height and DBH, to estimate the biomass of whole plants or their components (Haase & Haase, 1995). No matter how quick and common indirect methods are, every indirect method used the allometric equations from previous studies, first formulated following the destructive or direct biomass estimation methods. However, indirect biomass estimation methods, such as allometric equations and modern techniques such as remote sensing combined with different regression models and machine learning, are more common because they are less labor-intensive and faster (Babiy et al., 2022; Jenkins et al., 2003; Somogyi et al., 2007; Stovall et al., 2023). However, there are chances of error if such methods are not carefully used and validated since the equations may not be representative of local conditions (Somogyi et al., 2007). Since there is only one study thus far to develop an allometric equation to estimate the biomass of Callery pear in a small part of its range (Boyce & Ocasio, 2020), destructive sampling is required to do non-destructive estimates of biomass in future while monitoring biomass change over time, carbon accounting or making decision for invasive trees management.

Biomass tables and equations can be used to estimate total biomass (Nowak & Crane, 2002), which can then be used as a base by future researchers or government agencies to estimate the total C stored in that individual. Likewise, biomass and C estimates combined with the land cover land use changes studied in different periods are used to estimate C pools and fluxes (Cairns et al., 2003). Similarly, biomass can also be used in other studies such as water since water use efficiency can be calculated by the ratio of biomass growth (total dry matter produced).

1.4 ALLOMETRIC EQUATIONS

An allometric equation is a mathematical relationship between tree characteristics, such as DBH, height, wood density, and the total aboveground biomass (Jenkins et al., 2003). The equations are developed using regression analysis to gauge the relationship between the predictor variables (i.e., DBH, height) and the response variables (i.e., biomass, C:N, MC, wood density) obtained from destructive sampling, to develop regression models which can be broadly classified as either linear or non-linear (Ketterings et al., 2001; Návar, 2010; Picard et al., 2015).

Linear allometric models assume a linear relationship between the predictor and response variables and are often expressed in the form of a power-law equation with some transformation such as log (Sileshi, 2014):

$$\log(y) = \log(aX^b)$$

where y equals biomass, X is the predictor variable (e.g., DBH), and a and b are parameters estimated from the data. Linear allometric models are widely used in forestry and ecology due to their simplicity and ease of use. However, linear models can sometimes fail to capture the true nature of the relationship between the predictor and response variables, mainly when the relationship is nonlinear (Návar, 2010). Nonlinear allometric models allow for more flexible and complex relationships between the predictor and response variables. These models can take various forms, including exponential, logarithmic, or sigmoidal functions, depending on the specific relationship between the variables (Bi et al., 2012). In case of a complex relationship between the predictor and response variable, Nonlinear allometric models are often a better fit than linear (Sharma et al., 2017).

Ordinary least squares (OLS) regression is a form of linear regression commonly used to create the allometric equation of forest species. OLS regression estimates regression coefficients by minimizing the residual sum of squares between the predicted and observed values of the response variable (Loh, 2011) and can be expressed as:

$$y = \beta 1 + \beta 2x + \varepsilon, \qquad \text{where } \varepsilon \sim \mathrm{N}(0, \sigma_\varepsilon^2)$$

where y is the dependent variable (biomass for this study), x is the independent variable (DBH), $\beta 1$ and $\beta 2$ are the regression coefficients and ε is the error term (De Gryze et al., 2007).

The OLS approach has several benefits, including interpretability, simplicity, and computational effectiveness. The major limitation of OLS is that it does not consider autocorrelations in the data used to create the predictive model. Its drawbacks are it is highly susceptible to outliers and violating assumptions of normality, linearity, and homoscedasticity and cannot be used when these conditions are violated (Steele, 2008). Other techniques, such as Bayesian analysis (van de Schoot et al., 2021) and maximum likelihood estimation (MLE) (Zanakis & Kyparisis, 1986), can also be used depending on the particular study issue and data. However, the accuracy of these equations can be affected by factors such as stand density, site quality, and tree age, so the equations may need to be updated periodically to account for changes in forest conditions**.** Different statistical outputs such as R^2, root mean square error (RMSE), and relative bias can then be used to estimate the model's parameters and evaluate the goodness of fit and depend on the specific characteristics of the data and the research question being addressed (Sileshi, 2014).

1.5 CALLERY PEAR REPRODUCTIVE ECOLOGY

Callery pear is a perennial tree in the Rosaceae family and shares some common family-level characteristics such as self-incompatibility, a genetic feature that prevents self-fertilization through a flower's pollen–pistil recognition mechanism, resulting in rejection of conspecific pollen from the same stigma (De Nettancourt, 2001). Its flowers are hermaphroditic, protandrous (i.e., male reproductive organs become mature before female organs) and diploid, and they bloom in very early spring in clusters containing anywhere from five to 20 flowers (or more) per cluster, and each flower consisting of five petals (Culley & Hardiman, 2007). Callery pear is a prolific flowering species producing abundant seeds with high germination and survival rates (Serota & Culley, 2019). It also has a tendency toward interspecific hybridization, and high genetic plasticity leading to increased adaptability (Sapkota et al., 2022). The combination of these characteristics serves as an advantage for the spread of Callery pear (Culley & Hardiman, 2007; Sapkota et al., 2022).

Callery pear begins flowering approximately at age three and produces famously malodiferous flowers (Warrix et al., 2017); however, its flowering potential at an early age relies on optimal growing conditions with enough light, space, and reduced competition all of which may not be ideal in many places (Warrix et al., 2017). Callery pear flowers are assumed to be highly attractive to generalist insect pollinators such as honeybees (*Apis mellifera* L.), bumblebees (*Bombus terrestris* L.), and hoverflies (Diptera: Syrphidae) (Culley & Hardiman, 2007). However, there has been no detailed investigation of the pollinator community that uses Callery pear.

1.6 INTERACTIONS BETWEEN POLLINATORS AND INVASIVE PLANTS

The impacts of invasive plants on native plant species can directly and indirectly alter plant-pollinator interactions. Invasive plants substantially affect insect-based pollination in United States wildlands and agroecosystems by altering community composition of pollinators as well as their visitation rates to different flowering species (Poland et al., 2021). The reproductive success of invasive plants outside their native range may be due either to pollinators visiting them at similar, or greater, rates relative to surrounding native plants or because the invasive trees are self-fertilizing (Poland et al., 2021). Pollen not only needs to be moved among flowers but needs to be transferred among different individuals to achieve optimal fertilization. A more diverse pollinator community has been found to improve pollination services through increased pollinator diversity that causes changes in the foraging behavior of dominant pollinator species through species interaction (Brittain et al., 2013). Since Callery pear is not a self-fertilizing species, pollinator communities are likely contributing to its successful spread across the eastern United States.

Previous studies have demonstrated conflicting results regarding the effects of invasive species on native flowering plants and their respective pollinator communities. SPECIES may positively affect native flowering plants by increasing the number of pollinators due to increased resource availability, especially when that additional floral resource is at a different time than native species, or negatively by changing pollinator community composition, relative visitation rates, and/or heterospecific pollination of native flowers (Bjerknes et al., 2007). Invasive species can also negatively affect native plants through interspecific pollen transfer which can limit the fertilization of natives, thereby reducing fitness through altered pollen availability, eventually interfering with native seed production (Baskett et al., 2011). Hence, although invasive species may initially serve to attract pollinators to an area and positively affect native plants, over the long term, they are likely to reduce the overall reproduction of native plants by decreasing relative rates of pollinator visits to natives and increasing interspecific pollen transfer (Baskett et al., 2011).

Studies of pollinators of invasive species often have contrasting results among different plant species and habitat types, it is challenging to generalize conclusions. Hence, conducting taxa- and site-specific studies are needed to investigate these findings more broadly (Montero-Castaño & Vila, 2012). In the case of Callery Pear, the early flowering time may facilitate the pollination of native trees positively by providing additional resources for pollinators during times when floral resources are normally low

(Ollerton et al., 2012). Although the integration of non-native pollinators may not directly alter the pollinator network connectivity, their presence increases alien-pollinator interactions eventually resulting in decreased connectivity among natives (Aizen et al., 2008). This decrease in connectivity may cause a shift from native plant species' dependence on native specialist pollinators to super-generalist alien species (e.g., honeybees) for reproduction or survivorship (Aizen et al., 2008).

1.7 OBJECTIVES

Callery pear is one of the major invasive plant species in South Carolina and is rapidly spreading throughout the eastern United States. It can resprout vigorously after it is felled or damaged by abiotic factors such as ice or fire (Coyle et al., 2021; Maloney et al., 2023). The direct effects of Callery pear on natives through resource competition and alteration of soil properties means that it likely increases its biomass, and distribution, relative to native species. Additionally, its tendency for early and prolific flowering likely alters pollinator communities, also affecting its spread and future impacts.

Biomass is also widely used to measure plant fitness and fecundity (Younginger et al., 2017). It is an important variable used in the study of the ecological impact on the plant community level in order to assess if there has been an increase in overall community production and how it affects the abundance, diversity, and community composition of other native species (Vila & D’Antonio, 1998). The accurate estimation of Callery pear biomass in individuals, and on a landscape scale, will give a clearer picture of the level of invasion and help land managers develop different strategies for managing its spread. Quantifying Callery pear biomass could contribute to assessing how it impacts the overall ecosystems it invades and control its further spread by providing necessary data for management plans (Boyce & Ocasio, 2020).

Similarly, the study of the Callery pear pollinator community is essential to know which species are responsible for the reproductive output of the Callery pear through the transfer of pollen between the different species of Callery pear. Identifying possible pollinators of Callery pear will also give some insights into how it affects the ecosystem by attracting various species of pollinators, their relative abundance, and their community composition.

Currently, very few studies have been done to examine these important biological aspects of Callery pear, making it difficult to accurately quantify its abundance on the landscape or assess its impacts on native plant and pollinator communities. Creating a biomass equation for and characterizing the pollinator community of Callery pear will allow researchers and land managers to assess and predict its distribution and impacts on native species. Addressing these gaps in knowledge will help in the future management and control of Callery Pear by providing helpful information on two essential characteristics.

My overall goal with this research was to investigate these ecological relationships that contribute to Callery Pear's dominance on the landscape. To accomplish this goal, my specific objectives were to:

1) quantify the physiological properties (i.e., aboveground biomass, moisture content, wood density, and C:N) of Callery pear and use these variables to create and validate allometric equations, and to

2) assess the composition of the pollinator community visiting flowers of Callery pear.

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CHAPTER TWO

PHYSIOLOGICAL AND MORPHOLOGICAL PROPERTIES OF CALLERY PEAR

2.1 INTRODUCTION

Invasive plants [i.e., non-native plants likely to cause harm to the economy, environment, and human health; (Clinton, 1999)] negatively affect species richness and community composition of native plants by competing for light, water, and space (Gaertner et al., 2009; Gioria & Osborne, 2014). Quantifying the impacts of invasive plants can be difficult as they involve different components and ecosystem services, which are not always easily quantifiable (Eviner et al., 2012). Also, their impacts are largely context-dependent due to their varying growth and spread under local conditions, and varying community composition of recipient ecosystems (Kumschick et al., 2015). Management costs of invasive species in the United States have totaled $1.22 trillion since 1960, with management cost being $17.31 billion (second highest) in southeast alone (Fantle-Lepczyk et al., 2022). Invasive plants can often allocate resources more efficiently than native plants, especially in resource-poor conditions, leading to faster relative growth rates of the non-native (Pattison et al., 1998).

Biomass refers to the total dry weight (kg) per unit area of the whole tree and/or its components (i.e., leaves, flowers, fruits, branches, stems, and roots) (Brown, 1997). Biomass equations (i.e., allometric equations) are useful in predicting the overall weight of these components by correlating it with a physiological variable (e.g., diameter at breast height; DBH). In forestry, biomass equations describe the relative change in overall size with a change in the dimension of parts of the organism (Gayon, 2000) and have traditionally been used for quantifying commercial hardwood, pine, and other valuable timber species (Jenkins et al., 2003; Munsell & Fox, 2010; Poudel & Temesgen, 2016; Tahvanainen & Forss, 2008). Quantifying biomass is also necessary to assess ecosystems' productivity, energy, and nutrient flow through the system (Burkhart & Tomé, 2012). Because of the importance of biomass in various components of forest productivity and health, it is a critical variable to understand in proper forest management and scientific studies (Burkhart & Tomé, 2012).

With increasing interest and conscientiousness of private forest landowners, forest practitioners, and timber companies regarding an integrated perspective on forest health, biomass equations are increasingly used for different purposes, such as determining fire characteristics and impact by estimating the fuel load (Portela et al., 2009), deriving forest carbon stocks (Gertrudix et al., 2012), and estimating wood waste yield (Timilsina et al., 2014). Creating above-ground biomass models for invasive trees can give valuable insights into their resource allocation, providing insight into their success in the invaded area (Pattison et al., 1998). Furthermore, biomass models of invasive species can help researchers and land managers understand and visualize the abundance of invasive plants and strengthen management in various landscapes (Boyce & Ocasio, 2020; Haase & Haase, 1995; Mandal & Joshi, 2015; Rayamajhi et al., 2008).

Carbon (C) can be measured directly from dry biomass, usually taken as ~50% of dry biomass to estimate the C credits (Temesgen et al., 2015). It has been reported that some invasive trees such as *Melaleuca quinquinervia* trees are sequestering more C than native species (Escobedo et al., 2010). However, this depends highly on the species, their size, and locality (low-density residential area vs urban areas) on which they are growing (Horn et al., 2015). Therefore, biomass study in other invasive trees is essential to be done to predict C contribution of invasive species within that locality. The loss of C from their removal can then be included in the C budget and help decision makers factor C sequestration into management decisions.

Three critical factors affect tree biomass; namely, wood density, moisture content (%; MC), and the ratio of carbon to nitrogen (C:N). Therefore, these are also reliable for developing biomass equations (Khanna & Chaturvedi, 1982). Understanding how tree species allocate biomass and utilize water provides essential knowledge of the growth pattern and their resource allocation patterns (Sternberg & Shoshany, 2001). For example, the relationship between MC and biomass often determines fire intensity, as this directly relates to the flammability level of wood (Keyes, 2006). C:N ratio is associated with biomass allocation and decomposition and helps determine N uptake and loss rate within a forest system and also with C sequestration as well (Pacaldo et al., 2013). Wood density is used as a predictor of biomass ($Stem\ biomass = volume * wood\ density$) which varies among tree species. It is commonly calculated using increment borers for non-destructive methods and as the ratio of oven-dry weight to green stem volume for destructive methods (Baker et al., 2004). While these factors [wood density (gm/cm^3) and MC] also vary by tree height and age within individual trees (Ketterings et al., 2001; Tasissa & Burkhart, 1998), the within-species variation is often not significant compared to between-species variation.

Callery pear (*Pyrus calleryana* Decne.) was initially introduced to improve the commercial European pear (*P. communis*) industry by providing rootstock resistant to the bacterial fire blight disease (*Erwinia amylovora*), but later became a widely planted ornamental, with dozens of cultivars available for planting (Culley et al., 2011). After decades of widespread planting, it has become a dominant tree on the landscape and is currently reported as present in 37 states and listed as invasive in 13 of those states (Georgia - center for invasive species and ecosystem health, 2023). While there have been many efforts to control the spread of Callery pear (Flynn et al., 2015; Maloney et al., 2023; Vogt et al., 2020), management is challenging because it resprouts vigorously after cutting and burning (Maloney, 2021; Woods et al., 2021), large thorns on stems and branches damage tires and equipment (Coyle et al., 2021; Vincent, 2005), and it is avoided by many herbivores (Hartshorn et al., 2022).

Since Callery pear has become increasingly recognized as a major invasive species, there has also been increasing interest in assessing ecological variables such as above-ground biomass (Boyce & Ocasio, 2020). However, no study has quantified MC, C:N, or wood density of Callery pear. Having this information will make it easier for land managers to prepare effective plans based on infestation level and landowner objectives. Within the broader goal of effective Callery pear management, my objectives were to 1) quantify above-ground biomass, MC, C:N, and wood density of Callery pear, and 2) create and validate allometric equations of Callery pear biomass.

2.2 METHODS

2.2.1 SITE DESCRIPTION

Trees for this study were collected from six sites across four counties in South Carolina: Greenville Co. (1 site), Pickens Co. (1 site), Anderson Co. (3 sites), and Richland Co. (1 site) (Figure 2). All sites except Richland Co. site (public) were located on private property with road access that was actively infested with Callery pear and were at least two km from each other (Table 1). The average annual temperature of the sites was 16.7˚C (62˚F), and the average annual precipitation was 127.6 cm (50.25 in.) (SCDNR, 2020). The sites differed in species composition with some primary species being pine, oaks, sweet gums and some other species, such as fir and thorny grasses, present in some of the sites.

2.2.2. DESTRUCTIVE SAMPLING

We felled 40 single-stem Callery pear trees across a range of size classes in August and September of 2021 (n = 8) and 2022 (n = 32), at the ground level. For each tree, we recorded the diameter at breast height (cm; DBH) and total tree height using distance tape (THT; m). After harvesting the trees, we separated the tissue components into leaves, branches and stem. We plucked all the leaves individually and cut the stems and branches into small sizes so as to pack them in paper bags and bring them to the lab. We immediately measured the green weight of each tissue component in the lab using either a lab-scale RC6RS Bench Scale (Ohaus, Parsippany, New Jersey) for light samples

(<10 kg) or a platform scale (Fisher Scientific, Pittsburgh, PA) for heavy stem samples (>10 kg).

Then, we dried all tissue samples in a convection oven (Lindberg 146 series, Waltham, MA) to constant weight @ 100˚C for 48-72 hours (Khanna & Chaturvedi, 1982), and measured the dry weight of each component using the same scale. We then added the dry weight of each tissue component to get the total dry weight for each tree. To calculate MC (%), we used the following formula:

$$MC\ (\%) = \frac{Wet\ weight - Dry\ weight}{Wet\ weight} \times\ 100$$

We destructively sampled all tissues from small trees (DBH < 7 cm) while large trees (DBH > 7 cm) were subsampled as described below. After felling large trees, we divided the length of the whole tree into three equal sections (i.e., below the start of the canopy (main stem), the start of the canopy (main canopy), and the apical meristem) (Figure 3). We measured the total green weight (kg) of the whole tree in the field using a hanging scale (HME Digital, California) and then subsampled 10% of each tissue component from each section. We also cut a 2.54 cm (1 in.) thick cookie (i.e., cross-cut section) from the bottom of each stem section for all trees to remeasure the wet weight of the cookie. We then took those subsampled leaves and branches and three cookies from each tree to remeasure each of them again with lab-scale in order to maintain consistency in measurement for large and small trees. This way we got the proportion of each tissue components in 10% of sub-sample taken compared to the total tree weight. We then compared the cookie wet weight to the whole stem wet weight to obtain a ratio which could be extrapolated to the whole tree. We also extrapolated the results of dry-weight of sub-sample of each tissue components to the total weight of tree measured in the field to get their total dry weight. Finally, we calculated the moisture content using similar formula as that for small trees.

After the samples were dried and weighed, we took a sub-sample of each tissue component from each of the 40 trees and ground them separately into a fine powder using a Wiley electric mill (Thomas Scientific, Swedesboro, NJ) with 2 mm mesh. Each ground sample was individually packaged in a labeled paper bag and sent to the Clemson Agricultural Services Lab to quantify C and N. The amount of C was then divided by the amount of N to determine each component's C:N ratio for all trees surveyed. Then we calculated the volume of each cookie using the volume of cylinder formula:

$$V = \pi r^2 (cm^2) \times h(cm)$$

We then calculated the wood density of each cookie using the following formula:

$$Density = \frac{dry\ weight\ (gm)}{volume\ (cm^3)}$$

2.2.3 ALLOMETRIC EQUATIONS

We used all 40 destructively sampled trees to create the biomass model for Callery pear. We used dry weight as the metric for creating biomass models because it is consistent and does not change with the environment (Burkhart & Tomé, 2012).

Because resulting biomass data did not follow a normal distribution, values were log transformed to be able to use linear regression. Different forms of equations with (1) and without transformation (2, 3) are commonly used as the underlying allometric scaling relationship for biomass models. We finalized these equations to develop and test biomass models through a systematic literature review and data visualization (Jiang et al., 2013; Timilsina et al., 2014). The log-linear model was chosen as one of the three because data followed a normal pattern after data were log-transformed (Figure 4). We estimated the parameters for linear model (Model 1) using “lm” function and non-linear model (Model 2 and 3) using non-linear least squares through ‘nls’ function in the “vegan” package in R (Oksanen et al., 2013).

$$\log(W) = \beta 0 + \beta 1 * Log(DBH) \quad \text{……………………} \; (1)$$

$$W = ((DBH)^2)^{\beta 0} \quad \text{……………………………………………………} \; (2)$$

$$W = \beta 0 * (DBH)^{\beta 1} \quad \text{…………………………………} \; (3)$$

where weight (W) is the response variable, DBH is the predictive variable, and β0 and β1 are the allometric constants. Models were evaluated and the final equation was chosen based on three major goodness of fit parameters: lowest RMSE, lowest bias, and highest R^2 values. These variables were calculated as follows:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(y_i - y_i)^2}{n}}$$

$$Bias = \frac{\sum_{i=1}^{n}(y_i - y_i)}{n}$$

where y_i is the observed value and y_i is the predicted value.

2.2.4 STATISTICAL ANALYSES

We compared MC and C:N ratios across the tissue components and DBH using the Kruskal Wallis test since those data were not numeric. Similarly, generalized linear model (GLM) with classical logistic regression was done to see if biomass of tissue components differs significantly with DBH. Also, correlation test was done to see if the correlation exists between DBH and height and DBH and location variables.

2.3 RESULTS

2.3.1 BIOMASS

DBH of sampled trees ranged from 1.2– 17.7 cm, and THT ranged from 1.6 m – 10.3 m. Total wet biomass ranged from 0.3267 kg (1.2 cm DBH) to 160 kg (17.7 cm DBH), and total dry biomass ranged from 0.19-97 kg for the same DBH. The wet biomass ranged from 0.2-86 kg for stems, 0.06-87 kg for branches, and 0.06-36 kg for leaves. Similarly, dry biomass ranged from 0.11-51 kg for stems, 0.04-97 kg for branches, and 0.04-12 kg for leaves. The stem was the major contributor to the overall biomass of Callery pear (Table 2). Dividing the results into different diameter classes, we found that stem biomass proportion to total biomass was highest for trees of 10-15 cm DBH while branch and leaf proportion to total biomass was highest for trees of 5-10 DBH class (Table 3).

GLM results shows that total dry biomass was significantly related to DBH ($t = 14.172$, $p < 0.0001$) and was significant among the tissue components ($p=0.0002$) but not to height ($t=1.628$, $p=0.112$). Similarly, branch dry biomass, stem dry biomass, leaf dry biomass was also significantly related to DBH. The location and DBH variables are found to be significantly correlated to each other (p= 0.001413). Likewise, DBH and height variables are also found to be significantly correlated to each other as well (68%, $p<0.0001$).

2.3.2 MOISTURE CONTENT

Moisture content ranged from 16.23-48.36% for stem, 15.88-56.61% for branches, and 23.76-53.80% for leaves (table 2). Kruskal Wallis test shows that moisture content did not differ significantly with DBH ($p = 0.769$) but was significant among the tissue type (0.0035).

2.3.3 C:N

C ranged from 44.36-48.02% for stems, 43.92-47.48% for branches, and 44.68-49.23% for leaves. N ranged from 0.18-0.50% for stems, 0.22-0.80% for branches, 1.17-1.78% for leaves, and 0.42-1.63% for fruits. The average Total C:N ratio ranged from 65.63:1 (Campground site) to 154.13:1 (Piedmont site). Site-wise comparison of the average C:N ratio in each site shows that the Piedmont site has the highest ratio of carbon and nitrogen (154.13), while the campground site had the lowest (65.63). The Kruskal Wallis test shows that C:N ratio differs significantly with the DBH ($p=0.00149$) and among the tissue components ($p<0.0001$).

2.3.4 WOOD DENSITY

The average wood density of Callery pear was 0.88 gm/cm^3± 0.006 (range: 0.169-2.926 gm/cm^3). For the apical meristem alone, the wood average wood density was 0.455±0.008 gm/cm^3 (range: 0.169-1.351 gm/cm^3). Similarly for main canopy part, the average wood density was 0.835±0.014 gm/cm^3 (range: 0.213-1.730 gm/cm^3) and for main stem, the average wood density was 1.349 ± 0.022 gm/cm^3 (range: 0.410-2.926 gm/cm^3).

2.3.5 ALLOMETRIC EQUATIONS

Among the three allometric equations tested on total dry biomass, Model 1 (log-linear) was found to provide the best fit in terms of low RMSE (25.371) and low bias (0.36) and high R^2 0.91 compared to other two non-linear models (Table 4). Stem biomass had the highest R^2 value and lowest SE while leaf biomass had lowest R^2 value, and branches had highest SE (Table 5).

2.4 DISCUSSION

A total of 40 Callery pear trees ranging in DBH from 1.27-17.78 cm were destructively sampled to assess physiological characteristics and develop allometric equations for the invasive tree. Stem biomass contributed more to the total biomass of Callery pear compared to other tissue components. The average dry biomass of Callery pear is found to be 16.683 ± 0.05 kg. The log-linear model was more accurate in predicting Callery pear biomass which is $logW(g) = 5.302 + 2.07 * Log(dbh(cm))$.

Another study to examine Callery pear biomass also report that log-linear model provides better estimate of Callery pear biomass. However, they used diameter at stump height (25 cm from ground level) as the predictive variable for their allometric equation because Callery pear frequently forks below breast height (Boyce & Ocasio, 2020). Our study calculated allometric equations with DBH to be consistent with other biomass literature and to provide comparison. The log-linear allometric equations are also commonly used to calculate biomass of most of the forest trees (Jenkins et al., 2003; Mascaro et al., 2014). However, some studies are found to use non-linear models because specially to those datasets with normal additive error (Dong et al., 2015). In general, linear models are found to be better for smaller trees and non-linear models are better for larger trees because of heteroscedasticity and distribution of statistical error (Lai et al., 2013). In case of this study too log-linear model was considered better provided that smaller diameter range of Callery pear tree were used for this study.

Non-destructive methods such as using allometric-equations are widely used to estimate biomass of common tree species in USA. However, the accuracy depends on the variable used (Chave et al., 2014). Our biomass model explains approx. 91% of the variation in total aboveground with RMSE of 25.371 gm and bias of 0.36 gm which indicates our model's accuracy for prediction purposes. Positive bias value shows that the model underpredicts the data by 0.36 kg which is negligible in context of forestry. The equation developed from this study can be added to the database consisting numerous biomass equations developed and used for most of the trees in United States (Jenkins et al., 2003; Radtke, et al., 2015; Woodall et al., 2011). Tree biomass equations are mostly subject to error if they are used outside the range of study area (Chave et al., 2004). However, there have been some generalized equations developed for the tree with single variable; DBH which can be implied over comparatively large geographical range to estimate biomass (Chojnacky et al., 2014). Our results shows that the biomass did not differ significantly among the sites which provides some justification that generalized equations developed for estimating biomass for Callery pear can be used in wider range. Other variables such as site, age, taper and other environmental variables are found to affect the accuracy of biomass models (Temesgen et al., 2015) and equations including those factors can be tested in future if higher accuracy is desired. However, DBH alone has been justified to be the good estimator of the biomass by many studies (Jenkins et al., 2003). Using single variable for estimation will be less complicated and efficient to estimate biomass especially in case of invasive trees such as Callery pear where high accuracy is not required compared to costly timber species. Also, since biomass models with multiple variable such as height and site are subjected to error when are used outside the range than models with DBH only (Chave et al., 2004).

The Forest Investment and Analysis (FIA) protocols require estimation of the biomass using component ratio method (CRM) where the large scale above ground biomass is computed using volume, component ratio (ratio of individual component to total biomass) and wood density. (Woodall et al., 2011) . Our study reports average wood density of Callery pear to be 0.88 gm/cm^3 ± 0.006 ranging from 0.169-2.926 gm/cm^3 which is higher compared to other hardwood species in south eastern United States such oak spp., Elm spp., sweetgum, and red maple (Radtke et al., 2017). However, wood density can differ with laboratory methods for drying and geography, even for the same species (MacFarlane, 2015).

The average C:N ratio of Callery pear was 106.44. Among all the tissue components, the leaf had the lowest C:N (33.87), and the stem the highest (167.79). Similar studies by Boyce (2022) found a C:N of 36.0 of Callery pear leaves, which is close to C: N of leaves estimated from our study. The C:N ratio in Callery pear leaves is not found to be statistically different compared to other native species which implies that the Callery pear does not alter the leaf decomposition and affect the nutrient uptake of other species available in that site (Boyce, 2022). However, C:N of other invasive trees such as Chinese privet's litter in Southeastern US is found to be 34.6 which was lower than other native species in similar location and hence privet is found to be altering the carbon storage and primary productivity through faster decomposition (Mitchell et al., 2011). This higher soil nitrate availability and lower C:N ratio in invaded sites of private could negatively impact decomposers as well (Weand, 2020). Comparing that to our results, the C:N ratio in Callery pear leaf is even lower (33.87) which might increase the rate of decomposition and increase nitrogen availability as well in the forest floor where it is present. Also, since C:N ratio is the indicator of nitrogen use efficiency, where plants with higher C:N ratio are found to promote nitrogen use efficiency for survival and with lower C:N ratio supports growth of that particular plant species (Zhang et al., 2020). So, higher C:N ratio in the Callery pear stem might be promoting the survival of Callery pear even in the extreme soil conditions with limited N availability. Also, since, C:N is species dependent (Aubrey et al., 2012), the lower C:N ratio of Callery pear in leaves might have supported its productivity and larger crown size even in nutrients limited areas.

Moisture content might differ with time of year, tree species, age, size and site variables (Henderson, 1968). The moisture content of trees (15.24 cm) in Southeastern pine stands are reported to range from 50.5 % for white ash tree to 116% for sweetgum trees (Manwiller, 1975). Similarly, the average moisture content of pine trees in south eastern United States is reported to be approximately 50% (Greene et al., 2014). Although Callery pear is considered to be a hardwood tree, its average moisture content of 42% is consistent with the average for trees in the southeastern United States. The results show that Callery pear's moisture content is still lower than other native trees, so it might not be competing for water resources. However, more research on the comparison of Moisture content in the same site, time, and species of the same DBH and age is required to reach to this conclusion.

Our study will provide baseline information for other studies in the future about Callery Pear. The biomass equation developed will help monitor biomass change within an invaded area more accurately, especially post-fire, disease outbreaks, and other human-induced disturbances. Other applicability in the future includes the estimation of biomass for making management plans, carbon budgeting (Russell et al., 2015), and integrating biomass predictions of Callery pear using remote sensing technologies (Jucker et al., 2017).

2.5 CONCLUSION

Although there are different allometric equations for commercial trees, allometric equations for invasive trees such as Callery pear are not widely available (Hulshof et al., 2015; Jenkins et al., 2003; McPherson et al., 2016). The allometric equation developed from this study can be used for estimating the above-ground biomass of Callery pear at a landscape scale. However, caution should be taken while using this equation to estimate the biomass for multiple-stem Callery pear trees since this study's equations were developed from single-stem trees. Also, the model should be validated using different environmental (e.g., soil type, associated species, topography, region, etc.) and species (e.g., DBH, THT) characteristics. Validation of the model using the additional independent data in the future might increase the accuracy of the model's use for further assessments of biomass.

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CHAPTER THREE

POLLINATOR COMMUNITY COMPOSITION AND DIVERSITY IN CALLERY PEAR STANDS IN THE SOUTHEASTERN UNITED STATES

3.1 INTRODUCTION

Invasive plants are those that are introduced to an ecosystem outside of their native range have the ability to spread aggressively and displace native vegetation which can negatively impact the environment, economy, and human health (Clinton, 1999). Management costs of invasive species (plants and animals) in the United States is $1.22 trillion, which has risen from $20.6 billion annually between 1960 and 1969 (adjusted for inflation) to $21.08 billion annually between 2010 and 2020 (Fantle-Lepczyk et al., 2022). Of the 1,285 identified invasive plant species in the United States (Beaury et al., 2021), most of them have been introduced intentionally as horticulture and landscape plants (Mack & Erneberg, 2002; Reichard & White, 2001).

Callery pear (*Pyrus calleryana* Decne.) is an invasive tree introduced to the United States from Asia to save the European pear (*Pyrus communis* L.) industry from the bacterial disease, fire blight (*Erwinia amylovora*, but was later prized as an ornamental tree because of its pyramidal shape and beautiful flowers (Culley & Hardiman, 2007). It is currently recorded in 37 states (Figure 1) and listed as invasive in 13 (Georgia - center for invasive species and ecosystem health, 2023). Callery pear flowers as early as age three (Warrix et al., 2017) and bloom as early as the first week of February in South Carolina. It has malodorous, protandrous (male flowers maturing before female flowers), and hermaphroditic blooms with up to 20 (or more) white flowers per inflorescence (Culley & Hardiman, 2007). Although Callery pear was noted as being self-incompatible when it was brought to the United States and more importantly when it was marketed as a landscape tree (Culley & Hardiman, 2007), the development of different, genetically distinct cultivars (Sapkota et al., 2022), and their widespread plantings, have allowed it to escape cultivation (Culley et al., 2011). Despite efforts to control the spread and impacts of Callery pear (Flynn et al., 2015; Maloney et al., 2023; Vogt et al., 2020), management is challenging because of its tendency to vigorously resprout after cutting and burning (Maloney, 2021; Woods et al., 2021), its ability to damage tires and equipment with large thorns (Coyle et al., 2021; Vincent, 2005), and a lack of natural enemies (Hartshorn et al., 2022).

The interactions between pollinators and invasive plants may affect the ecosystem functions of invaded habitat (Stout & Tiedeken, 2017). Globally, 87.5% of flowering plant species rely on animal pollination (both vertebrates and invertebrates; Ollerton et al.,2011) resulting in pollinators having a global annual conservation value of more than $215 billion for their ecosystem services (Vanbergen & Initiative, 2013). Insect pollinators are defined as the species of insects that can carry pollen from male to female flower structures in flowering plant species and help in fertilization externally (Ollerton, 2017). Among the 350,000 known species of pollinators, insects in these four major orders contribute the most to pollination: Lepidoptera (moths & butterflies), Coleoptera (beetles), Hymenoptera (wasps, ants, & bees), and Diptera (flies) (Ollerton, 2017). Compared to pollinators, flower visitors may perform pollination as an ecosystem service but are not specifically identified as carrying pollen on their bodies to and from flowers of a particular species. Because of the nuance of these definitions, and the difficulty in identifying specific pollinators, both groups will be referred to as 'pollinators' in this study.

Pollinators can be generalists (no pollen preference) or specialists (prefer pollen from a certain species or genera) and past studies have found that most invasive plants tend to attract generalist pollinators while native plants tend to attract specialist pollinators (Jesse et al., 2006; Montero-Castaño & Vilà, 2017). Therefore, the presence of invasive plants may affect specialist pollinator populations having both negative and positive effects on certain native plants. The complex network of plant-pollinator interactions can be altered due to the introduction of invasive plants through floral competition or facilitation (Aizen & Morales, 2020). The effect of invasive plants on pollinators can manifest differently at different scales, ranging from individuals to communities, including effects on species richness, community diversity and composition, and plant-pollinator network connectivity (Stout & Tiedeken, 2017). Invasive plants may reduce the fitness of native plant species by obstructing stigmas of native flowers or competing for pollinator services (Baskett et al., 2011). Conversely, invasive plants may improve native plant fitness by attracting more pollinators to the site with abundant floral resources (Drossart et al., 2017). Even though invasive plants may initially attract more pollinators by providing more foraging benefits, this depends on the type of pollinator (i.e., generalist vs. specialist), as well as phenology (e.g., spring vs summer flowering), and type of plant (i.e., annual vs. perennial).

Invasive plants may also directly affect the pollinators in an ecosystem due to differences in nectar and pollen quality and quantity (Bartomeus et al., 2008). Eventually, any initial benefits likely decrease over time due to the displacement of native floral resources by invasives (Kovács-Hostyánszki et al., 2022). Competition for, or facilitation of, pollinators of native plants also depend on the spatial scale of invader in question (Albrecht et al., 2016), as well as its relative abundance (Flanagan et al., 2010), and phenotypic similarity relative to native plants (Morales & Traveset, 2009).

Species diversity takes into account species richness (i.e., the number of species in a habitat) and evenness (i.e., relative proportion of each species in the habitat) and can be measured using different metrices depending on the research objective (DeJong, 1975; Dolan et al., 2022; Fountain, Mateos-Fierro et al., 2019). However, species richness might have some shortcomings such as being highly sensitive to sampling intensity and not incorporating relative abundance if used independently. Hill numbers are the unified form of species diversity which includes other indices such as the exponential of Shannon diversity, the inverse of Simpson diversity and species richness together expressed in the unit of effective number of species (Chao et al., 2014).

Previous studies show that some invasive trees such as the Chinese tallow tree (*Triadica sebifera*) are able to attract more pollinators in contrast to other invasive trees such as privet (*Ligustrum sinense*) which is associated with reduced pollinator diversity(Hanula & Horn, 2011; Lalk et al., 2021; Ulyshen et al., 2020). Callery pear's flowers are assumed to be highly attractive to generalist insect pollinators such as honeybees (*Apis mellifera* L.), bumblebees (*Bombus terrestris* L.), and hoverflies

(Diptera: Syrphidae) because of the nature of flowers in this tree but this has not been investigated (Culley & Hardiman, 2007). Despite having crucial importance in the ecological context, only one study has investigated the diversity of pollinators of Callery pear in the U.S. (Dolan et al., 2022) but this work was performed in Ohio, representing only a small portion of the distribution of Callery pear. Hence, how Callery pear impacts pollinators at the landscape scale have not been well-established. Since the impact of invasive plants on pollinators, and vice versa is inconsistent and depends on different local conditions, it is critical to identify and assess the pollinator community visiting the invasive Callery pear in South Carolina. To address this knowledge gap, the objectives of our study were to 1) identify primary flower visitors of Callery Pear and 2) quantify diversity metrics.

3.2 METHODS

3.2.1 FIELD METHODS

Pollinators were collected at five sites (Figure 2), each at least two km apart, spanning three counties in upstate South Carolina (Anderson, Pickens, Greenville Counties), during Callery pear flowering in February and March 2022 from 23 collection events (Table 1). Each site was 50 x 50 m square and was located on private property actively infested with Callery pear trees that were not being treated or removed during the duration of this study.

Pollinators were collected at each site using three methods: painted bowl traps (i.e., white, blue, yellow), blue vane trap (Banfieldbio, Seattle, OH), and timed (5 min.)

sweep net sampling (Bioquip, Compton, CA). Bowl traps were made by coating 4 oz (~30 ml) clear disposable plastic cups with white, fluorescent blue, and fluorescent yellow spray paint (Krylon Colormax, Cleveland, OH) (Figure 5) (Campbell & Hanula, 2007; Hall & Ascher, 2010). At each site, one bowl trap of each color was placed at each of the four corners (12 bowl traps per site) (Figure 5). Bowl traps were filled halfway with soapy water (~15 ml) using two tablespoons of soap per gallon of water (Hall & Ascher, 2010) for insect preservation, and placed directly on the ground adjacent to a live Callery pear tree (Figure 5). A single blue vane trap was placed at the center of each site and secured either to a post or to a Callery pear branch (Figure 5) (Campbell et al., 2023). Blue vane traps did not contain preservatives, and all collected insects were photographed and then immediately released. Sweep net collections were conducted at midday for five minutes each at five representative Callery pear trees within each site (Spafford & Lortie, 2013).

Bowl traps and blue vane traps were set shortly after sunrise on clear, sunny days with low wind speeds when the temperature was forecasted to reach above 10°C (50°F). Bowl traps were collected 24 hours later, while blue vane traps were collected every 12 hours so live pollinators could be released after photographing for identification. Bowl trap samples were collected by pouring all contents through paint filters (190 microns, TCP Global, Lakeside, CA) and then storing paint filters in a labeled Ziploc bag which was then stored in an ice cooler during transportation to a freezer (-5°C) at the Clemson Forest Health Lab. Insects collected during sweep netting were placed in a labeled Ziploc bag and placed on ice in a cooler before being transferred to a freezer at -5°C at the Clemson Forest Health Lab.

3.2.2. LAB METHODS

All bowl trap and sweep net samples were processed in the Clemson Forest Health Lab using a five-step method: wash, rinse, dry, pin, and label (Jones, 2012). First, insects were washed twice in lukewarm soapy water for 120s, then rinsed with tap water for 60s. After rinsing, insects were pat-dried with a paper towel and dried further using a hair dryer on low heat (Hall & Ascher, 2010). Insects were then pinned, labeled, and stored in boxes for later identification. The specimens were identified under microscope to the lowest taxonomic level possible as morphospecies, species, genus, or family level with the help of expert taxonomist (J. Campbell, pers. comm.). We stored all the identified specimens of insects after final labeling at Clemson University entomology lab, and reference specimens were also stored in the USDA Northern Plains Agricultural Research Laboratory (Sidney, Montana).

3.2.3 STATISTICAL ANALYSIS

All statistical analyses were conducted using R version 3.6.3 (R Core Team, 2020) through the interface of RStudio, version 1.2.5033 (RStudio, 2019), with a significance alpha level 0.05. All statistical tests were performed only for those insects which were identified at least to the genus level and excluded insects identified only to the family level.

Our dependent variables (species and morphospecies of insects) and independent variables (site and collection methods) violated assumptions of normality using a Shapiro-Wilk test and assessing residual plots from “vegan” package (Oksanen et al., 2013). Therefore, we used a permutational multivariate analysis of variance (PERMANOVA) test to compare community composition among “treatments" (i.e., collection methods and sites) with ordinary least squares (OLS) estimation method using the “lm. rrpp” function in the “RRPP” package (Collyer & Adams, 2018). We then used pairwise comparisons with 999 permutations to compare between levels for each “treatment” identified as significant with the PERMANOVA. We then performed an indicator species analysis (ISA) using the “multipatt” function in the “indicspecies” package (Cáceres & Legendre, 2009) to identify which species were unique to each collection method.

To compare pollinator diversity among sites, we first calculated Hill numbers (Hill, 1973) across each site and collection method:

N_0 = species richness

N_1 = exp(H)

N_2 = 1/D

where, *H* is the Shannon index for each site and collection method. Shannon index is calculated with the following formula in excel:

$$H = -\sum_{i=1}^{s} \frac{n_i}{N} ln \frac{n_i}{N}$$

where, n_i= number of individuals per method and N= total number of species

Species richness (N_0) refers to the number of unique species in a given sample. N_1 is also referred to as the "effective species number," which is the number of equally-common species required to obtain the *H* value of a given sample (Jost, 2006). N_2 is calculated as the reciprocal of Simpson's index using "invsimpson" function in "abdiv" package (Bittinger, 2020). We then used a Chi-square (χ^2) test to compare Hill numbers across collection methods and sites.

3.3 RESULTS

A total of 756 insects were collected, representing three orders (Diptera = 7, Hymenoptera = 7, Coleoptera = 1) and 15 families across those orders. Among them, 412 individuals were identified to the family level, 264 to the genus level, and the remaining 80 individuals were identified up to the species level. The most common families collected were the Dipteran families Syrphidae, Sarcophagidae, Anthomyiidae, and the Hymenopteran family Andrenidae. Among the insects identified to species, the most common species collected were *Toxomerus* (Syrphidae), *Andrena* (Andrenidae), *Apis mellifera, Osmia,* and *Lasioglossum* (Apidae) (Table 6).

Pollinator community composition differed significantly among the different collection methods ($F = 9.1265$, $p < 0.001$). However, they did not differ significantly among sites ($F = 1.776$, $p = 0.125$). Pairwise comparisons showed that all combinations of collection methods were significantly different from each other except for the combination of blue and white bowl traps.

Among the 62 taxa of insects, that we collected and classified to species, morphospecies and genera, four were found to be uniquely associated with certain collection methods. Specifically, *Toxomerus* was found to be uniquely associated with yellow bowl traps ($F = 0.495$, $p < 0.001$), while *Andrena barbara* ($F = 0.45$, $p<0.0001$), *Andrena* morphospecies 1 (Female) ($F = 0.34$, $p < 0.005$) and *Apis mellifera* ($F=0.288$, $p<0.0088$) are associated with sweep nets.

Among all methods, the yellow bowl trap has the lowest Hill numbers in terms of N_1 (0.69) and N_2 (7.01) and has the lowest evenness (N_0) too (0.9). However, the yellow bowl trap's species richness (N_0) was the highest (Table 7). The χ^2 test among the hill numbers of sites shows that the Hill numbers are not significant with the sites ($\chi^2=10.29$, $p=0.5902$; Table 8) but are significant among the methods ($\chi^2= 17.122$, $p = 0.0468$).

3.4 DISCUSSION

Our study examined the flower visitor's community and diversity in Callery pear invaded areas. We found that out of total 756 individuals collected from 15 families belonging to three orders, *Toxomerus* species from the Syrphidae family (25 % of the total) was the most common pollinators of Callery pear tree, followed by *Andrena* (8.33%), *Apis* mellifera (1.85%) and *Syrphus* (1.85%).

As expected, pollinator diversity did not differ significantly among sites, likely because sites were not located in geographically distinct areas. However, pollinator diversity varied significantly among collection methods used, indicating the yellow bowl traps and sweep net collections as the better methods of insect collection because the yellow bowl trap collected higher number of species collected and sweep net collected unique species. The blue vane trap was not successful in capturing insects which might be due to the position of the blue vane, or the escape of insects from the trap since familiar visitors are flies and tiny bees. The blue vane trap method may not be suitable for trapping the insects in Callery pear sites or similar species where the major pollinators are tiny such as syrphids for future studies.

The study by Dolan et al. (2022) in Ohio also found that *Apis mellifera* and *Andrena* were two of the most common pollinators of Callery pear, which supports our results. However, they captured only one species (*Helophilus fasciatus,* Syrphidae) (Dolan et al., 2022), compared to our results of five species from the Syrphidae family. Pollinators of Callery pear in its native range include species from the Syrphidae, Andrenidae, and Apidae (in a similar order) family that are similar to our findings (Makimura et al., 2015). Also, because the study in Japan used a similar technique of collecting insects as ours (sweep net), this might have resulted in the capture of similar species.

Some other species closely related to Callery pear are *Pyrus* species and apple trees. Studies in comparison of pollinators in *Pyrus communis* L. vs apple tree (*Malus pumila*) shows that the species of syrphids to be more attracted to *Pyrus communis* vs more bees attracted to apple trees because of nectar composition with low sugar concentration in *Malus* spp. (Quinet et al., 2016). But it differs with the pear cultivar and higher pollen production despite of diluted nectar might be sufficient enough to attract generalist bees (Orosz-Kovács et al., 2000). Additionally, because of early blooming in pear trees, they might receive less pollinators (Orosz-Kovács et al., 2000) and more study are required to look at the nectar quality of Callery pear to suggest how it is affecting these pollinators' role in ecological level.

One limitation of the study was that the pollen testing was not done to confirm these flower visitors as the major pollinators of Callery pear. We are still unsure if the pollinators captured during this study are the major pollinators of Callery pear or were captured simply due to their high abundance in the community and our experimental design. However, the fact that species of insects caught in sweep net collected were from Callery pear flowers, it is logical to presume that they are likely pollinators. Also, the syrphids and bees that we recorded in the study are found to be major pollinators of agroecosystem in South Carolina (Jenkins, 2019). Another limitation is sample size since our study is based on the data collected for only one year.

This study provides a baseline of the pollinator community of the Callery pear in South Carolina. Future directions for this research include comparisons of pollen collected from insects and flowers to confirm their role as major pollinators of Callery pear. This would provide evidence as to effects of pollinators on Callery pear spread, pollen competition, and potential hybridization with natives. Comparative study between invaded and uninvaded areas by establishing control or removal plots can provide clearer picture of the effects of Callery pear on the pollinator community. Studies investigating nutrient content of Callery pear nectar and pollen can also be done to assess the effects of Callery pear on pollinators.

3.5 CONCLUSION

We collected a total of 756 insects across 15 families and three orders during Feb-March of 2022. The most common types of insects visiting Callery pear flowers was *Toxomerus* in order Diptera followed by *Andrena* (Hymenoptera: Andrenidae) and *Apis mellifera* (European honeybee; Hymenoptera: Apidae). Pairwise comparisons showed that community composition differed significantly among collection methods but not sites with the yellow bowl traps collecting the highest number of insects and sweep net collections resulting in the most unique insect species. Blue vane traps did not yield any pollinators and was considered inefficient for the purposes of this study. Future studies can be done on the pollen test between the flower and the insects to confirm these flower visitors as pollinators of Callery pear.

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APPENDICES

A: Tables

Site name	Latitude	Longitude	County	Tree Sample size (n1)	Insects sample size (n2)
Ingles	34.70363	-82.79423	Pickens	8	149
Water trt.	34.63873	-82.77371	Anderson	7	139
Campground	34.62554	-82.77981	Anderson	7	153
Piedmont	34.745865	-82.47123	Anderson	7	-
Greenville	34.82342	-82.46984	Greenville	6	177
Sandhill	34.138636	-80.87096	Richland	5	-
Lakewood	34.745865	-82.47123	Greenville	-	138

Table 1: Locations of study sites with latitude, longitude, county, ecoregions, and number of trees (N1) harvested and number of species (N2) collected on each site.

Tissue Component	Wet Biomass (kg) $(\boldsymbol{\mu} \pm \boldsymbol{SE})$	Dry Biomass (kg) $(\boldsymbol{\mu} \pm \boldsymbol{SE})$	Moisture Content (%± **SE**)	C:N $(\boldsymbol{\mu} \pm \boldsymbol{SE})$
Stem	15.255±0.4	9.150±0.2	41±0.001	167.79±0.98
Branch	10.339±0.4	6.400±0.2	39±0.001	117.69±0.72
Leaf	1.952±0.006	1.132±0.003	42±0.001	33.84±0.08
Total	28.619±0.9	16.683±0.5	42±0.001	106.44±0.52

Table 2 : Descriptive statistics of tissue components (stem, branch and leaf) including average wet biomass (kg), average dry biomass (kg), average moisture content (%) and average C:N (± SE).

DBH class	Wet Stem prop.	Wet Branch prop.	Wet Leaf Prop.	Dry Stem prop.	Dry Branch prop.	Dry Leaf Prop.	Total wet (kg)	Total dry (kg)	N
1-5	0.58	0.27	0.12	0.59	0.28	0.11	64.84	36.36	15
5-10	0.49	0.39	0.09	0.50	0.41	0.08	476.66	275.16	18
10-15	0.57	0.32	0.04	0.58	0.36	0.04	328.50	19.88	5
15-20	0.53	0.36	0.04	0.56	0.38	0.05	274.74	15.69	2

Table 3: Proportion of wet and dry biomass, total wet and dry biomass (kg), and number of trees in each diameter class.

Models	RMSE	BIAS	R-square	β0	β1
Model1	25.371	0.36	0.91	5.302	2.07
Model2	31.097	0.56	0.61	1.973	-
Model3	24.212	0.99	0.60	5.87	0.233

Table 4: Test statistics of three models tested for total biomass, the first being linear and the last two being non-linear models. The table lists the root mean square (RMSE) value, bias, $R^{2,}$ and estimate of parameters β_0 and β_1.

Tissue components	Model	R^2	Residual SE	p-value
Stem	$\log W(g) = 4.749 + 2.073 * \mathrm{Log}(dbh(cm))$	0.94	0.34	<0.0001
Branch	$\log W(g) = 4.011 + 2.033 * \mathrm{Log}(dbh(cm))$	0.66	0.95	<0.0001
Leaf	$\log W(g) = 4.026 + 1.331 * \mathrm{Log}(dbh(cm))$	0.53	0.83	<0.0001
Total	$\log W(g) = 5.302 + 2.07 * \mathrm{Log}(dbh(cm))$	0.91	0.42	<0.0001

Table 5: Final biomass model selected for total biomass of Callery pear and its individual tissue components with R^2, residual standard error (Residual SE), and p-value.

Order	Family	Genus and species	No.	%	Comments
Diptera	Syrphidae	*Toxomerus* sp.	189	25	
Diptera	Syrphidae	*Syrphus* sp.	14	1.85	
Diptera	Syrphidae	*Helophilus* sp.	2	0.26	
Diptera	Syrphidae	*Orthonevra* sp.	1	0.13	
Diptera	Syrphidae	*Pipiza* sp.	1	0.13	
Diptera	Conopidae	*Zodion* sp.	1	0.13	
Diptera	Bibionidae	*Bibio* sp.	1	0.13	m
Diptera	Sarcophagidae*	-	199	26.3	
Diptera	Anthomyiidae*	-	147	19.4	
Diptera	Calliphroidae*	-	31	4.1	
Diptera	Tachinidae*	-	25	3.3	
Hymenoptera	Andrenidae	*Andrena* sp. #	33	4.36	25(m),8(f)
Hymenoptera	Andrenidae	*Andrena barbara*	27	3.57	All (f)
Hymenoptera	Andrenidae	*Andrena violae*	3	0.39	All (f)
Hymenoptera	Apidae	*Apis mellefera*	14	1.85	All (f)
Hymenoptera	Apidae	*Ceratina strenua*	11	1.45	All (m)
Hymenoptera	Apidae	*Ceratina floridana*	6	0.79	5(m),1(f)
Hymenoptera	Apidae	*Ceratina* sp.	4	0.52	
Hymenoptera	Apidae	*Bombus bimaculatus*	2	0.26	
Hymenoptera	Apidae	*Nomada* sp. #	2	0.26	1(m),1(f)
Hymenoptera	Apidae	*Xylocopa virginica*	2	0.26	Both(m)

Hymenoptera	Apidae	*Habropoda laboriosa*	1	0.13	m
Hymenoptera	Megachilidae	*Osmia* sp. #	13	1.71	All m
Hymenoptera	Halictidae	*Lasioglossum (= Dialictus) imitatum*	7	0.92	All (f)
Hymenoptera	Halictidae	*Lasioglossum (Dialictus) leviense*	1	0.13	f
Hymenoptera	Halictidae	*Lasioglossum (Dialictus) pruinosum*	1	0.13	f
Hymenoptera	Halictidae	*Lasioglossum (Dialictus) zephyrum*	1	0.13	f
Hymenoptera	Halictidae	*Lasioglossum (Paralictus) cephalotes*	1	0.13	f
Hymenoptera	Halictidae	*Lasioglossum (Dialictus)*	1	0.13	f
Hymenoptera	Chrysididae	*Chrysura* sp.	1	0.13	
Hymenoptera	Colletidae	*Colletes inaequalis*	1	0.13	f
Hymenoptera	Vespidae	*Polistes exclamans*	1	0.13	
Hymenoptera	Vespidae	*Polistes* sp.	1	0.13	
Coleoptera	Nitidulidae*	-	8	1.05	

Table 6: Species of insects classified into order, family, genus/species level. The families with the (*) sign could not be further classified to species level because of missing body

parts, time constraints, or taxonomic complications. We combined all the morphospecies (with # sign) together into single species to list species in the table in order to save space.

Method	#	(N_0)	N_1	N_2	Simpson	Evenness
Blue bowl	49	13	1.32521	7.29787	0.86297	0.02165
White bowl	44	11	1.30607	13.08108	0.92355	0.02427
Yellow bowl	135	18	1.44216	7.01231	0.85739	0.02034
Sweep net	106	20	1.43942	17.18043	0.94179	0.01821

Table 7: Hill numbers of species by different method. Second column refers to total number of individuals collected by each method (#). N_0 refers to species richness, N_1 refers to exponential of Shannon index (exp(H)) and N_2 refers to reciprocal of Simpson's index (1/D). H and D, are Shannon and Simpson index respectively.

Site	#	(N_0)	N_1	N_2	Simpson	Evenness
Ingles	148	15	1.40299	6.33509	0.84214	0.02257
Lakewood	57	16	1.24034	4.63917	0.78444	0.01346
Water Res	127	19	1.42062	11.69439	0.91448	0.01350
Waffle	140	20	1.39439	8.04306	0.87566	0.01662
Tanglewood	168	26	1.37840	12.93007	0.92266	0.01689

Table 8: Hill numbers of species by different sites including individuals' number (#), species richness (N_0), Shannon index exponential (*N_1)*, reciprocal of Simpson's index *(N_2*), Simpson index value, and evenness value.

B: Figures

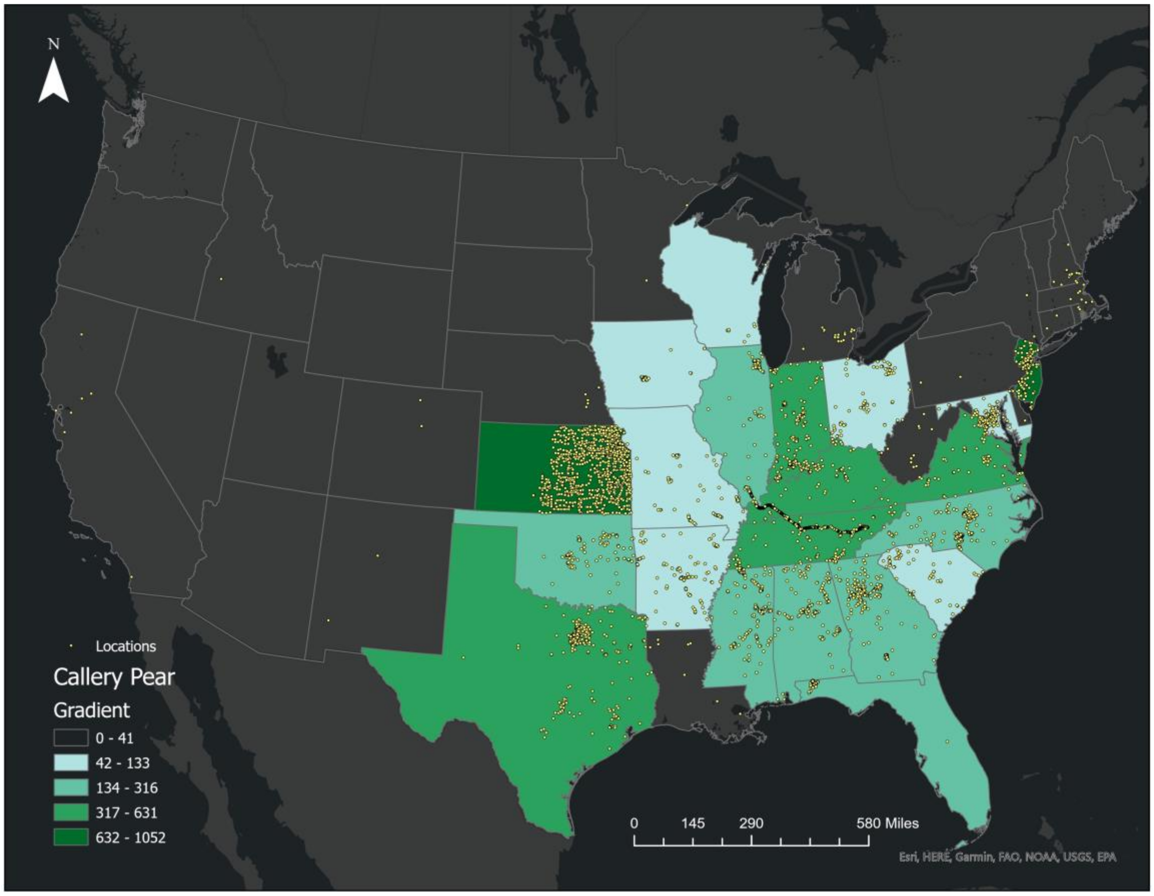

Figure 1: Callery pear distribution map with the points showing the presence of the Callery pear in the United States and the colors showing how number of the Callery pears are present as per the states (darkest color being the highest number of Callery pear trees present). The data for making this map was downloaded from (Georgia - center for invasive species and ecosystem health,2023).

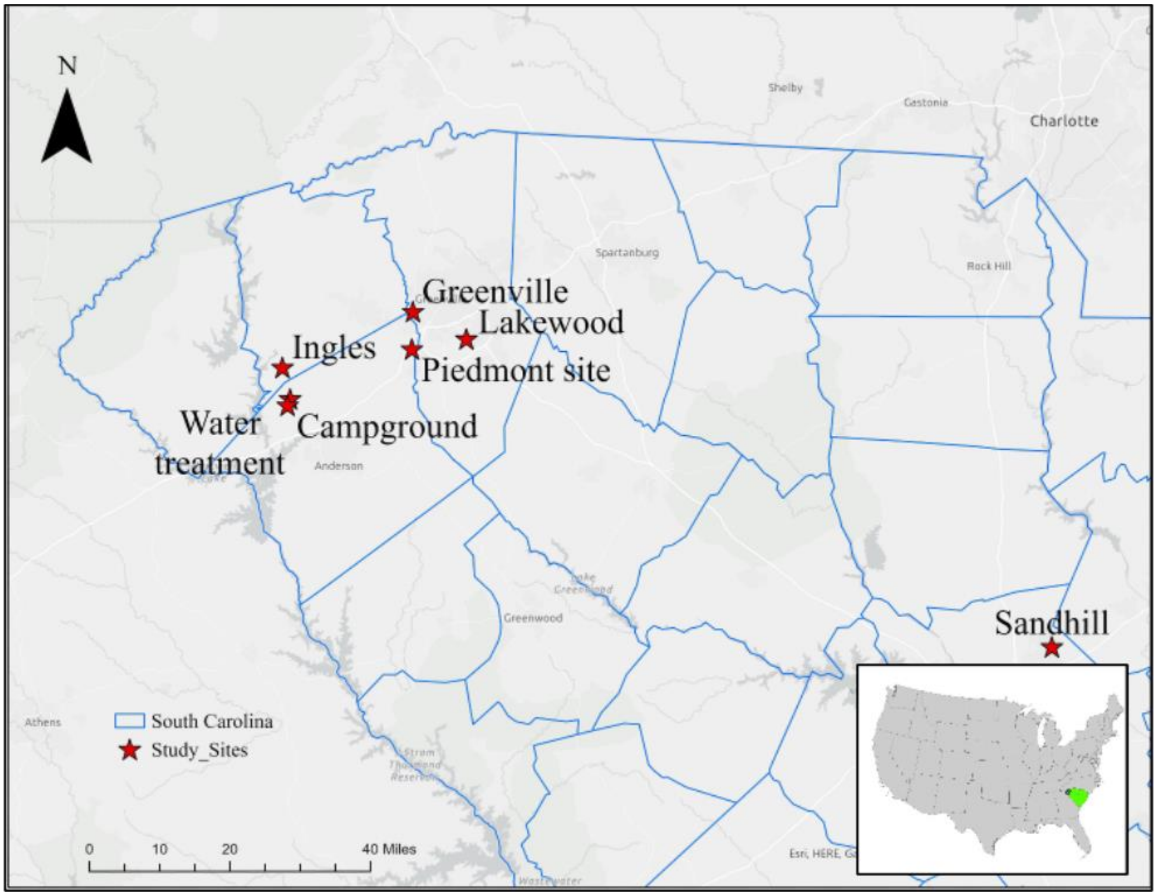

Figure 2: Location of sites on the map of South Carolina with red stars representing the study locations.

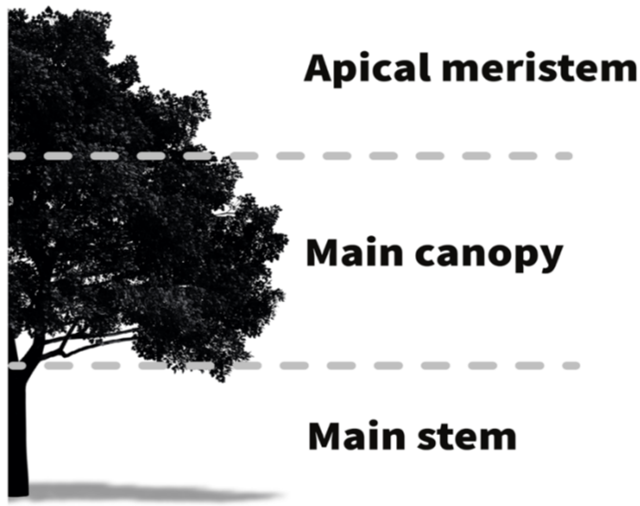

Figure 3: Tree divided into three different sections for collecting biomass and cookies from each section

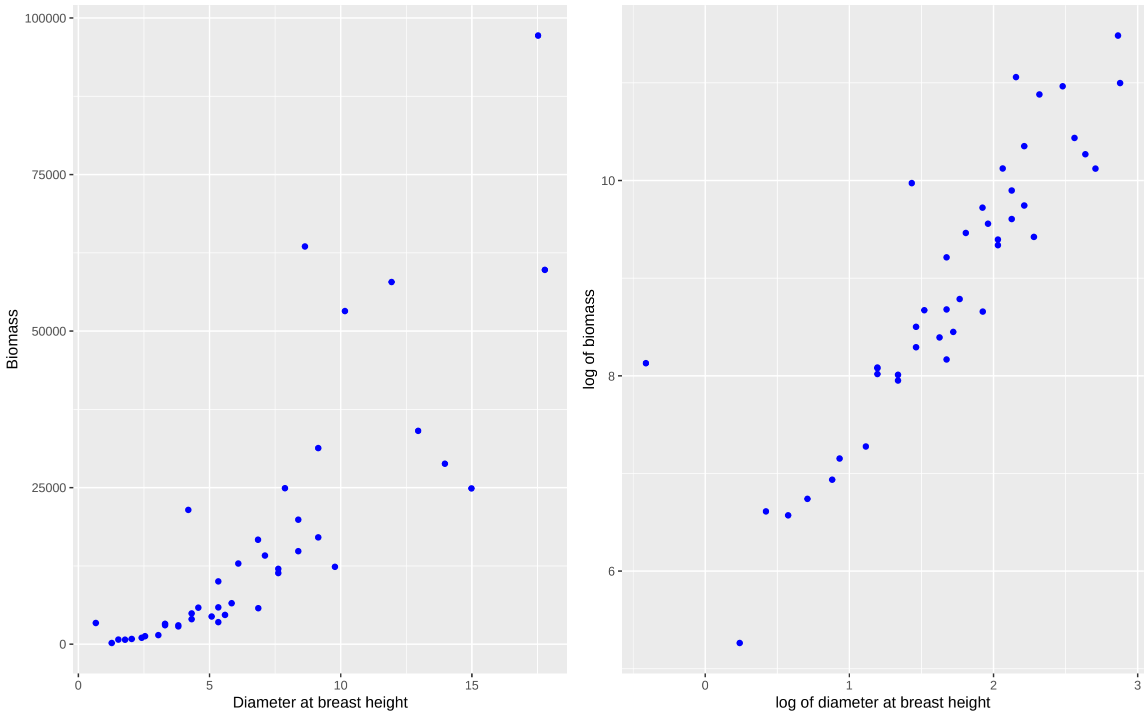

Figure 4: Comparison of plots with log transformation and without log transformation

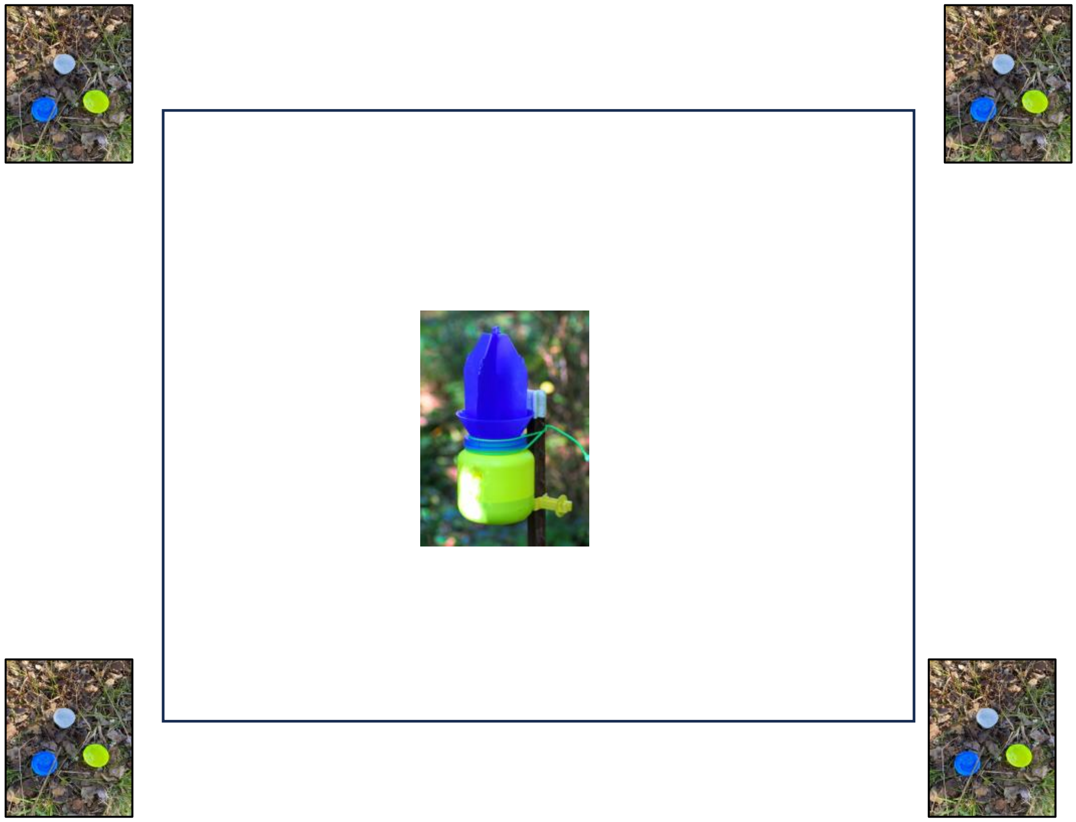

Figure 5: Diagram of pollinator collection sites, each containing four sets of three colored 2 oz. cups (blue, white, yellow painted) filled with soapy water placed on the ground adjacent to flowering Callery pear trees at four corners. A single blue vane trap attached at breast height (1.37 m) to a stake centrally located between several flowering Callery pear trees was placed in the center of the site.