

Developing Production Techniques and A Site Assessment Tool for Forest Farmed Ramps in
Appalachia

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ABSTRACT (ACADEMIC)

The *Allium tricoccum* Aiton (ramps, aka wild leeks), a native spring ephemeral, is a cultural keystone species in Appalachia, a mountainous physiographic region encompassing 205,000 square miles of the eastern United States. People in Appalachia have long harvested ramps in the wild. However, growing demand for the plant in and outside the region has increased harvesting, resulting in threats to native populations. Agroforestry cultivation techniques and technical support for sustained-yield forest farming practices are needed to conserve ramps and meet increasing demand. Various techniques for assessing suitable production practices for ramps were explored in this dissertation, particularly examining best-suited ramp ecotypes, mycorrhizal treatment, and habitat suitability determination. In the first study, bulbs and seeds from diverse Appalachian locations were gathered and transplanted to a common experimental site to investigate the effects of different ramp ecotypes on growth, survival, and stress responses. Plant characteristics and stress measurements were recorded before transplantation and post-transplantation assessments. Specifically, the study investigated the germination rate of three ramp seed ecotypes and the growth, survival, and stress responses of eight native ramp bulb ecotypes and three commercially obtained seedling samples. Results indicated that above-ground growth, survival, and stress response on the ramp ecotypes differed significantly.

The second study explored the impact of mycorrhizae on ramps and was evaluated by assessing the impact of Arbuscular Mycorrhizal Fungi (AMF) inoculation on *Allium tricoccum*. Four measurements, collectively referred to as parameter categories, were assessed. These included

measures of 1) above-ground plant growth: leaf length, and leaf width; 2) stress measurement: transplant stress after a few days of transplant, and photosynthetic performance stress after a year of transplant; 3) survival analysis; and 4) mycorrhizal colonization rate. For each parameter category, three treatment comparison categories were conducted: 1) Positive control treatments: bulbs were planted from their native environment without treatment; 2) Negative controls: bulbs were treated with fungicide before planting to eradicate existing AMF in roots; and 3) The test group: bulbs were inoculated with commercial AMF (Atriva 500). Results indicated that mycorrhizal inoculation could increase ramp leaf length ($P \leq 0.03$). However, the impact varied by ecotype, highlighting the importance of considering local environmental conditions and ramp ecotype. Mycorrhizal inoculation did not impact ramp growth at the seedling stage. Mycorrhizal treatment increased the transplanted ramp's survival and stress tolerance ($P \leq 0.001$).

The third study used multi-criteria decision-making (MCDM), the Analytic Hierarchical Process (AHP), and weighted linear combinations to model suitable habitats for ramps production. Ten habitat criteria were chosen (including five soil properties, three topographic parameters, and two land use properties) to assess the potential for growing ramps in seven counties in Virginia, West Virginia, Pennsylvania, and North Carolina. The percentage of highly suitable areas for ramps production in the studied counties ranges from 21.5% in Haywood County to 49.6% in Macon County. Similarly, moderately suitable areas range from 36.7% in Macon County to 54.5% in Lawrence County. Ground truthing was performed to validate the model. Ramp patch locations within each county were geocoded in the final suitability maps. Existing ramp patches were within the model's estimate of moderate to high site suitability ranges, suggesting the model is valid. Results of the study suggest that site suitability modeling could be useful for producers interested in growing ramps in forest farm settings across Appalachia.

Developing Production Techniques and A Site Assessment Tool for Forest Farmed Ramps in Appalachia

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ABSTRACT (GENERAL AUDIENCE)

The ramp, also known as the wild leek, is an important food, medicinal and cultural resource for the people of Appalachia. However, increasing demand for ramps beyond this region has led to over-harvesting and threatens the plant's native populations. Appropriate cultivation techniques and technical support for sustained-yield forest farming practices are needed to conserve ramps and meet growing demand. This dissertation explores various techniques for assessing suitable production practices for ramps, including examining ramp ecotypes, mycorrhizal treatments, and habitat suitability determination.

The first study evaluated the ecotypic variation among ramps collected from different geographic regions and their impact on plant performance. Ramp ecotypes displayed differences in above-ground growth, survival, and stress response. The second study examined the impact of mycorrhizae on ramps and found that mycorrhizal inoculation can increase ramp leaf length and survival and stress tolerance of transplanted ramps. However, this impact varied by ecotype, highlighting the importance of considering local environmental conditions and ramp ecotype. The third study involved developing a model to identify suitable habitats for growing ramps. The model was tested for seven counties across Virginia, West Virginia, Pennsylvania, and North Carolina. The percentage of land predicted as highly suitable for ramps production ranged from 21.5% in Haywood County, NC, to 49.6% in Macon County, NC. Moderately suitable lands ranged from 36.7% in Macon County, NC to 54.5% in Lawrence County, PA. Ground truthing confirmed the model's accuracy as geocoded existing ramp patch locations fell within the estimated suitable

ranges. Site suitability modeling could be useful for people interested in growing ramps in forest farm settings across Appalachia. Overall, this research provides insights into best practices for ramp cultivation that help conserve a cultural keystone species and meet the growing demand for ramps

DEDICATIONS

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1. CHAPTER I

INTRODUCTION

People have gathered food and medicine from forests for survival since ancient times. Unfortunately, unsustainable harvesting practices have led to concerns about the overexploitation of these plants, resulting in the threat or extinction of many high-value species (DeValue, 2013). For example, medicinal plants such as American ginseng root (*Panax quinquefolius*) and goldenseal (*Hydrastis canadensis*), which grow throughout the eastern United States, are threatened due to their commercial value and the failure to replace them after each harvest (Dworkin, 1999; DeValue, 2013). As wild food plants become more popular, sourcing them has become an essential aspect of the restaurant industry (Strand & DiStefano, 2010).

In the eastern United States, the wild onion, *Allium tricoccum* Aiton (ramps, also known as leeks), is a popular forest food of concern. A cultural keystone species, ramps have historically contributed to the region's identity (Garibaldi & Turner, 2004). Ramps are celebrated as a nutritious spring vegetable, and festivals centered on ramps bring tourism revenue (Rivers et al., 2014) to the Appalachian region. Where adapted, ramps once were abundant and widely distributed across the forest floor, but presently, foragers must go deeper into the woods to locate a single ramp (Sen, 2011). In order to conserve native populations and to meet the increasing consumer demand for ramps, improved cultivation techniques and technical support for sustained yield forest farming practices seem to be needed.

Ramps can be produced from seeds or bulbs in forest farming systems, but exploring the best methods is limited. Bulb propagation methods may be easier than growing from seed in terms of the time it takes for maturity, yet transplanting bulbs presents challenges, such as sensitivity to environmental changes (temperature, moisture, soil nutrient composition), the season of

transplantation, and stress, making long-term production questionable. To address these issues, we tested different seed germination methods, bulb propagation, and their compatibility for sustainable ramp production.

Sustainable forest farming for ramps requires understanding how plant materials respond to different site conditions. Ramps have a large native range along the Appalachian Mountain region from Canada to north Georgia and west to Missouri (Nault and Gagnon 1988; Nault and Gagnon 1993). Natural selection has led to the development of distinct ecotypes in various plant species growing in different site conditions, which enhance their ability to adapt to environmental stressors (Chaves et al., 2003; Hameed and Ashraf, 2008; Randin et al., 2009). Ramp seeds, seedlings, and bulbs are available commercially but from a few and quite diverse sites across this range (e.g., from North Carolina to Iowa and Minnesota). This raises questions about transplanting ramps from one region to another, and the suitability of commercial plant material for use in forest farming systems across this range should be explored. Thus, the starting point for this research was to investigate differences in production and adaptation for ramps collected from several distinct areas within Appalachia. We loosely described these as ecotypes and hypothesized that these plants might respond differently to various site conditions, including stress tolerance, growth, and survival.

Ramp's response to a given site may be further affected by association with mycorrhizal fungi. Some evidence suggests that plants colonized by arbuscular mycorrhizal fungi (AMF) have greater moisture and nutrient uptake and are more resilient to drought stress than non-colonized plants (Bowles et al., 2017). Mycorrhizal colonization can enhance forest herbs' growth, biomass, and phosphorus uptake compared to non-mycorrhizal conditions (Helgason *et al.*, 2002). For ramps, specifically, mycorrhizal colonization may affect resource acquisition since up to 60% of its root

length possesses mycorrhizal structures (DeMars 1996), and increases in bulb nutrient contents are observed when the plant is not actively photosynthesizing (Hewins et al., 2015). Mycorrhizal association may also offer potential benefits for ramps in the face of drought and extreme weather expected with climate change. However, limited research has been conducted on the relationship between AMF and ramps. Therefore, this study explored the impact of AMF colonization and a commercial AMF treatment on ramp production by examining relationships between growth and photosynthesis parameters and AMF.

Landowner interest in producing culinary and medicinal plants such as ramps has grown significantly as the herb and botanicals market has been outpacing the rest of the industry in recent years (Davis and Persons, 2014; Smith et al., 2021). These botanicals, like ramps, can be cultivated commercially in a forest farming system, benefiting manufacturers looking for new reliable sources of high-quality crops (Davis, 2007). Increasing interest in forest farming among farmers and landowners suggests that a significant amount of forestland could be dedicated to this practice. However, developing a model that predicts suitable locations for forest farming practices could greatly increase investment efficiency due to the diversity of forestlands and site conditions.

Assessing suitable sites for ramps can help identify key environmental factors required to support their growth. Site assessment can also help in evaluating the species' adaptation to local environments and may ultimately help determine if that causes ecotypic diversity. Assessing site suitability may also help conserve ramps in their natural habitat by identifying alternative lands suitable for this species' commercial production.

Factors that need to be considered for developing a forest farming site suitability model include soil pH, organic matter, mineral nutrients, and drainage, landforms such as slope, aspect, erosion, and surface drainage, climatic factors such as precipitation and temperature, overstory canopy

cover, existing forest vegetation, pests, pathogens, and beneficial organisms (Zamora and Wyatt, 2018). In this study, we attempted to assess habitat suitability for ramps across the Appalachian region with the help of a GIS and spatial modeling approach in combination with multi-criteria decision-making (MCDM) techniques.

Based on these issues, we identified three hypotheses to test to address research questions about ramp production and site suitability. The hypotheses test were:

- i. When transplanted to a common garden, ramps from different locations/ecotypes will differ in morphological characteristics, stress tolerance, and survival response.
- ii. Ramp inoculation with commercial AMF will reduce transplantation stress, improving survival, recovery, and growth.
- iii. Multi-criteria decision-making tools and GIS modeling techniques can effectively identify suitable areas for growing ramps in the Appalachian region of the United States.

2. CHAPTER II

LITERATURE REVIEW

Habitat and Phenology

Ramps are forest spring ephemerals with a short period of leaf growth; this is a specific adaptation to the high resource availability of light, moisture, nutrients, and relative lack of competition in the understory of deciduous forests (Muller, 1978). To allow rapid spring emergence, ramp leaves, and flower stalks begin within the bulbs in autumn (September). Ramp leaves emerge from a bulb in the soil during spring, and the plant grows during this season only to die back in summer and return to dormancy during winter. Shoot extension of ramps occurs even through snow, and the oblong-lanceolate leaves expand shortly after snowmelt (Nault & Gagnon 1993). Within the growing season, from late March to mid-May, carbohydrate and protein reserves are replenished and accumulated in bulbs, and the leaves senesce and quickly disappear (Nault & Gagnon 1988). *Allium tricoccum* undergoes distinct photosynthetic and reproductive phases (Nault & Gagnon 1993). A mature ramp plant produces a single seed stalk just before leaf senescence. The emerging stalk develops slowly and produces several self-compatible flowers (Nault & Gagnon 1987) that bloom in July. The stalk produces small green pods that open to reveal shiny black seeds, which then fall to the ground. Seed germination may take over a year and requires a period of cold temperatures after a warm spell to break dormancy. From their germination and initial growth, these plants may take five to seven years to mature and produce seeds.

Generally, the early development stages of forest perennials are very critical for survival, with high juvenile mortality and low annual growth that leads to a long pre-reproductive phase (Angevine & Handel 1986; Inghe & Tamm 1985; Meacher & Antonovics 1982). Although the seedling establishment is low with this high juvenile mortality, most forest perennials can be vegetatively

propagated to ensure the maintenance and expansion of local populations (Inghe & Tamm 1985; Angevine & Handel 1986). Ramps also reproduce asexually via bulb division resulting from lateral bud development. Ramps have monopodial growth form from germination to the initiation of flowering. After ramps develop a terminal inflorescence, they follow sympodial growth through lateral bud development (Nault & Gagnon 1993). *A. tricoccum* has 2-3 leaves with a short petiole at the base attached to the bulb (Sitepu, 2018). After the release of apical dominance with the initiation of flowering, ramps develop one or two (rarely three) daughter ramets and the rhizome branches. These rhizomes are at the bulb's base, composed of stems grown over several seasons, and function as an additional storage organ. When the bulb divides, the terminal bulb remains larger and is referred to as the mother bulb, while lateral bulbs are smaller and referred to as the daughter bulbs (Nault & Gagnon 1993). After five to eight years, these daughter ramets become independent, with the old rhizomes linking mother and daughter gradually decomposing. Ramps remain clumped, but the clone becomes fragmented and dense patches of ramps can be found in undisturbed populations (Nault & Gagnon 1993).

History and cultural significance

Ramps, native to the Appalachian region of eastern North America, were utilized by indigenous peoples prior to the arrival of the first colonists (Edgar et al., 2012). Ramps were used as a food and tonic by peoples of the region prior to European settlement (Cavender, 2006). The colonists utilized ramps as a food source because of their resemblance in appearance and taste to wild European species they were familiar with (Allegheny Leek Belt, 1899). The term "ramp" might have originated from the common name of its botanical relative, ramsons (*A. usinum*; wild garlic), a European counterpart to *A. tricoccum* (Rivers et al., 2014). The early settlers in the "Leek Belt" of New York initially attempted to eliminate the plant, but it proved resilient and eventually gained

recognition as a potentially valuable commodity (Allegheny Leek Belt, 1899). Surprisingly, ramps, known for their strong and distinctive smell, would evolve into a profitable crop that people would actively cultivate (Edgar et al., 2012). The people of the Appalachian Mountains region in eastern North America have a deep cultural connection to ramps, considering them an important "tonic" that provides vitamins and minerals unavailable during winter (Davis and Greenfield 2002). Despite their increasing popularity in culinary establishments, ramps remain significant in Appalachian cuisine, not just for their unique flavor but also because they encapsulate the region's meaning, pride, and tradition in a celebratory manner (Rivers et al., 2014).

Wild harvesting

Ramps are harvested throughout their range for various purposes, including personal use, commercial sales in roadside market restaurants, and during celebrations and ramps festivals (Edgar et al., 2012). Most ramps are harvested in their entirety; the whole plant is pulled from the ground, including its leaves, bulbs, and any rhizomes connecting the plants (Rock et al. 2004). The main harvesting season lasts from April through May. The market is mostly informal, making it difficult to gather an accurate count of the number of ramps harvested for each purpose per year. Additionally, people harvesting ramps on their private lands do not report their take (Edgar et al., 2012), so the number of ramps harvested yearly is likely much higher than estimates suggest. According to a specific estimate that solely considers ramp festivals, it is suggested that approximately 3,200 pounds of ramps are consumed during these festivals each spring (Edgar et al., 2012). Davis-Hollander (2011) found that at least two million ramps are harvested each spring in the United States for consumption. This figure includes both wild-harvest and purpose-grown ramps, but most are wild-harvested, and such scale puts populations at risk, and the impacts are visible among studied populations (Rock et al. 2004).

All wild populations are vulnerable to over-harvesting and poaching effects and may need years to recover, even from a one-time harvest (Rock et al. 2004; Davis and Greenfield 2002). Moreover, ramps can only recover from whole-plant harvest when left undisturbed for an extended period. Identifying critical stages of population maintenance is important for its sustainability (Nault & Gagnon 1993), as little is known about the population biology of ramps (Jones 1979). A study of simulated whole plant harvesting indicated that only 5-15% removal rates were sufficient to bring population growth rates below equilibrium and could ultimately result in a population decline (Nault & Gagnon, 1993).

Ramps production

Very little information is available on growing ramps in production settings as they are almost exclusively taken from wild populations (Davis & Greenfield, 2001). Information on best germination, site conditions, soil fertility, and harvest management, among other production practices, could improve their viability as a farmed crop (Davis & Greenfield, 2002, DeValue, 2013; Ritchey & Schumann, 2005). Some limited information can be found on the cultivation of ramps in small scale settings, such as how to grow ramps in a home garden (Iannotti, 2022, UNH Extension, 2020).

Potential production practices

Forest farming

Forest farming, a management approach to achieve environmentally and economically sustainable land-use, combines some gardening or farming practices with conventional forestry (Douglas and Hart, 1984; Hill and Buck, 2000; Mudge, 2009). Agroforestry practices such as forest farming follow the "four I's principle": intentional, integrated, intensive, and interactive (Mason et al.,

2012). These systems achieve diverse, profitable, sustainable land use by integrating trees with non-timber forest crops (Mudge, 2009).

As a land use system, forest farming combines production and conservation, creating a diverse and efficient approach to resource use. Despite its complexities and challenges, forest farming remains highly intriguing to many landowners (Trozzo et al., 2014, 2019; MacFarland et al., 2017). Trozzo et al. (2021) found significant interest in forest farming among Appalachian family forest owners in 14 counties in Southwest Virginia. These owners were more inclined to lease land for forest farming if they had prior experience with non-timber forest products (NTFPs) or held a positive attitude toward conservation.

Farmers and small landowners are increasingly interested in growing native, perennial, woodland medicinal, and culinary herbs like ramps; these crops can serve as supplemental income sources and a means of preserving their woodlands (Davis and Persons, 2014). As they are native to forested ecosystems, ramps have great potential for forest farming (Chamberlain et al., 2009).

Arbuscular Mycorrhizal Fungi (AMF) inoculation

Arbuscular mycorrhizal fungi (AMF) have gained much interest in enhancing plant growth and yield of many crops in agricultural systems (Thirkell et al., 2017). AMF improves plant growth through increased uptake of phosphorus, nitrogen, and other nutrients. The most effective AMF largely belongs to the recently described Phylum Glomeromycota (Redecker et al., 2000; Schußler et al., 2001; Säle et al., 2021). They have obligated symbiotic associations with 90% of all vascular plant roots. The extrametrical hyphae of AM fungi act as extensions of roots and increase the surface area of the root system, making it more efficient for absorption of water and diffusion-limited nutrients, this effect being more pronounced in P-deficient soils (Bagyaraj and Reddy,

2000). Other beneficial effects are biological control of root pathogens, biological nitrogen fixation, and increased ability to withstand abiotic stresses.

The benefits of AM fungi under field conditions have been reported for annuals, and perennials inoculated in the nursery (Bagyaraj, 1984; Chandrashekara et al., 1995). Mycorrhizal colonization has been shown to benefit forest herbs through increased biomass, growth, and phosphorus (P) acquisition compared to a non-mycorrhizal condition (Helgason et al. 2002). Many forests herbaceous plants, including trout lily (*Erythronium americanum*), mayapple (*Podophyllum peltatum*), and ramp (*Allium tricoccum*), maintain a mutual relationship with Arbuscular mycorrhiza (Brundrett and Kendrick 1990ab, DeMars 1996; Lapointe and Molard 1997; Watson et al. 2002). The AMF species, plant genotype, soil nutrient availability to plants, and environmental and stress factors determine the efficiency of AMF utilization of that plant (Chen et al., 2018).

Due to their less developed root system, *Allium* species are more sensitive to AMF application compared to other species (Gashaw Deressa and Schenk., 2008, Bowling et al., 1980, Greenwood et al., 1982, Sanders et al., 1992, Bever et al., 1996). Many *Allium* representatives are widely used in human nutrition, such as garlic (*Allium sativum*), onion (*A. cepa*), leek (*A. porrum*), shallot (*A. cepa* L. *Aggregatum* group), chive (*A. schoenoprasum*), bunching onion (*A. fistulosum*) and others for their pungency and flavoring value as well as medicinal properties (Fenwick et al., 1985). The resource capture of *A. tricoccum* ramps throughout the year may be influenced by mycorrhizal colonization as the roots of *A. tricoccum* are known to be colonized by AM fungi, with as much as 60 % of the root length possessing mycorrhizal structures (DeMars 1996). Although there are limited studies on AMF and ramps, there are few studies on the impact of AMF on other annual *Allium* species, which may provide insights into the forest farming of ramps.

Borde et al. 2009, in their study, mentioned that AM fungal inoculation increased the level of AMF root colonization of garlic which is very important for the growth and nutrient uptake by the plant. Many researchers have observed the enhanced growth of onion and leek plants inoculated with AMF. The increase in shoot growth of onion and leek plants inoculated with AMF could be due to increased uptake of nutrients, especially phosphorous (P) (Tinker 1984; El-Seoud 2012). Other studies also have stated that the increase in plant growth with AMF application may be due to the increase in nutrient uptake and increased tolerance towards drought stress (Metwally & Al-Amri, 2020). Although mycorrhizal inoculation is noteworthy in the physiology and biochemistry of the whole plant, it is in the root where the main changes are due to the symbiosis establishment and function. The mycorrhizal root acts as a more “active” organ than the root itself: the sphere of influence of the mycorrhiza (root+AMF) in the soil (the “mycorrhizosphere”) was wider and more obvious than that of the non-symbiotic roots (Bago & Azcón-Aguilar, 1997).

Site Suitability Modeling

Site suitability modeling is a valuable tool for forest farmers and landowners that helps to identify the optimal locations for forest farming. Site suitability models can be used to evaluate a wide range of factors, including soil, climate, topography, and market accessibility, to evaluate the potential and feasibility of forest farming in a given area. However, the choice of model factors depends on the specific project goals. Site suitability models can be developed using various methods, such as the multi-criteria decision-making method (MCDM), expert judgment, and GIS tools.

A study by Store and Jokimäki (2003) developed a GIS-based multi-scale approach by combining empirical evaluation models, expertise-based models, and GIS to model habitat suitability for a group of old-forest species and tested in a case study that highlights the strengths of the method.

Another study by Pramanik (2016) uses the Analytical hierarchy process (AHP) and GIS techniques to model the site suitability for agricultural land use in Darjeeling district, India, where he found that approximately 5.31 % area of the study site was highly suitable, 29.82 % moderately suitable, 24.27 % marginally suitable, and 40.60 % unsuitable for agriculture. Ahmad et al. (2017) used GIS-based mapping for agroforestry site suitability using nutrient availability as the major factor and demonstrated the capability of remote sensing in studying agroforestry practices and estimating the prominent factors for its optimal productivity.

The combination of GIS and multi-criteria decision-making (MCDM) method is highly effective for suitability modeling because these tools complement each other perfectly. GIS provides decision-makers or decision-making groups with the ability to analyze, manage, store, and visualize geospatial information, while MCDM techniques can be employed to structure decision problems and evaluate the various alternatives being considered (Malczewski, 1999). Over the past twenty years, MCDM has been applied with geographic information systems (GIS) to analyze spatial issues (Greene *et al.*, 2011).

3. CHAPTER III

RAMP (*ALLIUM TRICOCCUM*) ECOTYPIC VARIATION: GROWTH CHARACTERISTICS AND STRESS RESPONSES

ABSTRACT

Ramps, or wild leeks, are culturally significant spring ephemeral plants native to Appalachia and eastern North America. They are the earliest emerging edible plants and are consumed for tonic properties. However, overharvest of wild populations threatens their survival, necessitating improved cultivation techniques. This study examines the role of ecotypic population diversity in selecting ramp planting material for cultivation. Ramp bulb samples (n=50) and attendant soil from seven counties within Virginia, West Virginia, North Carolina, and Pennsylvania were collected randomly in Spring 2021 and were transplanted to a common location to investigate ecotype effects on growth, survival, and stress responses. Seed samples (n=500) were collected from different sites within Southwest Virginia during the Fall of 2020. In addition, seedlings samples developed from three seed sources (NC and two from MN) were purchased from a commercial nursery. Plant characteristics (Plant weight, bulb diameter, leaf length, and width) were measured just before transplanting into raised beds in Christiansburg, VA. Ramp survival, leaf morphological measures, and plant stress response (as determined by chlorophyll fluorescence) were measured in the second and third years after transplanting. Results showed that different ramp ecotypes exhibited variations in morphological characteristics, such as leaf length and width. The survival proportion of transplanted ramps in the second year varied among sites, with the highest rates observed in Montgomery County, VA, and the lowest in Giles County, along with a higher proportion of stressed plants. No significant differences in ramp seed germination were found across different site origins when grown in raised beds. However, ecotypic variation was evident among seedlings, with those from Clayton County, IA, displaying greater leaf dimensions. The survival proportion of transplanted seedlings in the second year was lowest for the seedlings

sourced from a private property in Wabasha County, MN, while those from Buncombe and Clayton counties in NC exhibited similar survival rates. These findings emphasize the importance of selecting suitable planting materials and sites to ensure optimal survival and growth of ramps. Understanding the ecotypic population diversity contributes to the conservation and cultivation efforts for this culturally significant plant species.

Keywords: ramps, cultivation techniques, ecotypic variation, sustainable forest farming, growth, and stress responses.

INTRODUCTION

Ramps (*Allium tricoccum* Ait. (Liliaceae), also called wild leeks, are spring ephemerals found primarily within deciduous forests of eastern North America (Greenfield and Davis 2001). Their rapid spring growth, followed by quiescence over the summer, represents a specific adaptation to a short period of high resource availability between snowmelt and canopy closure in the understory of deciduous forests (Muller 1978). Ramps, known for their tonic properties and rich in essential vitamins and minerals, have a strong historical and cultural significance in Appalachia, with traditional uses by Native American tribes such as treating colds, earaches, and worms (Davis and Greenfield 2002; Khan et al., 2017). These cultural ties have led to the establishment of spring ramps festivals that have shaped the Appalachian identity, contributed to the local economy, and garnered national attention as a gourmet food item (Rivers et al., 2014; Baumflek and Chamberlain, 2019; Khan et al., 2017). The growing awareness of and demand for ramps in and outside of Appalachia has spurred increased harvesting, and most are gathered from wild populations in public and private forests (Davis and Greenfield, 2002).

In many locations, overharvest now threatens native populations. In order to conserve native populations and meet the increasing consumer demand for ramps, improved cultivation techniques and technical support for sustained-yield forest farming practices are needed. Forest farming, an agroforestry practice that intentionally integrates elements of gardening or farming within a forest setting, has been identified as both commercially viable and environmentally sustainable (Mudge, 2009; Trozzo et al., 2021) and may work well for ramp production. This approach offers a unique opportunity to generate additional income from forested lands through the production of high-value specialty crops such as medicinal herbs (American ginseng (*Panax quinquefolius*), goldenseal (*Hydrastis canadensis*), and black cohosh (*Actaea racemosa*)) and foods (including ramps, several species of edible mushrooms (phylum *Basidiomycota*) and various fruits), while

also enhancing ecosystem services such as soil conservation, biodiversity, and carbon sequestration (Trozzo et al., 2021). Developing sustainable forest farming systems for ramps requires answering crucial questions about appropriate planting materials (including seeds, plantlets, and bulbs) and their sources. Specifically, "How do planting materials respond to different site conditions regarding growth and productivity?" Site conditions include soil type, moisture availability, sunlight exposure, temperature, and topography. Each site may have unique characteristics that can influence the growth and performance of ramp plants. By studying the responses of different planting materials to various site conditions, farmers can make informed decisions about which materials are best suited for specific growing environments. This knowledge allows them to maximize the chances of successful ramp cultivation and optimize the productivity of their forest farming systems.

In general, plant morphological, physiological, and chemical traits vary as a result of edaphic and environmental cues, which, over time, give rise to ecotypic population diversification (Oleksyn et al., 1992; Hancock et al., 2011; Reich et al., 2014). Ecotypes, genetically distinct populations of a given species, display phenotypic traits that maximize fitness to localized abiotic and biotic conditions (Stebbins 1950, Kawecki and Ebert 2004). The ecotypic response has been recognized in species distributed along climatic gradients (Clausen et al., 1940, 1948). Biogeographical gradients are expected to select for clinal adaptation in such traits (Woods et al., 2012). At the same time, natural selection has led to the evolution of different ecotypes in many plant species, which improves adaptation to environmental stressors such as salinity, drought, and temperature extremes (Chaves et al., 2003; Hameed and Ashraf, 2008; Randin et al., 2009).

To date, two varieties of ramps, *A. tricoccum* var. *tricoccum* and *A. tricoccum* var. *burdickii* (Hanes 1953; Jones 1979), have been identified, but comparisons of ecotypes from different

regions, and studies of their adaptations are limited. This study addresses this knowledge gap to explore the morphological and physiological characteristics of ramps collected along appropriate gradients (temperature, precipitation, elevation, soil composition) within seven counties in four states of Appalachia and subsequently transplanted to a single site for controlled observation and measurement. Measures of interest included morphological characteristics, stress tolerance, and survival response following transplantation. We hypothesized that these parameters would differ significantly among ramps collected from different locations/populations, shedding light on the diversity and adaptability.

METHODS

Ramp Samples Collection

The studies used natural bulbs, seeds, and commercial seedlings for analysis and experimentation.

Bulb samples

Bulb samples for this study were collected from eight different locations (Giles, Smyth, and Montgomery counties of VA, Pocahontas County of WV, Macon and Haywood counties of NC, and two sites in Lawrence County of Pennsylvania) in the Appalachian region of the eastern United States on the first week of April 2021 Figure 3-1. Sampling sites were selected based largely on collaborator locations, site access, and elevation gradient (195m to 1512m). At each site, 50 ramps and attendant soil samples were collected. The samples were collected by ramps experts in the respective locations and shipped in an ice box.

Seed samples

Three different seed samples were acquired during the Fall of 2020. Two sample sets were collected from wild ramp populations growing in Virginia (Montgomery and Giles Counties). One

set was purchased commercially (Prairie Moon Nursery, Wabasha, MN). Seeds from the nursery were collected from wild ramp stands in Wabasha County, MN.

Commercial Seedling samples

Seedlings were purchased from a commercial nursery (Red Root Nursery, Weaverville, NC) that sourced seeds from Weaverville, NC, Wabasha County, MN, and Clayton County, IA. Seventy-two (72) seedlings of each seed source were bought.

Climatic, Site Conditions, and Soil Sample Analysis

The study examined the climatic and site conditions, soil samples, and their analysis. Temperature and precipitation data from 2000 to 2022 were obtained from the International Research Institute for Climate and Society, specifically using county data ([NOAA, n.d.](#)). The summer and winter temperature averages and annual average precipitation were recorded for the sampling extraction and source sites (Table 3-1). Additionally, topographical variables such as elevation, slope, and aspect were noted for each sample extraction site during the sampling process (Table 3-1).

The soil series for each sample extraction site was determined using Web Soil Survey ([nracs.usda.gov](#)) and presented in Table 3-2: Values of soil properties for studied counties.. One composite soil sample was collected from each native site when ramp bulbs and seeds were collected. After a rainfall event, a minimum of four days before collecting samples was given to ensure the soil was as dry as possible. Soil samples were collected, removing the top layer with leaves, rocks, and sticks. Four sub-samples, taken at a fixed linear distance of 1.2 meters in cardinal directions from the randomized point of the ramp patch, were collected with a soil probe to 15 cm depth. The subsamples were thoroughly mixed to create a composite sample and transferred into

plastic sealable bags to avoid drying. Samples of constructed soils used for raised bed plantings were also collected.

Soil properties (pH, P, K, Ca, Mg, Zn, MN, Cu, Fe, % OM, and texture) for all samples were determined following the procedures of Maguire and Heckendorn (2019) at the Virginia Tech Soil Testing Laboratory.

A portion of each soil sample was placed in plastic bags, sealed, and shipped overnight to protect the mycorrhizal spores in the soil samples. Soil samples from each of the eight ramp sampling sites were sent to a commercial lab in California, USA (BIOMEMAKER) and analyzed for fungal and bacterial species presence using 16 rRNA and the ITS fungal identification method. There the microbiome analysis such as bio sustainability of the microbial community in the soil sample (bio-diversity, functionality, and stress resistance), major (C, N, P, and K) and minor (Fe, Zn, Mn, S, Ca, Cu, Mg and Cl) soil nutrition available for plants based on the microbial mobilization of these compounds, disease risks, crop health, and stress adaptation were analyzed for each soil sample.

Experimental Design

Site preparation and transplantation of bulb samples

A raised bed (1.22×2.44 m; 4×8 feet) was prepared in Christiansburg, VA. To make a standard growing medium, the raised bed was filled with 60% topsoil, 30% compost, and 10% potting mix from a local supplier. These materials were mixed thoroughly using shovels and rakes to ensure even distribution and homogeneity of the growing medium. The soil's properties (pH, P, K, Ca, Mg, Zn, MN, Cu, Fe, % OM, and texture) were determined following the procedures of Maguire and Heckendorn (2019) at the Virginia Tech Soil Testing Laboratory.

In order to evaluate the impact of geographic origin on ramp growth, 48 whole plants, including top growth, transplantable bulbs, and roots, were transplanted from each sampling site into raised beds. The plants were divided into Control, fungicidal, and mycorrhizal treatments. This study focused solely on the control treatment, where the ramps were transplanted as they were obtained from their native sites, without any additional treatments applied. The study followed a randomized block design, with the source of ramp origin being the main factor of interest. Each experimental unit contained four plants, and the study was replicated four times. Plants were measured before transplantation for baseline data and then placed within a defined matrix (with 2 inches (5 cm) of space between each) within each plot to facilitate tracking individual growth responses through time.

Site preparation and sowing of seed samples

For seed germination, two (0.91×1.52 m; 3×5 feet) raised beds were maintained under artificial shade (70% shade cloth), and another plot (0.91 × 2.13 m; 3 × 7 feet) on native soil under a forested canopy were prepared in a wooded area of the Catawba Sustainability Center, Catawba, VA. The raised beds were filled with a mixture of 60% topsoil, 30% compost, and 10% potting mix bought from a local supplier to make a standard growing medium. The soil in the wild-simulated site was Weikert-Berks complex (Loamy, shallow to moderately deep, well-drained soils (Table 3-2). Soil properties (pH, P, K, Ca, Mg, Zn, MN, Cu, Fe, % OM, and texture) for the soil in both sites were determined following the procedures of Maguire and Heckendorn (2019) at Virginia Tech Soil Testing Laboratory.

To examine the difference in total germination of ramps seeds collected from different locations across the species' native range, seeds (n=200) from each site (Montgomery, Giles, and Clayton Counties) were sowed in the raised beds, and 160 seeds of each origin were planted in the forest

simulated plot. The beds were divided into 4 different replicates, and the seeds were equally divided into two treatments: Control and mycorrhizal. This study focused solely on the control treatment, where the ramps seeds were sowed as they were obtained from their native sites, without any additional treatments applied.

Site preparation and transplantation of seedling samples

To investigate the ecotypic variation of commercially available ramps, we conducted a study where we acquired and transplanted seedlings from three distinct ecotypes. All seedlings were of the same age and were previously germinated and nurtured by a single commercial nursery. We transplanted them into an experimental raised bed measuring 1.22x1.22 meters (4x4 feet), prepared in Christiansburg, VA. The site preparation and transplantation process followed a similar procedure as described earlier for bulb transplantation.

Measurements

This study examines the growth of ramp bulb samples before transplantation in year one, representing their growth in their native site. It compares this growth to their growth in the experimental site in years two and three after transplantation, measuring the difference. The study also evaluates ramp survival and stress in years two and three after transplantation. Similarly, the growth of ramp seedlings is measured both before and after transplantation, along with their survival after transplantation. Furthermore, the total count of seed germination is recorded in years two and three.

Bulb Morphological Characteristics and Response to Transplantation

Measures prior to transplanting included plant weight, bulb diameter, leaf length, leaf width, and leaf number. Leaf length and width measurements were facilitated using gridded paper, and bulb

diameter was measured with digital calipers (Harbor Freight, Pittsburg, PA). Leaf length, width, and leaf number were measured in years 2 and 3.

Bulb Physiological Characteristics and Response to Transplantation

Stress measures

Transplantation stress

Ramps from all sampling sites were examined for signs of transplantation stress a month after, on May 3, 2021. At this time, some ramps' leaves were already wilted and starting to senesce. Chlorophyll fluorescence (OS-30, OPTI-SCIENCES) readings (Fv/Fm value) were taken on each plant and used as a proxy estimate of plant stress. Due to recent transplantation, the plants with 'zero' Fv/Fm value were considered and recorded as stressed plants.

Recovery analysis

The difference in the proportion of recovered plants among sampling sites was measured to make inferences about the impact of plant origin on stress recovery. Plants were categorized as 'recovered' or 'unrecovered' based on their active growth in year 2 after displaying substantial stress (Fv/Fm = 0) during year 1.

Photosynthetic performance stress

Various biotic and abiotic stresses (drought, cold, and heat) adversely affect plants' photosynthesis (Sherin et al., 2022). Chlorophyll fluorescence (OS-30, OPTI-SCIENCES) readings (Fv/Fm value) were taken for each plant to measure the plant stress. Excluding all 'zeros' (that were analyzed in transplantation stress in the first year), the value of Fv/Fm was used to compare the photosynthetic

stress in plants. Chlorophyll fluorescence was also measured in years 2 and 3, but with a different system (Handy PEA, HANSATECH) in year three.

Plant health analysis (Chlorophyll Content)

In order to analyze the impact of treatment on the overall plant health of ramps, the chlorophyll content was measured for each of the transplanted ramps in year three using a Chlorophyll Content Meter (Model CCM-300, OPTI-SCIENCES). Plant chlorophyll concentration is used in agriculture for monitoring plant health (Cortazar *et al.*, 2015).

Survival

Emerging plants were counted in years 2 and 3 to assess post-transplant survival. Plants with no observable emergence were considered dead and coded 0; surviving plants were coded 1.

Ramp Seed Germination Count

Counts of germinated seeds (exposed leaf tip) were made every 6 months, and data were recorded.

Seedling Morphological Characteristics and Response to Transplantation

One-year-old seedlings (n=24) of each ecotype were transplanted into raised beds. Before transplanting, leaf length, width, and seedling weight were measured to establish a baseline and observe differences in initial seedling growth parameters. Leaf length and width measurements were facilitated using gridded paper, and leaf length and width were measured in years 2 and 3.

Seedling Physiological Characteristics and Response to Transplantation

Post-transplant survival was assessed in spring in the two subsequent years following transplanting. Plants with no observable emergence were considered dead and coded 0; surviving plants were coded 1.

Data analysis

The data were analyzed in JMP software (16.0, SAS Institute Inc., 2021). Data distributions were visually inspected in a normal quantile and probability plot. Shapiro-Wilk normality test was performed to calculate a p-value and quantitatively determine the data's normality. For those variables (Fv/Fm data and survival data) whose data points did not have a normal distribution, a non-parametric test (Wilcoxon/Kruskal-Wallis tests) was performed. Ecotypes were arranged in a randomized complete block with four replications. Data were analyzed with a one-way analysis of variance (ANOVA) to test for differences between growth means among sites. A post-hoc test (Tukey's HSD) was used for source site differences among normally distributed data. A non-parametric post-hoc test (Pairwise Wilcoxon tests with multiple testing corrections) was performed for the data that were not normally distributed (Survival and stress analysis). For each study, the morphological features (leaf length (LL), leaf width (LW), and the difference between years, survival, and Fv/FM measures were considered dependent variables, with sites of origin as the independent variable. For each test, the significance level was $P=0.05$. Regression analysis was performed to calculate the correlation between these dependent variables. Multicollinearity was performed using Variance Inflation Factor (VIF) among the variables (no multicollinearity when VIF value less than 2 for each pair) to avoid overfitting the regression model between growth measures. To model the relationship between a set of predictors (baseline growth parameters (LL, LW, WT, and BD) and a nominal response (transplantation stress, survival, and recovery data), the Nominal Logistic Regression (NLR) analysis was performed that predicts the probability of an event with two or more outcomes (in this case: stressed or not, survived or not, and recovered or not) based on the values of several predictor variables (in this case: growth measures; BD, WT, LL, and LW).

An analysis of means for Proportions (ANOMP) was performed to analyze the proportion of surviving plants in year 2 and year 3. This method was used to test whether the proportion of surviving plants in years 2 and 3 for each site differed from the overall population proportion.

RESULTS

Climatic, Site Conditions, and Soil Sample Analysis

The temperature data ranges from a maximum of 24°C to 28°C and a minimum of -5.1°C to 7.7°C. The annual precipitation data ranges from 109cm to 163cm among native bulb and seed sampling sites located at different elevations, ranging from 195m to 1512m (Table 3-1). Similarly, these climatic data for the sample extraction sites vary from the sample transplantation sites, with a maximum temperature of 28.6°C and a minimum temperature of -4.2°C, an annual precipitation of 109.4cm, and an elevation of 658m (Table 3-1).

Soils collected from these native sites (Table 3-2) were very acidic to acidic, with pH ranging from 4.5 to 6.5. Similarly, macronutrients were quite variable (2 to 44 ppm P, 48 to 112 ppm K, and 306 to 2790 ppm Ca). The soil organic matter percentage in source sites varied substantially but was generally much greater than typically found in agricultural soils (i.e., 3.4%) (Loveland and Webb, 2003). Soils generally were finely textured and classified as silty clay loam, clay loam, or silty clay (Table 3-2). Based on the soil microbial activity, the available nutrients for plants for each sampling site were varied (Table 3-3)

Based on microbial biodiversity and function measures, these sites' microbial ecosystems were also quite variable and would potentially affect crop health and stress resistance (Van and Semenov, 2000) (Table 3-4 and Table 3-5).

Bulb Morphological Characteristics and Response to Transplantation

Plant weight and morphology at baseline

Average plant weights differed ($P < 0.0001$) as much as four-fold among sites of origin. Ramps from Pocahontas County (WV) and Haywood County (NC) weighted the most, followed by plants from Giles County (7.7g, 6.6g, and 5.8g). Ramps transplanted from Montgomery County (VA) weighed the least (2.0g; Figure 3-2).

The average bulb diameter significantly differed ($P < 0.0001$) two-fold among sites of origin when measured before transplantation. The plants from Pocahontas County and Haywood County had greater ($P < 0.0001$) bulb diameters, followed by plants from Macon County (8.8mm, 8.23 mm, and 7.43 mm). The plants from Montgomery County had the smallest bulb diameter (4.89 mm) (Figure 3-3)

Leaf Measures

Number of leaves

An average number of leaves differed ($P = 0.001$) among the sites before transplanting, with maximum leaves for ramps from Lawrence County, PA (1), while the minimum number of leaves were observed on ramps from Macon County, NC (Figure 3-5, a). The difference in leaf number with its base did not differ among the years (Figure 3-5,b).

Leaf length

Average leaf length differed ($P = 0.001$) among the sites before transplanting, with the longest leaf lengths for ramps from Haywood County, NC, and Giles County, VA (18.0 and 16.1 cm, respectively). Ramps with the shortest leaves were from sites in Montgomery and Smyth County, VA (9.5 and 11.3 cm; Figure 3-5, a).

The average leaf length for year 2 also differed significantly among sites ($P=0.01$) (Figure 3-5), with the highest average leaf length for Haywood County; 14.63 cm, followed by Pocahontas County; 13.65 cm and lowest for Macon County; 10.14 cm. Leaf length differed ($P < 0.001$) yearly, but the change was inconsistent across ecotypes. For example, ramps from Macon County, NC, and Giles County, VA, had shorter ($P = 0.002$) leaf lengths one year after transplanting than a length at baseline (decreasing by 24% and 12%, respectively). In contrast, leaf length increased ($P = 0.002$) yearly for ramps from Smyth County and Montgomery County (VA/ Figure 3-5, a).

Leaf length varied significantly among sites in year 3 ($P=0.001$), with Haywood County having the greatest average and Lawrence County, PA (1) having the lowest (Figure 3-5, a). Leaf length changed from year 2 to year 3 ($P < 0.001$), but this change was inconsistent across sites. Leaf length in year 3 was greater for Smyth and Montgomery County, VA, from the baseline length, while for other sites, leaf lengths are below the baseline length, although increased from year 2 for Macon and Haywood County, NC (Figure 3-5, b).

Leaf width

Ramps with the widest average leaf width were from Pocahontas County, WV (4.7 cm), Giles County, VA (4 cm), and Haywood County, NC (3.9 cm). The narrowest average leaf width measured came from plants from Montgomery County, VA (1.9 cm; Figure 3-6, a).

All ecotypes had narrower leaves one year after transplanting (Fig 3-6, a) Average decline in leaf width across all ecotypes was about 32% from baseline to the one-year width measure. The largest declines (39% and 60%) were observed for ramps from Lawrence Co., PA (1) and Macon Co., NC. In contrast, only a small reduction (9%) in width was observed for Montgomery Co, VA plants. The average width was 90% of the initial two years after transplanting. However, leaf widths of ramps from Macon, NC, remained less than two-thirds (60%) of their baseline measure,

and the Leaf width for Montgomery County, VA, was 40% more than the baseline measure (Figure 3-6, b).

Interaction among growth measures

Since the growth of one part or dimension of a plant is related to the growth of other parts and various plant activities, a correlation analysis was conducted to examine the relationship between different above-ground plant growth measures at baseline (year 1). The observations indicate that Plant Weight (WT) showed a strong positive correlation with Bulb Diameter (BD), Leaf Number (LN), Leaf Length (LL), and Leaf Width (LW). Additionally, LL and LW showed a strong positive correlation (72%). However, there were weak correlations below 50% between LL and LW with LN and LL and LN with BD, with no issue of multicollinearity (Figure 3-7).

Bulb Physiological Characteristics and Response to Transplantation

Stress measures

Transplantation stress

After less than a month of transplantation, ramps with a value 'zero' for chlorophyll fluorescence (Fv/Fm) were considered stressed plants, and it was assumed that the stress-causing factor was recent transplantation. Furthermore, the Fv/Fm values more than 'zero' were considered less stressed plants. The proportion of totally stressed plants was greatest for ramps from Giles County, VA (~70%) and Macon County, NC; (63.6%). Little stress was apparent for ramps from Pocahontas County, WV (~3%) and Smyth and Montgomery Counties, VA (~5% 10%, respectively; Figure 3-8)

Interaction of transplantation stress with other growth measures

The plant weight (WT) and Leaf length (LL) have the strongest effects on transplantation stress, as they have the highest log-worth values and lowest p-values ($P = 0.00001$ and $P = 0.00005$). The variables Leaf Width (LW) and Bulb Diameter (BD) also have a significant effect ($P = 0.012$ and $P = 0.022$), although their effects are weaker on transplantation stress. The Leaf Number (LN) does not significantly affect transplantation stress. Furthermore, with transplantation stress, the LL, LW, and BD had a positive coefficient estimate, indicating that as leaf length, leaf width, and bulb diameter at transplanting increase, the greater the likelihood of transplant stress. In contrast, ramp weight had a negative coefficient estimate, indicating that as plant weight increased, the likelihood of stress decreased (Figure 3-8).

Recovery analysis

The proportion of recovered plants (with Fv/Fm value greater than 'zero') among the stressed plants due to transplantation in year 1 was calculated here and found that the proportion of recovered plants was highest for Montgomery County; 99% followed by Macon County; 95% whereas the proportion for recovered plants was lowest in Haywood County and Lawrence County site-1 and Smyth County (Figure 3-10).

While performing Nominal Logistic Regression (NLR) analysis to model the growth measures with the recovery of ramps, no significantly fitted regression was found between recovery and other growth parameters.

Photosynthetic performance stress

The average Fv/Fm value differed between years ($P = 0.0001$), with maximum value for Haywood County, NC, in year 3 (0.82) followed by Montgomery County in the same year (0.81); however,

values did not differ significantly among sites and its interaction with year. The value for Fv/Fm significantly increased for all sites in Year 3 when compared to Year 1 and Year 2 ($P < 0.0001$) Figure 3-11.

Plant health analysis (Chlorophyll Content)

The chlorophyll content (CC) was measured for each plant to examine if the plant health differed due to its source/origin. The average value for CC was found to be significantly different among sites ($P = 0.001$), with a maximum value for site 8 (Haywood County; 442 mg/m²) and a minimum for site 5 (Lawrence-2; 357 mg/m²) Figure 3-12.

Survival

The proportion of ramp plants surviving in year 2 ranged from (0.94 to 0.60) and was the smallest ($P = 0.0001$) for plants from Giles County, VA, and Lawrence County, PA (1) (Figure 3-13, a). Among the survivors in the second year, 50% of ramps died in 3rd year from Giles County, VA, whereas the plants from Lawrence County, PA (1) survived 100% in 2nd year (Figure 3-13, b). However, the survival proportions were not significantly different among sites of plant origin in year three.

Interaction of survival with other growth measures

Among plant measures, ramp leaf length (LL) had the largest log-worth value and a significant p-value of 0.0001, indicating that it was the most important predictor of survival in the experiment. Similarly, ramp bulb diameter (BD) and leaf number (LN) with a p-value of 0.002 and 0.008 are other predictors of survival in the experiment. The plant weight (WT) and leaf width (LW) variables have log-worth values close to zero and non-significant p-values, indicating that they are not important predictors in the experiment. Furthermore, the LL and LN variables have negative

coefficient estimates, indicating that the probability of survival decreases as their value increases. The BD variable has a positive coefficient estimate, indicating that the probability of survival increases as its value increases. The LW and WT variables have coefficient estimates close to zero and non-significant p-values, indicating that they do not significantly affect the probability of survival. (Figure: 3-14).

Ramp seed germination count

The seeds in the constructed raised bed at Catawba germinated 18 months after sowing. Mean germination rates did not differ among sites, with similar germination among the source sites. Germination was 16% and 18% for Montgomery and Giles counties (Virginia) and 12% for seeds from Wabasha County, MN (Figure 3-15a). Similar results were observed for seeds sown in the forest simulated plots (14% and 13% germination for seeds from Montgomery and Giles counties and 9% for seeds from Wabasha County, MN (Figure 3-15b). Seedlings were counted again the following year to determine survival and whether or not additional seeds germinated. The mean germination/emergence percentages were 18% and 26% for Montgomery and Giles Counties' seed origins and 14% for Wabasha County's seed (Figure 3-15). However, the germination data were not significantly different among sites of origin and years of measurements.

In contrast, stratified seeds that were kept refrigerated started to germinate at 6 months. However, the germination percentages were the opposite when compared to outdoor beds. 16% of seeds from Wabasha County, MN, germinated after stratification. In comparison, 3% of Giles County, VA seeds and no seeds from Montgomery County, VA, germinated after 6 months of stratification (Figure 3-16).

Seedling morphological characteristics and response to transplantation

Baseline seedling weights (measured at transplantation) were similar among sites of origin (mean = 14g; Figure 3-17).

Leaf length

Leaf lengths at the baseline measure (before transplanting) were the shortest ($P = 0.0004$) for seedlings from Wabash, MN (Figure 3-18).

Leaf length one year after transplanting increased for seedlings from Buncombe County, NC, and Clayton County, IA, but decreased for seedlings from Wabasha County, MN. The trend in the difference was similar as in the first year (before transplanting) between sites (Site $P = 0.0009$, Year $P = 0.008$ and site X year interaction, $P = 0.1$; Figure 3-18). However, the average leaf length increased for Wabasha, MN, and Clayton, IA. However, it stayed similar for Buncombe County, NC (Site $P = 0.01$) in year 3 (Figure 3-18).

Leaf width

The average leaf width at the baseline measure (before transplanting) was greatest ($P = 0.0001$) for seedlings from Clayton County, IA, and Wabasha County, MN but differed in subsequent years (site $P = 0.001$, year $p = 0.01$, site X year interaction, $P = 0.77$; Figure 3-19). The year following transplanting, ramps from Buncombe County, NC, and Wabasha County, MN, had narrower leaves than at baseline, but this was not observed for Clayton County, IA seedlings. Two years after transplanting, leaf widths were equal to or greater than baseline measures for all ecotypes Figure 3-19.

Seedling Physiological Characteristics and Response to Transplantation

Survival

The survival of transplanted seedlings in year 2 was significantly lower ($P=0.03$) for seedlings sourced from Wabasha County, MN (62%) compared to seedlings from NC and IA, which had an average survival rate of 90% (Figure 3-20). From year 2 to year 3, some seedlings did not survive, but no differences in survival among the sites of origin were observed.

Furthermore, based on Nominal Logistic Regression (NLR) analysis, seedling survival was not significantly related to any growth parameters measured for seedlings.

DISCUSSION

Climatic, Site Conditions, and Soil Sample Analysis

Spatial heterogeneity is an inherent feature of soils and has significant functional implications, such as variation in nutrient transformation rates among different soil patches (Pajares *et al.*, 2016). Heterogeneity in soil influences plants' distribution and spatial pattern (Stoyan *et al.*, 2000). Soil properties may differ from one landscape position to another because of differences in soil-forming factors such as climate and vegetation, relief, and parent material (Badia *et al.*, 2016). Elevation is the major factor causing differences in temperature and soil properties, ultimately impacting plant species distribution (Ratier *et al.*, 2021). For example, Badia *et al.* (2016) found that increasing elevation significantly decreased pH and exchangeable potassium (K). Soil K values were lower at higher elevations in this study, too. Soil organic matter also increased with elevation, which matches the study findings (Table 3-1 and Table 3-2: Values of soil properties for studied counties.). Differences in these and other environmental and edaphic factors may contribute to the development of ramps ecotypes distributed over latitudinal and elevational gradients as ecotypes

are groupings of species populations with different environmental or habitat conditions (Turesson, 1922; Reichert, 1999).

Bulb Morphological Characteristics and Response to Transplantation

Ramps for this study, collected from eight sites along latitude and altitude gradients, differed in average leaf length and width, bulb diameter, and plant weight, indicating some ecotypic variability. However, certain limitations in this study may impact the ability to identify ecotypic variations definitively. These limitations include difficulties in obtaining precise measurements of plant age, as it was challenging to calculate the exact age of the ramps. Additionally, there were variations in sourcing and site quality, although some environmental variables related to these sites were examined.

Furthermore, a strong positive correlation is observed among WT, BD, and other leaf measures at baseline (in year 1), suggesting that the growth of one part of a plant is interconnected with the growth of other parts or that the selection for one trait can impact other traits. For instance, selecting plants with larger bulb diameters may also increase plant weight, indicating a potential relationship between these traits. However, it is important to note that the correlation between LN and LL with BD and LN with LL and LW is weaker, with correlations below 50%, suggesting that different genetic or environmental factors may influence these traits compared to other growth measures. This finding implies that the genetic and environmental influences on LN, LL, and LW may be distinct from those affecting bulb diameter, reinforcing the idea of ecotypic variation among ramp plants collected from different environmental gradients. This study is also limited in determining whether observed differences in the plants are due to genetic or environmental factors (i.e., nature vs nurture). Genetic testing is needed to verify trait differences between the plants to address the question of ecotypic variation fully.

This study observed the longest leaf lengths in samples collected from Haywood County, NC, with the highest elevation and soils with high organic matter and annual precipitation. Shorter leaf length for these ramps in the second year (as well as for site 6 (Macon County), could be attributed to transplantation stress, changes in environmental factors, or both. Site 6 (Macon County, had the greatest decrease in leaf length. Although transpiration was not measured, ramps from Macon County (Site 6) may have greater water demand given that their native site typically receives substantially more annual as well as spring precipitation (163 cm and 48 cm, respectively) than the research site received (109 cm and 19.7 cm respectively) during the study period (Table 3-1). However, the increased leaf lengths for these sites in year 3 highlight increased adaptation of plants in the experimental sites, possibly due to the availability of favorable growing conditions. During years 2 and 3, the observed increase in leaf length for plants from Montgomery County, VA (Site 3) might be attributed to the similarity of conditions between native and experimental sites (both in Montgomery County). Also, it was picked after its full maturation (9 years old), which might have allocated its energy for above-ground biomass than below-ground (though the below-ground biomass was not measured). Local phenotypes generally are better suited to their specific environment of origin, resulting in a higher level of fitness (ability to survive and grow) than individuals from other locations within the species' geographic range (Leimu, and Fischer 2008, Kawecki and Ebert 2004).

The highest leaf width was observed in samples collected from site 7 (Pocahontas County), which had the calmest summer temperature(24°C) and greatest organic matter (18.5) among all as well as great annual (128.7cm) and spring precipitation (26 cm). However, the leaf width decreased significantly in the second year for all sites, with the greatest decrease for plants from site 6 (Macon County; 2.3cm) followed by Pocahontas County (1.5cm), which could be a similar reason for the

decrease in leaf length. Although transpiration was not measured, ramps from Macon County (Site 6) may have greater water demand given that their native site typically receives substantially more annual as well as spring precipitation (163 cm and 48 cm, respectively) for Macon County and ~129cm and 26 cm for Pocahontas County, WV) than the research site received (109 cm and 19.7 cm respectively) during the study period (Table 3-1). The leaf length and width decrease in the second year may be due to plant transplantation stress. Similar studies have reported that the decrease in leaf size in plants alleviates the adverse effect of drought stress, thereby minimizing water loss (Seleiman *et al.*, 2021). The increased leaf width for all sites in year 3 from year 2 highlights the increasing adaptation of ramps in the experimental site may be due to the availability of favorable growing conditions such as temperature (less number of the snow day, cool and moist weather (average temperature for March and April: 10-15 °C, 7-9 days of rain) (WeatherTab, 2023). The increment in leaf width for all sites in year 3 suggests that leaf width would be a better marker for plant recovery.

Bulb Physiological Characteristics and Response to Transplantation

Stress measures

The proportion of plants experiencing stress in the month following transplanting was greatest (more plants with '0' Fv/Fm values) for the population from Giles County, VA. In contrast, populations from Pocahontas County, WV (the second furthest north from our experimental site) had the lowest stress (as determined by the lowest proportion of plants with '0' Fv/Fm values one month after transplanting), suggests that plants from Giles County, VA are more vulnerable to transplantation stress compared to other sites. The average values of Fv/Fm for plants from all sites were less than 0.79, indicating they were stressed. The optimal Fv/Fm range for many plant species is between 0.79 to 0.84, with lower values indicating plant stress (Maxwell and Johnson,

2000). Transplant stress often impacts plant growth and development, particularly during the first year (Pecknold, 2001). These results suggest that ramp ecotypes responded differentially and may have varying abilities to cope with stressors that occur with transplantation. Both genetic and environmental factors influence the ability of plant ecotypes to adapt and cope with stressors (Bakhtiari et al., 2019). Differences in the environmental conditions from the sites of origin to the study site (i.e., differences in site aspect, elevation, temperature, and precipitation) and edaphic conditions of the site could have affected (and stressed) the recently transplanted ramps (see Table 3-1).

Additionally, given the strong positive correlation between leaf length and transplantation stress, as well as the negative correlation between bulb diameter and transplantation stress in ramps, it is important to understand that plants with limited resources, such as a small bulb, but with high demands for water and nutrients, such as those with large leaves, are more prone to experiencing stress, could be due to the insufficient capacity of the plant to meet its metabolic requirements. Having a longer mean leaf length (16.1cm) and smaller mean bulb diameter (6.3mm) among transplanted ramps from all sites, Giles County might have experienced the strongest transplantation stress among all. Furthermore, the ramps from Pocahontas County, with the larger bulb diameter (8.8mm) among all and a medium leaf length (14.7cm), might have caused the lowest transplantation stress.

The proportion of recovered plants in the second year among the stressed plants in the first year was greater for ramps from Macon County, NC, followed by Montgomery County, VA, while recovery for other sites was close to the lower decision limit. Correlating this result with the resulting change in the number of leaves, leaf length, and leaf width in the second year of transplantation, the decline in both of the leaf growth parameters and the number of leaves were

greatest for Macon County, NC, among all (although the below-ground growth measures were not conducted in this study to look at the exact change). This indicates that plants from Macon County were more successful in adapting to the new environment than plants from other sites might be due to the phenotypic plasticity that the ramps from Macon County showed by decreasing their leaf size to adapt to the new environment. Phenotypic plasticity is a major way a plant can cope with environmental variability such as moisture and temperature (Gratani, 2014). This finding is consistent with the idea that the ability of plants to recover from stress is determined by their inherent traits, such as genetic diversity and plasticity (Strauss and Agrawal, 1999).

Greater F_v/F_m values in year 3 for all ramps suggest that the plants had begun to recover from the initial transplant shock. However, low (<0.79) F_v/F_m values for ramps from Lawrence County, PA, and Macon County, NC, indicate that those ecotypes had not fully recovered. Ramps from Lawrence County also had the lowest estimated chlorophyll concentration, indicating that these plants likely had lower nutrient concentration and green fluorescence (Cortazar *et al.*, 2015).

Survival

The proportion of transplanted ramps that survived the first year after transplantation varied significantly among source materials. The lowest proportion of ramps surviving came from the second closest source site (Giles County, VA; 0.12) and the furthest north source site (Lawrence County, PA (Site 4); 0.18). Their latitude of origin did not influence ramp survival in this study. Other factors that may have contributed to the observed differences in survival rates include altitude of the origin, soil nutrients, OM, temperatures, and precipitation (Table 3-6). These factors may further interact with the composition and structure of soil microbial communities across diverse geographic regions. Parent soils from these ramp sites had distinct variations in the soil

microbial communities (Table 3-6). These variations could potentially impact the growth and survival of plants, but the direct relationships were not determined in this study.

Furthermore, given the strong positive correlation between bulb diameter (BD) and survival, as well as the negative correlation between leaf length (LL) and the number of leaves (LN) and survival in ramps, it is important to understand the effect of longer leaf length, more leaf number and smaller bulb diameter on ramps as they could potentially impact the survival of ramps. The transplanted ramps from Giles County, VA, with larger leaf lengths and smaller bulb diameters, showed the lowermost survival proportion, followed by ramps from Lawrence County that have medium size leaf lengths and medium size bulb diameters but greater leaf number (LN) amongst transplanted ramps from all sites suggests that ramps with larger bulb diameter and smaller leaf length with lesser leaf number might have a higher chance of survival during transplantation. This finding might suggest that the plants mobilize the resource more soon after emergence so that the plants with larger leaf sizes and a high number of leaves have smaller bulbs and will not have enough storage capacity to help the survival of plants for next year. This finding might suggest that transplanting after senescence would be better than transplanting soon after emergence (before significant resources are expended). However, this has not been tested to prove the statement. As well as, if the smaller bulb size and larger leaf size, a morpho-physiological feature of an ecotype or a phenological difference is not clear from the findings of this study. It is important to consider these factors when selecting ramps for transplantation to increase their chances of survival. However, it is important to note that other factors not included in the model may also affect the survival of ramps during transplantation.

These results may have an important implication for forest farming and management. To maximize the success of ramp transplantation efforts, it may be necessary to carefully consider the bulb size

and leaf length ratio and environmental factors of the source of origin for those transplanted plants. Furthermore, genetic differences among plant populations should also be considered when selecting plants for transplantation.

Ramp seed germination count

One important aspect frequently overlooked in studies of seed dormancy is the possibility that populations in different environments will possess differing seed dormancy strategies (Dunbabin & Cocks, 1999). Seed germination and seedling growths are the first and the most vulnerable stages in the life cycle of plants (Ma et al., 2021), which suggests that seed vigor is an important trait for selecting important crop varieties (Eggert & von 2013). Therefore, it is very important to know the best technique for higher seed germination and its survival to secure many plant varieties in the next step. In the case of ramps, it is not easy to discover how to germinate seeds better as ramp seeds have double dormancy (radicle or hypocotyl and epicotyl). Seed dormancy is a mechanism that delays germination to ensure it occurs when conditions are suitable and the seedling's probability of survival and growth is high (DeValue, 2013). Stratification is a temperature treatment to break out the dormancy, and in the case of ramps, warm stratification breaks radicle dormancy, and epicotyl dormancy is broken by cold stratification, but only after the radicle has emerged (Baskin and Baskin, 2005). Root dormancy is broken if sufficient warm weather occurs after sowing in late summer or early fall, followed by the cold of the subsequent winter, breaking shoot dormancy and leading to plant emergence in spring; however, if there is not a suitable warm period after sowing, the seed will only germinate in the second spring (Davis and Greenfield, 2002).

Most importantly, temperature, moisture, and light availability significantly affect seed germination (Tang et al., 2009). The ramps seeds in the field (both in a forest simulated plot and

raised bed with an artificial shed) in this study that took entire 18 months to germinate as well as had very few germination percentages (below 26% from each site) may be because of not getting enough temperature to break radical and epicotyl dormancy at the right time. A similar trend followed in the second year of germination (in 2023).

On the other hand, the early germination of seeds from Wabasha County with larger looking size, shinier and healthier looking, when stored at room temperature for 60 days and refrigerated for another 90 days for stratification, indicated that the size and origin of the seeds as well as favorable temperature and moisture might have an important role to break the dormancy of the seeds. Some studies have suggested that seed dormancy-break (thus germination) can be affected by environmental cues experienced by the mother plant (Nguyen et al., 2021), seed size, that small seeds require more time to break out dormancy (Rodrigues-Junior et al., 2018).

Seedling Morphological characteristics and response to transplantation

This study evaluated the growth of ramps seedlings purchased from a nursery with seed sources from three locations. Significant differences in leaf length and width were found among the seedlings from the three different seed sources before and in the second year after transplanting. The highest average leaf length and width were found for seedlings from Site 3 (seed source: purchased from Prairie Moon Nursery, MN, collected from Clayton County, Iowa), followed by Site 1 (seed source: germinated in a nursery in Buncombe County, NC). The leaf growth patterns of the seedlings from the different sites also varied over the two years. The seedlings that seed sources from Buncombe County, NC (site 1) and Clayton County, IA (Site 3) grow better than the ones from Wabasha County, MN (site 2). The better growth of plant sourced from site 3, even though it is two units farther in the USDA hardiness zone, could be because of the previous mulching with PROMIX (that has mycorrhizae within it) by the supplier. The study further

revealed that seedlings with sources of seeds purchased from commercial nurseries performed better than those germinating from seeds sourced from a private ramp farm. This could be attributed to the seeds being well-matured with higher growth potential or intraspecific genetic variation among the ramp seeds collected from different geographic regions, leading to differences in leaf growth.

Intraspecific genetic variation may strongly influence plant leaf morphology, and this variation in plant size, growth, and leaf morphology corresponds with the species' total climate range and certain climatic limits related to temperature and moisture extremes (Mauseth, 2003). In addition, the study revealed the existence of considerable variation between the origin of seeds to seedling growth. The significant variation in seedling leaf growth within the origin of ramp seeds may be due to both environmental and genetic variation and their interaction (Van et al., 2020). Similar findings were reported by Fredrick et al. (2015), where they found differences in seed morphology, germination, and seedling growth of apple-ring acacia (*Faidherbia albida*) with seeds from different origins.

The increased leaf length and width of seedlings in year 3 for site 2 (indicates that they adapt well to the environment. Furthermore, the higher leaf length and leaf width for seedlings from site 3 (Seed source: Clayton County, IA) could be because of the initial application of PROMIX mixed with mycorrhizae in it by the supplier, indicating that the use of such growth-promoting additives in the early stage may help in the better growth of seedlings in future.

Seedling Physiological Characteristics and Response to Transplantation

Survival

The survival of ramp seedlings is crucial for the productivity and sustainability of forest farming. This study assessed the survival of ramp seedlings obtained from a nursery with seed sources from three locations. The seedlings were transplanted into a common experimental plot under a forest farming system. The results revealed significant variations in survival proportions among the sampling sites (p -value=0.03). The lowest survival proportion was observed in seedlings from Wabasha, MN (62%), while Clayton Co., IA, had the highest survival rate (93%), followed by Buncombe Co., NC (84%). Although the quality of the sourced seeds was not examined, the low survival rate in Wabasha, MN, may be attributed to poor seed quality, environmental changes, and inadequate seed adaptation capacity. As demonstrated in Clayton County, IA, these findings highlight the importance of selecting appropriate seed sources and implementing effective management practices, such as mulch with mycorrhizae. The study emphasizes the significance of these results for forest farming, highlighting the need for appropriate seed sources and management strategies to enhance the establishment and persistence of ramp seedling populations. Additionally, local seed sources adapted to the specific environment can increase the likelihood of successful establishment and reduce the risk of introducing genetic incompatibilities that could compromise population fitness (Rowe and Leger, 2012).

CONCLUSION

The study found significant differences in the growth and morphology of *Allium tricoccum* (ramps) collected from different geographical locations when transplanted to a common experimental site. The diversity in soil and climatic variables among sites could contribute to developing ramps ecotypes distributed among different geographical locations. Environmental factors, such as soil

moisture, soil nutrient levels, and competition for resources, may have significantly influenced the difference in the growth and survival of ramps. The results suggest that the growth of ramps is highly dependent on the variety of plants collected from different geographic locations and may respond differently to transplantation and changes in environmental conditions.

The study also demonstrates that ramp plants' survival rate and stress adaptation vary significantly among the plants collected from different geographic locations. When selecting plants for transplantation, soil type, moisture levels, temperature, and genetic differences among plant populations should be considered. Moreover, some ecotypes adapt more successfully to stress factors, while others are more vulnerable. The correlation analysis between plant morphology, transplantation stress, and survival in ramps reveals that ramps with larger leaves and smaller bulb diameters exhibit higher vulnerability to transplantation stress and reduced survival rates. This suggests a limited metabolic capacity and insufficient energy storage for future survival. Based on these findings, it is recommended to consider ramps with larger bulb sizes and shorter, narrower leaves for transplantation or transplant bulbs in the fall after leaf senescence rather than transplanting the entire plant soon after emergence. The findings of this study have implications for ramp conservation and management, as well as for forest farming programs aimed at improving stress tolerance in ramp plants.

Based on the result, it can be concluded that the ecotypic variation of ramps is evident in the variability of seed germination and growth responses in different environments. The size and origin of the seeds, as well as favorable temperature and moisture, could play a significant role in breaking dormancy. Similarly, based on the commercial seedling study result, it can be concluded that there is ecotypic variation in the growth and stress response of ramp seedlings. The study found significant differences in leaf length and width among seedlings from three different

geographical locations, and seedlings from commercial nurseries performed better than those germinated from seeds sourced from a private ramp farm. Intraspecific genetic variation and environmental variability among seed sources could be the potential contributors to this variation. The survival proportion of ramp seedlings also varied significantly among sampling sites, highlighting the importance of selecting appropriate seed sources to enhance the establishment and persistence of ramp populations in the wild. Further research is needed to explore the genetic diversity of ramp populations and the effectiveness of different management strategies in promoting population resilience and long-term sustainability.

Table 3-1: Average summer and winter temperature and average annual precipitation data for sampling counties.

Source: International Research Institute for Climate and Society (NOAA) AND Slope and Aspect data for wild sample source locations and experimental sites

Sample Collection & Transplantation Sites	Sample Sites	County, State	Avg Max Temp		Avg Min Temp		Annual average precipitation		Feb-May Pptn 2021		Elevation (m)	Slope%	Aspect	LAT	Hardiness Zone
			°F	°C	°F	°C	inch	cm	inch	cm					
Bulb sample collection	1	Giles, VA	82.2	27.8	23.0	-5.1	43.5	110.4	8.0	20.4	195	Flat	NE	37°N	6b
	2	Smyth, VA	81.2	27.3	22.8	-5.1	48.2	122.4	10.9	27.7	359	30-40	W & NW	36°N	6a
	3	Montgomery, VA	83.5	28.6	24.1	-4.2	43.1	109.4	7.8	19.7	513	30-40	NE	37°N	6b
	4	Lawrence, PA (1)	83.0	28.3	18.8	7.3	44	111.7	9.6	24.5	358	Flat	N	40°N	6a
	5	Lawrence, PA (2)	83.0	28.3	18.8	7.3	44	111.7	9.6	24.5	273	0-10	N	40°N	6a
	6	Macon, NC	80.6	27.0	25.2	-3.7	64.2	163.0	18.9	48	1097	27	NE	35°N	6b
	7	Pocahontas, WV	76.5	24.7	18.1	7.7	50.6	128.7	10.2	26	969	35	S	38°N	6a
	8	Haywood, NC	77.0	25.0	23.5	-4.7	58.2	147.8	14.6	37.1	1512	40	NW	35°N	6b
Wild seed sample collection	1	Montgomery	83.5	28.6	24.1	-4.2	43.1	109.4	7.8	19.7	636	<10	E & SE	37°N	6b
	2	Giles	82.2	27.8	23.0	-5.1	43.5	110.4	8.0	20.4	195	Flat	NE	37°N	6b
Commercial seedlings sources	1	Buncombe, NC	81.7	27.6	25.4	-3.7	50.8	129.1	12.8	32.5	N/A	N/A	N/A	35°N	7b
	2	Wabasha, MN	82.7	27.6	6.9	-13.9	35.5	90.17	N/A	N/A	N/A	N/A	N/A	43°N	4b
	3	Clayton, IA	82.4	28.0	9.7	-12.3	38.4	97.53	N/A	N/A	N/A	N/A	N/A	42°N	4b
Bulb Transplant.	Exp.	Montgomery	83.5	28.6	24.1	-4.2	43.1	109.4	7.8	19.7	658	<15	SW	37°N	6b
Seed planting	WSB	Roanoke, VA	85.2	29.6	25.8	-3.4	45.5	115.6	7.1	18.1	508	15-45	NE	37°N	7a
	ACRB	Roanoke, VA	85.2	29.6	25.8	-3.4	45.5	115.6	7.1	18.1	511	0-10	NE	37°N	7a
Seedling Transplant.	Exp.	Montgomery< VA	83.5	28.6	24.1	-4.2	43.1	109.4	7.8	19.7	658	Flat	Flat	37°N	6b

Table 3-2: Values of soil properties for studied counties.

Sample Collection and Transplantation Sites	Site	County/State	pH	P ppm	K ppm	Ca ppm	Mg ppm	Zn ppm	Mn ppm	Cu ppm	Fe ppm	% OM	Text. Class	Soil-Series
Bulb sample collection sites	1	Giles, VA	5.4	17.0	88.0	717.0	62.0	1.7	13.7	0.6	14.2	5.6	SiCL	Nolichucky
	2	Smyth, VA	4.8	4.0	48.0	306.0	39.0	1.5	7.4	0.2	14.5	17.1	SiC	Cullasaja-tuckasegee complex
	3	Montgomery, VA	5.7	44.0	77.0	1051.0	108.0	1.8	20.7	1.7	57.2	6.1	CL	Unison and Braddock complex
	4	Lawrence-1, PA	6.1	6.0	112.0	1332.0	104.0	7.3	14.1	0.7	11.8	5.5	SiCL	Bethesda
	5	Lawrence-2, PA	5.7	16.0	82.0	725.0	61.0	5.9	11.9	1.0	25.1	4.5	SiCL	Allegheny
	6	Macon, NC	5.3	6.0	78.0	723.0	79.0	3.2	9.8	0.3	13.3	14.6	SiC	Edneyville-Chestnut complex
	7	Pocahontas, WV	5.2	2.0	67.0	618.0	84.0	3.1	10.9	0.8	15.2	18.5	SiCL	Cateache
	8	Haywood, NC	6.7	4.0	81.0	2790.0	168.0	2.7	57.7	0.1	3.4	15.5	SiCL	Plott
Wild seed sample collection sites	1	Montgomery, VA	6.3	1.0	83.5	658.5	145.0	0.9	8.2	0.4	10.4	3.5	CL	Jefferson
	2	Giles, VA	5.7	7.0	67.5	1146.0	75.0	1.2	19.3	0.3	4.8	6.9	SiCL	Nolichucky
Bulb transplantation site	Exp. Site	Montgomery, VA	7.9	25.3	92.0	2238.7	462.0	6.5	35.3	0.5	12.3	12.7	SiCL	Groseclose-Urban land complex

Seed planting sites	WSB	Roanoke, VA	6.1	7.0	196.0	1099.0	143.0	1.2	18.5	0.4	7.3	6.5	L	Weikert-Berks complex
	ACRB		7.6	32.0	222.0	988.0	208.0	3.8	20.2	0.4	9.1	8.1	SiCL	Chilhowie
*WSB: Wild Simulated Bed, ACRB: Artificially Constructed Raised Bed, SiCL: Silty Clay Loam, CL: Clay Loam, SiC: Silty Clay, L: Loam														

Table 3-3: Plant, available nutrients in the soil of native ramp sampling sites, based on microbial activity present in the soil.

Site ID	County/State	Soil nutrition (Nutritional status based on the microbial mobilization of certain compounds)											
		Major Compounds				Minor Compounds							
		C	N	P	K	Fe	Zn	Mn	S	Ca	Cu	Mg	CL
1	Giles, VA	high	very low	high	high	Very high	Very low	medium	very high	very low	High	very high	Medium
2	Smyth, VA	high	very low	very high	very high	high	Very low	Low	very high	very low	medium	High	Medium
3	Montgomery, VA	high	very low	high	high	high	Very low	Low	High	very low	medium	High	very low
4	Lawrence-1,PA	high	Low	very high	very high	Very high	low	medium	very high	low	High	very high	Medium
5	Lawrence-2,PA	high	very low	high	high	high	low	high	very high	very low	High	very high	low
6	Macon, NC	medium	very low	very high	very high	Very high	Very low	Low	very high	low	very high	very high	high
7	Pocahontas, WV	high	very low	high	high	high	low	medium	very high	very low	medium	very high	low
8	Haywood, NC	high	very low	very high	very high	Very high	Very low	Low	High	Very low	High	High	Medium

Table 3-4:Microbial ecosystem function of sampling sites

(*very low=<20%, low=20-40%, Medium=40-60%, High=60-80%, very high=>80%)

Site	County/State	Microbial bio-sustainability			According to the pathogens detected		Stress adaptation	Hormone Production
		Biodiversity	Functionality	Stress resistance	Crop health	No. of Disease Risk		
1	Giles, VA	medium	very high	medium	high	2	low	very low
2	Smyth, VA	low	High	very high	medium	3	low	very low
3	Montgomery, VA	medium	Medium	medium	high	2	low	low
4	Lawrence-1,PA	medium	very high	medium	medium	3	Medium	low
5	Lawrence -2, PA	very high	very high	medium	medium	2	Medium	medium
6	Macon, NC	low	very high	very high	high	3	low	very low
7	Pocahontas, WV	very high	very high	low	medium	3	very high	medium
8	Haywood, NC	low	High	very high	medium	4	high	low

i

Table 3-5: Composition of soil microbial community in sampling sites

Site	County/State	Fungal Phylum Distribution (%)			Bacterial Phylum Distribution (%)		
		Ascomycota	Basidiomycota	Mortierellomycota	Proteobacteria	Actinobacteria	Verrucomicrobiota
1	Giles, VA	33.63	43.86	22.35	36.63	18.54	16.53
2	Smyth, VA	40.65	23.10	35.20	39.16	14.94	10.00
3	Montgomery, VA	63	19	18	38	38	9
4	Lawrence-PA-Co, PA	72.15	21.81	5.90	39.44	16.88	16.63
5	Lawrence -PA-Ne, PA	53.22	10.45	36.20	40.06	17.24	9.63
6	Macon, NC	33.53	19.02	46.91	40.26	18.49	13.59
7	Pocahontas, WV	39.08	13.16	47.46	47.05	17.81	8.18
8	Haywood, NC	43.77	41.55	14.53	42.17	17.43	8.44

Table 3-6: Correlation of survival of plants in the second year of transplantation with soil chemical, microbial and climatic parameters of the ramps source of origin

Soil and climatic parameters	Correlation with survival proportion
pH	-0.26144
P	0.11732
K	-0.65103
Ca	-0.10828
Mg	0.076864
Zn	-0.37325
Mn	0.013808
Cu	0.156414
Fe	0.351601
% OM	0.588229
FPD (%) Ascomycota	-0.21446
FPD (%) Basidiomycota	-0.51736
FPD (%) Mortierellomycota	0.60513
BPD (%) Proteobacteria	0.491283
BPD (%) Actinobacteriota	0.354233
BPD (%) Verrucomicrobiota	-0.75835
Elevation	0.519737
Annual pptn	0.486335
Max temp	-0.38552
The numbers in bold are significant values	

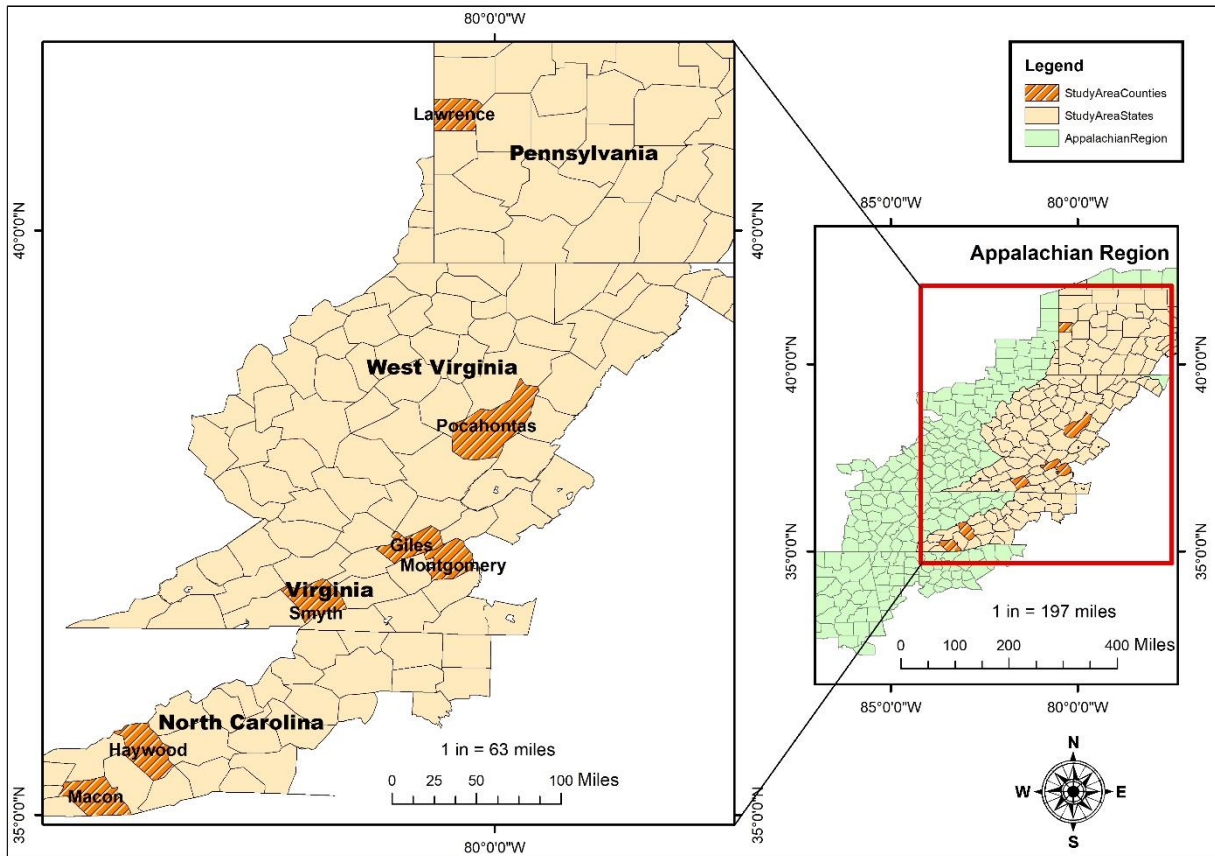


Figure 3-1: Study area map of the Appalachian region showing 7 different study counties in four states. The study area counties include Lawrence County of Pennsylvania; Pocahontas County of West Virginia; Giles, Montgomery, and Smyth counties of Virginia; Haywood and Macon County of North Carolina

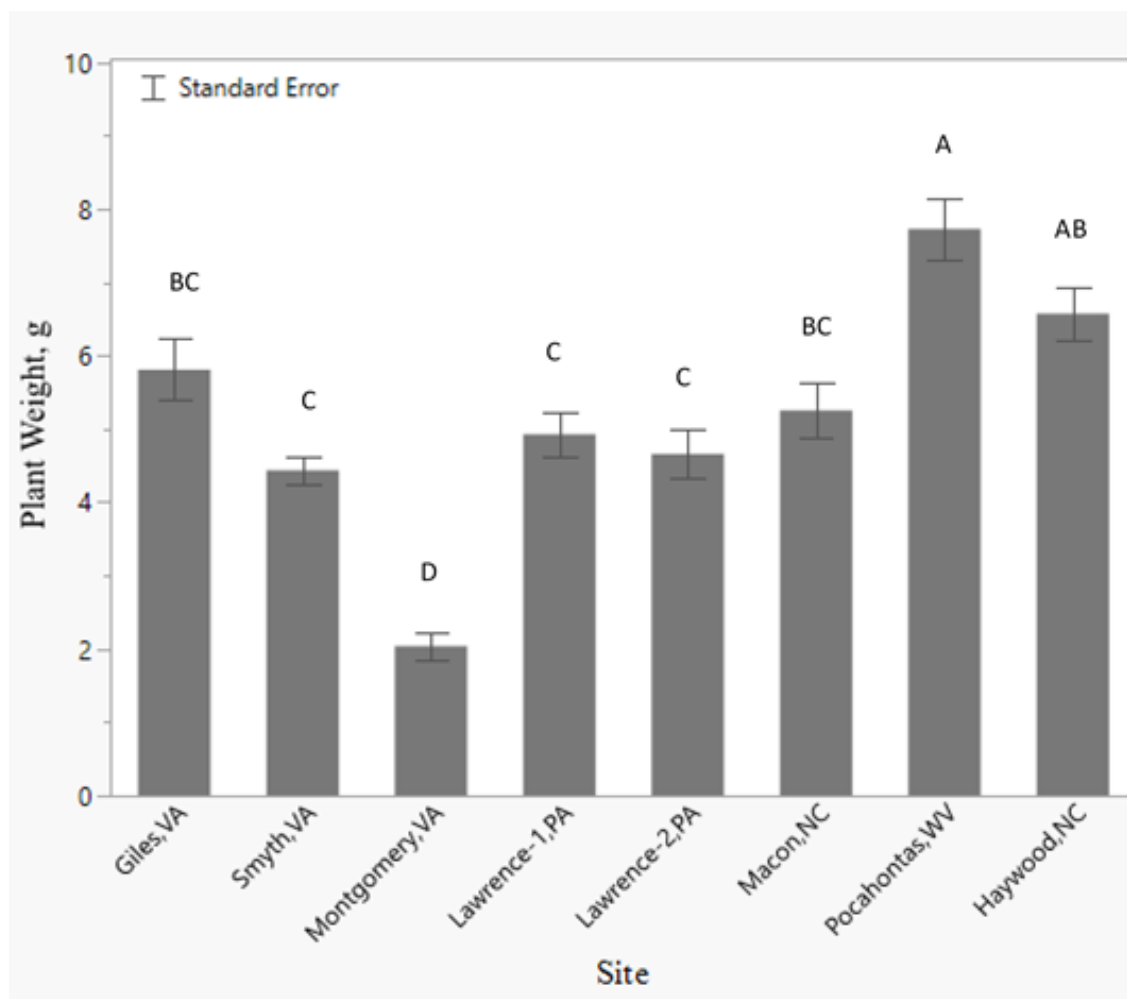


Figure 3-2: Mean plant weight of ramp (*Allium tricoccum*) ecotypes collected from each site across Appalachia before transplantation, p-value = 0.001. Sixteen ramps were collected randomly from each location. Mean, and standard bars are presented above each bar.

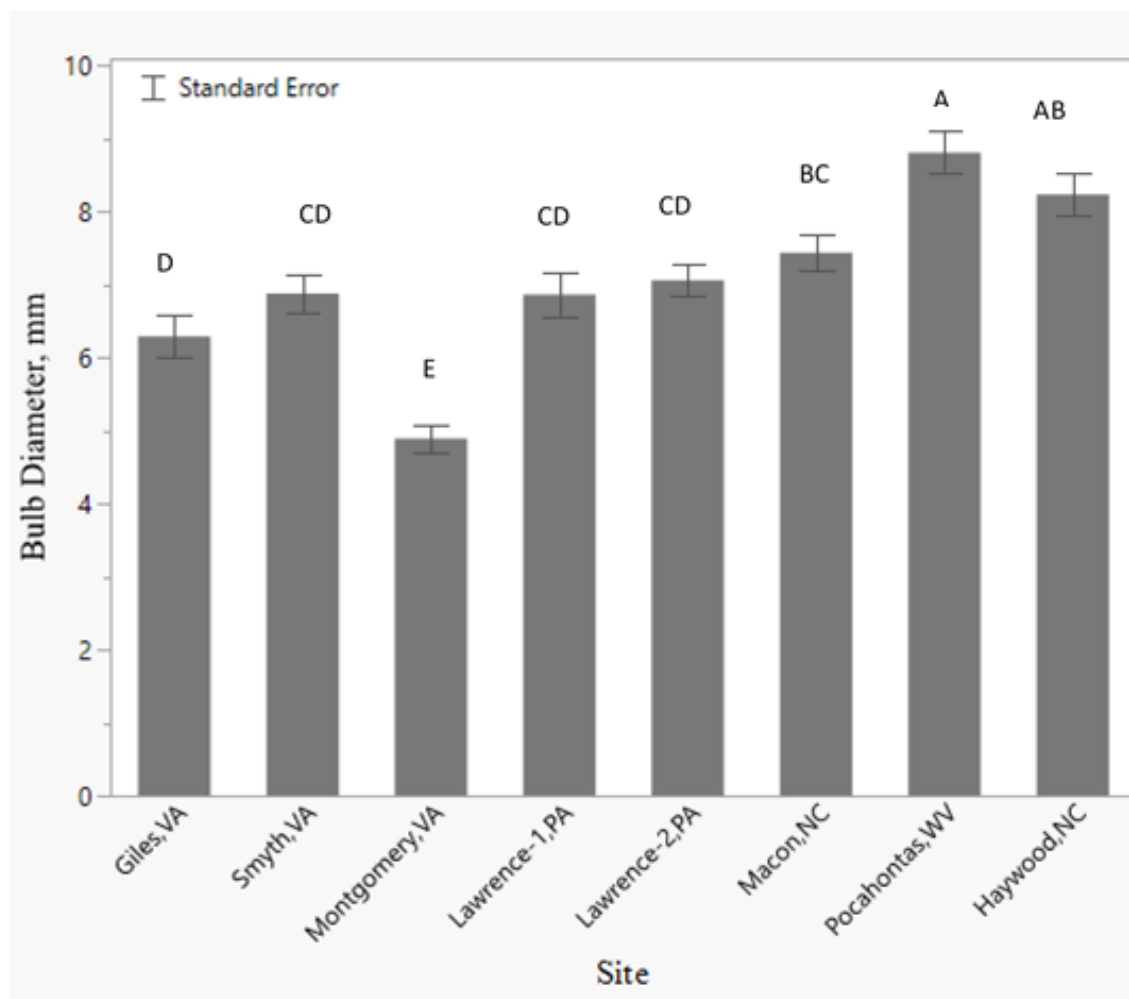


Figure 3-3: Mean bulb diameter of ramp (*Allium tricoccum*) ecotypes collected from each site across Appalachia before transplantation, p-value = 0.001. Sixteen ramps were collected randomly from each location. Mean, and standard bars are presented above each bar

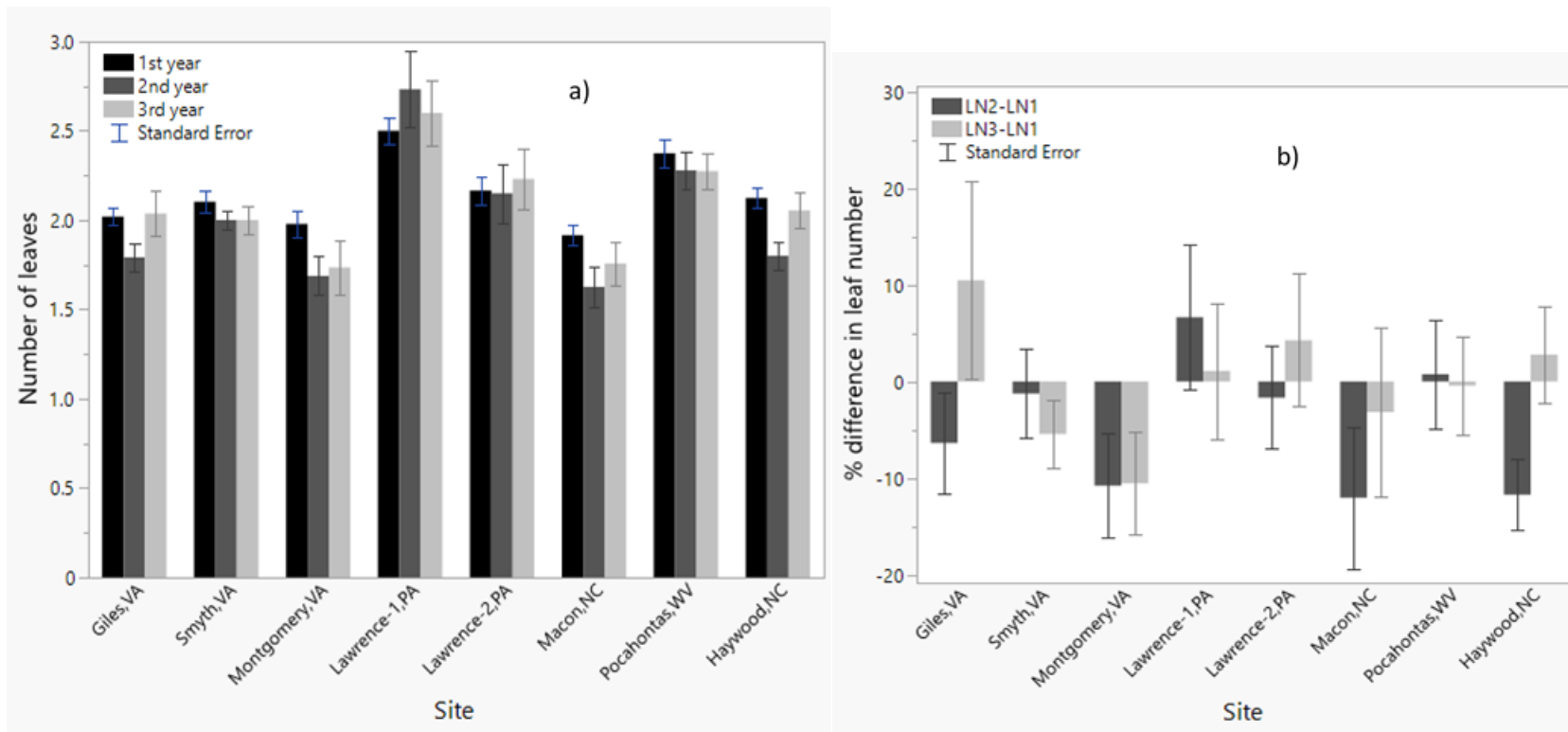


Figure 3-4: Average Number of leaves among ramp (*Allium tricoccum*) ecotypes collected from each site across Appalachia before transplantation (N1; p-value = 0.001), one year after transplantation (N2; p-value = 0.001), and two years after transplantation (N3; p-value=0.0001), and their change between years (Site p-value = 0.001, years p-value = 0.024 and site X year p-value = 0.4) Sixteen ramps were collected randomly from each location. b) the difference in leaf number in year 2 and year 3 from baseline data (p-value=0.3); Mean and standard bars are presented above each bar.

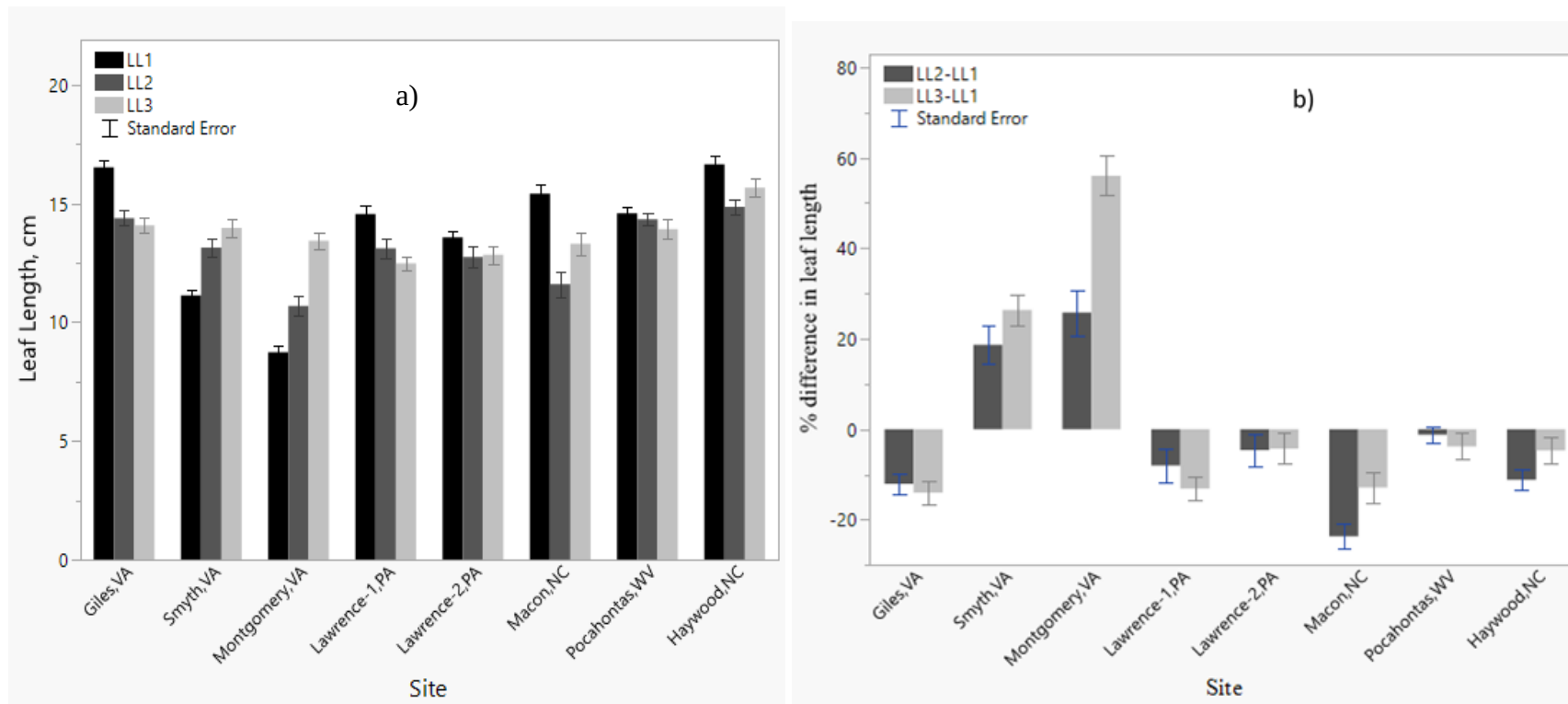


Figure 3-5: a) Mean leaf length of ramp (*Allium tricoccum*) ecotypes collected from each site across Appalachia before transplantation (LL1; p-value = 0.001), one year after transplantation (LL2; p-value = 0.001) and two year after transplantation (LL3; p-value=0.001) and their change between years (Site p-value = 0.0001, years p-value = 0.0006 and site X year p-value = 0.001). Sixteen ramps were collected randomly from each location. b) the difference in leaf length between from baseline (year 2- year 1 (LL2-LL1; p-value= 0.001) and year 3- year 1 (LL3-LL1; p-value=0.003)), Mean and standard bars are presented above each bar.

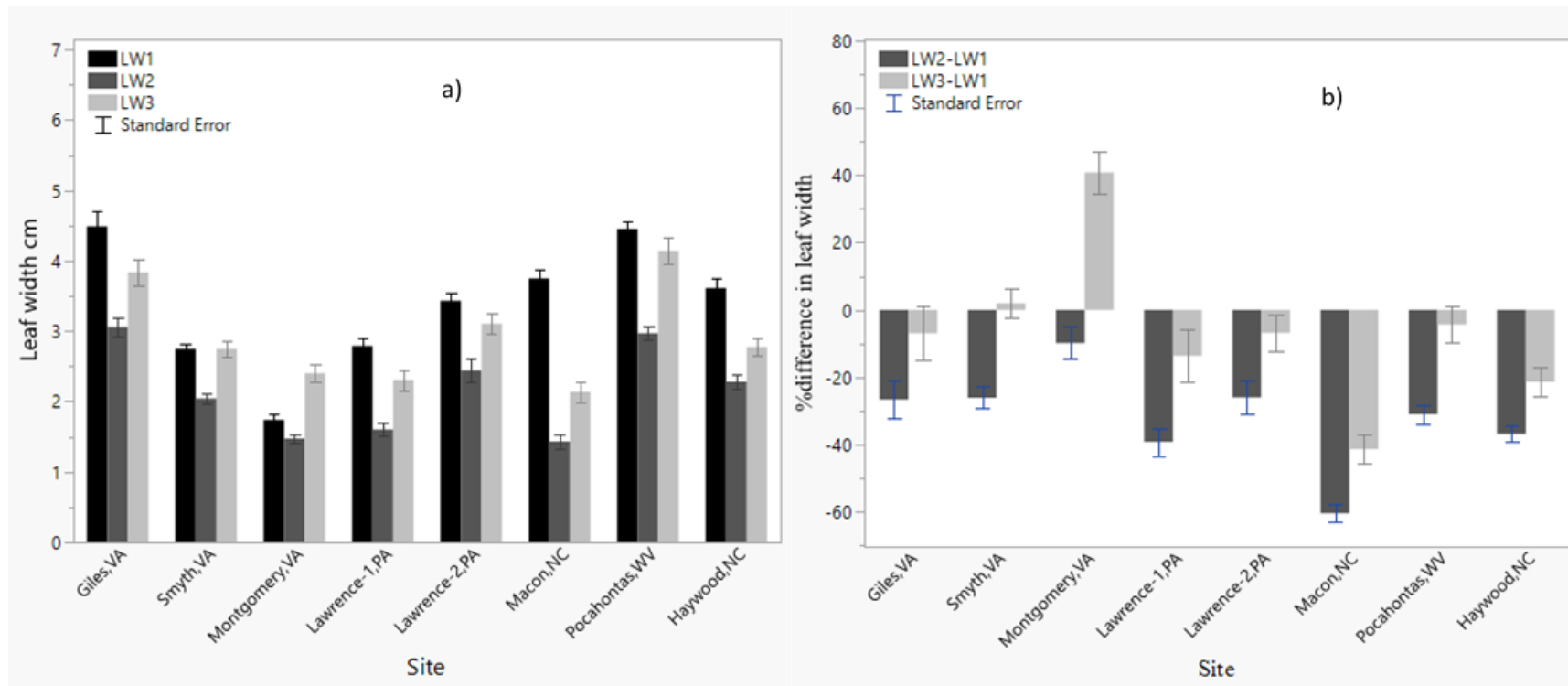


Figure 3-6: a) Average leaf width of ramp (*Allium tricoccum*) ecotypes collected from each site across Appalachia before transplantation (LW1; p-value = 0.001) and one year after transplantation (LW2; p-value = 0.001) and their change between years (Site p-value = 0.0001, years p-value = 0.0001 and site X year p-value = 0.0001). Sixteen ramps were collected randomly from each location. b) the difference in leaf width from baseline (year 2 - year 1 (LW2-LW1; p-value= 0.001) and year 3-year 1 (LW3-LW1; p-value=0.001)). Mean, and standard bars are presented above each bar

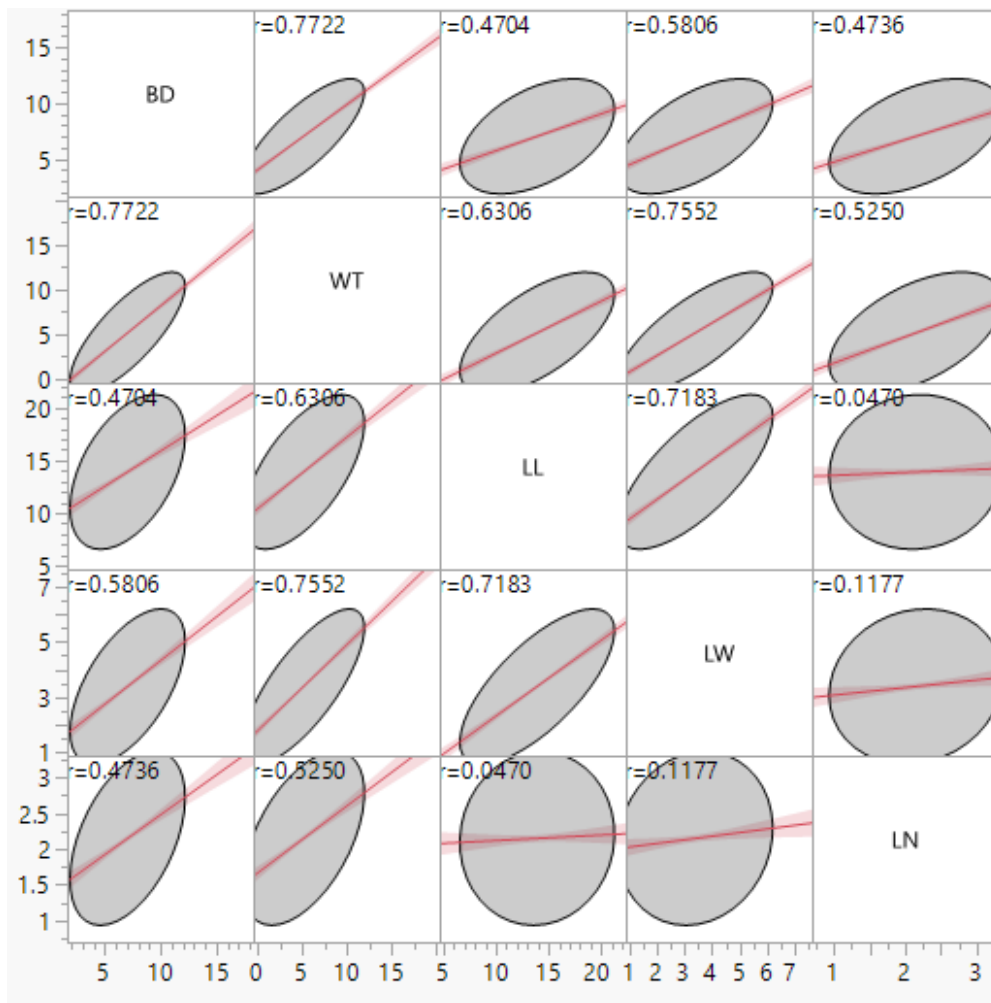


Figure 3-7: Scatter plot of correlations between five plant growth measures: Bulb Diameter (BD), Plant Weight (WT), Leaf Length (LL), Leaf Width (LW), and Leaf Number (LN).

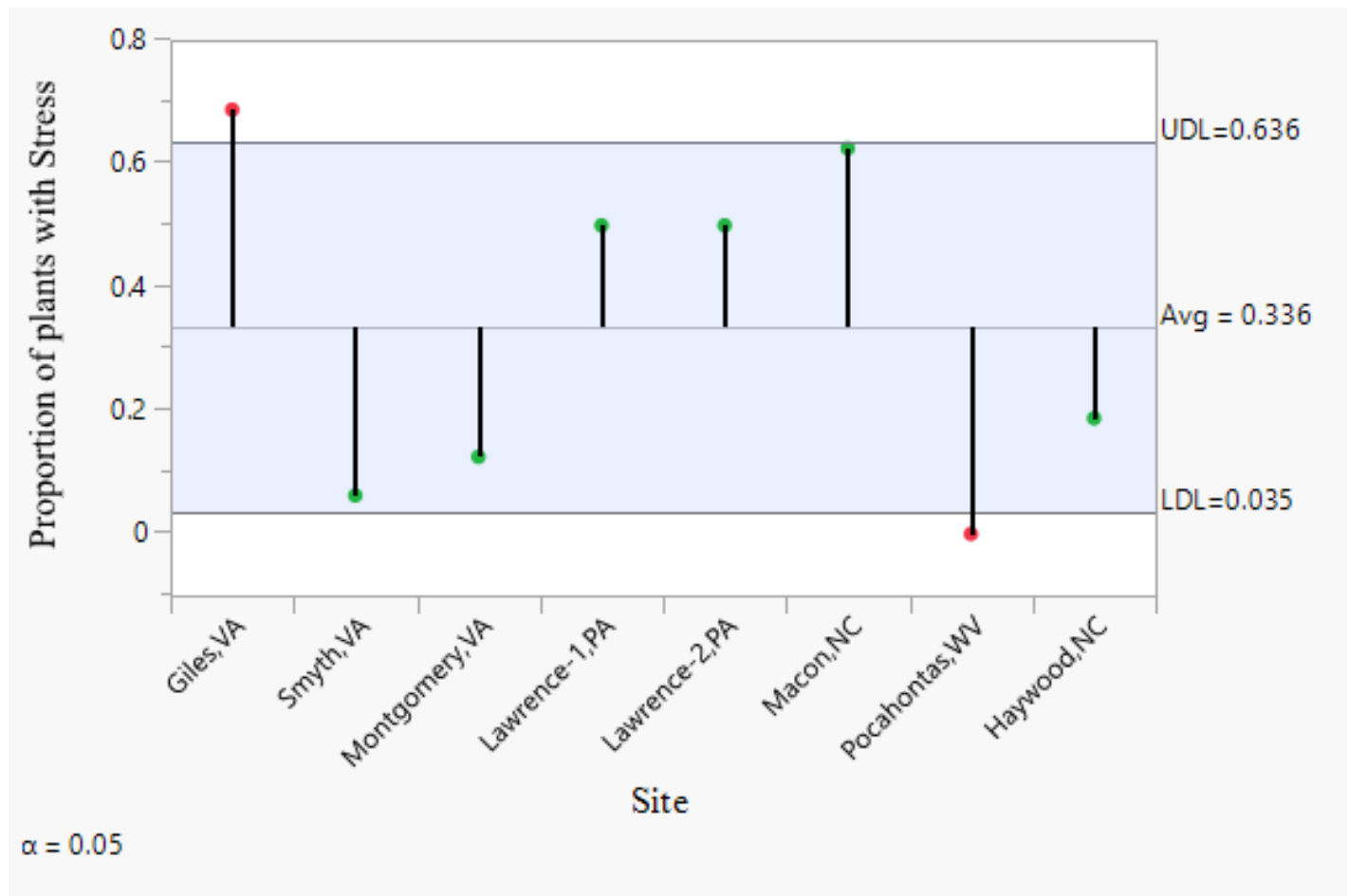


Figure 3-8: Stressed proportion of ramp (*Allium tricoccum*) ecotypes

Collected from each site across Appalachia after 1 month of transplantation, p-value = 0.001. Sixteen ramps were collected randomly from each location. The proportion of stressed ramps among total transplanted is displayed as an approximation to the binomial distribution (maximum stressed vs. less stressed) using the analysis of means for proportions (ANOMP), a multiple comparison method for testing if individual group proportions differ from the overall proportion. (LDL=Lower Decision Limit, UDL=Upper Decision Limit).

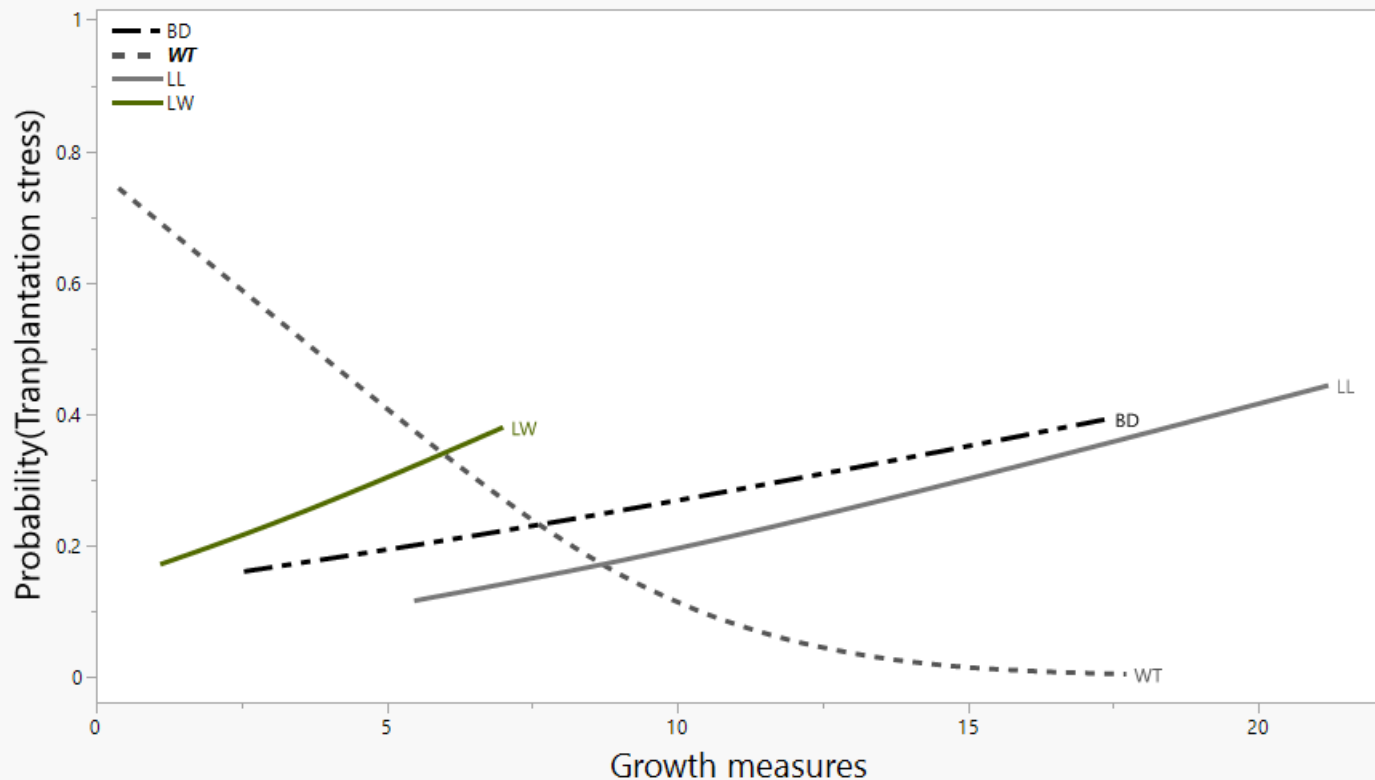
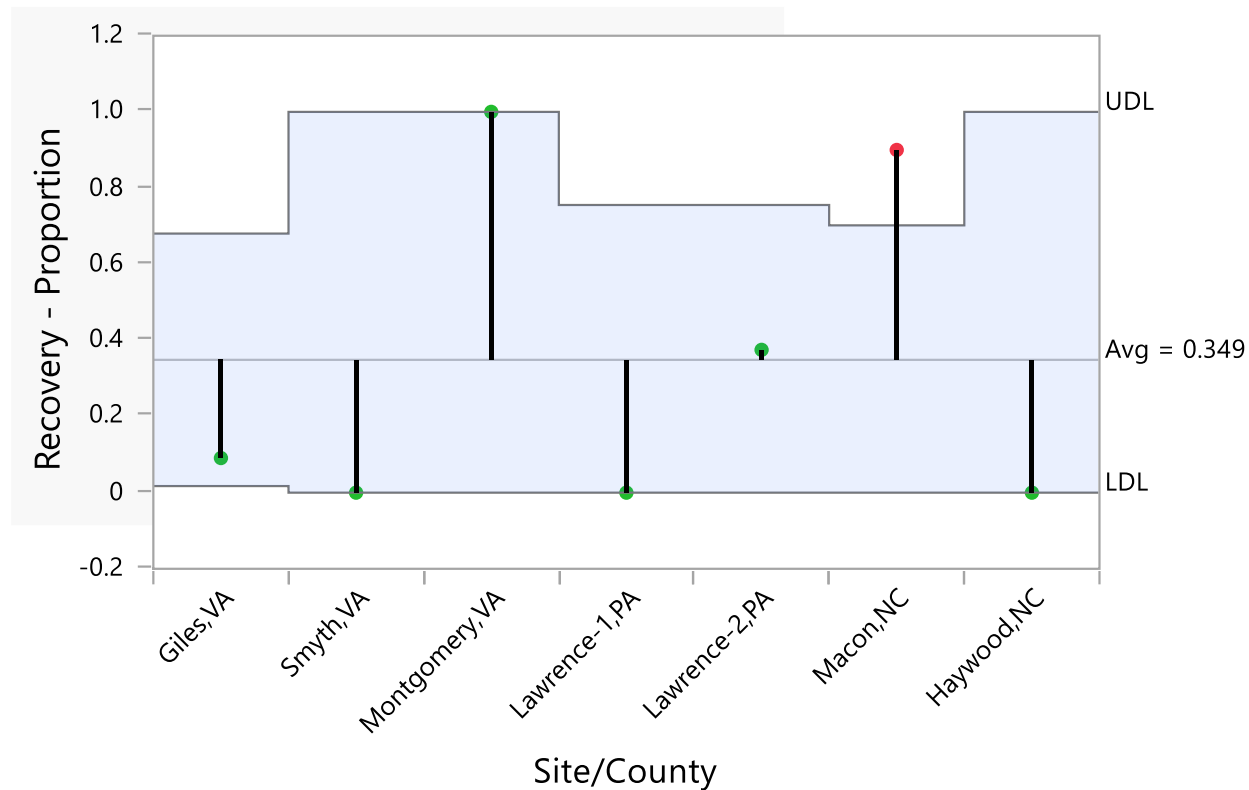


Figure 3-9: Interaction of Bulb Diameter (BD), Plant weight (WT), Leaf Length (LL), and Leaf width (LW) at baseline for ramp (*Allium tricoccum*) ecotypes Collected from eight different locations across Appalachia) on the stress right after one month of transplantation. Plant weight, with a significant p-value of 0.000001, is the most important predictor, followed by Leaf length ($p = 0.00005$) in the model, and Leaf Width (LW) and Bulb Diameter (BD) with significant p-value = 0.012 and 0.022 are also important predictors in the model. Sixteen ramps were collected randomly from each location. The influence of plant growth parameters (BD, LW, LW, and LL) at baseline with larger significant value was included in the Nominal Logistic Regression analysis, and the proportion of stressed ramps among total transplanted was calculated as binomial distribution (1=stressed and 0=not stressed). The stress probability grid table was produced using the probability profiler of the Nominal logistic Regression Model on JMP software and plotted against baseline growth measures (BD, WT, LL, and LW).



$\alpha = 0.05$

Figure 3-10: Recovery proportion of ramp (*Allium tricoccum*) ecotypes in 2nd year of transplantation among the stressed ramps after 1 month of transplantation, p-value = 0.02. Sixteen ramps were collected randomly from each site across Appalachia; transplanted and stressed plants were counted after one month of transplantation for each ramp ecotype. The proportion of recovered ramps *among total stressed in year 1 from each site is displayed as an approximation to the binomial distribution (recovered vs. not recovered)* using the analysis of means for proportions (ANOMP), a multiple comparison method for testing if individual groups proportions differ from the overall proportion. (LDL=Lower Decision Limit, UDL=Upper Decision Limit). * The data from Pocahontas County is absent here because there were no stressed plants from Pocahontas County in year 1 to be included in the recovery data.

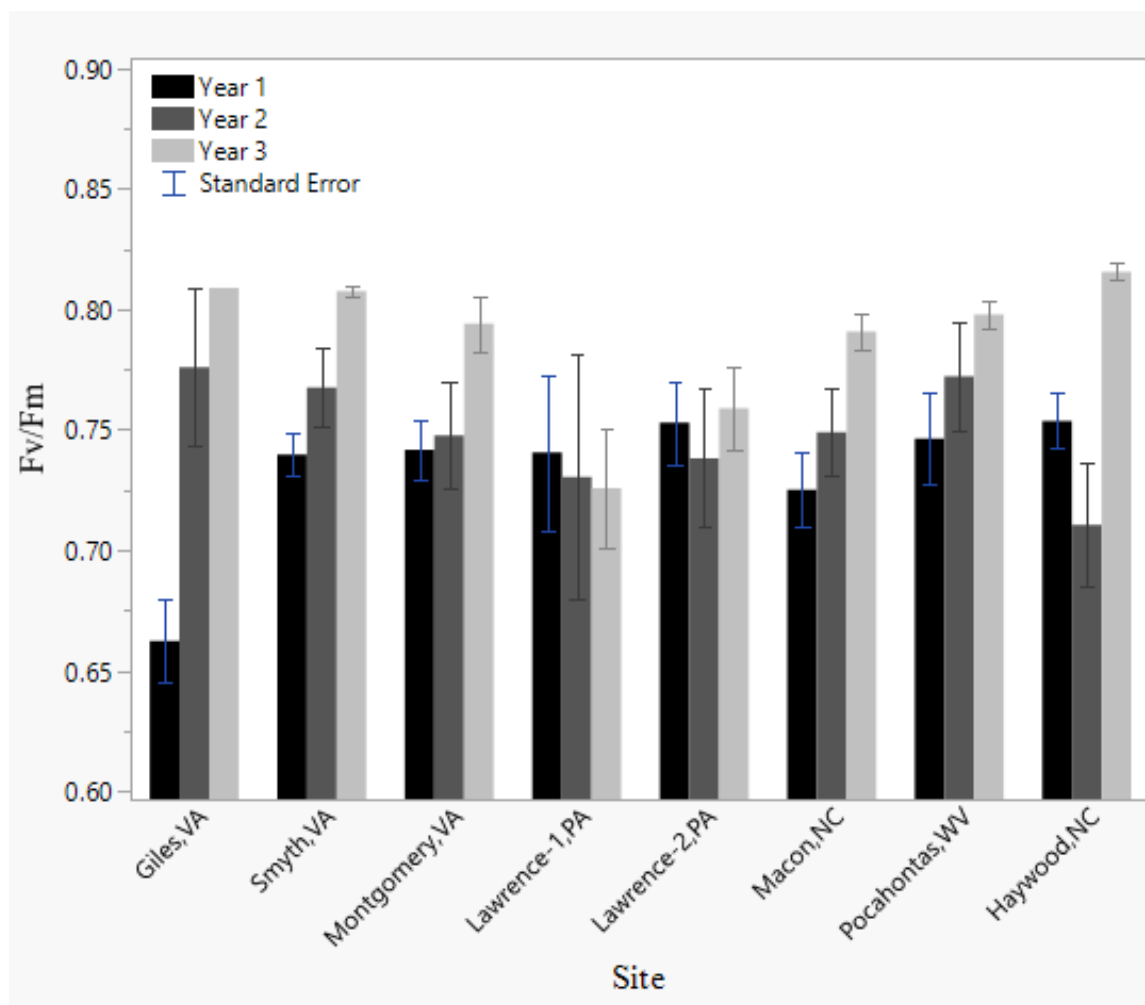


Figure 3-11: The average value of Fv/Fm ratio among the ramp (*Allium tricoccum*) ecotypes collected from each site across Appalachia after 1 month of transplantation (year 1, p-value = 0.11), after a year of transplantation (Year 2, p-value = 0.1), and after 2 years of transplant (Year 3, p-value= 0.003) and their change between years (Site p-value = 0.39, year p-value = 0.0001 and site X year p-value = 0.11). Sixteen ramps were collected randomly from each location, and Fv/Fm values were measured to examine the stress in plants. Mean, and standard bars are presented above each bar

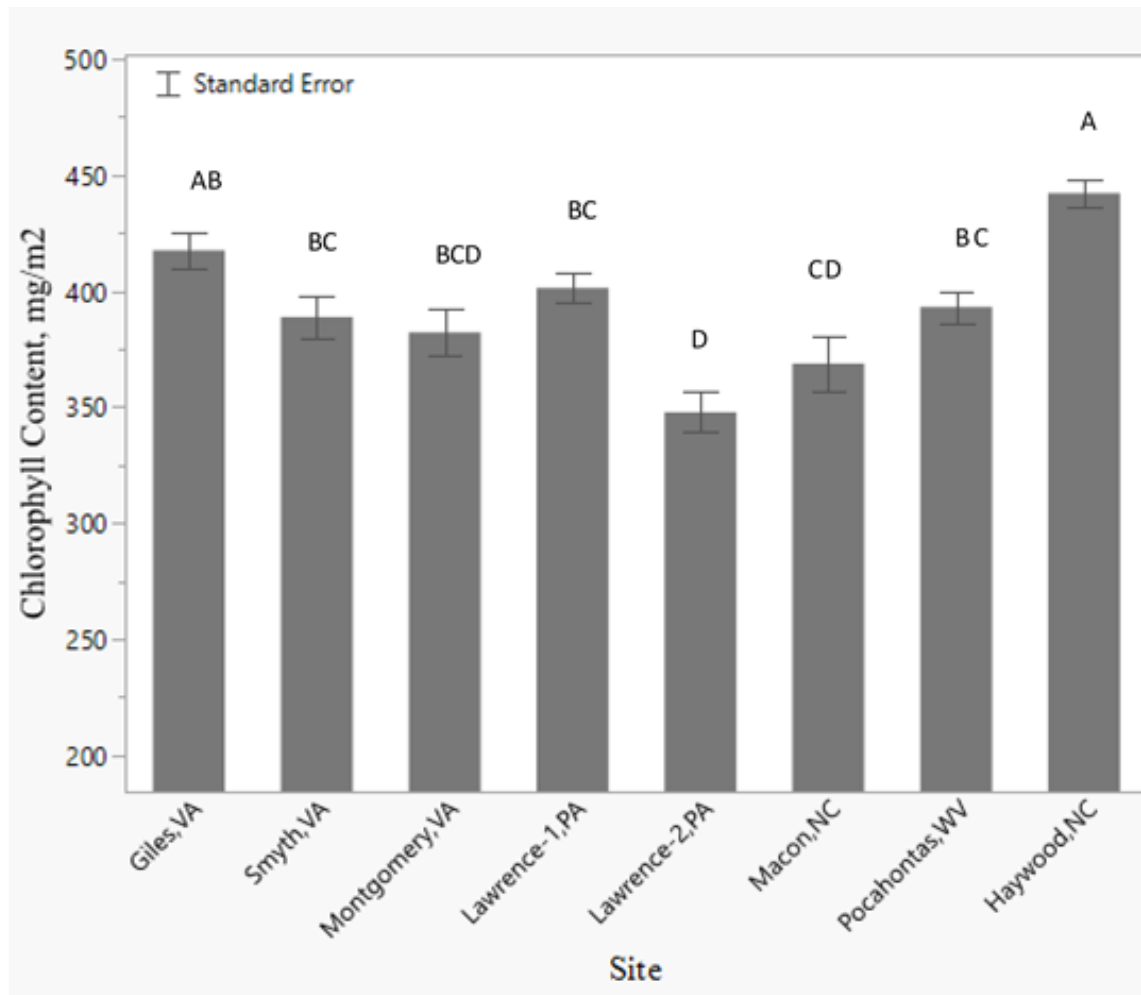


Figure 3-12: The average chlorophyll Content among the ramp (*Allium tricoccum*) ecotypes

collected from each site across Appalachia after 2 years of transplantation (year 3, p-value = 0.001). Sixteen ramps were collected randomly from each location, and chlorophyll content values were measured to examine the stress in plants. Mean, and standard bars are presented above each bar

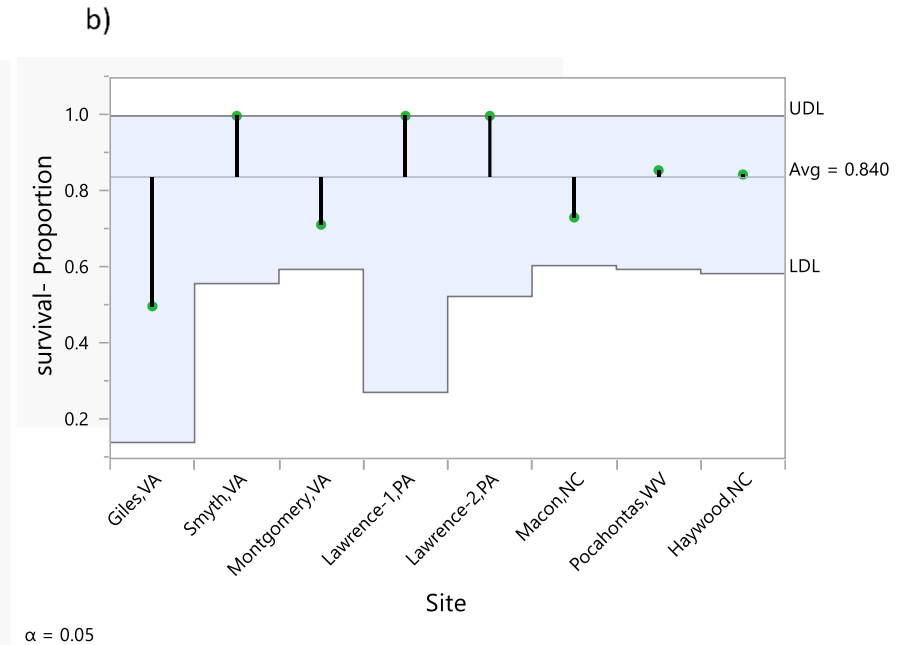
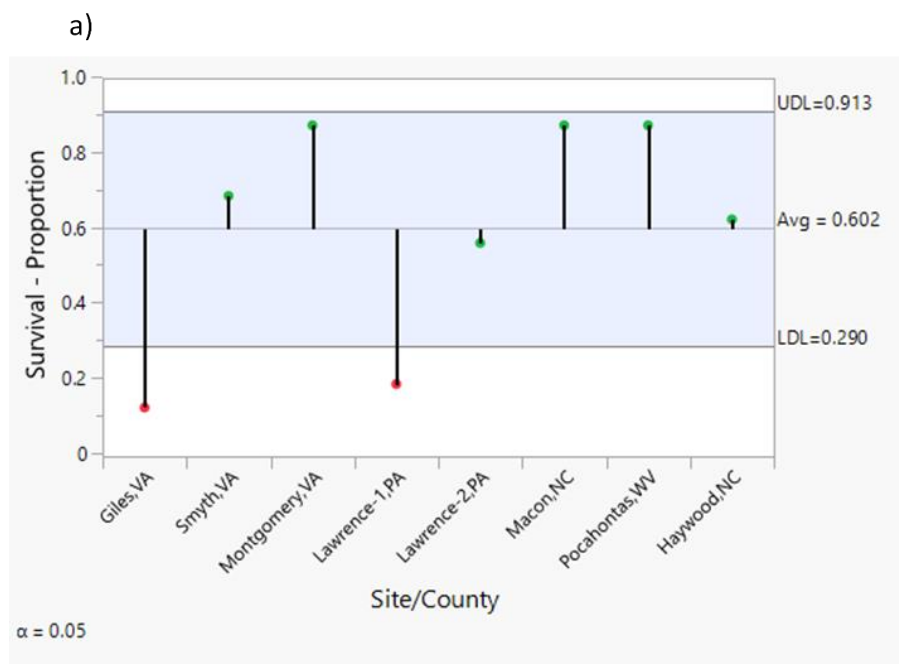


Figure 3-13: Survival proportion of ramp (*Allium tricoccum*) ecotypes

They were collected from each site across Appalachia after a year of transplantation. Sixteen ramps were collected randomly from each location. The proportion of survived ramps among total transplanted is displayed as an approximation to the binomial distribution (Dead vs. survived). (LDL=Lower Decision Limit, UDL=Upper Decision Limit). Survival in year 2 a) varied by site ($p = 0.001$) and was <20% for bulbs from Giles Co. and Lawrence Co. in year 3 b) survival of the remaining bulbs did not differ ($p = 0.3$) by the site.

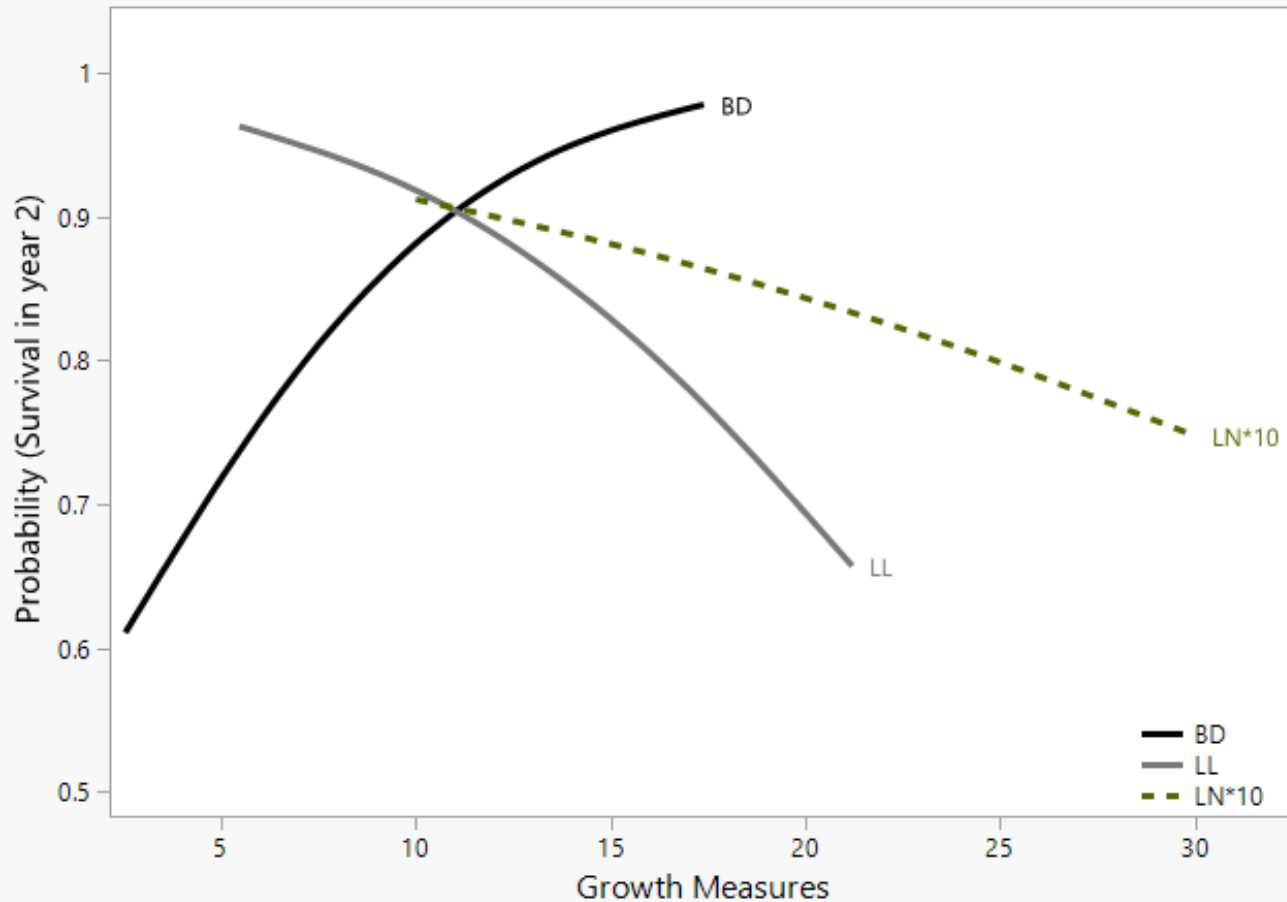


Figure 3-14: Interaction of Bulb Diameter (BD) and Leaf Length (LL1) of ramp (*Allium tricoccum*) ecotypes Collected from eight different locations across Appalachia) on the survival of ramps after a year of transplantation. LL1, with a significant p-value of 0.0001, is the most important predictor in the model, BD with a significant p-value = 0.002, and Leaf number (LN) with a significant p-value = 0.008. Sixteen ramps were collected randomly from each location. The influence of plant growth parameters (BD, LN, and LL) at baseline with a larger significant value was included in the Nominal Logistic Regression analysis, and the proportion of survived ramps among total transplanted was calculated as binomial distribution (1=Survived and 0=Dead). The survival probability grid table was produced using the probability profiler of the Nominal logistic Regression Model on JMP software and plotted against baseline growth measures (BD, LN, and LL) *LN was multiplied by 10 to match the scale of other parameters).

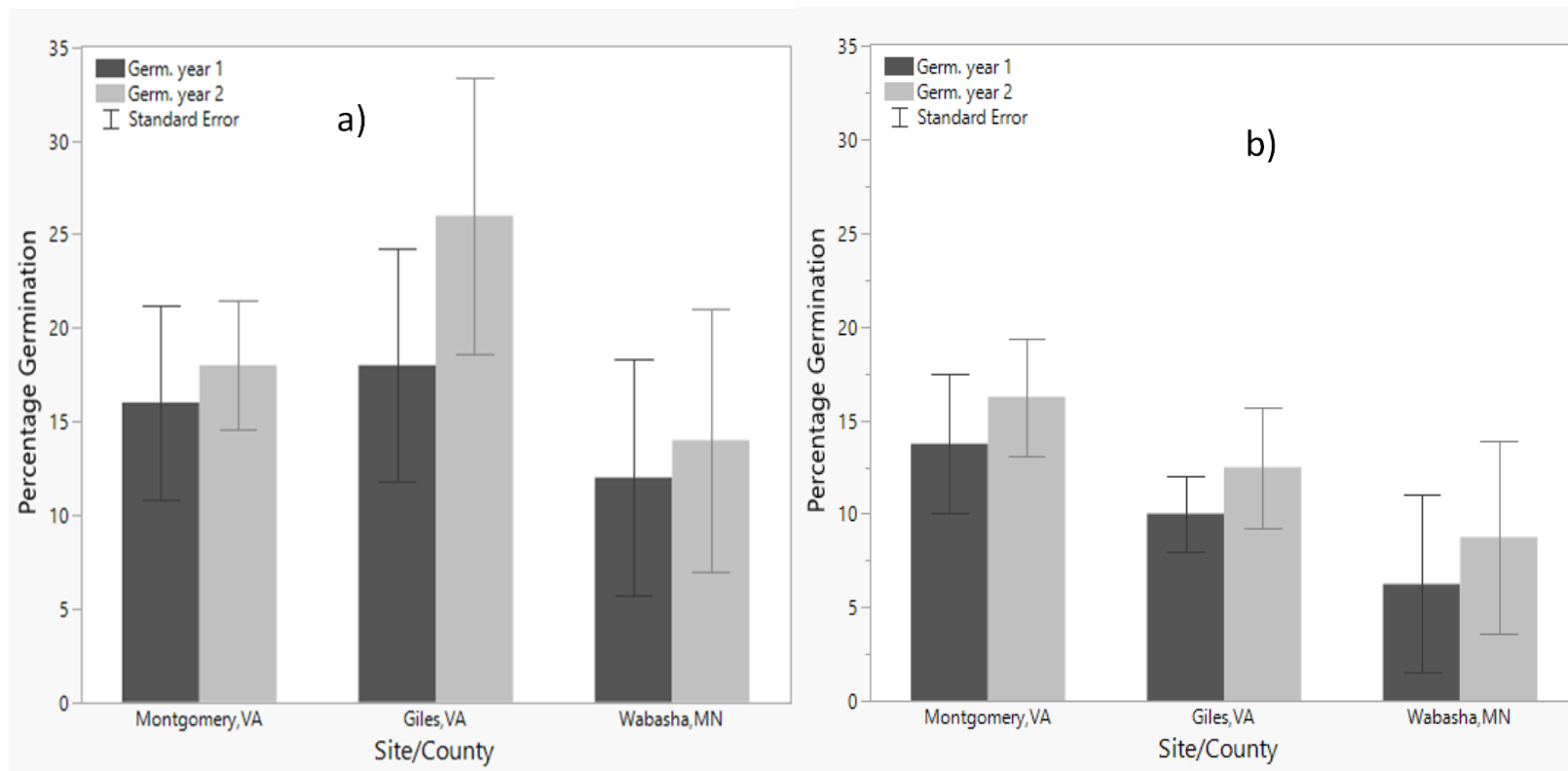


Figure 3-15: Seed germination percentage of ramp (*Allium tricoccum*) seeds collected from three seed sources and sowed to our experimental site in Roanoke County, VA. A) in Artificially Constructed Raised Bed (The germination percentage was measured after the first germination (Germ, year 1, p-value = 0.39) and one year after first germination (Germ, year 2; p-value = 0.44)) and b) in Wild Simulated Plot (The germination percentage was measured after first germination (Germ, year 1, p-value = 0.74) and one year after first germination (Germ, year 2; p-value = 0.43)). One Hundred ramp seeds from each seed origin (total of 300) were Sowed in ACRB, and eighty ramp seeds from each seed origin (total of 240) were Sowed in WSP. Mean, and standard bars are presented above each bar.



Figure 3-16: Germinated seeds after 6 months of stratification

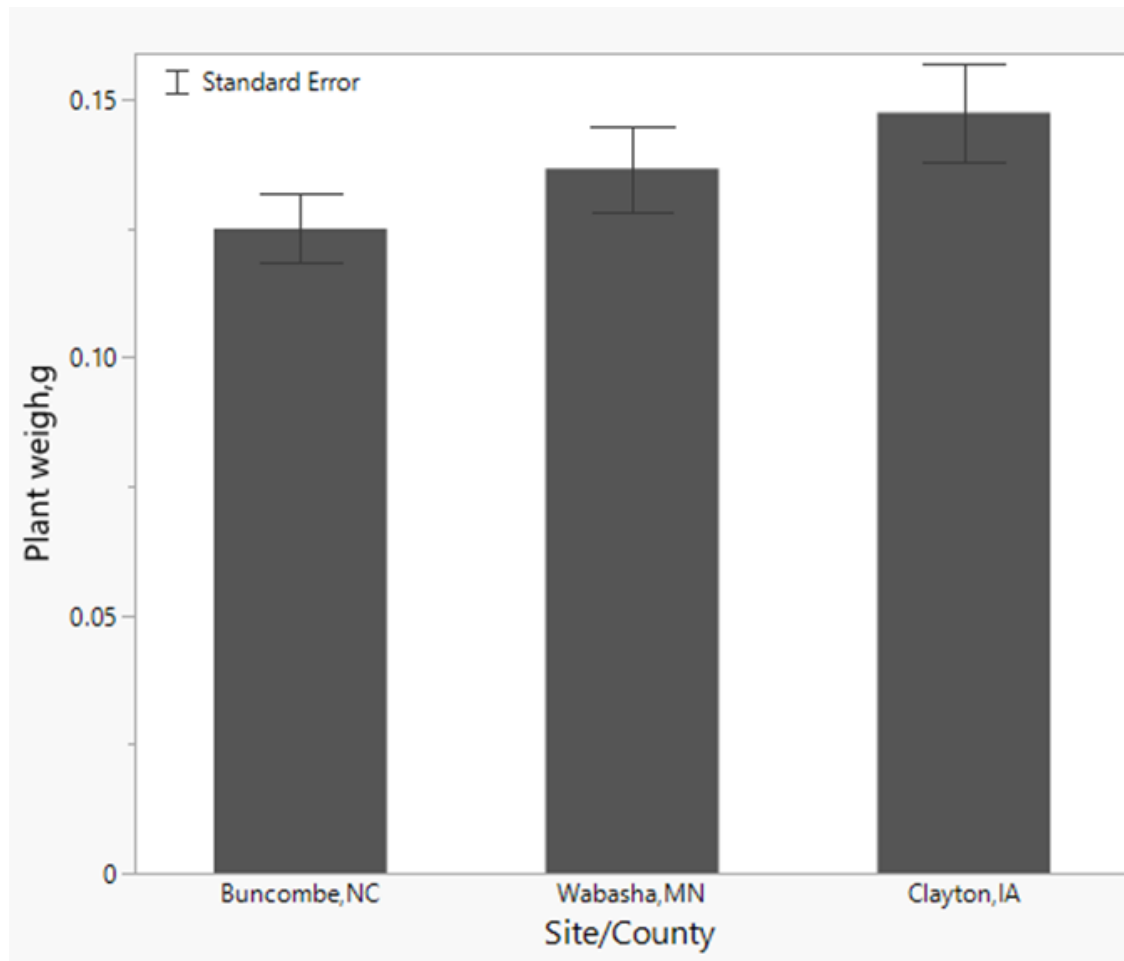


Figure 3-17: Mean plant weight (WT) of commercial ramp (*Allium tricoccum*) seedlings germinated from three different seed sources in a nursery in Buncombe County, NC, and transplanted to our experimental site in Montgomery County, VA. The plant weight was measured before transplantation and is not different among seed sources (p-value = 0.15)

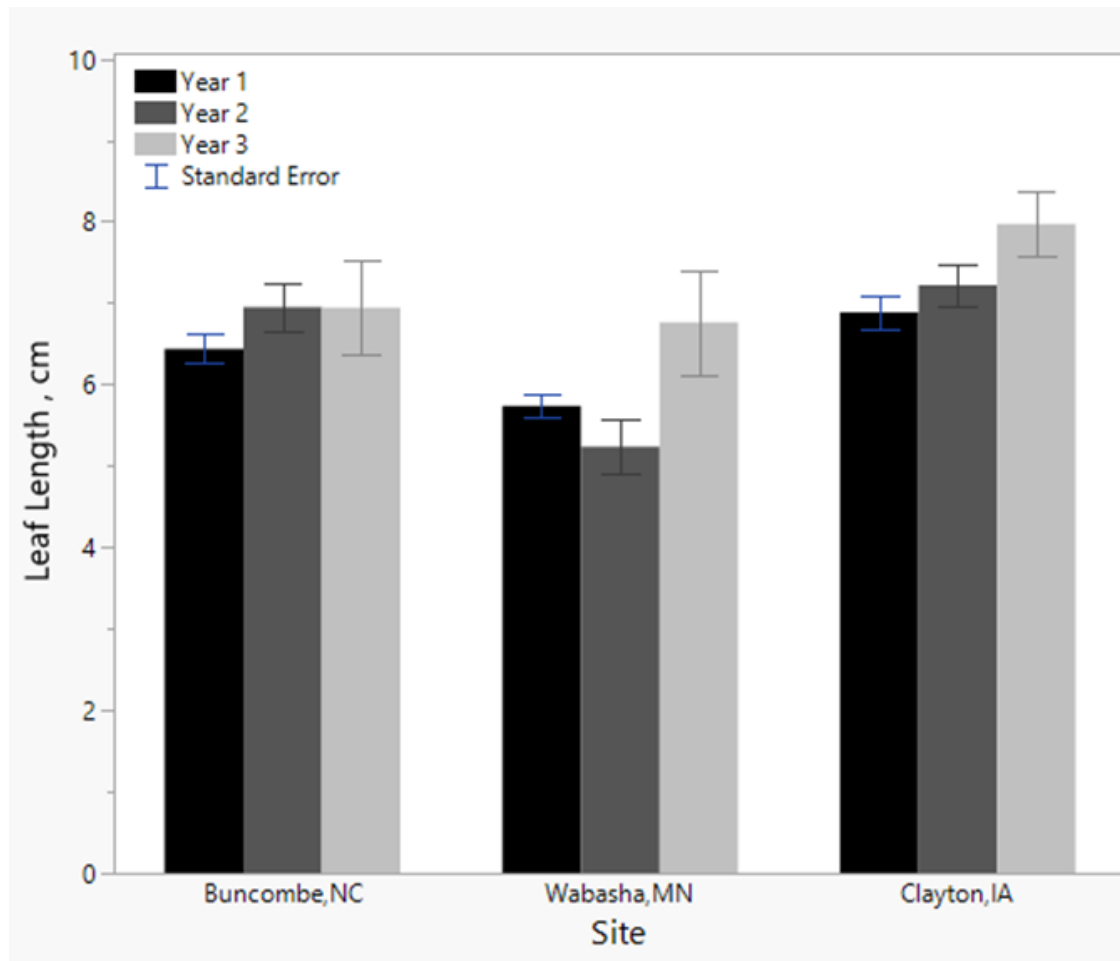


Figure 3-18: Mean leaf length of commercial ramp (*Allium tricoccum*) seedlings germinated from three different seed sources in a Buncombe County, NC nursery and transplanted to our experimental site in Montgomery County, VA. The leaf lengths were measured before transplantation (LL1; p-value = 0.0004), one year after transplantation (LL2; p-value = 0.025), and two years after transplantation (LL3; p-value = 0.21), and their change between years (Site p-value = 0.008, years p-value = 0.0011 and site X year p-value = 0.056). Twenty-four (1-year-old) ramp seedlings sourced from each seed origin (72) were transplanted from the nursery. Mean, and standard bars are presented above each bar.

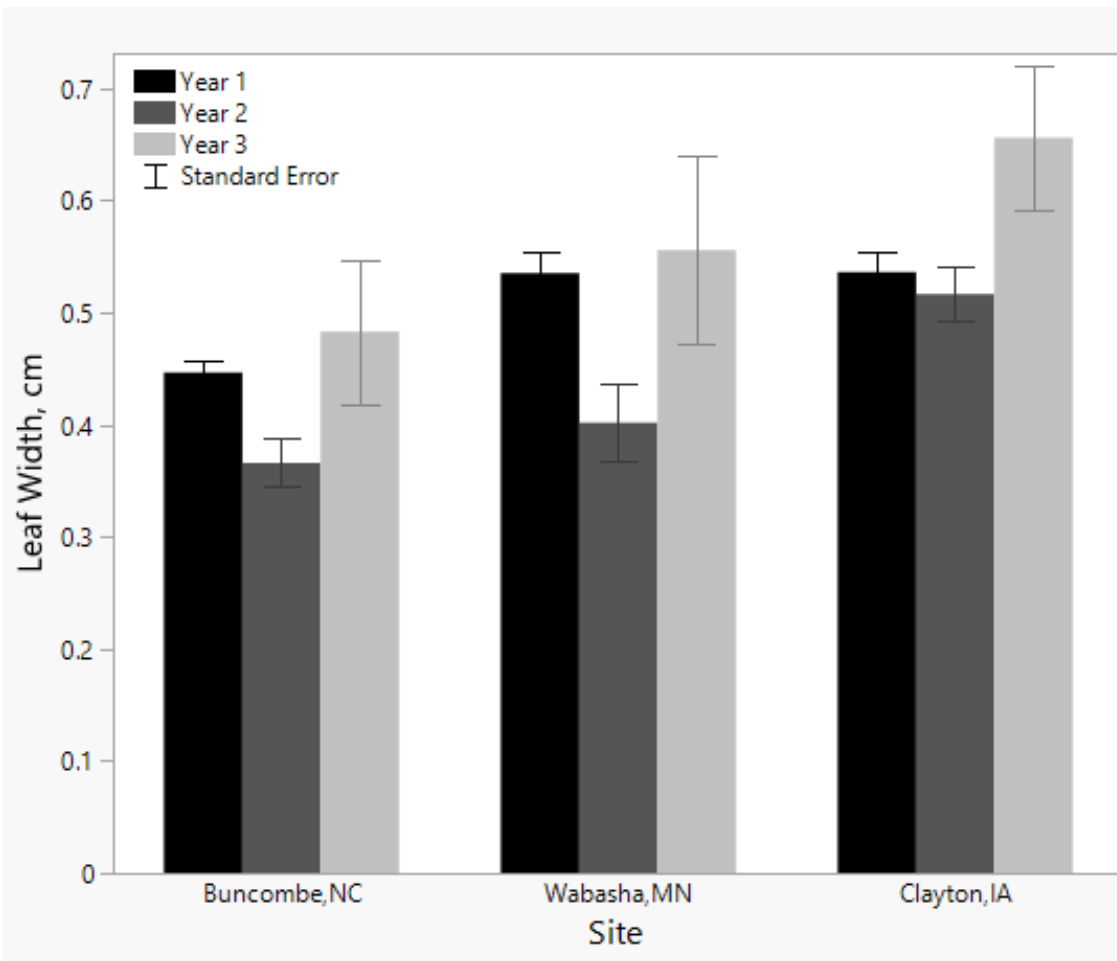


Figure 3-19: Mean leaf width of commercial ramp (*Allium tricoccum*) seedlings germinated from three different seed sources in a Buncombe County, NC nursery and transplanted to our experimental site in Montgomery County, VA. The leaf width was measured before transplantation (LW1; p-value = 0.0001), one year after transplantation (LW2; p-value = 0.048), and two years after transplantation (LW3; p-value = 0.21), and their change between years (Site p-value = 0.0012, years p-value = 0.0014 and site X year p-value = 0.7). Twenty-four (1-year-old) ramp seedlings sourced from each seed origin (72) were transplanted from the nursery. Mean, and standard bars are presented above each bar.

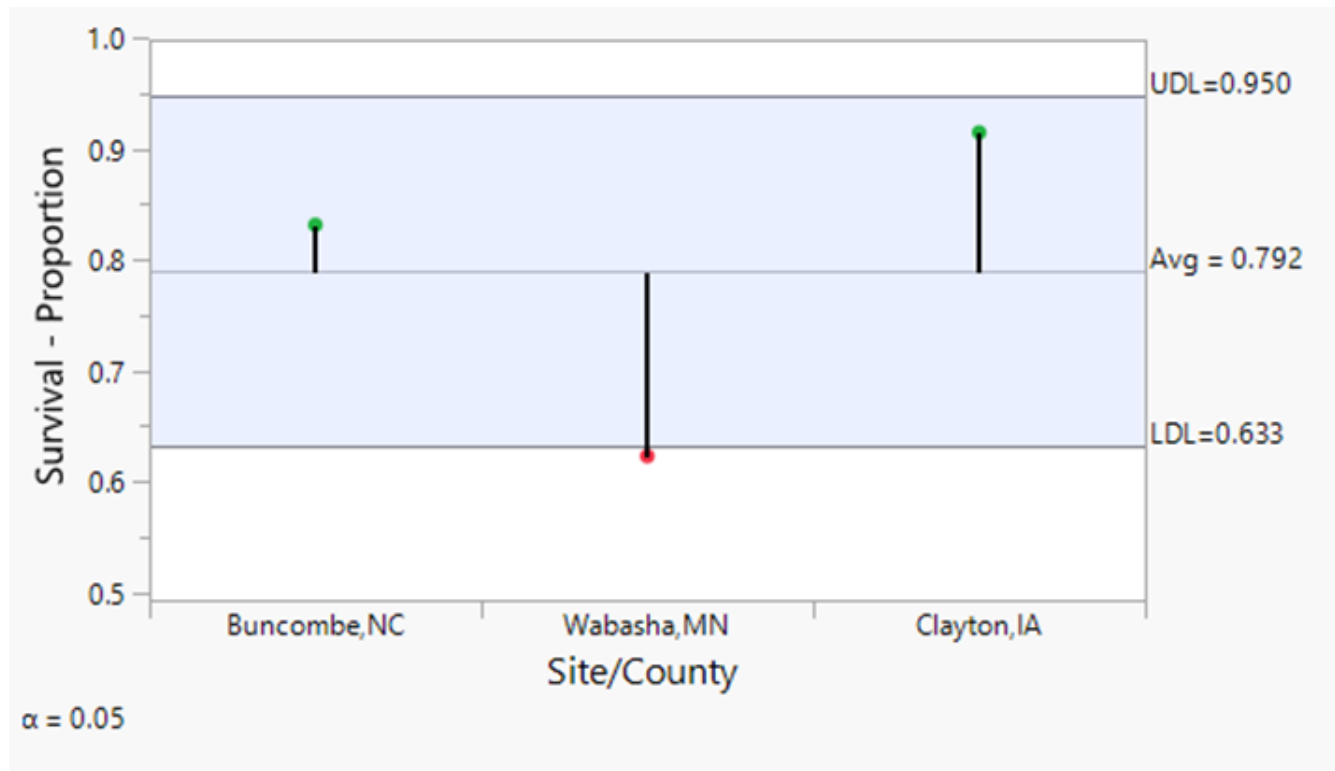


Figure 3-20: Analysis of Means for Proportions for the Survival of ramp seedlings in 2nd Year of Transplantation

The proportion of surviving seedlings among the total transplanted is displayed as an approximation to the binomial distribution (Dead vs. survived). (LDL=Lower Decision Limit, UDL=Upper Decision Limit). Survival in year 2 a) varied by site ($p = 0.002$) and was <65% for seedlings from Wabasha Co.

4. CHAPTER IV

ALLIUM TRICOCCUM GROWTH RESPONSES TO ARBUSCULAR MYCORRHIZAL FUNGI INOCULATION

ABSTRACT

Ramp (*Allium tricoccum*) is a spring-ephemeral forest herb and a wild-foraged plant of significant cultural value in Appalachia. The plant has gained popularity as a gourmet food beyond the region over the last 25 years, with supply largely met through wild harvest. Rising plant demand and harvest threaten the sustainability of these edible forest products. Forest farming may be a viable option to meet increasing global demand and conserve native populations, but little information is available regarding best practices for commercial production. Ramps typically grow in low-nutrient, high-organic matter soils, and their production may be facilitated through association with mycorrhizal fungi. Mycorrhizae improve plant growth and yield through increased nutrient uptake, particularly in nutrient-limited environments, and they provide biological control of root pathogens. Thus, ramps in forest farming systems may improve moisture and nutrient uptake and resilience to drought and disease stressors, but this relationship has received limited study. This work explored arbuscular mycorrhizal fungi (AMF) association with ramps to determine whether AMF can improve ramp growth and survival in forest farming systems. Ramps for the study were collected from eight locations across seven counties within four states, encompassing diverse climates, elevations, and latitudes – thus with potential for the difference in plant material (i.e., as potential ecotypes) and soil microbial communities. Soil properties (pH, nutrients, and texture) from these sites were assessed, and a commercial lab determined the presence of mycorrhizal species. Soil samples from the different sites had numerous (963) fungal species, with dominant species from Ascomycota, Basidiomycota, and Mortierellomycota phyla. However, only seven AMF species were found at very low frequency (0.06%), indicating low AMF abundance across

ramp habitats. For some ecotypes, treating ramp bulbs with commercial mycorrhizae (Atriva 500) increased ramp leaf length (Ecotype x trt interaction; $P = 0.03$). This inconsistent response highlights the need to understand and consider ecotype x AMF x environmental interactions. Mycorrhizal treatment also increased (Ecotype x trt interaction; $P \leq 0.003$) ramp survival and stress tolerance after transplanting. Mycorrhizae did not impact ramp growth or survival of ramp seedlings, however. This preliminary work suggests that AMF inoculation may provide several potential benefits for the growth and conservation of ramps when transplanting mature plants in forest farming systems. Future research should i) investigate the response of ramp ecotypes to a broader array of AMF strains across environments, ii) identify superior AMF for ramp forest farming; and iii) elucidate underlying mechanisms of AMF benefits to ramp survival and growth.

Keywords: Arbuscular Mycorrhizal Fungi (AMF), transplantation stress, stress recovery, photoinhibition, plant survival.

INTRODUCTION

Allium tricoccum, commonly known as ramps or wild leeks, are perennial forest herbs that serve as seasonal food sources and cultural symbols throughout the Appalachia region (Boehm, 2015). Their culinary value has garnered increasing national attention (Baumflek & Chamberlain, 2019), leading to elevated ramp harvests from wild populations and threatening their survival in certain areas. Developing improved cultivation techniques and providing enhanced technical support for sustainable forest farming practices is essential to conserve native populations and meet the growing consumer demand for ramps,

Ramps emerge as shoots briefly in spring before canopy tree leaves emerge when light availability is highest on the forest floor (Hewins et al., 2015). The bulbs of ramps persist throughout the year and act as the primary storage organ for the plant's nutrients. In autumn, new roots form on the rhizome at the bulb's base and follow an annual life cycle (Nault and Gagnon, 1988; Lapointe, 2001); and mycorrhizal colonization can influence resource capture.

Ramps, together with other forest-dwelling herbaceous plants such as *Erythronium Americanum* and *Podophyllum peltatum*, form mutualistic relationships with arbuscular mycorrhizal fungi (AMF) (Brundrett and Kendrick, 1990; DeMars, 1996; Lapointe and Molard, 1997; Watson et al., 2002). AMF, belonging to the Phylum Glomeromycota (Redecker et al., 2000; Schußler et al., 2001), establish mutually beneficial associations with approximately 90% of vascular plants (Bagyaraj and Reddy, 2000). These associations are often obligate, as AMF enhances plant growth by facilitating the uptake of essential nutrients, such as phosphorus and nitrogen (Bagyaraj and Reddy, 2000), while receiving carbohydrates from the plant for fungal development (Feng et al., 2019).

The commercial application of AMF in crop systems has garnered significant interest due to its potential to improve plant production and health while reducing nutrient and plant-protectant inputs. AMF's extrametrical hyphae, which are filamentous fungal hyphae extending from the mycorrhizae, effectively expand the root system, enhancing the surface area and facilitating efficient water and nutrient uptake, particularly in phosphorus-deficient soils (Bagyaraj and Reddy, 2000). Additionally, AMF can contribute to the biological control of root pathogens, biological nitrogen fixation, and the plant's ability to withstand abiotic stresses (Bagyaraj, 1984; Chandrashekara et al., 1995). Plants colonized by AMF exhibit improved moisture and nutrient uptake and enhanced resilience to drought stress compared to non-colonized plants (Bowles et al., 2017).

Allium species, including *A. tricoccum*, exhibit sensitivity to AMF application due to their less developed root systems (Gashaw Deressa and Schenk, 2008; Greenwood et al., 1982; Sanders et al., 1992). With over 900 species, primarily distributed in the Northern Hemisphere, Allium is recognized as one of the more diverse genera of flowering plants. Numerous Allium species, such as garlic (*A. sativum*), onion (*A. cepa*), leek (*A. porrum*), shallot (*A. cepa* L. Aggregatum group), chives (*A. shoenoprasum*), and bunching onion (*A. fistulosum*), hold significance in human nutrition (Putnik et al., 2019).

The colonization of ramp roots by AMF has been observed, with mycorrhizal structures present in up to 60% of the root length (DeMars, 1996). However, limited information on the interactions between ramps and AMF and the benefits of AMF colonization on aboveground ramp growth is available. The efficiency of a plant's utilization of available AMF is influenced by various factors, including the specific AMF species, plant genotype, soil nutrient levels, and environmental conditions (Gosling et al., 2016; Yang et al., 2015). Therefore, further research is needed to

understand the diversity of AMF within ramp study sites and to bridge the knowledge gap concerning the role of AMF in ramp growth and survival.

Given the limited previous work in this area, our research aims to investigate the potential benefits of commercial AMF inoculation on ramp survival and recovery from transplant stress, as well as its impact on ramp growth. We hypothesize that the inoculation of ramps with commercial AMF could enhance their survival, improve recovery from transplant stress, and promote overall growth. By enhancing ramp growth in forest farming systems, we can potentially contribute to the conservation of ramps while meeting the increasing global demand for this plant.

MATERIALS AND METHODS

Three experiments were conducted to test the response of ramps to AMF. The first experiment involved mature ramps collected from appropriate gradients across Appalachia and inoculated with AMF at transplanting. The second experiment tested ramp seeds collected from two wild locations in Appalachia and one commercial source from Minnesota. Lastly, seedlings obtained from a nursery with three different seed origins (NC, MN, and IA) were also included in the study and tested similarly.

Experiment 1. Ramp bulb ecotype response to AMF

Sample collection

Ramp samples for this study were collected from eight locations in the Appalachian region of the eastern United States Figure 3-1. Four hundred bulbs (50 bulbs per site) were dug randomly from larger patches at each location in Spring 2021. The ramp and attendant soil samples were collected by ramps experts in the respective locations and shipped overnight, keeping them moist in an ice box.

Soil nutrient analysis and Ramps-fungal association in native habitat

One composite soil sample was collected from each native site when ramp bulbs and seeds were collected. After a rainfall event, a minimum of four days before collecting samples was given to ensure the soil was as dry as possible. Soil samples were collected, removing the top layer with leaves, rocks, and sticks. Four sub-samples, taken at a fixed linear distance of 1.2 meters in cardinal directions from the randomized point of the ramp patch, were collected with a soil probe to 15 cm depth. The subsamples were thoroughly mixed to create a composite sample and transferred into plastic sealable bags to avoid drying.

Soil nutrients: P, K, Ca, Mg, Zn, MN, Cu, Fe, and % OM for all samples were determined following the procedures of Maguire and Heckendorn (2019) at the Virginia Tech Soil Testing Laboratory.

A portion of each soil sample was placed in plastic bags, sealed, and shipped overnight to protect the mycorrhizal spores in the soil samples. Soil samples from each of the eight ramp sampling sites were sent to a commercial lab in California, USA (BIOMEMAKER) and analyzed for fungal and bacterial species presence using 16 rRNA and the ITS fungal identification method. There the microbiome analysis such as bio sustainability of the microbial community in the soil sample (bio-diversity, functionality, and stress resistance), major (C, N, P, and K) and minor (Fe, Zn, Mn, S, Ca, Cu, Mg and Cl) soil nutrition available for plants based on the microbial mobilization of these compounds, disease risks, crop health, and stress adaptation were analyzed for each soil sample. The fungal data provided were screened manually for the presence of species from class Glomeromycota to determine the presence of AMF and their diversity among the sampling sites.

Experimental Site Preparation, Transplantation, and Mycorrhizal Experiment:

A raised bed (1.22 × 2.44 m) was prepared with treated lumber in Christiansburg, VA. The bed was filled with a standard growing medium of 60% topsoil, 30% compost, and 10% potting mix purchased from a local supplier.

Among the 50 ramp plants collected from each site, 48 were transplanted for three treatments to assess the impact of commercial AMF inoculum on ramp growth. For the positive control, 16 bulbs were planted in their natural state. The negative control involved treating 16 plants with Mancozeb fungicide (1,2-Ethanediybis(carbamodithioato) (2-)) manganese zinc salt) to eliminate any existing AMF. Mancozeb, a non-specific contact fungicide effective against various fungal pathogens, including potato blight, tomato leaf spot, and downy mildew (Gullino *et al.*, 2010), was

used by mixing 3 g of its powder with 1 liter of water and immersing the ramp roots before planting. The remaining 16 plants were inoculated with commercial AMF (Atriva 500, Itapollina USA, Inc., Anderson, IN), which contained over 7,000 spores each of *Funneliformis mosseae* and *Rhizoglyphus irregularis* AMF. The inoculum was applied as a root drench at the time of transplant in April 2021. Ramps were planted 5 cm apart within pre-delineated plots, and the study followed a randomized complete block design with four replicates.

Bulb morphological characteristics with AMF

Plants' morphological measures (leaf length and width, leaf number, plant weight, and bulb diameter) were recorded before treatment application and transplanting. Leaf length and width measurements were facilitated using gridded paper, and bulb diameter was measured with digital calipers (Harbor Freight, Pittsburg, PA). Leaf lengths and widths were measured in years 2 and 3 to examine the effect of AMF on their growth.

Bulb Physiological Characteristics with AMF

Transplantation stress and recovery

Ramps ecotypes within each treatment were examined for signs of transplantation stress on May 3, 2021, less than a month after transplanting. At that time, some ramps' leaves were already wilted and starting to senesce. Chlorophyll fluorescence readings were taken on each plant with a portable fluorometer (OS-30, OPTI-SCIENCES, Hudson, NH, USA). Fluorescence measures were used as a proxy estimate of plant stress. The plants with '0' Fv/Fm values were considered stressed as a response to transplanting.

Furthermore, some plants scored as stressed (Fv/Fm = 0) in year 1 and recovered (with fully emerged green leaves and a higher value for Fv/Fm) in year 2, and some did not recover and died.

Plants exhibiting transplantation stress at baseline (year 1) but verdant in year 2 were considered 'recovered' plants. Stressed plants from year 1 that failed to emerge were considered 'not recovered' due to potential mortality. The difference in the proportion of recovered plants from among the sampling sites was calculated and used to assess the impact of AMF on ramp stress recovery.

Survival

To assess post-transplant survival, we counted emerging plants in year 2. Plants with no observable emergence were considered dead and coded 0; surviving plants were coded 1.

Photoinhibition and chlorophyll estimates

Excluding all 'zeros' (that were analyzed in transplantation stress in the first year), the value of F_v/F_m was used to compare the photosynthetic stress in plants. Chlorophyll fluorescence was also measured in years 2 and 3, but with a different system (Handy PEA, HANSATECH) in year three. In year 3, chlorophyll content for each transplanted ramp was measured indirectly using a chlorophyll meter (CCM-300, OPTI-SCIENCES, City, STATE). Chlorophyll concentration is considered an indicator of plant health (Cortazar *et al.*, 2015). this measure served as a proxy for AMF effects on nutrient status and well-being.

Mycorrhizal root colonization study

Among the 50 plants received from each site, two plants (a total of 16) were randomly selected for analyzing AMF colonization in ramp roots in their native habitat. Roots were removed from each bulb tested (n=16), and three subsamples from each bulb were evaluated. Similarly, in year 2, destructive sampling of 3 roots per site (1 plant from each treatment), a total of 24 plants occurred. Roots were gently removed from each bulb tested (n=24), and two subsamples from each bulb

were evaluated by observation of root fragments following the method of Phillips & Hayman (1970). The staining protocol mentioned was modified for ramp roots using the trial-and-error method. Briefly, root segments (2-3 cm length) were submerged in 10% KOH overnight, and the following day they were transferred to another vessel of KOH and heated at 90 °C for 10-15 minutes. Afterward, roots were bleached in an alkaline H₂O₂ solution, prepared by mixing 3 ml of household ammonia, 9 ml of 30% H₂O₂, and 588 ml of distilled water, for 1 hour. Finally, the segments were acidified with 1% HCl in a petri dish for an additional hour. Then, the root segments were stained by soaking overnight in a 0.03% solution of trypan blue (the stock solution was prepared by dissolving 0.3 g of trypan blue in 100 mL of lactoglycerol, and then 0.03% was prepared by mixing 10 mL of stock solution with 90 mL of lactoglycerol). Frequencies of AMF hyphae were calculated as 100 x the number of fragments observed with AMF divided into the total number of fragments containing AMF (Giovannetti and Mosse, 1980).

Experiment 2. Ramp seed germination in response to AMF

Seed samples (n=3) were collected during Fall 2020; seeds were collected from ramp patches in Montgomery County and Giles County of Virginia, and a third sample was purchased from a commercial nursery (Prairie Moon Nursery, MN) which collected seeds from Wabasha County, MN.

Experimental Site Preparation Seed Sowing and Mycorrhizal Experiment

For seed germination, two 0.91×1.52 m (3×5 feet) raised beds with 70% shade and another two 0.91 × 2.13 m (3 × 7 feet) forest simulated plots without soil amendments were prepared in a wooded site at Catawba Sustainability Center, Catawba, VA. To make a standard growing medium, the raised bed was filled with 60% topsoil, 30% compost, and 10% potting mix from a

local supplier. The soil in the simulated forest site was Weikert-Berks complex (Loamy, shallow to moderately deep, well-drained soil (Table 3-2).

In October 2020, to test the effect of commercial AMF on ramp seed germination, we imposed two treatments on the collected ramp seed samples. Seeds (n=200) from each site (Montgomery, Giles, and Wabasha Counties) were sowed in the raised beds, and 160 seeds of each origin were planted in the forest simulated plot. The beds were divided into 4 different replicates. For the raised bed study, 100 seeds from each seed source were inoculated with commercial AMF (Atriva 500, Italtollina USA, Inc., Anderson, IN). The seeds were lightly dampened and rolled in the mycorrhizae powder for attachment immediately before planting. The remaining 100 seeds from each source were not inoculated (control treatment). This procedure was repeated for planting in native soil in the simulated wild site, but 80 seeds per source site were inoculated with commercial AMF, and 80 were not inoculated. Following treatment application, ramp seeds were sown in the raised bed and forested site. For an indoor stratification trial, 200 seeds from each site were placed in sealed plastic bags with moist vermiculite and refrigerated (4°C) for six months to stratify. Half (100) seeds were inoculated with commercial AMF, and half were kept uninoculated.

The total number of germinated seeds was measured 18 months after planting seeds in the raised bed and simulated wild sites and after six months for the stratified seed.

Experiment 3. Ramp seedling response to AMF

Seedlings for this study were purchased from a commercial nursery (Red Root Nursery, Weaverville, NC). The nursery grew seeds sourced from Buncombe County, NC, Wabasha County (private land), MN, and Clayton County, IA (wild population). A total of 216 seedlings (72 / source) were used for this study.

To test the effect of commercial AMF on-ramp seedling growth and survival, we imposed three treatments on the collected ramp seedling samples following the same procedure applied to ramp bulb samples. Following treatment application, seedlings were planted into raised beds assembled as described previously for bulb study. Seedling growth measures (leaf length and width) were measured as described for the bulb study.

The number of live ramp seedlings was counted in years 2 and 3 to determine if AMF inoculation affected seedling survival.

Data analysis

Data were analyzed in JMP software (16.0, SAS Institute Inc., 2021). Data distributions were visually inspected in a normal quantile and probability plot. The Shapiro-Wilk normality test was performed to calculate a p-value and quantitatively determine the data's normality. For those variables (Fv/Fm data) whose data were not normally distributed, a non-parametric test (Wilcoxon/Kruskal-Wallis tests) was performed. Data were analyzed with a two-way analysis of variance (ANOVA) to test for differences between growth means within treatments among sites. A post-hoc test (Tukey's HSD) was used to determine which sites differed from each other for normally distributed data. A non-parametric post-hoc test (Pairwise Wilcoxon tests with multiple testing corrections) was performed for the data that were not normally distributed. For each study, the morphological features of leaf length (LL) and leaf width (LW) and the difference between years, survival, and Fv/Fm measures were considered dependent variables, with sites of origin and AMF treatments as independent variables. For each test, the significance level was $P=0.05$.

Analysis of means for Proportions (ANOMP) was performed to analyze the proportion of surviving plants in year 2 and year 3. This method was used to test whether the proportion of surviving plants in years 2 and 3 for each site differed from the overall population proportion.

RESULTS

Ramp bulb ecotype response to AMF

Soil nutrient analysis and Ramps-fungal association in native habitat

The nutrient parameters of the soil varied across the sampling sites. There were wide ranges observed for various nutrients, including phosphorus (P) with levels ranging from 2 to 44 ppm, K: 48-97 ppm; Ca: 306-2790 ppm; Mg: 39-168 ppm; Zn: 1.5-7.3 ppm; Mn: 7.4-57.7 ppm; Cu: 0.1-1.7 ppm and Fe: 3.4-57.2 ppm. Furthermore, the percentage of organic matter in the study areas was higher than that typically found in agricultural soils Table 3-2. Evaluating the nutrient availability for plant growth based on soil microbial activity, it was observed that the native ramp sites exhibited low release of nitrogen available for plants, while phosphorus availability was high to very high, and potassium availability was also high to very high Table 4-1.

Nine hundred sixty-three different fungal species from eight phyla were present in the soil samples from the eight native ramps sites. Fungal species from three different phyla (Ascomycota, Basidiomycota, and Mortierellomycota) were dominant in soils from all sites, although there were differences in phylum distribution among sites (Table 4-2). Only seven AMF species (phylum Glomeromycota) with very low abundance (total of 0.06%) were present and with varying distribution in the soil samples Table 4-3.

Bulb morphological characteristics with AMF

Leaf length

AMF affected ramp leaf lengths one year after transplanting, but this response varied by site (treatment X site; $P = 0.001$) but not in year 3 ($P = 0.15$). In both years, leaf lengths were longer for fungicide-treated ramps from Haywood County Figure 4-1, Ai and Bi). Ramps from most sites

had shorter leaf lengths from year 1 to year 2 and 3, but leaf lengths were longer in year 2 for ramps from Smyth and Montgomery Counties (year x site interaction; $P = 0.03$) Figure 4-1, Aii). Leaf lengths were longer with AMF treatment for ramps from Lawrence (1 & 2) and Pocahontas counties, whereas fungicide treatment increased leaf length for Giles and Haywood counties, Figure 4-1, Aii and Bii).

Leaf width

As with leaf length, leaf width response to AMF varied by site (treatment x site interaction; $P = 0.001$) in both years post-transplant. Leaf widths were greater with mycorrhizal treatment for ramps from Macon and Haywood counties, but for plants from Montgomery County, leaf width was greater with control treatment in both years Figure 4-2, Ai and Bi).

Similarly, the difference in leaf width in year 2 was positive (3.1%) in control ramps in Montgomery County and negative (-63%) in control ramps in Macon County, although they have borderline significance (treatment X site $P = 0.07$). By year 3, leaf widths were greater than at baseline for some sites (treatment X site; $P = 0.01$). Control ramps from Montgomery County were 50% wider, while control ramps from Macon County were smaller-44%) than at the baseline measure (Figure 4-2, Aii and Bii).

Number of leaves

The number of leaves per plant differed by size ($P = 0.01$) in both post-transplant years. Leaf numbers declined (slightly) from year one to year two but increased in year three (year effect; $P = 0.01$). Most ramps maintained about two leaves per plant, but this was not differed by treatment (treatment X site; $P = 0.1$), Figure 4-3.

Bulb Physiological Characteristics with AMF

Transplantation stress and recovery

One month after transplanting, the proportion of stressed plants was greater (treatment X site; $P = 0.001$) for plants in the control treatment (about 34%) than for ramps treated with fungicide or AMF (average of 18%; Figure 4-4). However, the proportion of stressed plants was inconsistent with sites. The proportion of stressed plants was greater with fungicidal-treated ramps from Macon and Pocahontas County, but for plants from other counties, the proportion of stressed plants was greater with the control treatment.

Recovery of stressed plants from year one to year 2 was approximately 60% for ramps treated with fungicide or AMF, while 35% for ramps in the control treatment. However, the recovery of ramps was inconsistent with sites and did not significantly differ with treatment (treatment X site $P=0.4$). (Figure 4-5).

Survival

Treating ramps with fungicide increased (treatment $P = 0.0001$, site X treatment $P = 0.01$) survival to almost 92%, followed by ramps with AMF treatment (87%) in the year after transplanting. Survival for untreated ramps was 65% in year 2 (Figure 4-6a). Treatment effects on surviving plants carried over into year 3, with lower (treatment $P=0.02$) survival rates within control plants (83%) than in two other treatments (94%; Figure 4-6b). However, the survival was not differed by the interaction of site and treatment (treatment X site $P=0.4$).

Photoinhibition and chlorophyll estimates

Treatment with AMF supported greater ($P=0.001$) F_v/F_m values (0.77) one month after transplanting, indicating lower stress. Ramps from Giles and Montgomery counties (VA) and

Lawrence County (1), PA, had greater Fv/Fm values with the mycorrhizal treatment (site X treat. $P = 0.02$; Figure 4-7, Ai).

Fv/Fm values generally increased (year $P=0.001$) in the second year but were not affected by treatment (Figure 4-7, Aii). The Fv/Fm was greater (>0.80) in ramps from Montgomery and Lawrence (sites 1 and 2) counties (site X treat. $P = 0.04$) treated with AMF in year 3 (Figure 4-7, Aiii).

Along with Fv/Fm, chlorophyll content was measured in year 3 for each plant within each treatment. Only site ($P=0.001$) effects were observed but did not differ with treatment (Figure 4-7, B).

Mycorrhizal root colonization

Mycorrhizal colonization frequency (MCF) was measured in a subset of ramps before transplanting and one year afterward. For all sites except Giles and Pocahontas counties, MCF was greater in year 2 (site $P = 0.0004$; year $P < 0.0001$, site X year $P = 0.1$) (Figure 4-8).

In year 2, ramps treated with fungicide tended ($P < 0.08$) to have more MCF ($>80\%$) than the other treatments (Figure 4-9).

Ramp seed germination in response to AMF

Neither the source site nor AMF treatment affected seed germination in raised beds under artificial shade in year 2 (18 months after sowing) or year 3 (Figure 4-10, Ai and Aii). In contrast, in the wild-simulated plots, more AMF-treated seeds tended ($P=0.07$) to germinate (19.2%) than untreated seeds in year 3 (12.5%; Figure 4-10, Bii). Germination of stratified (refrigerated) seeds was 10% more with AMF treatment for the seeds from Wabasha County and 3% more from Giles

County; however, no statistical analysis was conducted for this as this was trial germination with AMF.

Ramp seedling response to AMF

Leaf length and width

Leaf lengths differed ($P = 0.006$) among sites of origin but only in year 2 (Figure 4-11, Ai).

Leaf widths generally were narrower ($P = 0.0004$) for ramps from Wabasha, MN, one year after transplanting (year 2). Neither treatments nor treatment x site interaction was observed (Figure 4-11, Bi). Leaf widths did not differ by site and treatment in year 3 (Figure 4-11, Bii).

Seedling survival

The proportion of transplanted seedlings that survived in year 2 differed with treatment ($P=0.0001$). More transplanted seedlings (79.2%) within control treatments survived ($P < 0.001$) than those treated with AMF (28%) or fungicide (67%; Figure 4-12 a). In year 3, more seedlings died among the survivors in year 2. Survival for the proportion of surviving seedlings from year 2 was similar across treatments in year 3. Among all treatments, approximately 50% of seedlings that survived in year 2 were still alive in year 3 (Figure 4-12 b).

DISCUSSION

Soil nutrient analysis and Ramps-fungal association in native habitat

Due to variations in climate, elevation, and latitude, Appalachia is a botanical diversity hotspot (Pickering et al., 2003). The bi-directional relationship between plant diversity and mycorrhizal fungi diversity has been shown by some studies (Kernaghan, 2005). Only seven AMF species were identified across all source site soil samples, indicating their relatively low abundance in ramps habitats.

AMF is one of the most prevalent organisms in the rhizosphere (Lee *et al.*, 2013), colonizing multiple plant species, indicating that the organisms generally lack host specificity (Smith and Read, 2010). AMF has a low species richness, with only about 240 species officially recognized (Schüßler & Walker, 2010; Krüger *et al.*, 2012). The spores of AMF in the spores in soil depend on the sampling timing, physical environment, and soil chemistry. Especially soil organic matter, Nitrogen, Phosphorus, Potassium, and Calcium are important factors for the abundance of AMF spores in soil (Alori *et al.*, 2020, Lin *et al.*, 2020, Casazza *et al.*, 2017). A study by Alori *et al.* (2020) reported a decrease in the abundance of arbuscular mycorrhizal fungi (AMF) with high levels of phosphorus and organic matter in the soil, while Casazza *et al.* (2017) reported an increase in AMF abundance with available calcium in the soil. Our study found that source site soil had very high levels of phosphorus and organic matter (Table 3-2) but low levels of available/extractable calcium (Table 4-1), which may indicate a low abundance of AMF spores in the soil. This aligns with our findings. However, mycorrhizal spores do not necessarily indicate mycorrhizal infection's abundance (Alori *et al.*, 2020). In our ramp samples collected from Giles, Smyth, and Montgomery Counties, VA, we found no AMF spores but did find AMF colonization in roots (Figure 4-4).

Bulb morphological characteristics with AMF

Several studies have investigated the relationship between arbuscular mycorrhizal fungi (AMF) and *Allium* species, which have shown high responsiveness to soil mycorrhiza due to their smaller root system surface area (Golubkina *et al.*, 2020). Priyadharsini *et al.* (2012) found that shallot roots (*Allium cepa* var. *aggregatum*) were colonized by *Glomus* and *Scutellospora* fungi, with a higher concentration of *Glomus*. In another study, Mayr and Godoy (1990) observed that the roots of *Allium ursinum* were predominantly colonized by AMF. Furthermore, Jansa *et al.* (2008)

demonstrated that the inoculation of *A. porrum* roots with *Glomus mosseae* resulted in the fastest colonization. Galván et al. (2009) reported a direct relationship between the amount of AMF colonization and the number of onions produced, emphasizing the importance of a diverse blend of AMF species for onion cultivation in organic and conventional systems.

However, in our study, the response of ramp leaf growth to AMF treatment (a blend of *Funneliformis mosseae* and *Rhizoglyphus irregularis*) was significant but inconsistent among ramps from different sites, potentially indicating differences in ramp-AMF compatibility or limited ability to colonize roots under our protocol. For ramp leaf length, an increase was observed in response to AMF treatment for ramps from Lawrence 1 and 2 and Pocahontas counties, while fungicide treatment increased leaf length for ramps from Haywood County. These findings underscore the importance of considering the interaction between treatments and sites when studying the impact of mycorrhizal treatment on plant growth.

Similarly, AMF treatment affected ramp leaf width, but the effects varied among sites. Mycorrhizal treatment resulted in greater leaf widths for ramps from Macon and Haywood counties, whereas leaf width was greater with control treatment for plants from Montgomery County. These results indicate that AMF treatment can positively impact ramp leaf lengths and widths; however, the effects depend on the source material. The effect of AMF treatment may depend on factors such as the specific AMF strain utilized and its compatibility with the plant tested. Further research is necessary to understand better and manage these interactions for consistent and positive outcomes.

Differences in leaf length and width (in years 2 and 3 following baseline leaf measures) varied among treatments and sites. The increased leaf lengths and widths for ramps from Smyth and Montgomery Counties in years 2 and 3 may be due to their having favorable environmental

conditions relative to their source sites, which were close to the experimental site. Variable differences in leaf measures within treatments among sites and years highlight the importance of evaluating a broader range of plant growth (such as bulb diameter) and physiological measures (such as transpiration) in future studies. These findings further highlight the importance of monitoring plant growth over time and considering the impact of environmental conditions on growth when interpreting the results, as symbiotic organisms adapt to both their biotic and abiotic environments (Johnson *et al.*, 2010).

Bulb Physiological Characteristics with AMF

Transplantation stress and recovery

The impact of mycorrhizal fungi on plant stress has been a topic of interest in recent years as plants inoculated with AMF can withstand environmental stresses such as salt, drought, nutrient deficiency, and extreme temperatures, increasing yields for various crops (fruits, vegetables, and grains) (Begum *et al.*, 2019). This study investigated the impact of mycorrhizal fungi on the stress of transplanted ramps plants after 1 month. The results indicated that the proportion of stressed plants was significantly higher in the controlled treatment than in the mycorrhizal treatment. However, the effect was inconsistent across sites. This finding suggests that mycorrhizal fungi may play an important role in reducing plant stress after transplantation; however, the effect depends on the source of plant material. This finding has important implications for the transplantation of ramps in forest farming settings and conservation efforts, as transplanted plants often experience high levels of stress that can reduce their survival and growth. By inoculating transplanted plants with mycorrhizal fungi, it may be possible to improve their chances of survival and growth, particularly in stressful environments.

Similarly, this study also examines the impact of mycorrhizal inoculation on the recovery of stressed plants from 1st year to the second year and found that mycorrhizal treatment did not have a significant impact on the recovery of stressed transplanted ramps plants in the second year. However, it is important to note that there was a trend towards lower recovery proportions of plants within the controlled treatment compared to the other two treatments. This suggests that mycorrhizal treatment may potentially enhance plant recovery, although further research is needed to confirm this.

Survival

Mycorrhizal fungi form symbiotic relationships with plant roots, providing them with nutrients and improving their growth and survival (Akhtar and Siddiqui, 2008). The present study investigated the impact of mycorrhizal fungi on the survival of transplanted ramps plants in the following years. The results showed that treating ramps with AMF increased survival to almost 90% in the year after transplanting and 94% in two years after transplanting, where survival for untreated ramps was <60% in a year after transplanting, among which just 83% survived in the following year. These findings are consistent with other studies showing mycorrhizal fungi's positive impact on plant survival. For example, Middleton *et al.* (2015), in their study on the impact of AMF on native prairie plant species, found that mycorrhizal inoculation significantly improved the survival of *Allium cernuum*. Another study by Moraes *et al.* (2004) found that inoculation by AMF significantly improves the survival of Mayapple plantlets.

This study also showed that the fungicidal treatment positively impacted the survival of ramps plants. That may be due to the risk of fungal disease (Black mold, blue mold, fusarium foot, and Botrytis leaf spot); these ramps samples already faced in their native habitat (Table 3- 4), and they benefited from fungicidal treatment before transplantation.

Photoinhibition and chlorophyll estimates

This study also examined the impact of mycorrhizal treatment on the photosynthetic performance of transplanted ramps, as AMF has been shown to facilitate host plants to grow well under stressful conditions by enhancing photosynthetic performance (Birhane *et al.*, 2012). The results showed that mycorrhizal treatment had a significant positive impact on the photosynthetic performance of ramps after 1 month of transplantation and two years after transplantation. Ramps from Giles and Montgomery Counties (VA) and Lawrence County (1), PA, had greater Fv/Fm value with the mycorrhizal treatment in the first year, while the Fv/Fm was greater in ramps from Montgomery, Lawrence (1)(2) Counties treated with AMF in year three. This result showed that the positive impact of mycorrhizal treatment varied among individual sites, which highlights the importance of considering plant sources when implementing mycorrhizal treatments on transplantations of such plants from varying environmental conditions. The present study also found that the photosynthetic performance of ramps varied between the first, second, and third years of transplantation. This suggests that ramps are highly sensitive to environmental changes and that transplanted ramps may require ongoing monitoring and management. On the other hand, the chlorophyll content on-ramp plants did not differ with treatments.

Mycorrhizal root colonization with AMF

Mycorrhizal fungi are important in nutrient acquisition and plant health, especially in natural ecosystems. However, many agricultural and horticultural practices, such as soil sterilization and fungicide application, can negatively impact the establishment and maintenance of mycorrhizal associations. Therefore, understanding the impact of mycorrhizal inoculation on plant growth and mycorrhizal colonization frequency (MCF) is essential for developing sustainable agricultural practices. Mikiciuk *et al.* (2019) reported that plants inoculated with mycorrhizal fungi increase in

mycorrhizal colonization frequency when studied on the strawberry plant. With *Allium* species, there have been reports that the inoculation with a combination of two or more AMF species resulted in more effective colonization of the plant roots than inoculation with only one species. For example, *A. porrum* seedlings, when treated with three different species of beneficial fungi (AMF), namely *Rhizophagus intraradices* (RI), *Claroideoglomus claroideum* (CC), and *Funneliformis mosseae* (FM), the colonization level of the fungi on the plant roots increased significantly (up to 59%) when two of the fungi were used in combination (RI+FM or RI+CC) compared to using a single AMF species (Golubkina *et al.*, 2020). This study initially hypothesized that the mycorrhizal inoculation (combination of *Funneliformis mosseae* (formerly *Glomus mosseae*) and *Rhizogloium irregulare* (previously known as *Glomus intraradices*)) could significantly increase MCF in transplanted ramps.

Interestingly, the present study found that fungicide-treated plants had a significantly higher MCF than control plants while not significantly different from the colonization of plants in mycorrhizal treatment. This unexpected result may be due to removing mycorrhizal competitors or pathogens that may have inhibited mycorrhizal colonization in the fungicidal-treated plants. The increase in root colonization and spore production by AM fungi have been reported using non-systemic fungicides, such as difolatan and sodium azide (Nemec, 1980). Furthermore, Mancozeb used in this study is a non-systemic fungicide; there could have been the same effect. Although the mechanisms by which the fungicides stimulate AM fungi are unknown, non-systemic fungicides are thought to act indirectly to reduce competition in the rhizosphere and create a favorable environment for the colonization of host roots by AM fungi (Jabaji-Hare *et al.*, 1987).

Although it was not assessed before the study, it is possible that the commercial inoculant did not adequately colonize the roots. Previous studies have demonstrated instances where commercial

mycorrhizal fungi failed to colonize roots properly. The research by Tarbell and Koske, 2007; Basiru and Hijri, 2022 and Elliott et al. 2021 has highlighted instances where commercial mycorrhizal fungi failed to colonize roots adequately. Thus, conducting a preliminary test of the commercial mycorrhizal inoculant before application to the plants could be a prudent approach. Additionally, the success of AMF inoculation depends on the adaptation of the inoculated species to the soil. For example, in a study by Schlaeppi et al. in 2016, *R. irregulare* inoculation was successful in soil predominantly dominated by the same species, while *F. mosseae* did not establish. However, since there was no testing of the soil AMF species before inoculation in the current experiment, it cannot be concluded whether the lack of AMF colonization was due to the inoculated species not being adapted to the soil. Further investigations are necessary to understand the biogeography of AMF better and effectively use AMF inoculation in the future.

Ramp seed germination in response to AMF

There was no significant impact of either site or treatment and their interaction on seed germination in wild-simulated plots and raised beds with artificial sheds at first germination (year 2). In contrast, in the wild-simulated plots, more seed tended ($P=0.07$) to germinate when treated with AMF (19.2%) than did untreated seed in year 3 (12.5%), which suggests that mycorrhizal inoculation may have a positive impact on seed germination. In addition, the seeds kept for indoor stratification also showed slightly higher germination (10%) among the seeds from mycorrhizal treatments. Also, the stratified seedlings germinated one year before the seeds were in the field. The study by Gutowski (2015) on the impact of mycorrhizae on seed germination of Rapid Gro Radish found that mycorrhizae help plants grow by reducing the time it takes for seeds to germinate; however, the reason was unknown. The author also suggests that plants and mycorrhizae release chemicals that can influence each other's growth, and some of these chemicals

have been shown to promote plant growth and fungal metabolism and may be responsible for earlier germination. The present study and comparable studies highlight the complex and context-dependent nature of the impact of mycorrhizal inoculation on seed germination. While mycorrhizal inoculation may positively impact seed germination under certain conditions, it is not always effective and may have different impacts on different species. Although mycorrhizal inoculation did not significantly impact seed germination, it could enhance plant growth and nutrient uptake in the later stages of plant development.

Ramp seedling response to AMF

Together with the growth of ramps plants, the impact of mycorrhizal colonization of the growth of ramps plantlets (seedlings) was also examined in this study and appeared dependent on on-site conditions. Results suggest that AMF inoculation may not impact the leaf length and width of *Allium tricoccum* seedlings, at least in the short term. Tracking over longer periods may still be warranted, however.

On the other hand, the survival of ramp seedlings seems to be impacted by treatment in this study, with a higher survival proportion in the controlled treatment. This could be because mycorrhizal colonization was not well established to work for seedlings, allowing the natural growth and establishment.

CONCLUSION

The study focused on mycorrhizal fungi's role in ramps' growth and survival. The study found a rich fungal diversity in the soil of native ramp sites, with Ascomycota, Basidiomycota, and Mortierellomycota phyla being the dominant species. However, the abundance of arbuscular mycorrhizal fungi (AMF) was relatively low, with only seven species in the soil samples. Mycorrhizal treatment significantly impacted ramp leaf length and width in the following years of transplantation, but only when considering the interaction with sites. The study highlighted the importance of considering the variety of plants collected from different sources with their local environmental conditions, soil properties, and other site-specific factors. This study also suggests that mycorrhizal inoculation may not significantly impact the leaf growth of ramps seedlings in the short term, and its effects may be more apparent in the long term. Mycorrhizal treatment significantly increases survival and stress tolerance, and the photosynthetic performance of ramps after transplantation indicates the positive impact of AMF inoculation with transplantation efforts. The positive result from the fungicidal treatment on the survival of ramps is potentially due to the risk of fungal diseases in their native habitat. In contrast, the higher survival proportion for control seedlings indicates that mycorrhizal colonization was not well established to work for seedlings. Additionally, the trend towards lower recovery proportions of plants within the controlled treatment suggests that mycorrhizal treatment may have some potential for enhancing plant recovery, although further research is needed to confirm this.

Overall, the study concludes that mycorrhizal fungi play a crucial role in the growth, stress tolerance, and survival of transplanted ramps, and their impact varies significantly depending on the source of transplanted ramps. The study suggests that careful monitoring and management of mycorrhizal treatment are essential for ensuring the best possible results in ramp growth and survival. The study also highlights the need for further research on the potential role of AMF in

ramps' growth and their interaction with other dominant fungal species in the soil. The findings of this study have significant implications for forest farming with transplanted ramps and the conservation of native ramp populations and other spring ephemeral plant species, emphasizing the importance of understanding their ecological relationships with fungi and other soil organisms. Future research on ramp should focus on investigating the response of ramp ecotypes to a broader range of arbuscular mycorrhizal fungi (AMF) strains across different environments. Additionally, identifying superior AMF for ramp forest farming and elucidating the underlying mechanisms of AMF benefits to ramp survival and growth should be prioritized. By conducting these studies, we can better understand the role of AMF in ramp production, which could lead to the development of more effective strategies for cultivating and managing this important plant species.

Table 4-1: Plant available nutrients in the soil of native ramp sampling sites, based on microbial activity present in the soil.

(*very low=<20%, low=20-40%, Medium=40-60%, High=60-80%, very high=>80%)

Site	Site name	Soil nutrition (Nutritional status based on the microbial mobilization of certain compounds)											
		Major Compounds				Minor Compounds							
		C	N	P	K	Fe	Zn	Mn	S	Ca	Cu	Mg	CL
1	Giles, VA	high	very low	High	high	Very high	Very low	medium	very high	very low	High	very high	Medium
2	Smyth, VA	high	very low	very high	very high	high	Very low	Low	very high	very low	medium	High	Medium
3	Montgomery, VA	high	very low	High	high	high	Very low	Low	High	very low	medium	High	very low
4	Lawrence-1, PA	high	Low	very high	very high	Very high	low	medium	very high	low	High	very high	Medium
5	Lawrence-2, PA	high	very low	High	high	high	low	high	very high	very low	High	very high	low
6	Macon, NC	medium	very low	very high	very high	Very high	Very low	Low	very high	low	very high	very high	high
7	Pocahontas, WV	high	very low	High	high	high	low	medium	very high	very low	medium	very high	low
8	Haywood, NC	high	very low	very high	very high	Very high	Very low	Low	High	Very low	High	High	Medium

Table 4-2: Fungal phylum distribution in soil from each study site

Site	Site Name	Fungal Phylum Distribution (%)		
		Ascomycota	Basidiomycota	Mortierellomycota
1	Giles, VA	33.63	43.86	22.35
2	Smyth, VA	40.65	23.10	35.20
3	Montgomery, VA	63	19	18
4	Lawrence-1, PA	72.15	21.81	5.90
5	Lawrence – 2, PA	53.22	10.45	36.20
6	Macon, NC	33.53	19.02	46.91
7	Pocahontas, WV	39.08	13.16	47.46
8	Haywood, NC	43.77	41.55	14.53

Table 4-3: Distribution of AMF species in ramps native sites

taxa [%]/Sites	Giles, VA	Smyth, VA	Montgomery, VA	Lawrence-1,PA	Lawrence-2,PA	Macon, NC	Pocahontas, WV	Haywood, NC
Archaeospora sp.	0	0	0	0	0.00736829	0	0	0
Diversispora epigaea	0	0	0	0	0	0.00325018	0.00264831	0.0108599
Diversispora sp.	0	0	0	0	0	0	0	0.00542993
Entrophospora sp.	0	0	0	0	0.0196488	0	0	0
Claroideoglomus sp.	0	0	0	0	0	0	0	0.00698134
Glomus sp.	0	0	0	0	0	0	0	0.00310282
Paraglomus sp.	0	0	0	0.00106034	0	0	0	0

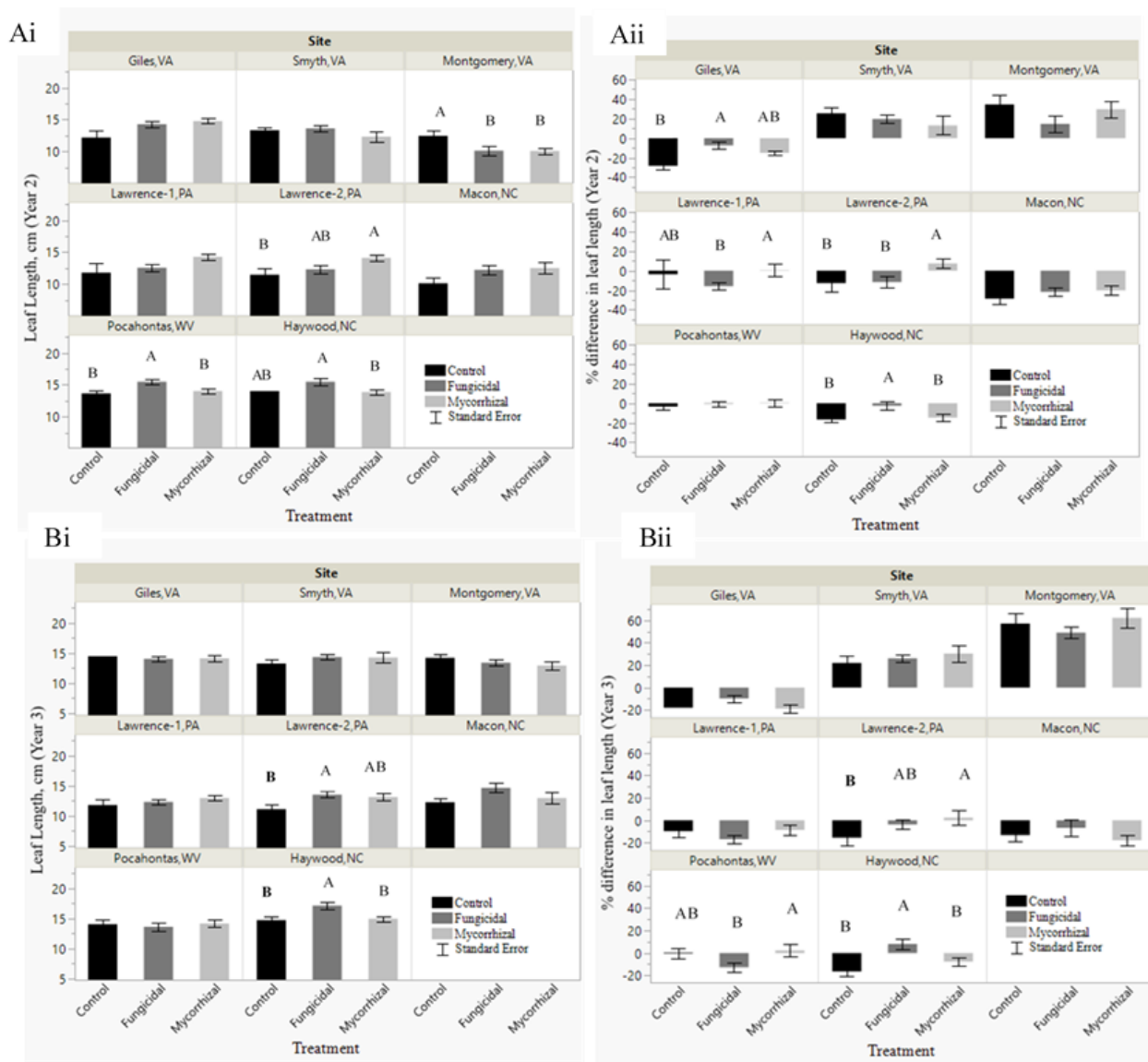


Figure 4-1: A) Mean leaf length and B) its change from baseline leaf length of ramp (*Allium tricoccum*) ecotypes. Collected from each site across Appalachia with three different treatments in year 2 Ai) Sites $p = 0.001$, treatment $p = 0.09$, site*treat $p = 0.001$ Aii) Site $p < 0.0001$, treatment $p = 0.3$, site X treat $p = 0.03$, with greater increase (40%) in Control ramps from Montgomery Co. and greater decrease (-40%) in Control ramps from Macon Co. And year 3 Bi) Site $p = 0.001$, treat. $p = 0.09$, site*treat. $p = 0.15$, Bii) Site $p < 0.0001$, treatment $p = 0.4$, site X treat $p = 0.04$, with greater increase (62%) in AMF treated ramps in Montgomery Co. and greater decrease (-18cm) in AMF treated ramps in Giles Co. Mean and standard bars are presented above each bar.

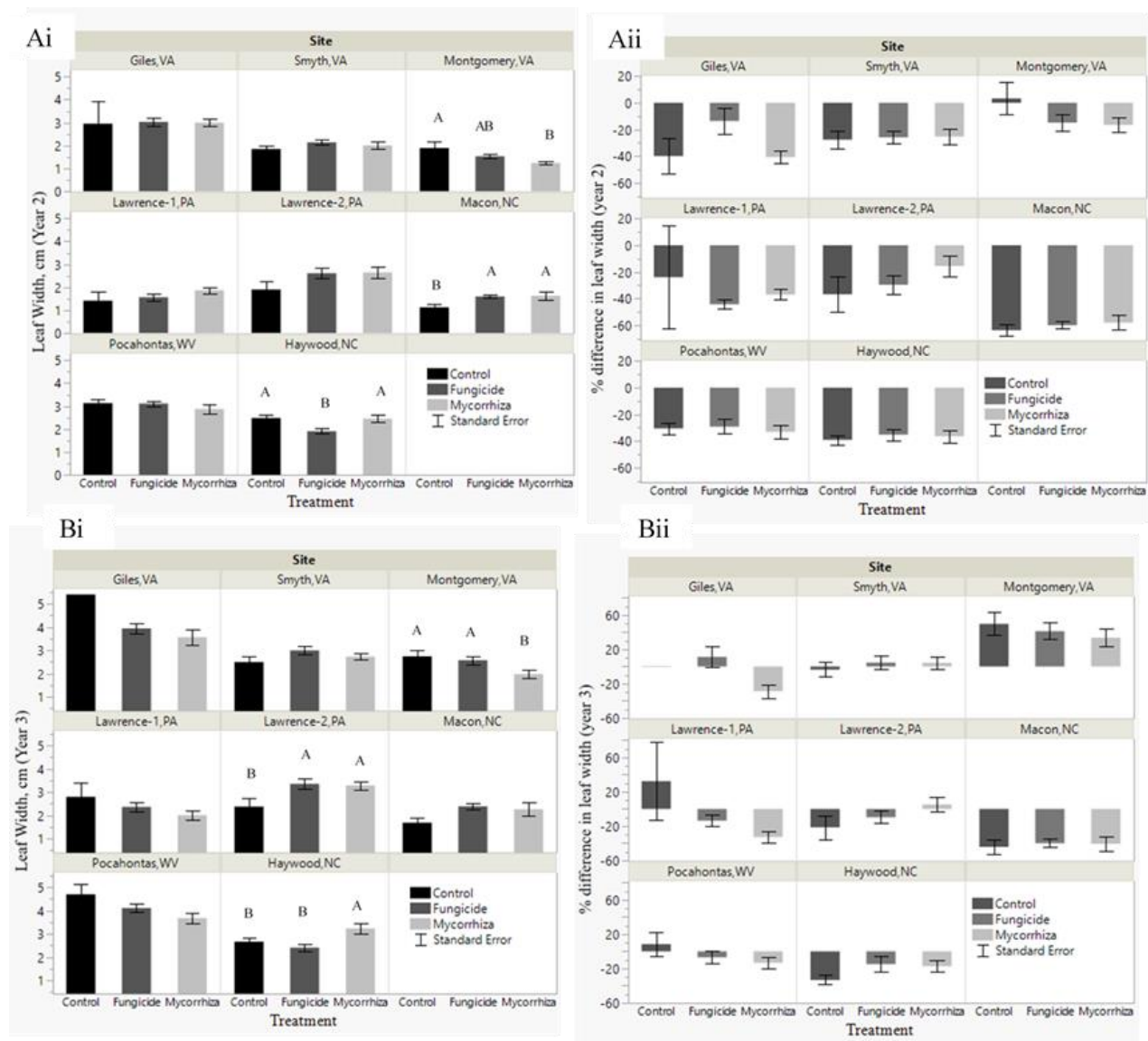


Figure 4-2: A) Mean leaf width and B) it changes from baseline leaf width of ramp (*Allium tricoccum*) ecotypes collected from each site across Appalachia with three different treatments in year 2 Ai) Sites $p = 0.001$, treatment $p = 0.5$, site*treat $p = 0.003$, Aii) Site $p < 0.0001$, treatment $p = 0.9$, site X treat. $p = 0.07$, with a greater difference (3.1%) in control-treated ramps in Montgomery Co. and the smallest (-63%) in control ramps in Macon Co. and year 3 Bi) Site $p = 0.001$, treat. $p = 0.15$, site*treat. $p = 0.0001$, Bii) Site $p < 0.0001$, treatment $p = 0.1$, site X treat $p = 0.01$, with a greater difference (50%) in control-treated ramps in Montgomery Co. and smallest (-44%) in control ramps in Macon Co. Mean and standard bars are presented above each bar.

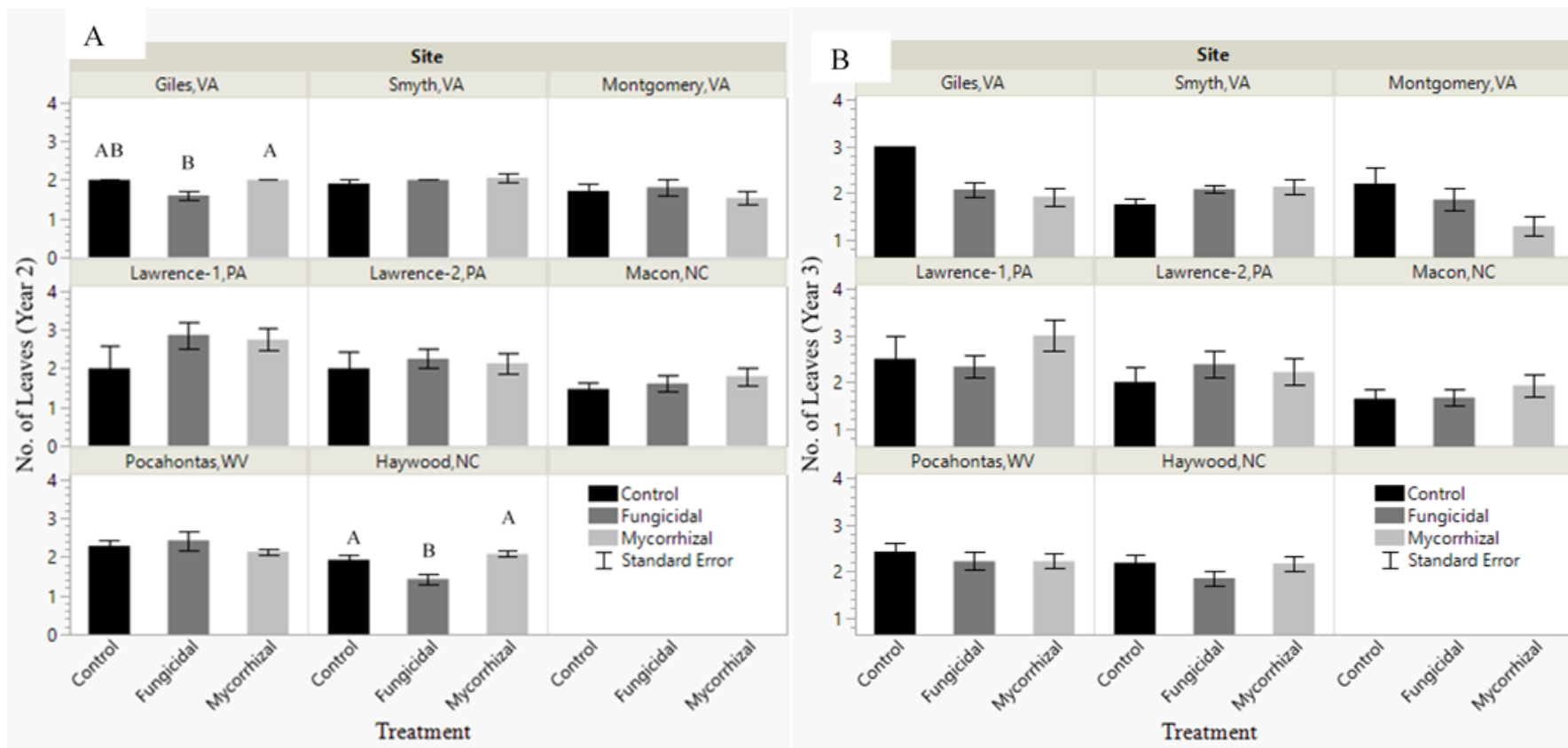


Figure 4-3: Mean leaf number of ramp (*Allium tricoccum*) ecotypes collected from each site across Appalachia with three different treatments in A) year 2 (Sites $p = 0.0001$, treatment $p = 0.5$, site*treat $p = 0.3$) and B) year 3 (Site $p = 0.0005$, treat. $p = 0.5$, site*treat. $p = 0.1$). Mean, and standard bars are presented above each bar.

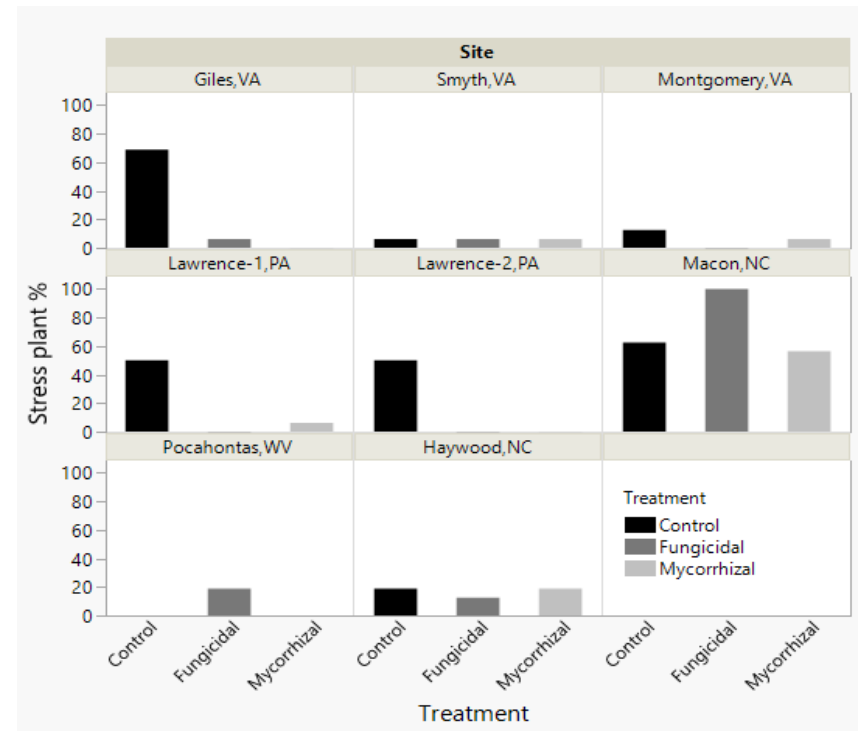
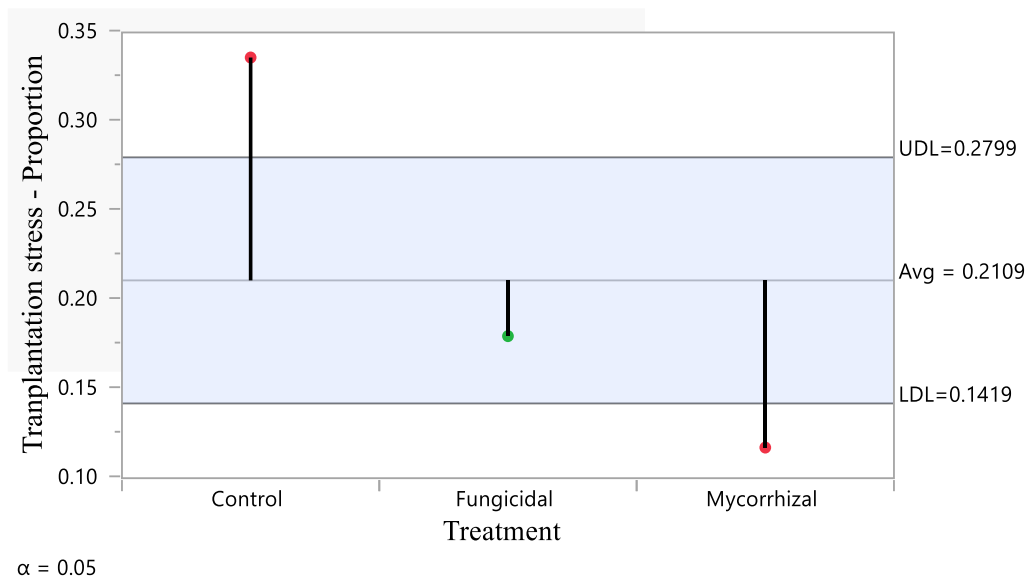


Figure 4-4: Proportion of plants with recent transplantation stress of ramp (*Allium tricoccum*) ecotypes among treatments after 1 month of transplantation. (n=48) ramps were collected randomly from each location across Appalachia. The proportion of stressed ramps among total transplanted is displayed as an approximation to the binomial distribution (maximum stressed vs. less stressed) using the analysis of means for proportions (ANOMP), a multiple comparison method for testing if individual group proportions differ from the overall proportion. (LDL=Lower Decision Limit, UDL=Upper Decision Limit). The proportion of stressed plants varied by treatments ($p = 0.001$) but was inconsistent with sites (Site $P > 0.00001$, treat. $P = 0.9$, Site X treat. $P > 0.0001$).

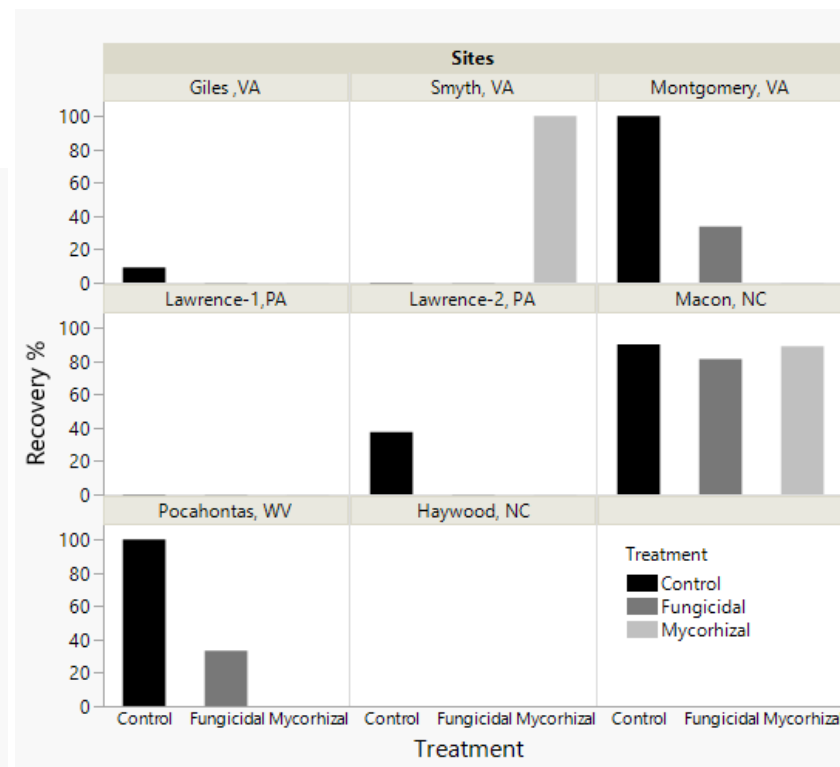
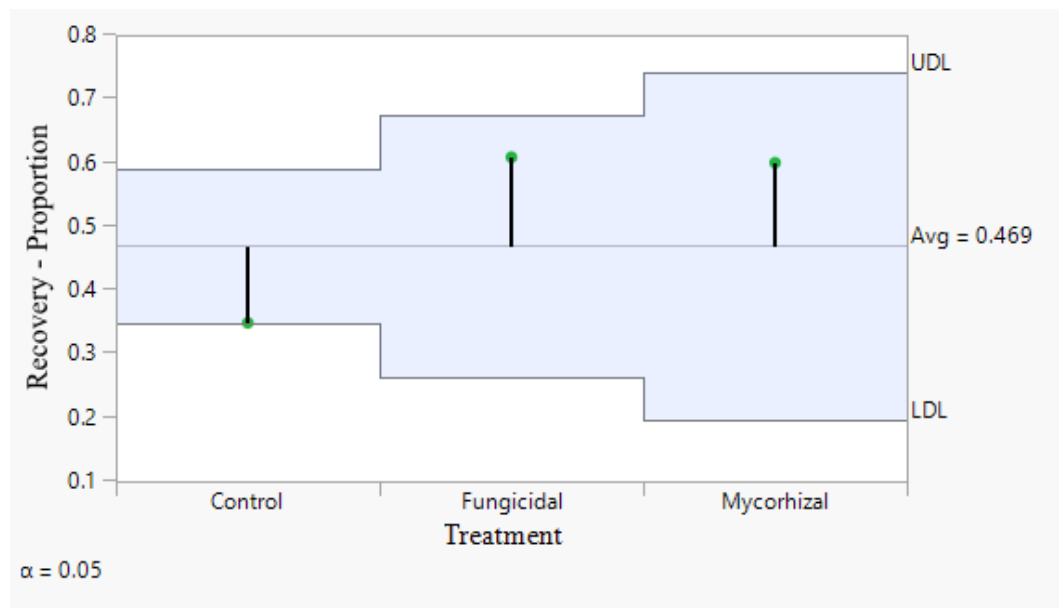


Figure 4-5: Recovery proportion of ramp (*Allium tricoccum*) ecotypes among treatments in 2nd year of transplantation among the stressed ramps after 1 month of transplantation. (n=48) ramps ecotypes were collected randomly from each site across Appalachia, transplanted with treatments, and stressed plants were counted after one month of transplantation within each treatment. The proportion of recovered ramps among total stressed in year 1 from each site is displayed as an approximation to the binomial distribution (recovered vs. not recovered) using the analysis of means for proportions (ANOMP), a multiple comparison method for testing if individual groups proportions differ from the overall proportion. (LDL=Lower Decision Limit, UDL=Upper Decision Limit). The recovery was not significantly different among treatments ($p = 0.06$) but differed with sites (Site $P = 0.00001$, treat. $P = 0.1$, Site X treat. $P = 0.4$).

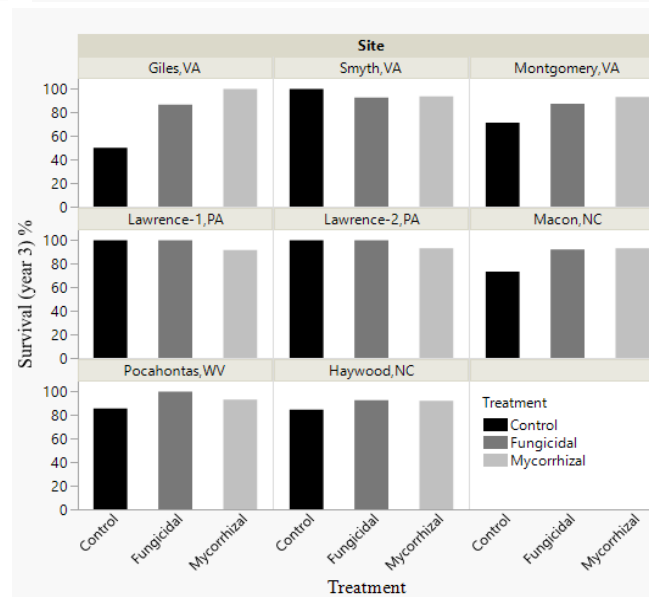
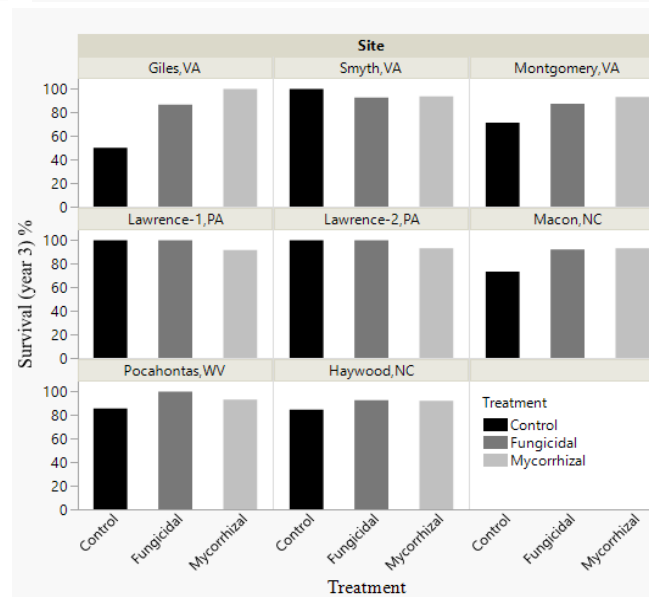
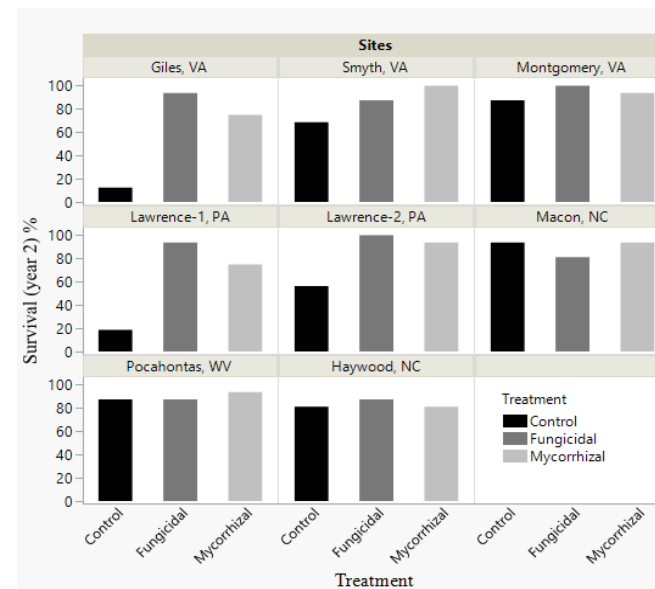
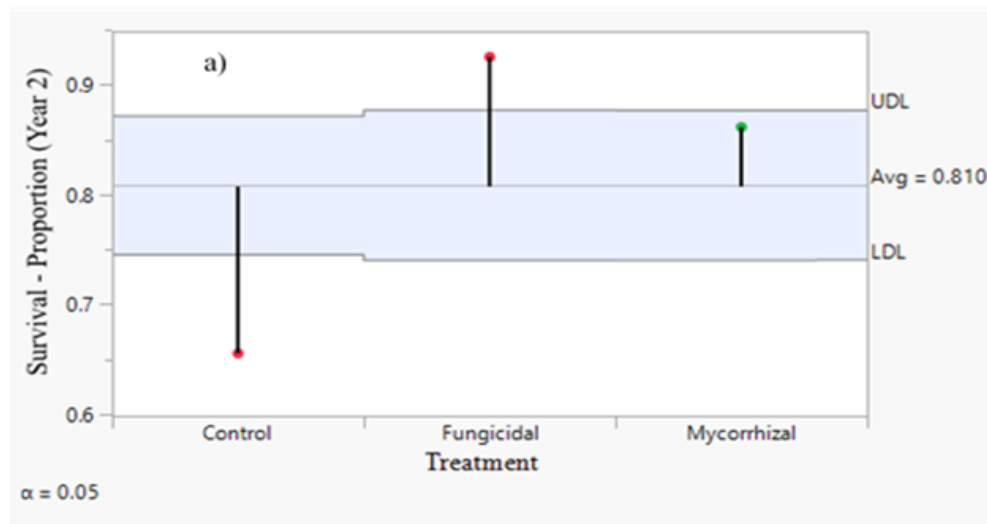


Figure 4-6: Survival proportion of ramp (*Allium tricoccum*) ecotypes between treatments after a year of transplantation. (n = 48) ramps were collected randomly from each location across Appalachia. The proportion of surviving ramps among total transplanted is displayed as an approximation to the binomial distribution (dead vs. surviving). (LDL=Lower Decision Limit, UDL=Upper Decision Limit). Survival in year 2 a) varied by treatment and site (Site $P = 0.01$, treat. $P = 0.00002$ Site X treat. $P = 0.01$) and was 65% for bulbs in control treatment b) in year 3, the survival of the remaining bulbs was varied by treatment ($p = 0.02$) and was <85% for the control treatment; however, did not differ when interacted with sites (Site $P = 0.1$, treat. $P = 0.9$ Site X treat. $P = 0.4$)

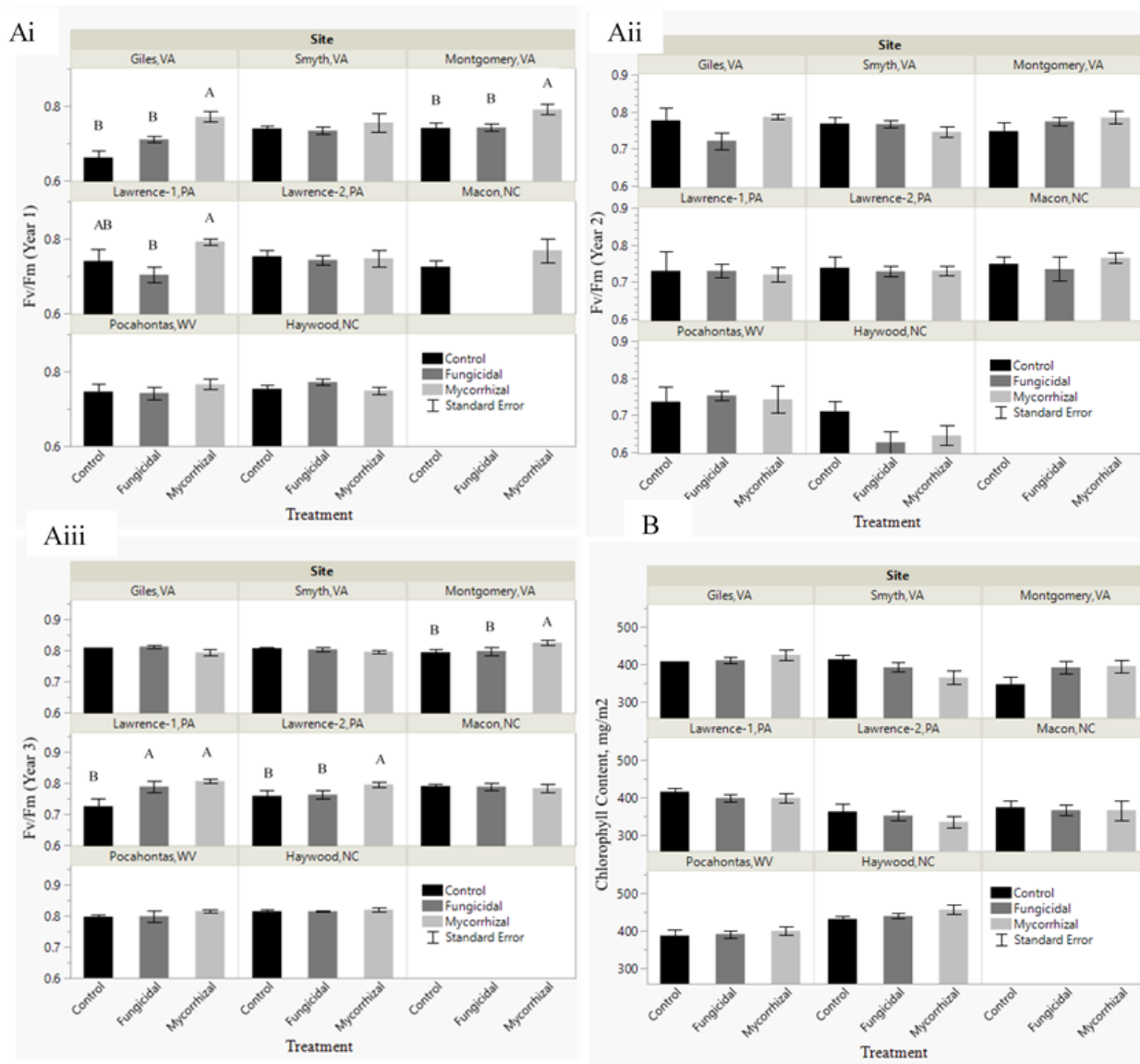


Figure 4-7: A) The average value of Fv/Fm and B) the average value of chlorophyll content (measured in year 3) among the ramp (*Allium tricoccum*) ecotypes within treatments. Fv/Fm differ among site and treatment in Ai) year 1 (after 1 month of transplantation) (site $p = 0.06$, treatment $p = 0.0001$, Site X treat $p = 0.02$) Aii) in year 2 (site $p = 0.001$, treat. $p = 0.4$, site X treat. $p = 0.5$) and Aiii) in year 3 (site $p = 0.001$, treat. $p = 0.04$, site X treat. $p = 0.04$). They were greater (0.77, 0.80) in plants within mycorrhizal treatment (A) in year 1 and year 3. B) Chlorophyll Content differs among sites but not with treatment (site $p = 0.001$, treatment $p = 0.9$, site X treatment $p = 0.3$). Mean, and standard bars are presented above each bar.

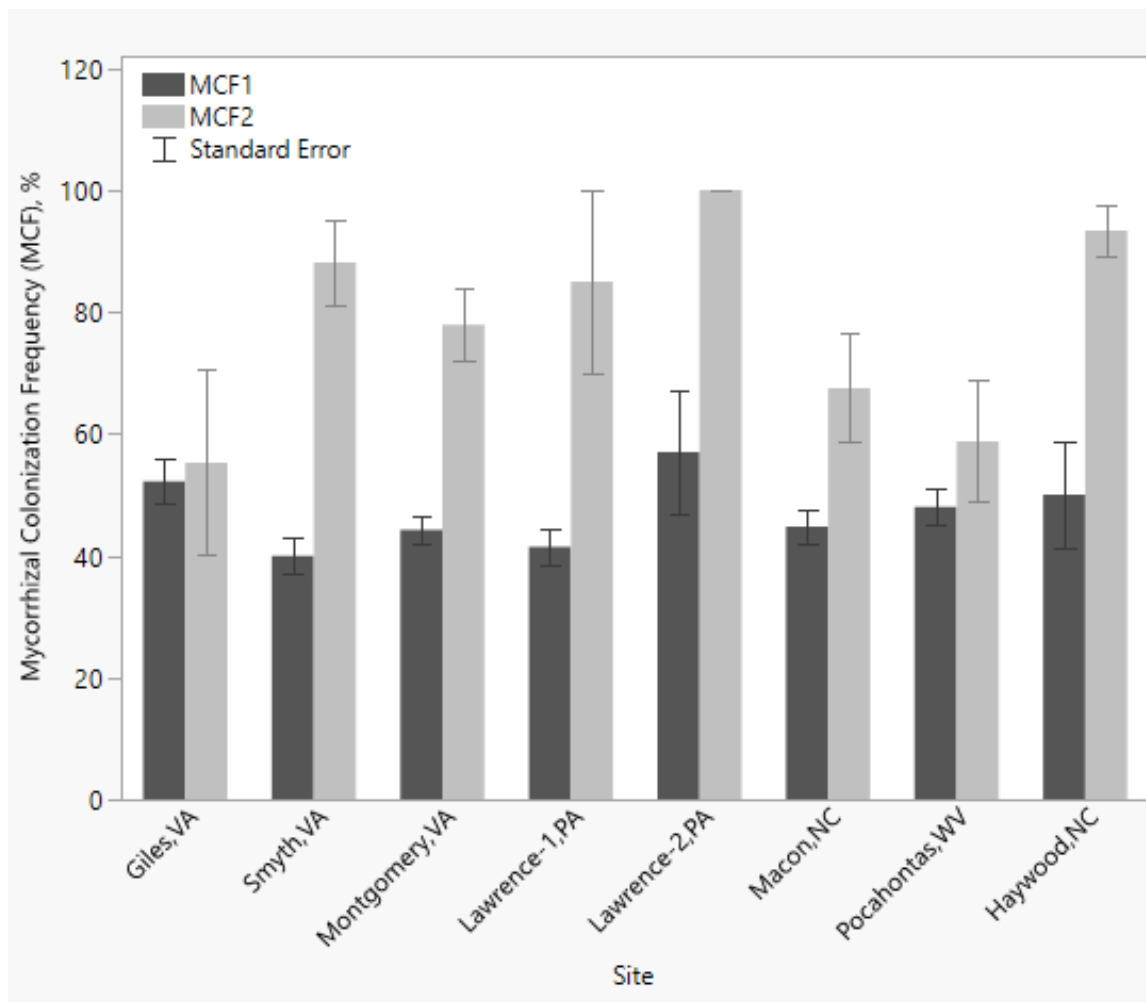


Figure 4-8: Difference in mycorrhizal frequency among sites from year 1 to year 2. MCF between year one and year 2 differ among sites and years but no interaction between site and year (site $p = 0.0004$; year $p < 0.0001$, site X year $p = 0.1$).

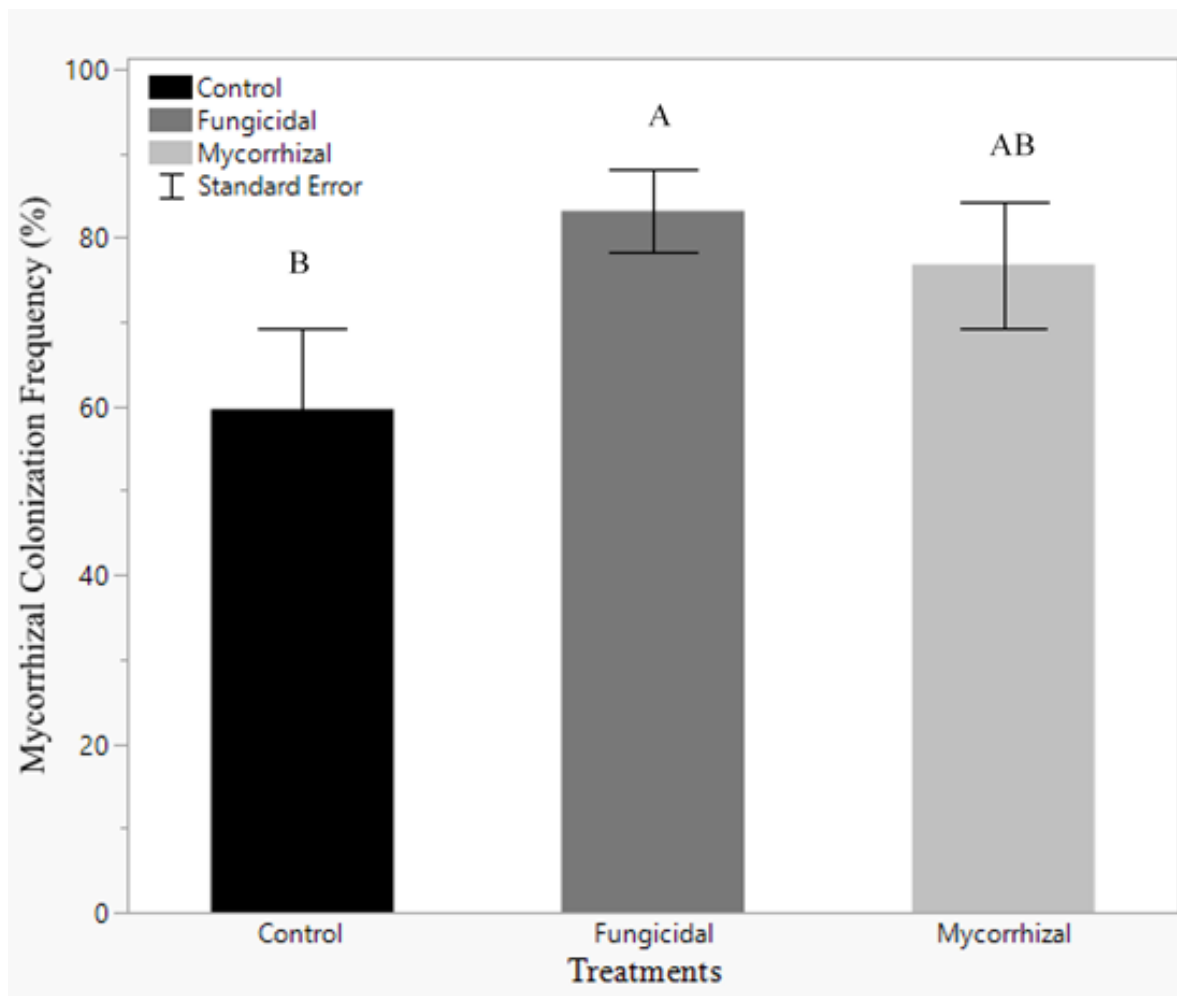


Figure 4-9: Mycorrhizal colonization frequency in year 2 among ramp roots transplanted with three treatments. Mycorrhizal colonization frequency was greater (>80%) in ramps within fungicidal treatment (B) than in control ($p = 0.03$) in year 2; however, it did not differ with mycorrhizal treatment ($p = 0.5$). The overall model has a borderline significance value ($p = 0.08$).

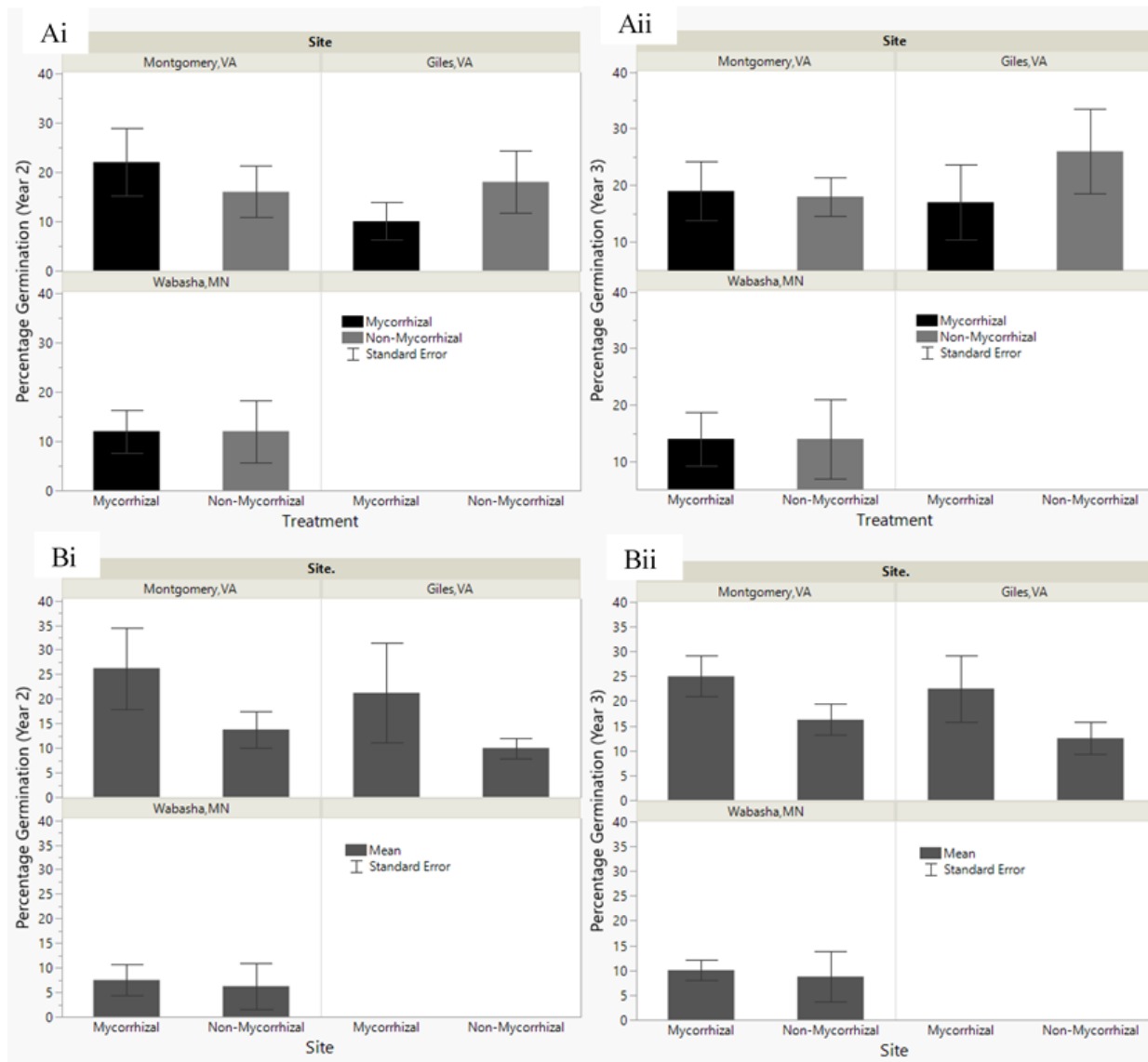


Figure 4-10: Impact of site and treatment on seed germination at A) raised bed and B) at the wild-simulated site.

For raised bed: Seed germination did not differ among site and treatment in Ai) year 2 and Aii) year 3 (site $p = 0.4$, treatment $p = 0.8$, site X treat. $p = 0.4$)

For Wild simulated site: Seed germination did not differ among site and treatment in Bi) year 2 (site $p = 0.1$, treatment $p = 0.1$, site X treat. $p = 0.6$), Bii) seed germination differ with sites but not with treatment in year 3 (Site $p = 0.04$, treatment $p = 0.07$, Site X treatment $p = 0.5$) with greater (25% and 22 %) germination in mycorrhizal treated seeds from Montgomery Co. and Giles Co., though have borderline significance ($p = 0.07$). Mean, and standard bars are presented above each bar.

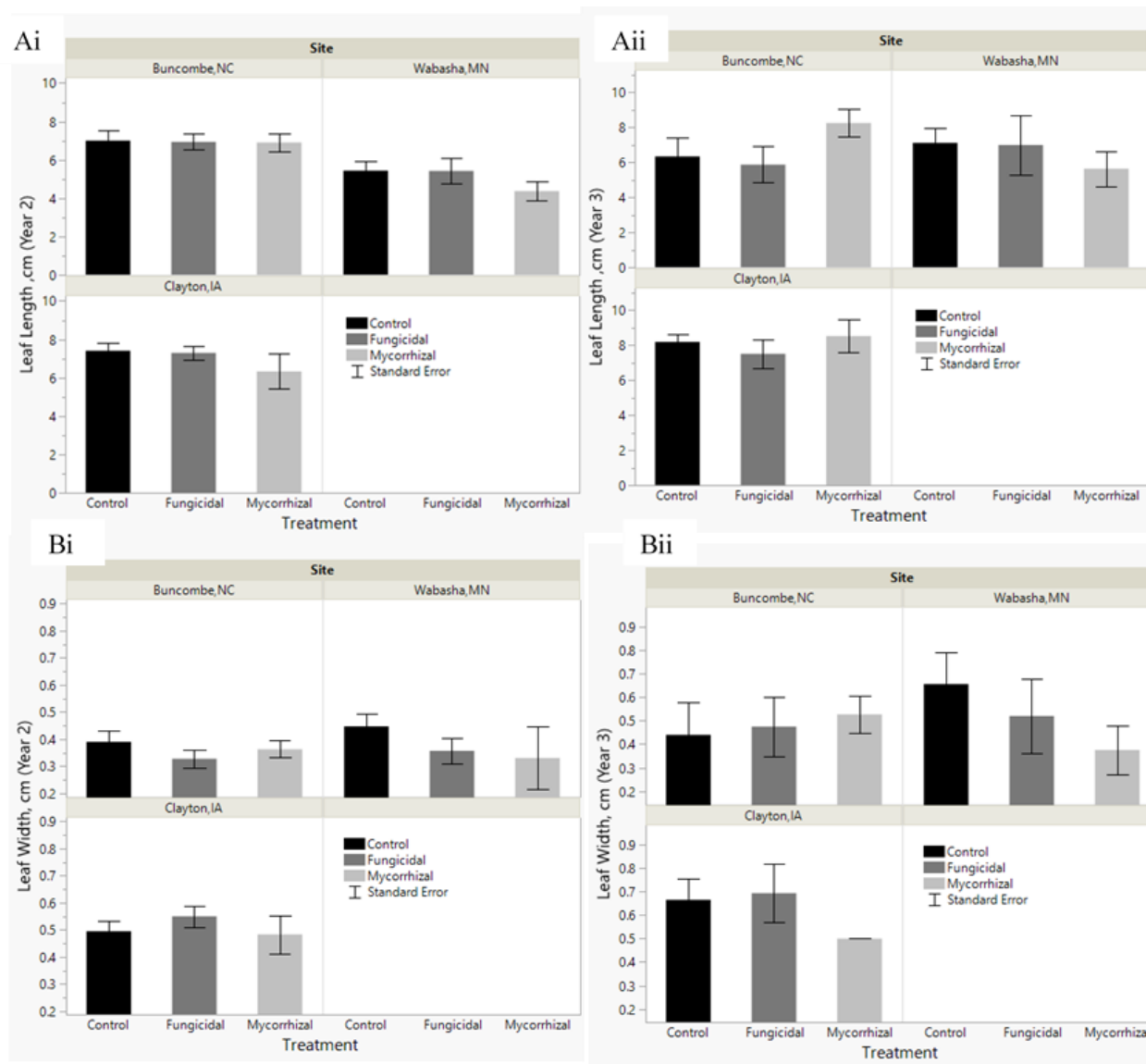


Figure 4-11: Impact of site and treatment on seedlings A) leaf length and B) leaf width; developed using three different seed sources in a nursery. Leaf length differs among sites but not with treatments in Ai) year 2 (site $p = 0.0005$, treatment $p = 0.2$, site X treat. $p = 0.8$). Moreover, in Aii) year 3, they did not differ (site $p = 0.1$, treatment $p = 0.7$, site X treat. $p = 0.4$). Leaf width differs among sites but not with treatments in Bi) year 2 (site $p = 0.0004$, treatment $p = 0.4$, site X treat. $p = 0.4$). And in Bii) year 3, they did not differ with site and treatment (site $p = 0.3$, treatment $p = 0.5$, site X treat. $p = 0.6$)

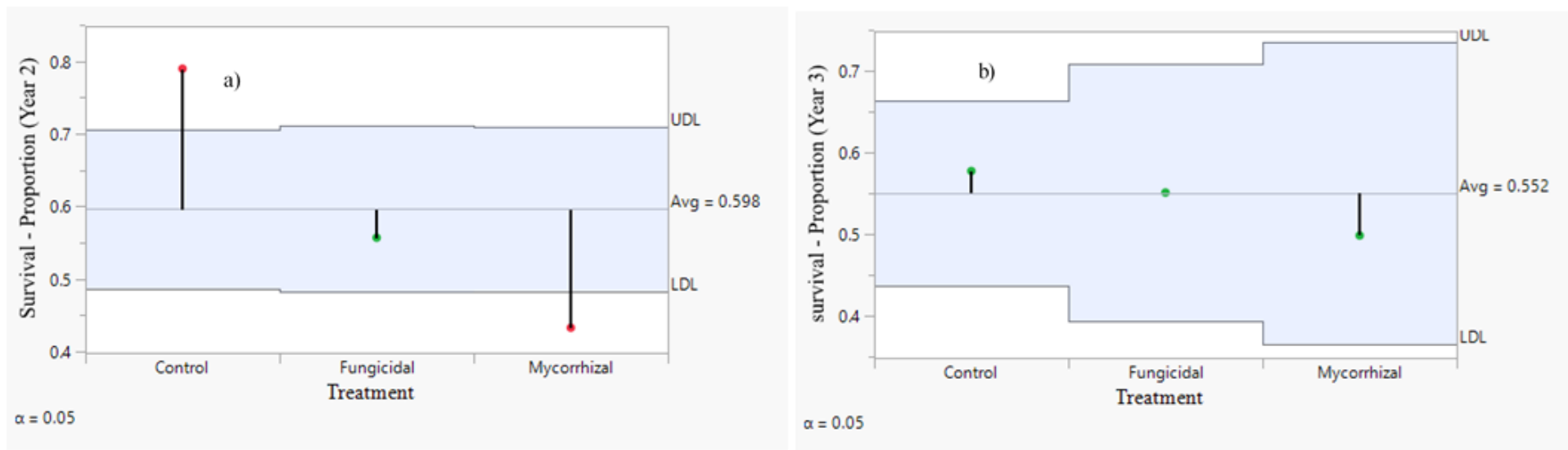


Figure 4-12: Impact of treatment on survival proportion of ramp (*Allium tricoccum*) seedling

Purchased from nursery after years of transplantation. (n= 72) ramps were purchased from a nursery. The proportion of surviving seedlings among total transplanted is displayed as an approximation to the binomial distribution (Dead vs. surviving). (LDL=Lower Decision Limit, UDL=Upper Decision Limit). Survival in year 2 a) varied by treatment ($p = 0.001$) and was <40% for seedlings with mycorrhizal treatment; b) in year 3, survival of the remaining bulbs was ~ 50% for seedlings with mycorrhizal treatment and 60% for seedlings with control treatment but was not significantly different ($p = 0.3$) by treatment.

5. CHAPTER V

MODELING FOREST FARMING SITE SUITABILITY FOR *ALLIUM TRICOCCUM* IN APPALACHIA

ABSTRACT

Site suitability analysis and modeling based on environmental resources (climatic and edaphic conditions) can provide critical information to support better management. Modeling techniques can help improve decisions on where to grow plants. The present study used suitability analysis and modeling to identify potential areas for *Allium tricoccum* Aiton (ramps, aka wild leeks) in the Appalachian region of the United States. Ramps, a wild-foraged plant of significant cultural value in the region, have gained popularity with audiences outside of Appalachia over the last 25 years. Increased wild harvesting and rising plant demand threaten the sustainability of these edible forest products. Forest farming may be a viable option to meet increasing global demand and conserve native populations, but a better understanding of site suitability is a critical need. This study used multi-criteria decision-making (MCDM), the Analytic Hierarchical Process (AHP), and weighted linear combinations to model suitable habitats for growing ramps. Ten habitat criteria were chosen (including five soil properties, three topographic parameters, and two land use properties) to assess the potential for growing ramps in seven counties in Virginia, West Virginia, Pennsylvania, and North Carolina. Sites were sampled at 10-m spatial resolution. Approximately 22% to 50% of the total land area in the studied counties was considered highly suitable for ramps production, with 36% to 54% moderately suitable. Ground truthing was performed to check the validity of the model. Ramp patch locations within each county were geocoded in the final suitability maps. The existing ramp patches were within the model's estimate of moderate to high site suitability ranges, suggesting that the model is valid. The geospatial approach and the results generated from the study suggest that site suitability modeling could be a useful tool for people interested in growing

ramps in forest farm settings across Appalachia. This study also provided insights for geospatial modeling and site suitability analysis for other woodland botanicals over a broader habitat range, although care must be taken so that such models are not used to increase sites for wild harvest by botanical poachers.

Keywords: Analytical Hierarchy Process (AHP), forest farming, geospatial approach, Multi-Criteria Decision-Making (MCDM).

INTRODUCTION

Allium tricoccum Aiton, or ramps or wild leeks, grow in mesic, hardwood forest ecosystems throughout the eastern United States and Canada. People living in the Appalachian Highlands in eastern North America have strong cultural ties to ramps (Davis and Greenfield, 2002) which play vital roles in food systems and local economies (Baumflek and Chamberlain, 2019). Because of its mountainous terrain and extensive forests, more than half of Appalachia's lands are unsuitable for traditional open-field agricultural production (Coltrane and Baum, 1965). In addition, the region's economy and communities face an uncertain future as its manufacturing and coal industries are in decline (McIlmoil and Hansen, 2010), and poverty and drug use are widespread (Moody *et al.*, 2017). At the same time, an influx of amenity seekers and the out-migration of multigenerational inhabitants in search of economic opportunities have shifted the region's social landscape (Trozzo *et al.*, 2019).

People in Appalachia must utilize their strengths to overcome these challenges. One such strength is the deep connection between people and scores of woodland understory plant species that grow abundantly in the biodiverse forests of the region. The people of the region have a rich history of harvesting these non-timber forest products (NTFPs), and several of these species offer opportunities as salable food (e.g., ramps, mushrooms (e.g., morels (*Morchella esculenta* Fr.), medicine (e.g., American ginseng (*Panax quinquefolius* L.), and ornamental crops (e.g., galax (*Galax urceolata* (Poir.)) (Vaughan *et al.*, 2013).

The growth in herb markets has significantly increased landowners' interest in growing medicinal plants and other NTFPs (Davis and Persons, 2014; Smith *et al.*, 2021). According to the Nutrition Business Journal (2006), consumer sales of herbs and botanical products were US\$4.41 billion in 2005. Sales increased by 17.3% from 2019 to 2020 (Smith *et al.*, 2021), a year of record revenues

(US\$11.2 billion). Sales growth of these products also exceeded that of the overall supplement industry in 2022 (9.7% for botanicals vs. 7.5% for the broader supplement industry; Nutrition Business Journal, 2022). This report also indicated that the share of herbs and botanicals in the supplement market is projected to increase from 18.6% in 2017 to 22.0% by 2025 (Nutrition Business Journal, 2022).

Most raw materials for forest-based herbal products are wild-harvested native woodland botanicals. In addition, the Appalachian bioregion serves as a supply hub for some of the most widely traded plants native to the region (Chittum *et al.*, 2019). Increasing pressure on native plant populations is thus an unfortunate consequence of the robustly growing herb market. Many uncertainties surround the long-term impacts of wild harvest on the ecological sustainability of these herb species.

Some botanicals may be cultivated commercially in forest farming systems as an alternative to wild harvest. Forest farming, a commercially and environmentally viable land-use strategy, deliberately combines some gardening or farming practices with conventional forestry (Mudge, 2009, Trozzo *et al.*, 2021). Although indigenous people have practiced forest farming and other agroforestry practices for centuries, Hill and Buck (2000) introduced the term ‘forest farming’ to describe contemporary cultivation of non-timber forest products, such as medicinal plants (e.g., ginseng), edible fungi (e.g., shiitake mushrooms) and decorative ferns, beneath existing tree canopy (Mudge, 2009). Such an approach could be advantageous for reducing pressures on native populations and for farmers and industries looking for new consistent sources of high-quality crops (Davis, 2007). Sustainably grown native plants in forest farms could create greater product value through source verification while reducing pressures on native plant populations and meeting the demands of global markets (Chamberlain, 2009).

Increasing interest in forest farming among landowners implies extensive acreage could be dedicated to the practice (Trozzo *et al.*, 2021), although growers may have little basis for making site selection decisions for such practices. Technology applications offer great promise to support and realize this potential growth. Effective forest farming systems will require forest management plans based on an owner's goals, inventory of resources, and detailed business plans (Zamora and Wyatt, 2018). Selecting suitable sites for the forest farm crop is important before developing forest management plans. A forest farming site suitability assessment should consider factors such as land formation (e.g., slope, aspect, erosion, surface drainage), climatic factors (e.g., precipitation, temperature), soil quality factors (e.g., pH, organic matter, mineral nutrients, and drainage), overstory canopy cover and existing forest vegetation (Zamora and Wyatt, 2018). One approach to better and faster assessment might involve using Geographic Information System (GIS) and spatial modeling technologies that are readily available and could be applied across Appalachia.

Why GIS and spatial modeling

Traditional methods of studying biophysical parameters, such as manual land surveys and point-based methods, are inefficient, time-consuming, and expensive. Often, such methods do not have sufficient information for the end users (Ahmad *et al.*, 2017). GIS data such as a digital elevation model (DEM) or land use land cover (LULC) classification usually have greater spatial coverage, efficiently handle data and computation, and are more reliable than traditional, point-based data. GIS and remote sensing data have been used in agricultural and forest systems to analyze land, visualize field data, and ultimately assist in precision farming (Abdullahi *et al.*, 2015, Kingra *et al.*, 2016 & Šedina *et al.*, 2017). A GIS-based tool can aid farmers and landowners in identifying suitable sites for crop production and in the application of site-specific management purposes.

Spatial modeling of the land for agricultural purposes can help farmers get the most out of the land without detriment to the environment.

GIS and forest farming

A modeling approach to site assessment can help rapidly screen and identify lands suitable for forest farming ramps and other NTFPs. This could ultimately aid in the conservation and preservation of their natural habitat. This study evaluated habitat suitability for ramps across selected landscapes within the Appalachian region. To accomplish this, we used the Analytical Hierarchical Process (AHP), a method of multi-criteria decision analysis using pairwise comparison matrices and incorporating them into a GIS platform. To correlate predictions from our suitability maps with species existence across the study locations, we compared the model results with known locations of ramps populations in natural settings.

METHODOLOGY

Study Area

We conducted this study in the Appalachian region of the eastern United States, which includes 420 counties across 13 states (Martin, 2013). The Appalachian region of the Eastern United States is a cultural and geographical area that spans from northern Alabama and Georgia (latitude 32° N, longitude -88°W) to the Southern Tier of New York State (latitude 42° N, longitude -74°W) (Appalachian Regional Commission, 2008). This region is characterized by its mountainous terrain, diverse flora and fauna, and unique cultural traditions (Appalachian Regional Commission, 2021). For this study, we selected seven counties from four states, i.e., Montgomery, Giles, and Smyth Counties of Virginia; Macon and Haywood Counties of North Carolina; Pocahontas County of West Virginia; and Lawrence County of Pennsylvania (Figure 3-1).

The study sites have diverse climates. Climatic variability (i.e., maximum and minimum temperature and precipitation) among the study counties were obtained using historical (2000 to 2022) annual gridded data from the International Research Institute for Climate and Society (NOAA NCEI) and presented in a table (Table 3-1).

Soil structure

Given the region's topography, climate, and parent material, the soils of Appalachia are generally thin and acidic, making the region unsuited for farming (Daniels and Zipper, 1995; Sena *et al.*, 2015). According to the soil survey, Montgomery County, Virginia, consists of deep, well drained, gentle to very steep soils that have clayey and loamy subsoil, texture ranging from silty clay loam, clay, silty clay to very gravelly sandy loam, and pH ranging from 3.6 to 7.8 (Porter *et al.*, 1985). Smyth County soils are acidic, predominantly brown, with textures ranging from silty clay loam and silt loam to loam and fine sandy loam consisting of slightly low organic matter (Jurney *et al.*, 1948). The soil of Giles County, Virginia, typically is well drained loamy and sandy sub soil, although many are stony and have steep slopes (Swecker *et al.*, 1985). Lawrence County of Pennsylvania has very deep, poorly drained soils composed of channery loam, shaly silt, clay loam, and silt loam mixed with unweathered bed rock (Smith, 1982). Similarly, the soils in Pocahontas County, West Virginia range from deep to very deep, gently sloping to extremely steep, well drained loamy soils formed in siltstone, limestone, shale, and some sandstone, and texture is dominated by channery silt loam and silt loam (Flegel 1999, USDA (NRCS) 2008). Haywood County, North Carolina soils are composed of silty clay, loam, clay loam, silty clay loam, and some fine sand (Goldston, 1954), while Macon County has loamy textured, very deep, well drained or moderately well drained gently sloping to very steep soils (Joy and Power, 2002; Thomas 1996).

Soil samples collected from each study County were subjected to routine analysis (including pH, P, K, Ca, Mg, Zn, MN, Cu, Fe, %OM and texture) at the Soil Testing Laboratory at Virginia Tech. The soil texture for the samples was analyzed using the hydrometer method. The soil physical and chemical parameters in the study Counties were diverse: soil pH ranged from 4.78-6.72, and nutrients P, K, and Ca ranged from (2-44 ppm), (48-97 ppm) and (306-2790 ppm), respectively. The percent of organic matter in the study area was very high (5-18.5). The soil texture in our study area is mostly silty clay loam with a combination of clay loam and silty clay Table 3-2.

Geological components and landforms

The Appalachian Mountain range, one of the world's oldest continental mountain systems, is divided into various physiographic provinces (Mitchell, 2020). The four main physiographic regions of Appalachia include the Piedmont, Blue Ridge, Ridge and Valley, and Appalachian Plateau provinces (Raitz, 2019). Sandstone, limestone, and shale are predominant parent materials of the southern reaches of the Appalachian Plateau and Ridge and Valley provinces, whereas igneous and metamorphic rocks predominate in the Blue Ridge (Mitchell, 2020). Montgomery County comprises mountains and valleys formed by erosion (Porter *et al.*, 1985), while Smyth County, in Appalachian Highlands, comprises steep mountain ridges between which lies wide valleys (Jurney *et al.*, 1948). The Giles County area is mostly in the Valley and Ridge province of Virginia and West Virginia (McDowell and Schultz, 1990). Haywood County and Macon County are with in Blue Ridge Province of Appalachian Highlands and Southern Appalachian Mountains with rolling, hilly and mountainous relief (Goldston, 1954; Thomas 1996; Wooten et al., 2008), while Lawrence County is in the Alleghany Plateau of Appalachian Plateau Province (Smith, 1982).

Multi-Criteria Decision Making

Multi-criteria decision-making (MCDM) offers methods and measures for evaluating decision problems, designing, and assessing alternative decisions (Malczewski, 2010). In GIS, multiple geographic data inputs are combined and transformed by MCDM to provide a decision output (Drobne and Lisec, 2009). In a multi-criteria decision-making technique, every criterion is weighed to indicate its significance to the phenomena (Chow and Sadler, 2010). Input data, the decision maker's preference, and manipulating information using predetermined decision rules are all components of MCDM (Ayehu and Besufekad, 2015). For this study, the input data for the MCDM approach were geographical, with topography, soil physical and chemical properties, and forest type and canopy cover selected for ramps habitat suitability analysis based on local and expert knowledge. Ten contributing factors—aspect, slope, elevation, soil moisture, soil drainage, organic matter, soil texture, soil pH, forest types, and canopy cover—were chosen after expert knowledge, literature review, and data analysis.

Developing habitat suitability model

Along with MCDM, AHP and weighted linear combinations methods were used to develop a habitat suitability model for ramps in this study. The conceptual framework for the study is presented in Figure 5-1 and described below.

Preliminary evaluation

Literature review and expert interview

We reviewed journal and newspaper articles, websites, and blogs related to ramp habitats. The review provided insights into ramps and their general habitat. Targeted subject matter experts were identified using the snowball sampling method. A total of 10 subject matter experts from different

states of Appalachia were interviewed to get preliminary information on their ideas regarding the major factors affecting the ramp's growth and the plants' general habitat. Based on the information collected, 10 habitat criteria were chosen: aspect, elevation, slope, soil moisture, soil organic matter, soil drainage, soil texture, soil pH, land cover, and forest type.

Study site selection

We chose seven counties from four states for this study (Figure 3-1). The counties selected have diverse climates, elevations Table 3-1, and latitudes. We had location and habitat information of at least one ramp population from each county as ramps and soil samples from these locations had been collected for other studies. The location-based information on ramp availability provided crucial validation sites for the suitability maps.

Data Collection and GIS Layer Preparation

Topographic factors: Elevation, Slope, and Aspect

Topography is a major controlling factor in the spatial distribution of plants (Jin *et al.*, 2008). The three primary topographic parameters that control the microclimate (which in turn affects the large-scale spatial distribution and vegetation patterns) are elevation, aspect, and slope (Allen and Peet 1990; Busing *et al.* 1993; Day and Monk 1974; Geiger 1965; Johnson 1981; Marks and Harcombe 1981). For elevation data, we downloaded a 1 arc-second (30 meters) digital elevation model (DEM) from the United States Geological Survey (USGS) National Elevation Dataset. Slope, one of the basic topographic elements for crop land suitability mapping, was generated from the DEM using the Slope tool under the spatial Analyst Tool of ArcGIS Pro 2.9. Slope and other factors can help with site suitability assessments (Wilson and Gallant, 2000). Every cell in the output raster had a slope value calculated as a percentage, with lower values indicating flatter terrain and higher

values indicating steeper terrain. Aspect provides information about the direction to which a slope faces which is significant in analyzing the light availability, temperature, and moisture for habitat suitability of the targeted crop. Aspect data for this study was created from the DEM downloaded from the USGS National Elevation Dataset.

Soil physical and chemical properties:

The foundational raw material for the growth of any plant is soil. From soil, plants acquire nutrients, secure anchorage, and interact with other beneficial (and detrimental) lifeforms. Analyzing and mapping any crop habitat suitability requires thoroughly understanding the soil's physical, chemical, and biological aspects (Ayehu and Besufekad, 2015). Important soil characteristics such as moisture (Water holding capacity), drainage, organic matter, texture, and pH were selected and analyzed for mapping suitable habitats of ramps. High-resolution (30-m) soil data were attained from the Soil Survey Geographic database (SSURGO), Natural Resources Conservation Service (NRCS) Web Soil Survey (WSS). The data were then extracted with Soil Data Viewer 6.2, which provided vector polygon layers of soil physical and chemical properties. Using the polygon attribute table, thematic layers of soil factors were retrieved and rasterized according to their field value using the conversion function in the ArcGIS platform.

Forest type and Forest Canopy

Ramps grow best in deciduous forests. To extract information on forest type, we downloaded national land cover data (NLCD) for 2016 (Dewitz, 2019). As with soil properties, the data have a spatial resolution of 30 m.

The forest canopy data were also created from the 2016 National Land Cover Database (REF). The U.S. Forest Service analytical canopy product represents the percent canopy cover for a given pixel. These data were downloaded at 30 meters spatial resolution.

All of these data were then resampled to 10 meters to improve performance because such resolution can show finer details of an image than 30-m per pixel resolution and be consistent with the spatial resolution of other layers.

Data interpolation

In some small patches of Giles and Smyth Counties (VA) and Haywood County (NC), soil data were missing. To complete soil data coverage across the counties, we applied Kriging, a spatial interpolation technique (Remy *et al.*, 2009), to estimate the soil values at unknown patches from samples of soil data at known nearby locations. Kriging, a technique for local weighted averaging evaluation (Oliver and Webster, 1990), uses z-scores to create an approximated raster surface from the spatial description of a dispersed collection of data points and has been widely used for predicting and mapping different soil properties (Govaerts and Vervoort, 2010; Yasrebi *et al.*, 2009). The regionalized variables theory is the foundation for the Kriging interpolation method (Meng, 2021). For calculating the regionalized variable value with $Z(\mathcal{X})$ being the region change quantity of the unsampled point of the study area and $Z(x_0)$ a projected value of any point x_0 , the sample point observation value ($\mathcal{X}i$) might be used with n number of known observation sample points. The projected point values can be obtained by a linear combination of observed values of known sample points (Meng, 2021):

$$Z(x_0) = \sum_{i=1}^n \lambda_i Z(\mathcal{X}i) \text{ (Eq1)}$$

Where,

$Z(X_0)$ = estimated output value of predicted location point X_0

λ_i = unknown weights for the measured value at each location, the sum of which is one

$Z(X_i)$ = the measured values at each location

n = number of known values

For this study, we used the ‘Geostatistical Wizard’ function available in ArcGIS Pro and chose the ordinary Kriging with prediction analysis and spherical model function for our interpolation analysis.

Every raster data layer prepared was projected to the Universal Transverse Mercator (UTM) coordinate system Zone 17N and had a spatial resolution of 10 m. The literature, experts' knowledge through in-person interviews, and the author's field-based knowledge were used to define the suitability ranges for all criterion layers (Table 5-1). Each variable was reclassified to extract the values corresponding to suitable habitats for ramps.

Assessing weights for each criterion using the analytic hierarchy process (AHP)

Selected factors for ramps suitability assessment had different levels of importance, which varied with other factors. For example, the importance of soil organic matter could differ relative to slope or aspect. Thus, one needs to compare the importance of a factor among the criteria chosen. Many decision-making methods evaluate the relative importance of criteria with their alternatives (Islam *et al.*, 2018). MCDM offers several procedures to solve decisional problems and prioritize alternative decisions (Ayehu and Besufekad, 2015). AHP is the most extensively used and widely regarded MCDM technique, as many scholars consider AHP to be the most consistent (Triantaphyllou and Mann, 1995) and are widely used when working with GIS-related suitability studies (Marinoni, 2004). Using the AHP method, weights for each criterion are calculated by

comparing two criteria at a time in a pairwise comparison matrix (Saaty, 1980). The scale developed by Saaty (1980) was used to determine the values for the pairwise comparisons in the AHP method. The values in the scale range from 1 to 9 (Table 5-2), where 9 indicates that the row factor is more important than the column factor, and a value of 1 indicates that column and row factors are equally important. A rating of 1/9 indicates that the row factor is less important than the column factor (Mustafa *et al.*, 2011). Using this scale ratio, a value of importance was given to each criterion relative to other criteria in a pairwise comparison matrix (Table 5-3). In this study, the assignments of the value of importance were based on the subject matter experts' judgments and the literature. The pairwise comparison matrix A ($n \times n$ order) with elements 'a_{ij}' was formed (Ayehu and Besufekad, 2015) (Table 5-3) where:

$$A = [a_{ij}], i, j = 1, 2, 3, 4, \dots, n \text{ (Eq2)}$$

Furthermore, the reciprocal values are such that $a_{ij} = 1/a_{ji}$. This matrix was then normalized as matrix B (Feizizadeh *et al.*, 2014) (Table 5-4);

$$B = [B_{ij}], i, j = 1, 2, 3, 4, \dots, n \text{ (Eq3)}$$

with elements 'b_{ij}.' Where b_{ij} was calculated by dividing a_{ij} with the sum of a_{ij} in each column as:

$$b_{ij} = \frac{a_{ij}}{\sum_{i=1}^n a_{ij}} \text{ (Eq4)}$$

A weight value 'w_i' for each criterion was calculated by dividing the sum of normalized row values for each criterion by the total number of observations, such as:

$$w_i = \frac{\sum_{j=1}^n b_{ij}}{\sum_{i=1}^n \sum_{j=1}^n b_{ij}}, i, j = 1, 2, 3, 4, \dots, n \text{ (Eq5)}$$

The AHP determines the weighting (w_i) for each criterion using this pairwise comparison matrix by picking the Eigenvector corresponding to the biggest Eigenvalue of the matrix and normalizing the total of the components to unity (Feizizadeh *et al.*, 2014) as: $\sum_{i=1}^n w_i = 1$ (Eq6)

To be consistent with the weights calculated from the pairwise comparison, as suggested by Saaty (1977) and Prakash (2003), we calculated the consistency ratio (CR) that indicated random generation of matrix judgments.

CR is calculated by dividing the consistency index by the random index

$$CR = \frac{CI}{RI} \text{ (Eq7)}$$

Where:

$$CI = \frac{(\lambda - n)}{(n-1)} \text{ (Eq8)}$$

Furthermore, λ denotes the maximum eigenvalue (Table 5-5); n is the number of criteria in each pairwise comparison matrix, and RI is the average of the resulting consistency index (CI) depending on the order of the matrix (Saaty, 1980; Table 5-6). The maximum eigenvalue for the matrix is the average ratio between the individual weighted sum and the weight of each factor. The weighted sum of the factors is calculated by multiplying their normalized values in each row by their weights and adding these resulting values.

There is a reasonable amount of consistency when the consistency ratio (CR) is ≤ 0.10 (Ayehu and Besufekad 2015, Saaty 1977, Prakash 2003). In this study, CR was 0.08, indicating that the comparisons of habitat criterion were consistent and that the relative weights were appropriately chosen. On this basis, we decided to use the calculated weights from the AHP method as the final weights for the factors used in the habitat suitability analysis (Table 5-5).

Overlay Analysis

The weighted sum overlay technique was used to prepare the final suitability map. The weighted overlay is a method for creating an integrated analysis by applying a common scale of values to various and distinct input data (Kuria *et al.*, 2011). The decision-maker can define the observed importance of each criterion with the others considered in the evaluation using weighted priority (Carver, 1991). The weighted sum overlay method creates a suitability map by multiplying the cell values of each input raster with their respective criteria weights and then adding the resulting values together (Munene *et al.*, 2017). Weights derived from the AHP process (Table 5-7) were given to the different map layers for different factors characterizing habitat suitability for ramps in this study. Then, the cell values of each input raster layer are multiplied by their respective weights, and the resulting products are added together to obtain a weighted sum value for each cell in the final suitability map. After generating a final suitability map, the suitability range was classified into 3 classes low, moderate, and high, using the natural break classification method of ArcGIS Pro 2.9.

Suitable area calculation:

Suitable areas for ramps in each studied county were estimated by using a raster calculator in ArcGIS Pro. The number of pixels covered by each suitability class was calculated using a reclassified tool and raster calculator tool of ArcGIS Pro. Using that pixel number, the total area covered by that suitability class was calculated by multiplying the pixel number by the spatial resolution (cell size in m²) for that map.

Validating the map

Ground-based observation, a significant method to validate the information developed using the geospatial technique, was made to validate the results produced from our final model. For ground truthing, ramp patches were visited for each county to determine the coordinates of these patch boundaries using GPS (Garmin eTrex 10). After adding the GPS coordinate data for known locations to our final map, we determined whether or not the known locations fell within the suitability range of our final map. In addition, for each patch location, the environmental characteristics were recorded.

RESULTS

Suitability area calculations

The weighted overlay of soil data, land cover data, and DEM data elements are substantial in outlining the suitable areas for ramps. The suitability maps for ramps, generated by the weighted sum overlay technique using Spatial Analyst tools in ArcGIS Pro, are shown in Figure 5-2 to Figure 5-5. Suitable land cover areas for ramps in each studied county were calculated and are presented in Table 5-8.

Montgomery County (Figure 5-2): The final suitability map indicates that a considerable part (70%) of the study area is suitable for ramps. The highly suitable areas (24%) roughly correspond to the northeastern portion of the county, with high moisture soil and high organic matter content facing the landscape's eastern and northeastern aspects. However, some high and moderately suitable areas are located on the western and northwestern aspects of the landscape. These sites have moderate to high soil moisture and high organic matter content. The county's highly suitable areas are mostly between 1100 ft (325m) and 2250 ft (686m) in elevation.

Smyth County (Figure 5-3, *a*): Around 28.7% of Smyth County was in the high suitability range for ramps. Almost all areas of the mountains and creeks that are expanded through the eastern to the western part of the county are highly suitable for ramps. Most of the county's highly suitable areas for ramps were found between 2500 ft (762m) to 3500 ft (1067m) elevation and mostly in eastern and northeastern facing slopes.

Giles County (Figure 5-3,*b*): Around 27.8% of the county appears to be highly suitable for ramps. Based on the final map, the area comprising approximately 75% of the county, which includes mountains and creeks, is the most appropriate for ramps. The highly suitable sites for ramps were found between 1995 ft (608 m) to 2410 ft (734 m) elevation, whereas most of the moderately suitable sites were located between 2400 ft (731 m) to 3425 ft (952 m) elevation. Most of the slopes on the suitable sites faced east or southeast and had high soil moisture and organic matter content.

Pocahontas County (Figure 5-4, *a*): More than 80% of the county was considered suitable for ramps based on our model, with 44.5% of the area within the very high suitability range. These areas, predominantly within the northwestern part of the county, are largely mountainous with abundant creeks. These highly suitable sites have well-drained soils with high organic matter content and soil moisture. Most highly and moderately suitable sites were located between 2650 ft (807 m) to 4850 ft (1478 m) elevation.

Macon County (Figure 5-4,*b*): The final suitability map indicated that more than 85% area of the county is suitable for ramps (high to moderate), with about half (49.6%) of the county within the highly suitable range. These sites predominated in the eastern and southwestern parts of the county on mountains and ridges, with moderate to high soil moisture, high soil organic matter content, and good soil drainage capacity. High suitability areas were located between 2500 ft (762 m) to

4000 ft (1219 m), while some high and moderately suitable sites were also located around 1200 ft (365 m) elevation.

Haywood County (Figure 5-5, *a*): More than 2/3rd of the county is suitable for ramps, with around 21.5% of the area's very high suitability range. Looking at the final map, the area with mountains, ridges, and creeks in the county appears to be highly suitable for ramps. The highly suitable sites for this county are between 2950 ft (899 m) to 5445 ft (1659 m). The suitable sites at higher altitudes were characterized by high soil moisture.

Lawrence County (Figure 5-5, *b*): The final suitability map showed that more than 4/5th of areas of the County are highly or moderately suitable for ramps, with approximately 33% of the area under the very high suitability range. The forested area with small streams and creeks is highly suitable for ramps. The suitable sites were mostly located towards eastern and northeastern-facing slopes with high soil moisture and very high organic matter. Most highly suitable areas are located between 750 ft (228 m) to 1275ft (388 m) elevation.

Ground truthing for validation

For this study, we used one location point of known ramp patches within each county except for Montgomery County, VA, Haywood County, NC, and Lawrence County, PA, where two known ramp patches were used for ground truthing the model. These sites were geocoded in the final maps using the GIS platform. Ground truth plot locations fell under the high and moderately suitable site ranges in all landscapes of our final maps (Figure 5-2 to Figure 5-5). In addition, our field observations of the habitat conditions for each ramp patch were consistent with the characteristics of suitable sites the model generated for each studied county. On the other hand, we did observe some differences in habitat conditions of these existing patches compared to the ranges of environmental variables used to develop the suitability map. For instance, the wild ramp

patch in Macon County was located at an elevation of 3600 ft (1100 m), which falls slightly outside of the elevation range we used (1000-3500 ft (305-1067) m; Table 5-1). The sloping landscape of this patch was 27% with a northeastern aspect, while the soil was well-drained and moist. Similarly, the wild ramp patch in Pocahontas County, West Virginia, was located at 3180 ft (970 m), with a slope landscape of 35% in the southern aspect (aspect ranges used were North, East, Northeast, and Northwest, Table 5-1), and the soil was highly drained and moist, (Table 5-9).

DISCUSSION

Suitability modeling

Results indicate that the suitability mapping approach can identify suitable forest habitats to grow ramps. Site suitability mapping illustrated that unsampled parts of the region might be hotspots for ramps. Soil moisture, soil organic matter, and forest type were reliable for identifying suitable habitats for ramps. Vasseur and Gagnon (1994) presented a similar assessment of site conditions and declared that “ramps can be found in mesic deciduous forests where soil moisture and nutrients are the most important factors in their growth.” Their findings supported the continued use of these parameters in modeling and selecting appropriate habitats for conserving and cultivating ramps. In our model, soil moisture was given the greatest weight, which is considered the most important factor for ramps growth. Vasseur and Gagnon (1994) corroborate this by reporting that ramps could be produced in open agricultural fields with sufficient soil moisture. Stark (2022) also reported that soil moisture content was greater in the ramp’s habitat. Together with high soil moisture, this study considered soil organic matter as a critical factor for the habitat suitability of ramps, as previously identified by Beatty (1984), Bernatchez et al. (2013), Chamberlain et al. (2009), and Davis and Greenfield (2002). Our findings indicate that areas with high soil organic matter within each county are highly suitable for ramps.

As a shade-tolerant herbaceous species, ramps grow well under a forest canopy of deciduous trees such as beech, birch, and maple (Chamberlain, 2009). After the growing season, shade from the overstory maintains conditions suitable for ramps by conserving soil moisture and keeping soil temperatures low (Chamberlain *et al.*, 2014). However, moisture appears to be more important than canopy cover, as ramps can grow and survive well in conditions of high light availability as long as there is adequate soil moisture (Dion *et al.* 2017). This is consistent with our approach, as canopy cover was given less weight than soil moisture in the habitat suitability analysis for ramps based on expert opinion. Among the topographical variables used in this study, aspect was given the highest weightage to model suitable ramps habitat. North, east, northeast, and northwest aspects were considered suitable for ramps. The results showed that the highly suitable areas for ramps were predominantly located in each county's eastern and northern aspects, as these aspects provided more shade from the south. This shade created an environment with optimal moisture and temperature conditions conducive to the growth of ramps. In a study on the ramps population in southern Appalachia, the highest densities of natural ramps patches were on east through north aspects (Walker and Knapp, 2010). In this study, although most of the highly suitable sites for ramps were found in sites with eastern to northern aspects, western and northwestern aspects were also suitable in sites with high soil moisture and high soil organic matter content. Our findings indicate a connection between high-suitability sites and soil moisture supported by high soil organic matter for ramps. These, in turn, were more likely on cooler, shaded sites found on eastern, northeastern, and northern aspects of deciduous forests. The relationship between the study area's topography, soil quality, and landcover maps and the map showing the ramps' suitability is better understood using the weighted overlay technique.

Model validation with ground truthing

Ground-based validation has always been of utmost importance in geospatial science. Without ground-truthing, the issue is how much of the representation of the data we have provided is an abstraction of the real world and how much that undermines accuracy (Carp, 2008). Ground truthing compares evidence specified in official documents or maps with the actual situation at a location (CPR-Namati, 2018). As a method for the physical confirmation of the created maps, ground truthing can be used as a powerful method to produce evidence by gathering readily observable information about the incidence, presence, or absence of the factors presented in the map. For this study, location points of known ramp patches in each county were used to ground the truth of our model and geocoded into our final map using the GIS platform. The ground truth plot locations fell within the high and moderately suitable site ranges in all landscapes of our final maps (Figure 5-2 to Figure 5-5).

Furthermore, the habitat conditions of each ramp patch matched the characteristics of suitable sites generated by the model for each studied county. However, some ground-truthed site conditions measure slightly outside the ranges we used to develop the suitability map. In particular, some existing ramp patches' elevation values and aspects were slightly outside the range we used to develop the model. For example, the ramp patch in Giles County is located at 641ft (200 m) elevation, and the ramp patch in Smyth County faces west and northwest. However, these patches are in deciduous forests with moist, well-drained loamy soil. This indicates that soil moisture and drainage are more important than elevation and aspect for modeling the habitat of ramps. Ground-based verification of known ramp patches in each study site ensured the applicability of the habitat suitability map and provided validation for the model.

Since the accuracy of the ground truth data (obtained from dependable sources) verified the habitat suitability map (Jacquin et al., 2005), the approach and method presented in this study can be an important tool for monitoring targeted species. Suppose further information, such as socioeconomic variables, were accessible and taken into consideration. In that case, the accuracy of the final mapping may be increased even more.

Practicality of the Model

The overall model developed in this study could be useful to determine the locations where ramps may grow well, but it cannot necessarily predict that sites will contain wild populations. This model can serve those to establish new populations through forest farming for economic benefit or reintroducing the species as a conservation practice. Modeling site suitability for ramps primarily depended on the soil characteristics of the landscape, particularly soil moisture and soil organic matter.

This approach can also determine suitable habitats for other forest woodland botanicals such as goldenseal, ginseng, and blue cohosh. This could be accomplished by adjusting the weights of variables used in accordance with the needs of the targeted species. For example, this approach could be useful to model the American ginseng habitat, which prefers moist, well-drained soil with high organic matter on north and east-facing slopes (Vaughan *et al.*, 2009). Similarly, this approach can be useful for modeling goldenseal habitat, considering its preference for forested slopes facing north, northeast, and east under the mature forest canopy (Persons and Davis, 2005). The model may need to be adjusted to give greater importance to canopy cover, as Goldenseal cannot grow in open fields (Eidus, 2021).

We integrated spatial information about ramp habitat for our study area with GIS to model site suitability. Variable weights were assigned to each criterion using AHP methodology that

incorporates objective criteria and the people's subjective judgment (Jianyuan, 1992). Some errors may occur while determining these weights, affecting the model's precision. Since ramps maintain a vegetative canopy for a few months each year and take five to seven years to reach maturity, it is not easy to assess the impacts of individual parameters on its growth and survival in a certain habitat. Estimating the impacts of individual parameters and their interaction with each other in various locations may help improve habitat suitability modeling.

CONCLUSIONS

Habitat suitability analysis of forest farming botanicals involves major decisions at various stages of model development. Such analysis has the potential for discerning suitable sites for the efficient production of these botanicals in forest farming systems and for conserving their natural populations. Suitability mapping could contribute to forest farming production to improve biodiversity conservation and food security. Data from many sources, such as soils and land cover maps, are systematically integrated to identify the optimal habitat appropriateness of a region. However, mapping criteria will vary in their contribution to estimating suitability. When criteria are categorized and arranged in hierarchies, it is possible to address the relative degree of contribution of different criteria. The relative significance of these elements can be assessed to determine suitability using weighted overlay techniques and multi-criteria decision-making.

This study used multi-criteria decision-making, the AHP method, and GIS-integrated weighted overlay methods to investigate the habitat suitability of ramps. According to the findings, the total amount of land across the studied counties' highly suitable ramp habitat ranged from 21% to 49%, while moderately suitable habitat ranged from 45% to 64%. This study showed the potential of soil data, land cover data, and DEM in the site suitability mapping of ramps. In conclusion, this study has highlighted the efficacy of choosing the right variables, giving reasonable weights to

them, and using AHP and GIS techniques to find a suitable site for the proposed herb. By choosing similar variables and methodology, site suitability mapping of other woodland botanicals that share a similar habitat with ramps can be done. Incorporating other environmental and socioeconomic variables can further help to understand ramps' habitats and their significant impacts on forest farming.

Table 5-1: Ranges of variables assessed using literature review and expert interviews
It was used to develop suitability maps for ramps.

Aspect	Slope (gradient)	Elevation (ft)	Soil Moisture	Soil Drainage	Organic matter	Soil Texture	Forest types	Canopy Cover	Soil pH
North, East, Northeast, and Northwest	6-40	1000-3500	Medium to High	Moderate to Well drain	High	Silty loam and loam	Deciduous	30-90	4.7-6.7

Table 5-2: Scales for pairwise comparison

Intensity of importance	Description
1	Equal importance
3	Moderate importance
5	The strong or essential importance
7	Very strong or demonstrated importance
9	Extreme importance
2,4,6, 8	Intermediate values
Reciprocals	Values for inverse comparison

Table 5-3: Pairwise comparison matrix

(Assigning the value of importance to each criterion relative to other criteria based on the subject matter experts' judgments and the literature)

Criteria	Soil OM	Soil pH	Soil Moisture	Texture	Drainage	Elevation	Slope	Aspect	Canopy cover	Forest type
Soil OM	1	7	1/7	5	3	7	6	5	3	2
Soil pH	1/7	1	1/9	1/3	1/5	1/2	1/3	1/4	1/5	1/5
Soil Moisture	7	9	1	5	3	9	5	7	5	3
Texture	1/5	3	1/5	1	1/3	3	2	1/3	1/4	1/5
Drainage	1/3	5	1/3	3	1	5	2	3	1/2	1/5
Elevation	1/7	2	1/9	1/3	1/5	1	1/2	1/3	1/4	1/5
Slope	1/6	3	1/5	1/2	1/2	2	1	1/2	1/3	1/5
Aspect	1/5	4	1/7	3	1/3	3	2	1	1/4	1/5
Canopy cover	1/3	5	1/5	4	2	4	3	4	1	1/2
Forest type	1/2	5	1/3	5	5	5	5	5	2	1

Table 5-4: Normalized Pairwise comparison matrix.

Criteria	Soil OM	Soil pH	Soil Moisture	Texture	Drainage	Elevation	Slope	Aspect	Canopy cover	Forest type	Weight	Rank
Soil OM	0.10	0.16	0.05	0.18	0.19	0.18	0.22	0.19	0.23	0.26	0.18	2
Soil pH	0.01	0.02	0.04	0.01	0.01	0.01	0.01	0.01	0.02	0.03	0.02	8
Soil Moisture	0.70	0.20	0.36	0.18	0.19	0.23	0.19	0.26	0.39	0.39	0.31	1
Texture	0.02	0.07	0.07	0.04	0.02	0.08	0.07	0.01	0.02	0.03	0.04	7
Drainage	0.03	0.11	0.12	0.11	0.06	0.13	0.07	0.11	0.04	0.03	0.08	5
Elevation	0.01	0.05	0.04	0.01	0.01	0.03	0.02	0.01	0.02	0.03	0.02	8
Slope	0.02	0.07	0.07	0.02	0.03	0.05	0.04	0.02	0.03	0.03	0.04	7
Aspect	0.02	0.09	0.05	0.11	0.02	0.08	0.07	0.04	0.02	0.03	0.05	6
Canopy cover	0.03	0.11	0.07	0.15	0.13	0.10	0.11	0.15	0.08	0.06	0.10	4
Forest type	0.05	0.11	0.12	0.18	0.32	0.13	0.19	0.19	0.16	0.13	0.16	3

Table 5-5: Eigen value computation matrix

Criteria	Soil OM	Soil pH	Soil Moisture	Texture	Drainage	Elevation	Slope	Aspect	Canopy cover	Forest type	WS	Weight	Ratio
Soil OM	0.18	0.12	0.04	0.21	0.25	0.16	0.22	0.26	0.30	0.32	2.07	0.18	11.66
Soil pH	0.03	0.02	0.03	0.01	0.02	0.01	0.01	0.01	0.02	0.03	0.20	0.02	11.02
Soil Moisture	1.24	0.16	0.31	0.21	0.25	0.20	0.18	0.37	0.50	0.47	3.90	0.31	12.59
Texture	0.04	0.05	0.06	0.04	0.03	0.07	0.07	0.02	0.03	0.03	0.44	0.04	10.22
Drainage	0.06	0.09	0.10	0.13	0.08	0.11	0.07	0.16	0.05	0.03	0.89	0.08	10.82
Elevation	0.03	0.04	0.03	0.01	0.02	0.02	0.02	0.02	0.03	0.03	0.24	0.02	10.63
Slope	0.03	0.05	0.06	0.02	0.04	0.05	0.04	0.03	0.03	0.03	0.38	0.04	10.40
Aspect	0.04	0.07	0.04	0.13	0.03	0.07	0.07	0.05	0.03	0.03	0.56	0.05	10.56
Canopy cover	0.06	0.09	0.06	0.17	0.16	0.09	0.11	0.21	0.10	0.08	1.14	0.10	11.34
Forest type	0.09	0.09	0.10	0.21	0.41	0.11	0.18	0.26	0.20	0.16	1.82	0.16	11.57
λ							11.08						

Table 5-6: Random Index (RI) matrix (Saaty, 1980)

Order Matrix	R.I.	Order Matrix	R.I.
1	0.0	6	1.24
2	0.0	7	1.32
3	0.58	8	1.41
4	0.9	9	1.45
5	0.12	10	1.49

Table 5-7:Decision matrix for criteria weights

Criteria	Soil OM	Soil pH	Soil Moisture	Texture	Drainage	Elevation	Slope	Aspect	Canopy cover	Forest type
Weight of criteria	0.18	0.017	0.31	0.042	0.0822	0.023	0.036	0.0528	0.1	0.157

Table 5-8:Suitability area for ramps in each county

Counties	Suitability (%)	
	High	Moderate
Montgomery	24	46
Smyth	28.7	49.8
Giles	27.8	46.7
Pocahontas	44.5	38.1
Macon	49.6	36.7
Haywood	21.5	48.9
Lawrence	33	54.5

Table 5-9:Habitat properties of existing ramp patches (studied for model validation)

Ramp patch	Location		Elevation (m)	Slope%	Aspect	Canopy Cover %	Soil Moisture	Soil Drainage
	State	County						
1	VA	Giles	195.4	Flat	North East	70-90	Wet	Drained
2	VA	Smyth	359.1	30-40	West and North west	70-80	Moderately wet	Drained
3	VA	Montgomery	513.0	30-40	Northeast	70-80	Moderately wet	Well drained
4	VA	Montgomery	609.6	<10	East and southeast	75-90	Wet	Well drained
5	PA	Lawrence	273.4	Flat	North	40	Slightly wet	Slightly drained
6	PA	Lawrence	357.8	0-10	North	70	Slightly wet	Slightly drained
7	NC	Macon	1097.3	27	North East	75-90	Slightly wet	Drained
8	WV	Pocahontas	969.3	35	South	75-90	Wet	Highly drained
9	NC	Haywood	1335.0	40	Northwest	70-90	Moderately wet	Well drained
10	NC	Haywood	1499.6	30-40	Northwest	75-90	Moderately wet	Well drained

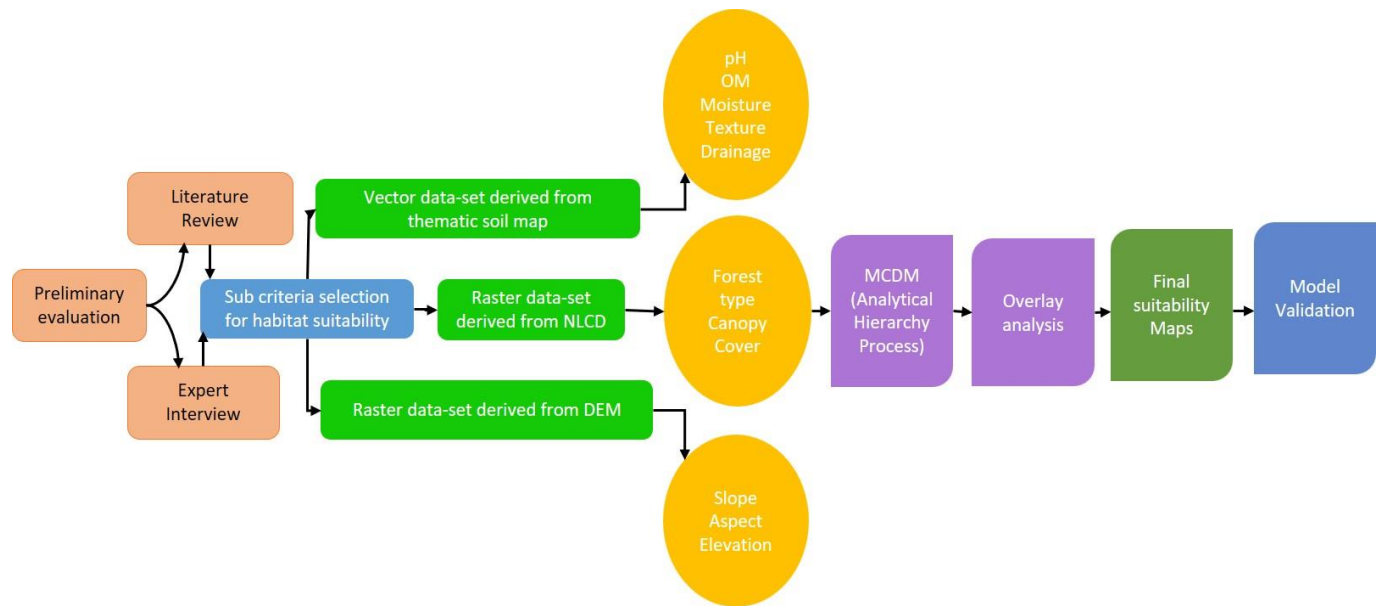


Figure 5-1: Conceptual framework of the study

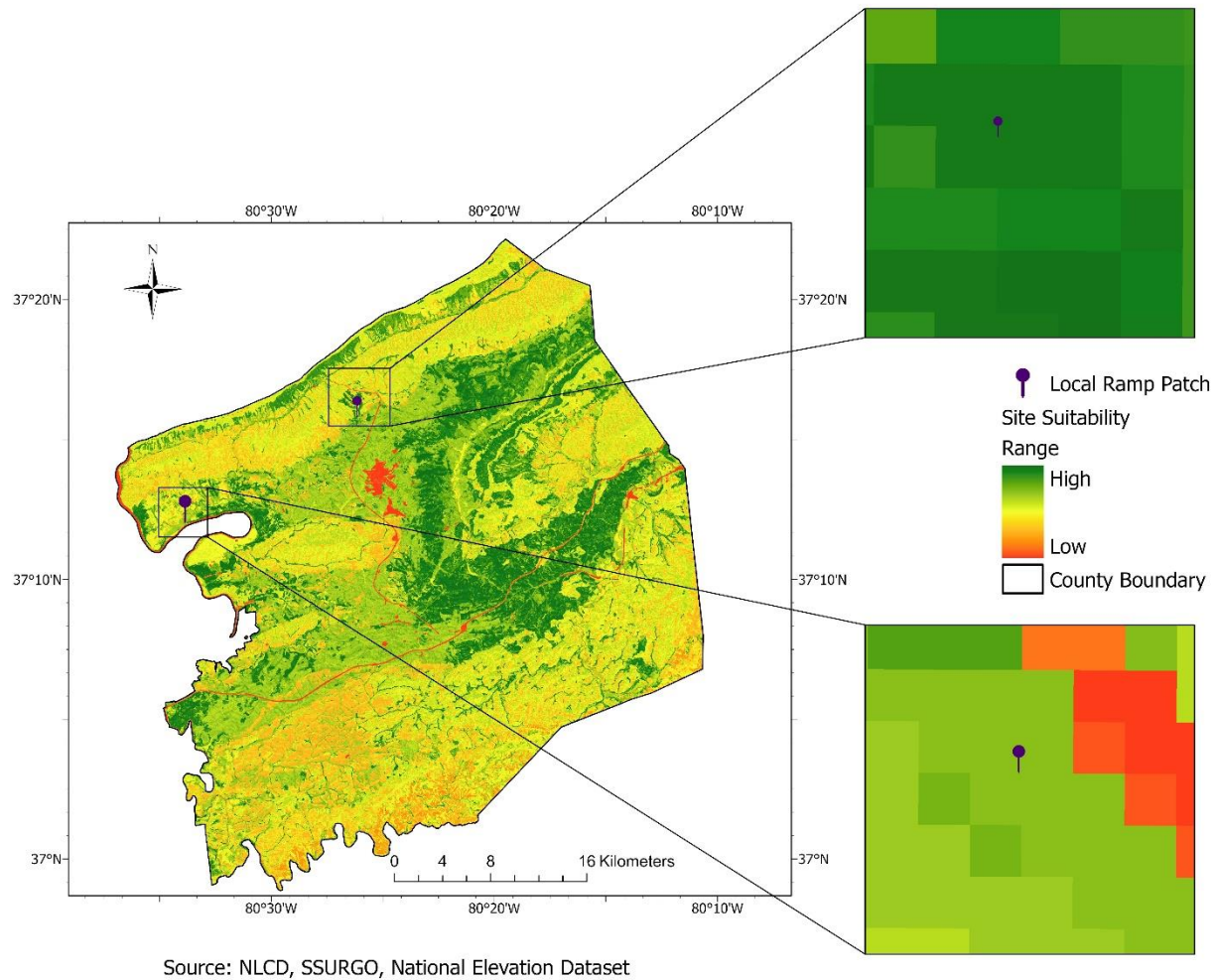
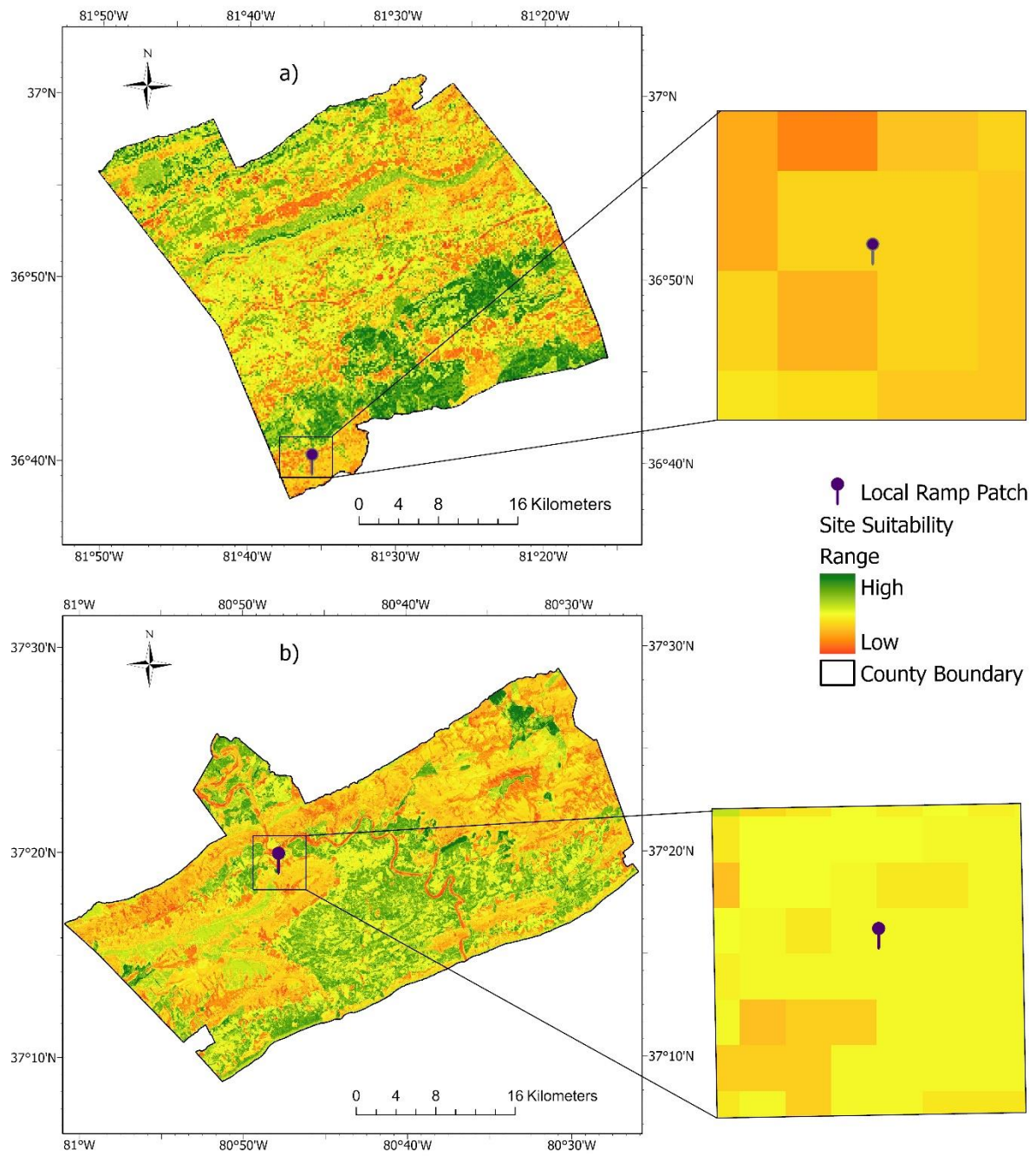


Figure 5-2: Habitat suitability map of ramps in Montgomery County, Virginia.
(The map also includes the existing plot locations of ramps)



Source: NLCD, SSURGO, National Elevation Dataset

Figure 5-3: Habitat suitability map of ramps in a) Smyth County and b) Giles County of Virginia. (The map also includes the existing plot locations of ramps)

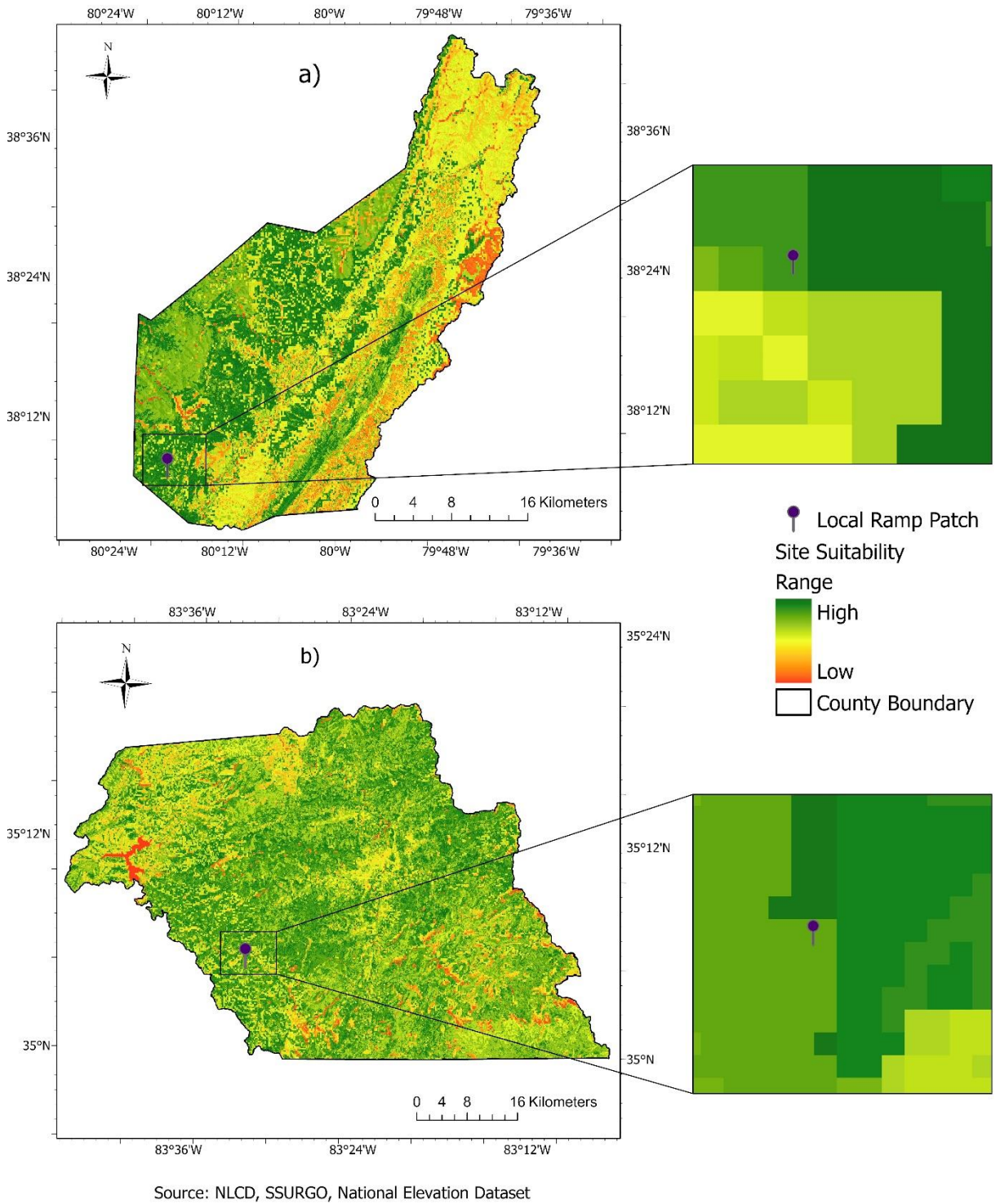
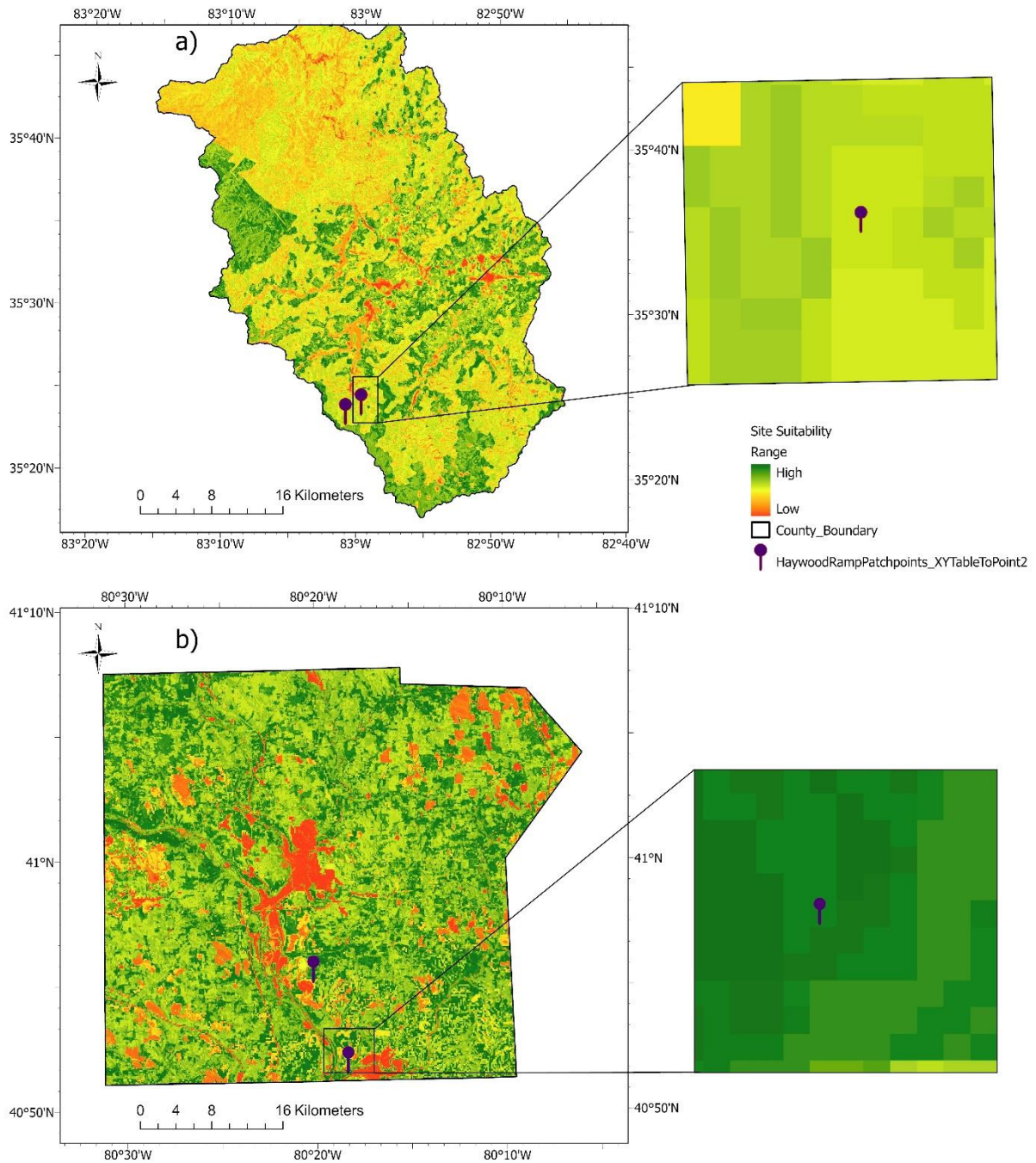


Figure 5-4: Habitat suitability map of ramps in a) Pocahontas County, West Virginia, and b) Macon County, North Carolina.

(The map also includes the existing plot locations of ramps)



Source: NLCD, SSURGO, National Elevation Dataset

Figure 5-5: Habitat suitability map of ramps in a) Haywood County, North Carolina, and b) Lawrence County, Pennsylvania.

(The map also includes the existing plot locations of ramps)

6. CHAPTER VI

SUMMARY

Ramps, a cultural keystone species for Appalachia, have historically contributed to the region's identity and economy, but the growing demand for the plant as a specialty food has resulted in increased harvest, which threatens native populations. Improved cultivation techniques and technical support for sustained yield forest farming practices seem to be needed to conserve native populations and meet the increasing demand. This dissertation investigated techniques to evaluate suitable production practices for ramps, examining the effects of ecotypic variation and mycorrhizal treatment on growth, survival, stress, and recovery of transplanted ramps and determining the suitability of habitats for forest farming of ramps. The first study examined the importance of ecotypic population diversification in selecting locally adapted planting materials for sustainable forest farming of ramps. The study investigated the growth, survival, and stress responses of native ramp bulb ecotypes from eight locations among appropriate gradients in the Appalachian region of the eastern United States. The growth response of native ramp ecotypes varied depending on the source site, highlighting the importance of the plant's source site environmental factors, such as temperature, precipitation, and soil nutrient composition, and those conditions at the recipient (transplanting) site for optimal growth and survival.

Ecotypic variation affected the growth and survival of ramp seedlings along with the proportion of survived plants in the years following transplanting. The highest and lowest proportion of stressed ramps surviving in the common garden site was moved from one of the locations closest to the garden. Clear relationships were observed between plant morphology, transplantation stress, and survival in ramps. Ramps with larger leaves and smaller bulb diameters demonstrated greater susceptibility to transplantation stress and had lower survival rates, suggesting limited metabolic capacity and inadequate energy storage for future survival. These findings imply that ramps with

larger bulb sizes and shorter, narrower leaves may be more suitable for transplantation or that transplanting bulbs in the fall after leaf senescence could be a preferable approach compared to transplanting the entire plant soon after emergence.

The second study investigated AMF association with ramps and their potential to improve growth and survival in forest farming systems. Ramps were collected from eight locations in four states, and soil properties were assessed to determine AMF presence. Ramps and soils had a relatively low abundance of AMF across habitats. Inoculation with a commercial AMF strain increased leaf length, survival, and stress tolerance of some transplanted ramps ecotypes. Overall, the study suggests that the association with AMF may offer potential benefits for the growth and conservation of native plants in forest farming systems. Nevertheless, before implementing AMF inoculation on a larger scale, preliminary tests should assess the adaptability of the inoculated species to the soil and source plant material. Further research is needed to understand the role of AMF in supporting ramp growth, particularly the host plant-fungal strain interactions and the interactions of AMF with other soil fungal species.

The third study identified suitable habitats for ramps in Appalachia using site suitability analysis and modeling techniques. MCDM, AHP, and weighted linear combinations were used to assess potential habitats for ramp growth based on ten habitat criteria. The model was applied to seven counties across Virginia, West Virginia, Pennsylvania, and North Carolina. The site suitability model indicated that 22% to 50% of the total land area in the studied counties was highly suitable for ramp production, with 36% to 54% moderately suitable. The model's validity was confirmed through ground-truthing, as existing ramp patches were within the model's estimate of moderate to high site suitability ranges. Site suitability modeling could be useful for those interested in growing ramps in forest farm settings across Appalachia and provides insights and a framework

for geospatial modeling and site suitability analysis for other woodland botanicals over a broader habitat range.

In conclusion, the findings of this dissertation highlight the importance of implementing improved cultivation techniques and technical support to conserve native populations of ramps, a cultural keystone species in Appalachia. The first study emphasized the significance of ecotypic population diversification in selecting locally adapted planting materials for sustainable forest farming, considering environmental factors for optimal growth and survival. The second study demonstrated the potential benefits of arbuscular mycorrhizal fungi (AMF) association in enhancing ramp growth and stress tolerance, although further research is needed. The third study showcased the utility of site suitability modeling for identifying suitable habitats for ramp production in Appalachia. These findings provide valuable insights into the conservation and sustainable management of ramps and the geospatial modeling of other woodland botanicals in diverse habitats.

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ⁱ **Biosustainability** indices are biomarkers of the ecosystem from where the soil sample is based, and are related to agricultural management practices. Three indices are calculated based on the detection of all the microorganisms in the samples (**Biodiversity** of microorganism, **Functionality**/capability of soil microbial communities to perform multiple functions and the ability of microorganism community to remain unchanged when stressed by a disturbance//**stress resistance**). Low values indices are indicators of aggressive practices, while high indexes are linked to sustainable practices.

Crop health was determined according to the detected pathogens and the vulnerability of the microbial ecosystem analyzed. The **risk level** is calculated based on the presence and proportion of microorganisms, as well as the ecology of the sample (≥ 3 is High and 2 is moderate).

Stress adaptation: Microbial species grouped according to their ability to produce metabolites that help plants withstand stress conditions. The presence of the right metabolites and microorganisms that use them ensures a balance on the microbial ecosystem.

Hormone production: Microbial species grouped according to the type of phytohormone they generate. Hormone producers' microorganisms are considered plant growth promoters, as they improve the general health status and development of plants. The presence of hormone producers, according to the level detected, implies a greater potential for positive action of the plant.