RESPONSES TO VARIED LIGHT ENVIRONMENTS AND PROPAGATION TECHNIQUES OF THE FEDERALLY ENDANGERED SHRUB, *LINDERA MELISSIFOLIA* (LAURACEAE)

by

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(Under the Direction of L. Katherine Kirkman)

ABSTRACT

Increasing vigor of existing populations and reintroduction of propagules will be necessary for persistence of the federally endangered shrub, *Lindera melissifolia*. The objectives of this study were to examine growth, morphology, and photosynthesis of plants under natural and controlled light regimes (19%, 42%, and 100% sunlight). Stem propagation, reintroduction, and seed germination were also investigated relative to re-establishment potential. Plants showed typical sun-shade morphological responses, and photosynthetic capacities (3-6 μmol $CO_2$ $m^{-2}s^{-1}$) of a shade-tolerant species. Plants grown under 100% sunlight had reduced photosynthesis and plant biomass. Canopy conditions at irradiance levels below 42% sunlight appear to be adequate for maintaining plant growth. Low success with stem propagation indicates further investigations are needed. Young transplants into field conditions can tolerate some degree of flooding but cannot survive submergence, suggesting plants may require dry down periods for establishment. A high percentage of seed germination is possible in containers without scarification.

INDEX WORDS: biomass, endangered species, light response, *Lindera melissifolia*, photosynthesis, pondberry, propagation, reintroduction, seed germination

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# DEDICATION

To my parents for their hard work and selfless devotion that has provided me with the education and opportunities I have today.

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# CHAPTER 1

# INTRODUCTION AND LITERATURE REVIEW

*Lindera melissifolia* (Walt.) Blume, pondberry, is a federally endangered (U.S. Fish and Wildlife Service 1986) deciduous, aromatic shrub in the Lauraceae. It occurs in seasonally flooded wetlands and is extant in only six states (Fig 1.1). The type of wetland habitat in which this species occurs varies regionally. In the southeastern Coastal Plain (GA, NC, SC), pondberry is found along the edges of limestone sinks, ponds or other depressional wetlands, whereas, populations in Arkansas, Missouri and Mississippi are associated with bottomland hardwood forests. *Lindera melissifolia* is believed to be extirpated from Alabama, Florida and Louisiana and has always been considered a rare species throughout its historical range (Steyermark 1949). The major threats to this species include habitat loss or alteration through drainage modification, timber cutting, or the conversion of land for pine plantations, agriculture and urban development (USFWS 1993). The following three subsections of the introduction review pertinent literature on the life history and habitat requirements of *L. melissifolia*, the response of plants to light, and the rationale and techniques for reintroduction of rare species. The final subsection outlines specific research objectives and thesis format, indicating contents of the remaining chapters.

***Life history and habitat requirements***

*Lindera melissifolia* is a rhizomatous, clonal shrub, predominately reproducing asexually. Plants typically grow in colonies of numerous stems averaging 0.5-2.0 m in height. Stem dieback occurs commonly in this species with new stems continually sprouting from rhizomes. A fungus (*Phomopsis*) and a weevil (*Heilipus squammosus*) have been found on dieback stems and could be contributing to the decline of pondberry populations (USFWS 1993). Devall et al. (2001) isolated fungal pathogens from diseased pondberry stems but believe the dieback phenomenon is a natural aging process and not a result of disease.

Plants are dioecious (separate female and male plants) and most populations are dominated by male plants (Wright 1994, Wright 1990a, USFWS 1993, Godt and Hamrick 1996). Many populations consist of one or two clones of a single sex, suggesting little potential for successful sexual reproduction or adaptation to environmental change (Godt and Hamrick 1996). *Lindera* is described as either dioecious or polygamodioecious (Radford et al. 1968). Plants that are polygamodioecious can have a few perfect flowers or a few flowers of the opposite sex on the same plant (Zomlefer 1994). This may explain why a few fruits are occasionally produced in populations that appear to be all female (pers. obs.). Pale yellow flower clusters appear in late February to mid-March before leaf-break, and bright red drupes develop from August to early October (Patrick et al. 1995). Staminate flowers occur in dense clusters, whereas pistillate flowers are fewer and less conspicuous. Flowers are believed to be insect pollinated, but there is little opportunity for pollen transfer among isolated populations (USFWS 1993).

Flower and fruit production is sporadic and can vary greatly from year to year (Morgan 1983, Wright 1990a). Flowers are sometimes damaged by late frosts resulting in reduced fruit set (Tucker 1984). Limited information is available on seed dispersers of *L. melissifolia*, but the bright red drupes suggest that they are dispersed by birds or mammals (USFWS 1993). Hermit thrushes (*Catharus guttatus*) have been observed gathering fruits in Mississippi (Devall pers. comm.). Germination of *Lindera benzoin* (spicebush; a widespread congener) seed was greatly reduced when fruit pulp remained intact, compared to seed with fruit pulp removed (Meyer and Witmer 1998). This observation suggests that frugivores may play a critical role by removing fruit pulp, and aiding in germination, as well as dispersal. In populations associated with floodplains, fruits may have been dispersed by floodwaters before riverflows were actively managed (Devall et al. 2001). Seeds may be also dispersed by simply falling from the parent

plant. Some *L. melissifolia* populations have produced numerous viable seeds, but seedlings are rarely observed in the wild (Wright 1989, USFWS 1993, Devall et al. 2001, Smith 2003). Even though viable seeds are produced, it is unclear why germination is extremely limited in natural populations (USFWS 1993).

The absence of seedling establishment, and thus the limited ability to establish new colonies, may be another factor contributing to the decline of this species (USFWS 1993, Morgan 1983). It is possible that the lack of seedling establishment is due to the loss of suitable habitat required for seed germination. Pondberry often occurs on the margins of seasonally flooded wetlands, and it is unclear how the hydrologic cycle influences seed germination and seedling establishment. Little information is available on the germination requirements for this species, but it has been found that seeds germinated quickly in petri dishes when the seed coat was ruptured and temperatures were elevated (Wright 1990a). In addition, germination occurred after seeds were pushed below the soil surface following disturbance of the forest floor by fruit harvesting (Wright 1989). It is possible that leaf litter inhibits seedling emergence (Werner 1975, Carson and Peterson 1990, Peterson and Facelli 1992), because a dense canopy and litter accumulation may prevent seeds from receiving light needed for germination. In the southeastern Coastal Plain, fire may have played a role in the past providing suitable microsites for germination by removing leaf litter and decreasing the abundance of competing hardwoods.

Habitats occur across a wide range of light conditions from extreme shade to full sun environments (Devall et al. 2001, Tucker 1984). Most populations have been observed under closed canopies and consequently, *L. melissifolia* has been considered a shade-tolerant species (Klomps 1980b, Devall et al. 2001). One anecdotal report, with little supporting evidence, suggests that *L. melissifolia* is shade-dependent (Klomps 1980a). This species also occurs in an

ecotonal habitat, adjacent to fire-maintained uplands and depressional wetlands in the southeastern Coastal Plain. Populations have survived and vigorously recovered following fire (Morgan 1983, Wright 1989). The frequency of fire (or absence of fire) in this ecotone would influence the canopy cover and could be a long-term management concern. However, little information is available on how this species responds to canopy cover, or if light is an important factor influencing growth and distribution. Plants observed in Arkansas under closed canopies had typical shade leaves and photosynthetic responses characteristic of shade-tolerant species (Wright 1990b). In addition, plants in large canopy gaps had thicker sun leaves with greater photosynthetic capacities than shade leaves (Wright 1990a). Tucker (1984) noted that plants can persist in full sun environments, but growth is often stunted. Therefore, populations may be suppressed by either full sun or by competing vegetation that is favored in more open habitats. Opinions regarding the light requirements of pondberry appear to be based on the light environment in which a population was observed (e.g., full sun or dense shade) (Wright 1989, 1990, Glitzenstein pers. comm.). Therefore, differences in observations have led to conflicting management recommendations (Klomps 1980a, USFWS 1993). Although plants occur in dense shade and full sun environments, the optimal light conditions for growth remain unclear.

The U.S. Fish and Wildlife Service Pondberry Recovery Plan (1993) states a need for understanding the relationship between light exposure and vigor as one of the recovery objectives. Elucidating the relationship between colony vigor and light exposure will provide useful information for developing management strategies and determining if removal of competing vegetation is necessary. Furthermore, infrequent sexual reproduction and seedling recruitment suggests that reintroduction of new populations will be necessary for species recovery (Godt and Hamrick 1996, Devall et al. 2001). Therefore, a better understanding of the

habitat requirements is essential in identifying suitable sites for new population establishment and for increasing vigor of existing populations.

***Responses to light***

Light availability is one of the major environmental factors influencing plant growth and distribution. Low light levels may limit photosynthesis, resulting in reduced net carbon gain and plant growth. Conversely, high light levels may cause damage to the photosynthetic apparatus (Lambers et al. 1998). Therefore, plants have developed physiological, anatomical, and morphological strategies to cope with these environmental stresses. General differences in leaf morphology, anatomy, and physiology have been attributed to species adapted to sun or shade environments. Often, plants adapted to either full sun or deep shade are unable to survive or adjust to the other light environment. Alternatively, some plants are very plastic in their response to light. Leaves of the same plant may display different traits depending on their position in the canopy and whether they developed under high or low light intensities (Beaudet and Messier 1998). Therefore, shade and sun plants refer to those individuals that are either adapted or acclimated to low and high light environments; whereas, shade leaves and sun leaves refer to leaves that developed in either low or high light environments (Lambers et al. 1998).

Measuring leaf-level photosynthetic rates can be useful in determining how light influences a plant's photosynthetic response. Photosynthetically active radiation (PAR) is the region of the electromagnetic spectrum between 400 and 700 nm that is used by plants for photosynthesis. Photosynthetic photon flux density (PPFD; μmol $m^{-2}s^{-1}$) can be used to measure PAR and the terms are often used interchangeably. By exposing a leaf to high irradiance and then reducing light incrementally while recording photosynthetic rate at each irradiance level, a light response curve can be generated. At low light levels, there is net $CO_2$ release by the plant

because respiration exceeds photosynthesis. The y-intercept of the curve represents the rate of dark respiration ($R_d$). The light compensation point (LCP), the x-intercept, is where photosynthetic uptake and respiratory $CO_2$ release are in equilibrium. At low light levels the rate of photosynthesis increases with increasing irradiance and photosynthesis is light limited. The initial slope of the curve or quantum yield ($\Phi$) refers to the efficiency in which light is converted into fixed carbon. The asymptote of the curve represents the maximum rate of $CO_2$ assimilation ($A_{max}$) and the light saturation point (LSP), where any further increase in light does not result in an increase in photosynthesis. In this range, photosynthesis is considered $CO_2$ limited (Larcher 1995, Taiz and Zeiger 1998) (Figure 1.2).

Generally, sun leaves have a high maximum rate of $CO_2$ assimilation, high light compensation point, high saturation irradiance, and a high rate of dark respiration (Boardman 1977, Björkman 1981, Givnish 1988, Lambers et al. 1998). Plants adapted to high light environments have a greater capacity to assimilate carbon as light levels increase. Higher photosynthetic rates are achieved by greater amounts of enzymes and an increase in leaf thickness (Boardman 1977). Some leaves of obligate sun plants may never reach light saturation (Loach 1967). Conversely, shade leaves characteristically have a low maximum rate of $CO_2$ assimilation, low light compensation point, low saturation irradiance, and high photosynthetic efficiency at low light because of their lower respiration rates per unit leaf area (Boardman 1977, Björkman 1981, Givnish 1988, Lambers et al. 1998). Shade plants adjust to low light by decreasing dark respiration rates and light compensation points in order to maintain a positive carbon budget (Björkman 1981, Thomspson et al. 1988). Consequently, shade leaves are less energetically costly than sun leaves. Shade plants often have a lower capacity for photosynthetic light acclimation than sun plants (Björkman 1981, Osmond 1987, Bazzaz and Carlson 1982,

Osborne et al. 1994, Awada and Redmann 2000, Valladares 2002), perhaps as a functional trade-off between light acclimation and lower respiration rates (Canham 1989).

Although most plants have similar quantum yields because they use identical biochemical pathways and means for converting energy, differences in apparent quantum yield may be a result of reduced leaf absorptance, water and temperature stresses, or damage to the photosystem (photoinhibition) (Boardman 1977, Dean et al. 1982, Singsaas et al. 2001). In particular, obligate shade species may have lower quantum yields and exhibit lower $A_{max}$ in high light (Björkman 1981). Shade-intolerant species often maintain similar quantum yields under high and low light conditions and increase photosynthetic rates under high light (Boardman 1977, Dean et al. 1982).

Growth rates are determined not only by leaf-level photosynthesis, but also by other plant characteristics that may be strongly influenced by light level, such as total plant leaf area, resource allocation, respiration, leaf life-span and construction, defense mechanisms, and developmental processes (Givnish 1988, Lambers et al. 1998, Körner 1991). For example, plants grown under low light intensity often increase allocation to stems and leaves at the expense of roots or other storage organs (Björkman 1981, Givnish 1988, Lentz and Cipollini 1998). Therefore, a whole-plant perspective is necessary in order to evaluate net carbon gain and plant growth.

Most plants have some ability to acclimate to changes in light. Some species exhibit distinctive leaf morphology and anatomy when grown in sun versus shade environments. Sun leaves achieve a high rate of $CO_2$ assimilation by producing thicker leaves resulting from greater development of the palisade and spongy mesophyll areas of the leaf (Boardman 1977, Givnish 1988, Ashton and Berlyn 1994). Typically, sun leaves have a higher stomatal density. Reduced

stomatal density observed in shade leaves may be explained by an increase in leaf area (Holmes and Cowling 1993). Alternatively, shade leaves have greater specific leaf area to maximize the photosynthetic surface area, often have thinner blades, and have larger chloroplasts containing more chlorophyll (Boardman 1977, Goulet and Bellefleur 1986, Givnish 1988). Some sun-adapted plants do not have the phenotypic plasticity to produce both sun and shade leaves (Goulet and Bellefleur 1986); whereas, shade plants may be more plastic, forming both sun and shade leaves depending on light environment (Wang and Klinka 1994). A partial list of characteristic differences between sun and shade leaves is presented in Table 1.1.

Full acclimation of an individual to a new light environment and the expression of a different phenotype generally requires the development of new leaves (Wallace and Dunn 1980, Sims and Pearcy 1992, Lambers et al. 1998). Apparently, differences in leaf expression are not predetermined in the bud, but occur during leaf expansion and development (Cormack and Gorham 1953, Goulet and Bellefleur 1986). Anatomical characteristics of fully developed leaves are stable, but alterations in photosynthesis may occur by the synthesis or breakdown of enzymes in response to light conditions (Boardman 1977).

Measuring photosynthetic and morphological responses can reveal information regarding tolerance and growth of a species to a range of light conditions (Loach 1967, Dean et al. 1982, Givnish 1988, Walters et al. 1993, Walters and Reich 1996, Olsen et al. 2002, Valladares et al. 2002). This approach is particularly useful in assessing optimal habitat conditions for the conservation of rare species that occur in only a few populations or in extremely varied conditions such as *L. melissifolia.*

***Reintroduction of rare species***

Reintroduction of rare species has become an increasingly necessary management tool in plant conservation. Approximately one-fourth of the recovery plans for all plants listed under the Endangered Species Act include reintroduction as a recovery objective (Falk and Olwell 1992), and *Lindera melissifolia* is included among those plants (USFWS 1993). Most rare species have small population sizes and are more prone to localized extinction by genetic, demographic, environmental or catastrophic stochastic events (Shaffer 1981, Falk 1992). Consequently, establishment of several healthy populations of rare species safeguards against extinction.

The terminology used to describe the establishment of individuals of a rare species in a natural environment (e.g., reintroduction, introduction, augmentation, translocation, and outplanting) have been used inconsistently in the conservation literature. Falk et al. (1996) suggest the following clarification: (a) Reintroduction typically refers to bringing a species back into a site where it occurred historically; (b) introduction involves establishing a population in an appropriate site where the species did not occur historically; (c) augmenting, or enhancing a population is achieved by adding new individuals in order to increase genetic diversity or vigor; (d) translocation involves moving plants from *in situ* (on-site) to another location; and (e) outplanting refers to taking plants from an *ex situ* (off-site) location and planting them in the wild. Reintroduction and introduction are the two terms most commonly used interchangeably. For the purposes of this review, a broad definition of reintroduction will be used as the establishment of individuals within the historical range of a rare species.

Although the reintroduction of rare plants is somewhat controversial, and conserving natural populations should still remain the first objective, reintroduction may be necessary to

ensure species survival under certain circumstances (Gordon 1994, Falk et al. 1996). Valuable information can be obtained from planting rare species in the wild, such as learning more about species biology, habitat requirements, survivorship, and establishment. Some of the concerns regarding reintroduction efforts include extending the historical range of species, confusing natural and planted populations for future research efforts, and the misuse of translocation as mitigation in place of conserving original habitat (Reinartz 1995). Often there is a lack of proper monitoring and documentation, making it difficult to evaluate long-term successes of reintroduction projects. In addition, increasing the opportunity for gene flow is disputable and often depends on the historical rarity of a species.

The decision to use single versus multiple sources for reintroduction efforts is strongly case dependent (Barret and Kohn 1991, Guerrant 1996). Mixing source populations can increase genetic diversity, which in turn, increases the capacity of a species to adapt to changing environments. Conversely, mixing different ecotypes may lead to outbreeding depression, or the breaking up of coadapted gene combinations that result in reduced fitness (Templeton 1986). Using single sources could maintain these gene combinations specifically adapted to a given environment.

Based on its historic rarity (Steyermark 1949), *L. melissifolia* may have experienced evolutionary genetic bottlenecks that have resulted in small population sizes and low genetic diversity of extant populations (Godt and Hamrick 1996). The dioecious and clonal nature of this species probably contributes to its low within-population diversity because populations are often dominated by one or two clones. Species that occur in small populations are prone to genetic risks, such as genetic drift and inbreeding depression (Ellstrand and Elam 1993). However, reduction in fitness and vigor is expected to be less evident in populations that have

undergone a long history of inbreeding. Those species, such as *L. melissifolia*, which historically occurred in restricted populations and have been rare for many years, may have genetic systems adapted to inbreeding (Barret and Kohn 1991, Maunder 1992). These naturally rare species may be more likely to suffer from outbreeding depression than those populations that have become small due to more recent anthropogenic causes.

Little information is available to guide propagation of *L. melissifolia* for new population establishment. Previous experiments have used seedlings, seeds or stems dug from clones (Wright 1989, Devall et al. 2001, Smith 2003), but few attempts to propagate stem cuttings for use in reintroduction efforts have been reported. Although genetic homogeneity has obvious disadvantages, clonal material provides greater opportunity to manage population sex ratios, to evaluate growth responses to varied environments, and may have the least impact on small populations.

The optimal stage of stem growth for obtaining cuttings for propagation and the usefulness of rooting hormones varies by species (Dirr and Heuser 1987). For propagation purposes, three basic growth stages of woody stems are softwood, semi-hardwood (greenwood) and hardwood. Softwood cuttings are taken early in the growing season when tissue is young and tender. Greenwood cuttings are obtained after leaves are mature, generally later in the summer, and hardwood cuttings are taken late fall or winter after leaf fall when plants are dormant (Dirr and Heuser 1987). Although, Bailey and Bailey (1976) recommend taking cuttings of *Lindera* spp. in the semi-hardwood (greenwood) stage, the optimal conditions and application of rooting hormones have not been reported in the vegetative propagation of *L. melissifolia*.

***Research objectives and thesis format***

A better understanding of the ecological requirements and species biology of *L. melissifolia* are needed if active management is necessary to ensure population growth and species survival. This thesis examines several aspects of the species biology of *L. melissifolia* through a combination of observations in natural populations, and shade-house and field experimentation. Chapter 2 investigates the growth and photosynthetic responses of plants under natural and controlled light environments. Chapter 3 examines the success in outplanting and propagating this species by stem cuttings. Chapter 4 investigates seed germination in both the field environment and greenhouse containers. Lastly, Chapter 5 synthesizes the results of these studies and discusses implications for recovery and management.

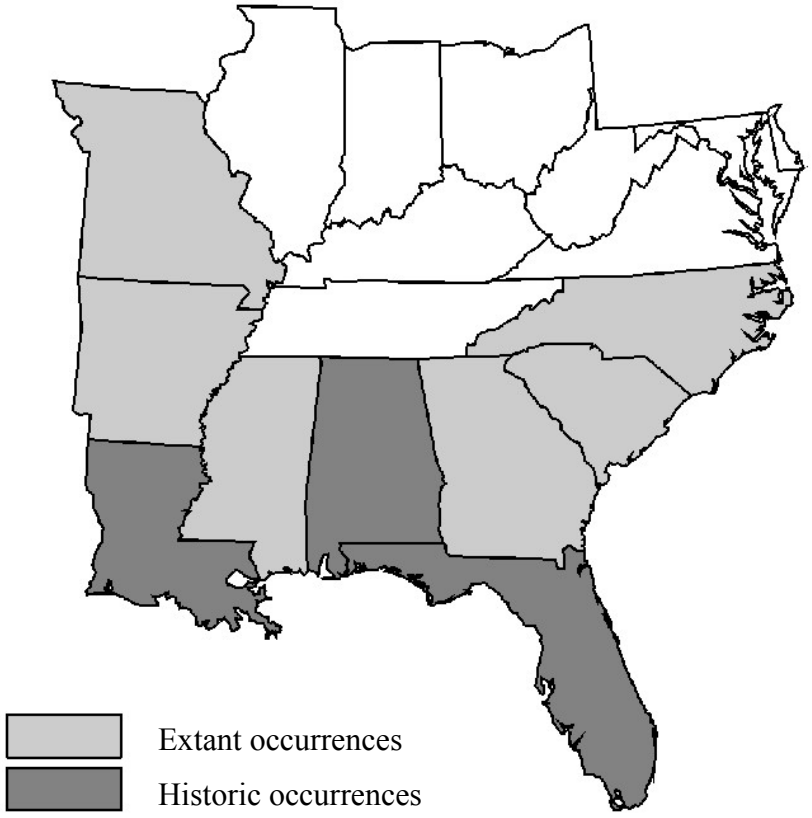



Figure 1.1. Extant and historic state occurrences of *Lindera melissifolia.*

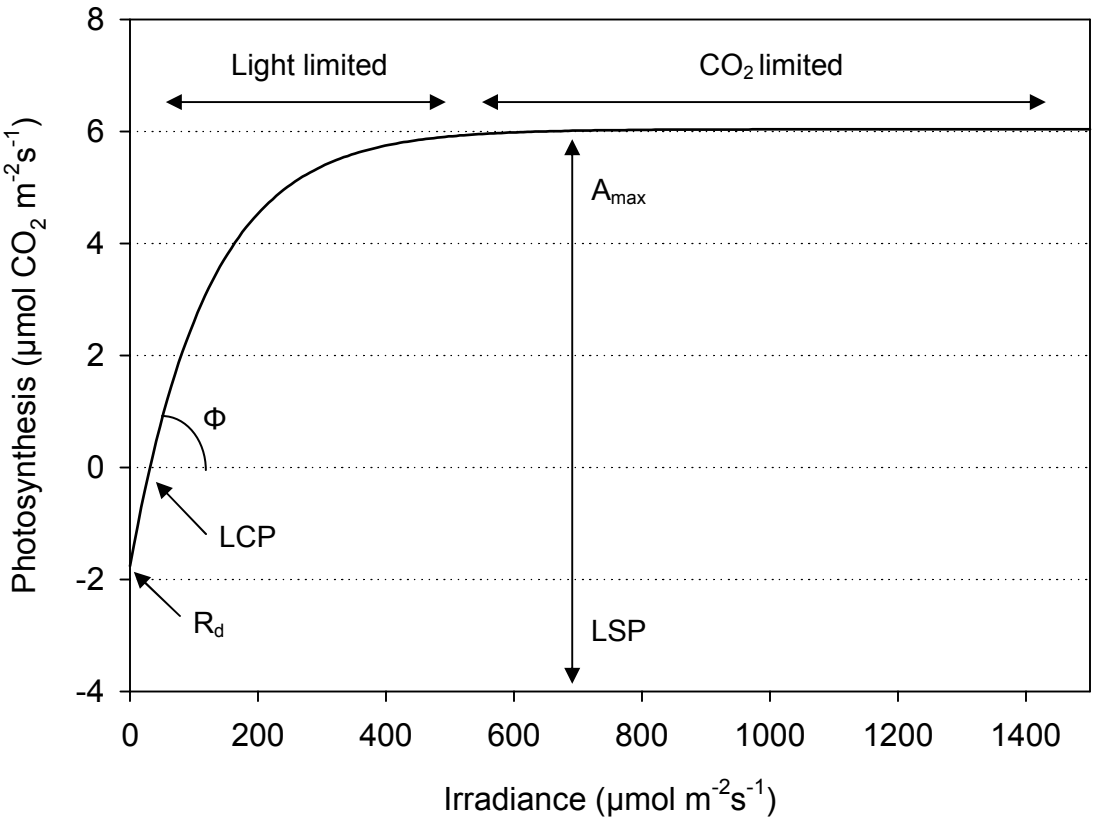



Figure 1.2. Light response curve of photosynthesis as a function of irradiance. The y-intercept represents the rate of dark respiration ($R_d$). The light compensation point (LCP), the x-intercept, is where photosynthetic uptake and respiratory $CO_2$ release are in equilibrium. The initial slope of the curve or quantum yield (Φ) refers to the efficiency in which light is converted into fixed carbon. At low light levels the rate of photosynthesis increases with increasing irradiance and photosynthesis is light limited. The asymptote of the curve represents the maximum rate of $CO_2$ assimilation ($A_{max}$) and the light saturation point (LSP), where any further increase in light does not result in an increase in photosynthesis. In this range, photosynthesis is considered $CO_2$ limited.

Table 1.1. General characteristics of sun and shade leaves (Givnish 1988, Lambers et al. 1998).

| Trait | Sun | Shade |
|---|---|---|
| **Leaf-level** | | |
| *Photosynthetic light response* | | |
| Maximum rate of $CO_2$ assimilation ($A_{max}$) | High | Low |
| Light compensation point (LCP) | High | Low |
| Light saturation point (LSP) | High | Low |
| Rate of dark respiration ($R_d$) | High | Low |
| Quantum Yield ($\Phi$) | Similar | Similar |
| *Structural response* | | |
| Leaf thickness | High | Low |
| Leaf area | Low | High |
| Specific leaf area (SLA) ($cm^2/g$) | Low | High |
| Stomatal density | High | Low |
| **Plant-level** | | |
| Fractional allocation to leaves | Low | High |
| Fractional allocation to roots | High | Low |

# CHAPTER 2

## EFFECTS OF LIGHT ENVIRONMENT ON GROWTH AND PHOTOSYNTHESIS IN THE FEDERALLY ENDANGERED SHRUB, *LINDERA MELISSIFOLIA* (LAURACEAE)[1]

[1]Aleric, K. M. and L. K. Kirkman. To be submitted to American Journal of Botany.

ABSTRACT

*Lindera melissifolia* (Walt.) Blume, a federally endangered shrub, occurs in seasonally flooded wetlands in the southern United States. Although populations exist in dense shade and full sun environments, the optimal conditions for growth remain unclear. A better understanding of the relationship between light exposure and colony vigor is needed to develop management strategies to ensure population growth and species persistence. We examined the light requirements for growth by comparing morphological and photosynthetic responses of plants under natural and controlled light regimes, as well as biomass and growth under three light treatments: 100%, 42%, and 19% full sunlight. Plants showed typical sun-shade morphological responses by decreasing stomatal density, increasing specific leaf area, and leaf area ratio with decreasing light levels. Photosynthetic capacity (3-6 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) was consistent with other shade-tolerant species. Light saturated rates of photosynthesis of experimental plants increased with increasing light up to 42% sunlight, and then declined at 100% sunlight. The 100% light treatment also had lower plant biomass resulting primarily from a reduction in root biomass. Our results indicate that canopy conditions at levels below 42% sunlight are adequate for growth.

INTRODUCTION

Light is one of the major environmental factors influencing growth and distribution of plant species. Insufficient light levels may cause stress on plants by limiting photosynthesis, resulting in reduced net carbon gain and plant growth. Conversely, high light levels may cause damage to the photosynthetic apparatus (Lambers et al. 1998). Therefore, plants have developed various strategies to deal with these stresses, such as leaf plasticity for sun/shade acclimation, light avoidance, and photoprotective means to dissipate excess energy (Demmig-Adams and Adams 1996). Differences in leaf morphology, anatomy, and physiology have been well

documented for species adapted or acclimated to sun or shade environments (Boardman 1977, Björkman 1981, Givnish 1988). Shade and sun plants refer to those individuals that are either adapted or acclimated to low and high light environments; whereas, shade leaves and sun leaves refer to leaves that developed in either low or high light environments (Lambers et al. 1998).

Generally, sun leaves have higher light saturated photosynthetic rates, light compensation points, and rates of dark respiration compared to shade leaves (Boardman 1977, Björkman 1981, Givnish 1988, Lambers et al. 1998). Shade leaves adjust to low light by decreasing dark respiration rates and light compensation points in order to maintain a positive carbon budget (Björkman 1981, Thompson et al. 1988). Shade-adapted plants often have a lower capacity for photosynthetic light acclimation than sun-adapted plants (Björkman 1981, Osmond 1987, Bazzaz and Carlson 1982, Osborne et al. 1994, Awada and Redmann 2000, Valladares et al. 2002). In particular, shade-adapted species may have lower quantum yields or efficiencies and exhibit lower maximum rates of photosynthesis in high light (Björkman 1981). Although most plants have similar quantum yields because they use identical biochemical pathways and means for converting energy, differences in quantum yields may be a result of reduced leaf absorptance, water and temperature stresses, or damage to the photosystem (photoinhibition) (Boardman 1977, Dean et al. 1982, Singsaas et al. 2001). Typically, shade leaves have lower stomatal densities and greater specific leaf areas (i.e. thinner) than sun leaves. Furthermore, shade leaves often have greater leaf areas in order to maximize light capture for photosynthesis (Boardman 1977, Goulet and Bellefleur 1986, Givnish 1988).

Measuring photosynthetic and morphological responses can reveal information regarding tolerance and growth of a species to a range of light conditions (Loach 1967, Dean et al. 1982, Givnish 1988, Walters et al. 1993, Walters and Reich 1996, Olsen et al. 2002, Valladares et al.

2002). This approach is particularly useful in assessing optimal habitat conditions for the conservation of rare species that occur in only a few populations or in extremely varied conditions. *Lindera melissifolia* (Walt.) Blume, pondberry, is a good example of a federally endangered species (USFWS 1986) that occurs across a wide range of light conditions from extreme shade to full sun environments, but the optimal conditions for growth remain unclear (Devall et al. 2001, Tucker 1984).

*Lindera melissifolia* occurs in seasonally flooded wetlands and is extant in only six states. The type of wetland habitat in which this species occurs varies regionally. In the southeastern Coastal Plain (GA, NC, SC), pondberry is found along the edges of limestone sinks, ponds or other depressional wetlands, whereas, populations in Arkansas, Missouri and Mississippi are associated with bottomland hardwood forests. *Lindera melissifolia* is believed to be extirpated from Alabama, Florida and Louisiana and has always been considered a rare species throughout its historical range (Steyermark 1949). Currently, the major threats to this species include the loss or alteration of its habitat through drainage modification, timber cutting, or the conversion of land for pine plantations, agriculture and urban development (USFWS 1993). The limited distribution and life history characteristics suggest that reintroduction of new populations will be necessary for species recovery (Godt and Hamrick 1996, Devall et al. 2001).

*Lindera melissifolia* is a rhizomatous, clonal shrub, predominately reproducing asexually. Plants are dioecious with many populations consisting of one or two clones of a single sex, suggesting little potential for successful sexual reproduction or adaptation to environmental change (Wright 1994, Wright 1990a, USFWS 1993, Godt and Hamrick 1996). Flower and fruit production is sporadic and seedling establishment is rarely observed in the wild

(Morgan 1983, Wright 1990a, USFWS 1993, Devall et al. 2001, Smith 2003). Therefore, increasing vigor of existing populations is also critical for species persistence.

Opinions regarding the light requirements of *L. melissifolia* appear to depend on the light environment in which a population was observed (e.g., full sun or dense shade). Most populations have been observed under closed overstory canopies of bottomland forests and consequently, *L. melissifolia* has been considered a shade-tolerant species (Klomps 1980b, Devall et al. 2001). This species also occurs in an ecotonal habitat, adjacent to fire-maintained uplands and depressional wetlands in the southeastern Coastal Plain. The frequency of fire (or absence of fire) in this ecotone would influence the degree of canopy cover and thus the maintenance of appropriate light conditions could be a long-term management concern. However, little information is available on how this species responds to canopy cover, or if light is an important factor influencing growth and distribution. Therefore, differences in observations have led to conflicting management recommendations (Klomps 1980a, USFWS 1993).

A better understanding of the ecological requirements and species biology are needed if active management is necessary to ensure population growth and species survival. The U.S. Fish and Wildlife Service Pondberry Recovery Plan (1993) states a need for understanding the relationship between light exposure and colony vigor as one of the recovery objectives. Such information is needed to develop management strategies, particularly for determining if removal of competing vegetation will be necessary, and in identifying suitable sites for new population establishment. The purpose of this study was to determine the optimal light requirements for growth of the federally endangered shrub, *L. melissifolia,* by comparing growth, morphological, and photosynthetic responses of plants under natural and controlled light environments. The following questions were addressed: (1) How does *L. melissifolia* adjust photosynthetically to

different light environments? (2) Does morphology, growth, and biomass differ for plants growing in varied light conditions?

## MATERIALS AND METHODS

### *Shade-house experiment*

We compared photosynthetic and growth responses of rooted cuttings of *L. melissifolia* across a light gradient in a shade-house experiment. Rooted plant material (90 cuttings), obtained from natural populations in North Carolina, South Carolina, and Missouri, was grown in pots for two years by Woodlanders Nursery, Aiken, South Carolina. Greenhouse tables (230 cm × 90 cm) were assembled in a randomized complete block design of six blocks outdoors at the J.W. Jones Ecological Research Center, Newton, Georgia. Each block contained a row of three tables that were randomly assigned one of three light treatments (100%, 42% and 19% full sunlight). Treatment light levels selected were within the range of light conditions we measured in existing populations (although not necessarily the lowest light conditions observed). We used neutral density shade cloth over support frames attached to greenhouse tables to achieve desired light conditions. Shade cloth covered all four sides of the frame except for a 15 cm gap from the base of the table to permit air circulation, and to help moderate temperature and relative humidity differences among treatments (Loach 1967). Containerized-plants were randomly assigned to tables before bud break on February 3, 2003. Each table contained five containerized plants, stratified according to source to control for locally adapted genotypes or size differences. Plants were watered to maintain field capacity and all plants were fertilized biweekly using Peters 20-20-20 liquid fertilizer with micronutrients (Scotts Company, Marysville, Ohio, USA) at a rate of 5 mL per 4 L of water. We applied Banrot fungicide (Scotts Company) at a rate of 3 g per 4 L of

water every 4 wk. Each plant received 237 mL of the fertilizer and fungicide solution when applied.

Light availability of each treatment was determined with the LI-191SA line quantum sensor (Li-Cor, Inc., Lincoln, Nebraska, USA). The average of three readings was recorded at plant height on a clear day in April for each treatment. Photosynthetic photon flux density (PPFD) ± standard error for the three treatments was: (100 % sunlight) 1728.67 ± 0.33, (42 % sunlight) 717.77 ± 0.46, and (19 % sunlight) 324.27 ± 2.64 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Light response curves were measured on a leaf area basis with the LI-6400 portable photosynthesis system (Li-Cor, Inc.) using the following irradiance levels: 1000, 700, 500, 300, 150, 80, 20, and 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Measurements were made on three randomly selected, well watered plants from each treatment from each block on separate days. Photosynthetic measurements were taken from approximately 10:00 am to 2:30 pm under clear to partly cloudy skies in mid-May on the second or third fully expanded leaf from the apex. The order of treatments and plants used were randomly selected each day.

To quantify differences in growth response to light environment a subset of three plants from each treatment replication were randomly selected for morphological and biomass measurements. We measured final stem height, stem length as the total length of the main stem and all branches, and stem diameter at a marked point at the base of the stem at the beginning and end of the experiment (Montgomery and Chazdon 2002). To determine mean stomatal density, we used the epidermal leaf peel technique (Winn 1996). We applied clear nail polish to the abaxial surface of the leaf, and once dry, we peeled the nail polish from the leaf and mounted the peel in distilled water on a microscope slide. We counted the number of stomata in a grid

(0.0625 $mm^2$) under a compound 400x microscope and recorded the average number of stomata observed under three fields of view for each peel.

We determined above and below ground patterns of biomass allocation by harvesting plants the first week of September 2003, and separating them into leaves (including petioles), stems, and roots. Plant material was dried at 70°C and weighed. We measured leaf area with the LI-3100 area meter (Li-Cor, Inc.) prior to drying. The following measures were calculated for each plant sampled: (a) Specific leaf area (SLA) = total leaf area divided by total leaf mass; (b) Leaf area ratio (LAR) = total leaf area divided by total plant mass; (c) Leaf mass ratio (LMR) = total leaf mass divided by total plant mass; (d) Stem mass ratio (SMR) = total stem mass divided by total plant mass; and (e) Root mass ratio (RMR) = total root mass divided by total plant mass. Response variables used in data analyses were the means of photosynthetic and growth measurements made on three plants.

***Field study***

Four naturally occurring populations of *L. melissifolia* in the southeastern Coastal Plain were selected for field studies because they occur in habitats that represent the range of light conditions in which this species occurs. Populations were located in Baker Co., Georgia (Ichauway), Worth Co., Georgia (Aultman Forest), and Berkley Co., South Carolina (Francis Marion National Forest; FMNF). Each of these populations occurred along the margin of a seasonally flooded depressional wetland dominated by *Nyssa sylvatica* var. *biflora* (Walt.) Sarg. and *Taxodium ascendens* Brongn. One population at Ichauway (I-Shade) occurred in dense shade of *Quercus laurifolia* Michx*., Acer rubrum* L*., and Liquidambar styraciflua* L. Two populations occurred in the Aultman Forest; one (A-Sun) in an herbaceous zone with little canopy cover adjacent to a cypress dome surrounded by pine plantations, and the other (A-

Shade) beneath a canopy of *Acer rubrum*, *Nyssa sylvatica* var. *biflora* and *Taxodium ascendens*. The fourth population in the FMNF occurred across a gradient of canopy cover including: full sun (FM-Sun), intermediate shade (FM-Int), and full shade (FM-Shade). The margin of this wetland is dominated by species such as *Ilex glabra* (L.) Gray, *Persea palustris* (Raf.) Sarg., *Cyrilla racemiflora* L., *Lyonia lucida* (Lam.) K. Koch, *Myrica cerifera* L.*, Acer rubrum, Magnolia virginiana* L., and *Liquidambar styraciflua*. All study sites were inundated when measurements were taken in June and July of 2003, with water levels ranging from 5.9 to 31.3 cm.

To determine differences in stem and leaf morphology among populations as a response to light conditions, we measured a minimum of 20 plants in each population. All plants within randomly placed $1m^2$ frames were selected for measurements until the minimum number of plants was sampled. For each plant, we measured stem height and diameter (base of the stem), and two leaves (second or third fully expanded leaf from the apex) were collected for specific leaf area and stomatal density measurements made in the lab using the same procedures mentioned above.

To compare photosynthetic responses of plants growing in different light conditions, we generated light response curves using the same light levels and conditions as those in the shade-house experiments. To make comparisons between the Georgia populations, a light curve was taken at each site on each of four days, alternating site order each day. This method was also used at the South Carolina population (three sites along a light gradient), except that two light curves were taken at each site on each day (6 light curves per day) for four days. By measuring light response curves on separate days, we were able to account for any daily factors potentially influencing photosynthetic measurements.

We characterized the light environment at each site by leaf area index (total leaf area per unit area of ground; LAI) with the LAI-2000 plant canopy analyzer (Li-Cor, Inc.). In addition, we determined percent light availability for each site with the LI-191SA line quantum sensor and LI-1000 dataloggers (LiCor, Inc.) by recording light levels at plant height and in an adjacent area located in full sunlight under clear sky conditions.

***Statistical analyses***

Light curves were fitted by nonlinear regression using the Mitscherlich model equation (Sigma Plot 8.0, SSPS Inc., IL) (Peek et al. 2002, Potvin et al. 1990):

$$A = A_{max}\,[1\text{-}e^{-Aqe\,(PPFD\text{-}LCP)}]$$

Where A is net photosynthesis, $A_{max}$ refers to the asymptote of photosynthesis, $A_{qe}$ represents the apparent quantum yield or initial slope of the curve, PPFD is the incident photosynthetic photon flux density, and the LCP refers to the light compensation point that corresponds to the x-intercept (where photosynthetic uptake and respiratory $CO_2$ release are in equilibrium). This model was used to obtain the following parameters: light saturated rate of photosynthesis ($A_{max}$), light compensation point (LCP), and apparent quantum yield ($A_{qe}$). The rate of dark respiration ($R_d$) was calculated as the y-intercept of the curve, and light saturation points (LSP) were estimated as the approximate light level where $A_{max}$ was reached.

All shade-house experiment and photosynthetic data for field studies were analyzed using a randomized complete block analysis of variance (PROC GLM; SAS 9.0, SAS Institute Inc., North Carolina, USA). Field studies were analyzed in two groups based on geographical location and sampling dates (i.e., A-Sun, A-Shade, I-Shade; and FM-Sun, FM-Int, FM-Shade). In this case, block was the date photosynthetic data was collected. The morphological field data

collected was analyzed using a one-way analysis of variance (PROC GLM; SAS Institute Inc.). Means comparisons were made using Tukey's Honestly Significant Difference test ($P > 0.05$).

## RESULTS

### *Shade-house experiment*

Light saturated rates of photosynthesis ($A_{max}$) increased as light levels increased from 19% to 42% full sunlight, but decreased as light levels increased from 42% to 100% full sunlight ($F = 16.50$; $P < 0.001$). Maximum rates of photosynthesis of plants grown in intermediate light (42% full sunlight) were 28-48% greater than either of the extreme light treatments (Table 2.1, Fig. 2.1). These plants also had greater light saturation points than plants grown under full sun. The apparent quantum yield differed among treatments; mean values for plants in full sun was greater than that of the lowest light condition (19% sunlight) ($F = 5.05$; $P = 0.03$). Plants in the 100% light treatment had significantly greater light compensation points and rates of dark respiration than the other two light treatments ($F = 11.30$; $P = 0.003$ and $F = 38.44$; $P < 0.0001$ respectively; Table 2.1, Fig. 2.1).

Growth, as measured by total plant biomass, was 30% less in the full sun treatment than that of the other two light treatments ($F = 5.41$; $P = 0.03$), primarily as a result of decreased root biomass associated with full sun conditions ($F = 5.95$; $P = 0.02$; Table 2.2, Fig. 2.2). Although not statistically significant, a trend for reduced stem and leaf biomass contributed to the lower total biomass in the full sun treatment ($F = 3.53$; $P = 0.07$ and $F = 3.10$; $P = 0.09$, respectively; Table 2.2, Fig. 2.2). There was no evidence of different biomass partitioning among the three light treatments where leaf mass ratio (LMR), stem mass ratio (SMR), and root mass ratio (RMR) did not differ. Final stem height decreased with increasing light levels (Table 2.2). Total stem growth did not differ among the three light treatments, whereas stem diameter growth was

significantly lower in full sunlight treatment relative to the low light treatment ($F = 4.30$; $P = 0.04$; Table 2.2).

Leaf morphology changed in response to light conditions. Total plant leaf area was greater in response to low and intermediate light treatments than to full sunlight ($F = 19.41$; $P < 0.001$; Table 2.2). Leaf area ratio (LAR) was greater in the lowest light treatment relative to the other treatments ($F = 33.82$; $P < 0.0001$). Specific leaf area (SLA) decreased with increasing light levels indicating that shade leaves were thinner than sun leaves ($F = 127.38$; $P < 0.0001$). Stomatal density was significantly lower only in the lowest light environment ($F = 12.68$; $P = 0.0018$). No significant block effect was observed for any of the response variables analyzed ($P > 0.05$).

***Field study***

The South Carolina study sites occurred as a single population that was distributed across a wide gradient of light conditions. FM-Sun occurred under little canopy cover with a leaf area index (LAI) of 0.71 and 95% light availability. FM-Intermediate, with a LAI of 2.19 and 28% light availability, occurred along the margin of the wetland. FM-Shade was located under a dense shrub thicket with a LAI of 4.42 and only 3% light availability (Table 2.3b). The Georgia populations represented only the extreme light conditions (i.e., no intermediate condition). A-Sun was located in an herbaceous region of a wetland with a LAI of 0.12 and 93% light availability. A-Shade and I-Shade had similar tree canopies with a LAI of 2.93 and 3.32 and 7% and 6% light availabilities, respectively (Table 2.4b).

Patterns of photosynthetic responses to varied light conditions in field sites were generally similar to that of plants grown under experimental light treatments. In the South Carolina sites, light saturated photosynthetic rates ($A_{max}$) in FM-Intermediate exceeded that of

FM-Shade by 45%, but did not differ from FM-Sun (Table 2.3a). Although light saturated photosynthetic rates were similar for plants in two of the Aultman forest sites (i.e., A-Sun and A-Shade) (Table 2.4a), the Ichauway population (I-Shade) had greater $A_{max}$ rates than A-Sun. The apparent quantum yield was greatest for FM-Shade plants compared to the intermediate and sun sites ($F = 16.90$; $P = 0.003$). Apparent quantum yields were similar for the three Georgia populations (Table 2.4a). Light compensation points (LCP) and rates of dark respiration ($R_d$) increased with increasing light for all sites.

Plant height did not differ among populations in South Carolina; however, stem diameter was nearly 50% less under dense shade than in the more open habitats (Table 2.3b). Basal stem diameter did not differ between the three Georgia populations (Table 2.4b), although for these populations, mean plant height in the A-Sun population was less than that of other two shaded sites. Specific leaf area decreased and stomatal density increased with increasing light regardless of geographic location (Table 2.3b and 2.4b). As in the shade-house experiment, no significant block effect was observed for any of the photosynthetic parameters ($P > 0.05$).

## DISCUSSION

Our results indicate that *L. melissifolia* is capable of acclimating to varied light conditions through plasticity in leaf morphology and physiology. Plants showed typical sun-shade morphological responses that have been reported in numerous other studies including decreased stomatal densities, increased specific leaf area (i.e., leaf thickness) and leaf area ratio with decreasing light levels (Dean et al. 1982, Midgley et al. 1992, Holmes and Cowling 1993, Groninger et al. 1996, Beaudet and Messier 1998, Sack and Grubb 2002). The reduced stomatal density that we observed in shade leaves may be explained by the increase in leaf area. Greater leaf area, which is often associated with shade leaves, allows a plant to maximize the

photosynthetic surface area under low light conditions (Boardman 1977, Goulet and Bellefleur 1986, Givnish 1988). Similar to responses of other shade-tolerant species, leaves of *L. melissifolia* adjusted physiologically to shading by lower light compensation points and rates of dark respiration (Midgley et al. 1992, Hamerlynck and Knapp 1994, Groninger et al. 1996, Sims and Pearcy 1991, Olsen et al. 2002). Maximum rates of photosynthesis for populations ranged between 3-6 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$, a response consistent with shade-tolerant trees and shrubs from temperate regions, but less than typical rates of 10-15 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ reported for sun leaves (Loach 1967, Larcher 1995).

A response pattern of increasing maximum rates of photosynthesis with increasing light up to intermediate light levels followed by a decline in $A_{max}$ at high light levels, similar to that of our experimental observations, has been reported for other shade-tolerant species (Loach 1967, Chabot and Chabot 1977, Oberabauer and Strain 1986, Tani et al. 2001). However, these results are inconsistent with that of a study by Wright (1990b) in which the thicker sun leaves of *L. melissifolia* plants occurring in large canopy gaps (PAR > 970 $\mu mol \cdot m^{-2} \cdot s^{-1}$) had maximum rates of photosynthesis up to 56% greater than that of shade leaves. Photosynthetic responses of plants measured in the field study were also not in complete agreement with our experimental observations. These discrepancies may be due other environmental factors such as temperature, relative humidity, soil moisture and nutrient availability, which influence photosynthesis under natural conditions (Thompson et al. 1988, Kozlowski et al. 1991). For example, inundated conditions have been shown to reduce photosynthesis, particular for leaves in full sunlight, and may have been a confounding factor in our study (Gravatt and Kirby 1998, Gardiner and Krauss 2001). Consequently, comparisons between field studies and controlled experiments can be difficult to interpret.

The pattern of low maximum rates of photosynthesis without loss of efficiency (i.e., apparent quantum yield) observed for the high light treatment has been observed for long-lived plant species usually associated with densely shaded environments (Anderson and Osmond 1987). Furthermore, photoinhibition, or damage to the photosystem, is often identified by a decrease in efficiency and the inability to increase light saturated photosynthetic rates under high light (Anderson and Osmond 1987). Greater rates of dark respiration and light compensation points observed in the high light treatment did not result in greater photosynthetic capacities, but plants maintained efficient photosynthesis. Therefore, *L. melissifolia* appears to have the ability to acclimate to high and low light conditions by maintaining efficient photosynthesis. However, the slight decrease in photosynthesis after light saturation is surpassed for the 100% light treatment (Fig. 2.1.) can also be an indication of photoinhibition (Smith et al. 1993). The repairs of damage and avoidance strategies due to photoinhibiton are energetically costly, and can result in increased rates of respiration (Raven 1989). Potentially, increased rates of respiration for sun leaves were a result of other factors such as photoinhibitory costs, light environment and/or carbon gain, and little was associated with the maintenance of photosynthetic capacity (Sims and Pearcy 1991). Additionally, we observed that leaves of *L. melissifolia* growing under extremely high light environments, in the field as well as experimental conditions, tended to be rolled longitudinally. Leaf rolling is considered a light avoidance strategy used by plants to minimize leaf temperature and transpiration (Lambers et al. 1990). Thus, the leaf rolling habit that we observed may be an indicator of stress under high light conditions.

Even though measurements of leaf-level photosynthetic rates can be useful in determining how light influences a plant's photosynthetic response, these measures do not necessarily corroborate growth rates or plant vigor (Givnish 1988, Lambers et al. 1998, Körner

1991). For example, although plants in the 19% light treatment had low maximum rates of photosynthesis compared to the intermediate light treatment, differences were not measured in biomass and growth patterns. The fact that all experimental plants survived the duration of the study, but significant reduction in growth and photosynthesis occurred in full sun, indicates that *L. melissifolia* can persist in a range of light environments, but high light environments do not appear to be optimal for growth. This conclusion is consistent with previous anecdotal observations that growth of this species is often stunted under high light levels (Tucker 1984), and that most populations of *L. melissifolia* have been observed in shade to partial shade. In addition, low root production could limit competition for belowground resources under high light conditions. It is unclear how this species would respond to more extreme shade conditions than that of our experimental treatment.

Plants in light-limiting environments often increase allocation to stems and leaves at the expense of roots or other storage organs (Boardman 1977, Lentz and Cipollini 1998, Niinemets 1998, Van Hees and Clerkx 2003). However, we did not observe differences in biomass partitioning between above and below-ground parts similar to other studies (Pattison et al. 1998, Groninger et al. 1996). These inconsistencies could reflect species-specific responses in biomass partitioning to light environment. The degree of partitioning has been shown to differ among species and may change as a plant grows (Niinemets 1998, Longbrake and McCarthy 2001, Montgomery and Chazdon 2002, Van Hees and Clerkx 2003).

Carbon allocation patterns may also reflect tolerance of species to irradiance. Allocation to shoot development is often observed for shade-intolerant species growing in low light environments reflecting a light-seeking strategy (Walters et al. 1993, Beaudet and Messier 1998, Lentz and Cipollini 1998). Leaves under dense canopies are greatly affected by red to far-red

ratios (R/FR). As shading increases, the R/FR values decrease (Taiz and Zeiger 1998). Shade tolerant species respond differently to R/FR than do shade-avoiding species. Morgan and Smith (1979) found plants that normally grow in open field habitats (sun plants) to have a higher rate of stem elongation with increasing far-red light. Conversely, shade tolerant species did not demonstrate stem elongation with changing R/FR (Morgan and Smith 1979). Generally, shade tolerant species do not exhibit increased stem elongation, but respond to increasing far-red light by increased leaf area (Lambers et al. 1998). Even though large differences in stem height were not pronounced among natural populations of *L. melissifolia,* sun plants were typically shorter than shade plants for both the Georgia populations and the experimental plants. It is likely that stem height was overestimated for FM-Sun plants; shorter plants were submerged and could not be located.

***Management implications***

Although controlled experiments offer information regarding a particular resource, a suite of environmental factors interact and influence plant establishment and growth in natural environments. Therefore, it is important to consider limitations of results from shade-house experiments in drawing conclusions for management of natural populations. For example, plants growing under shade cloth do not experience brief periods of direct solar radiation known as sunflecks that commonly occur under a forest canopy. Depending on the type of canopy, sunflecks can greatly increase total daily irradiance, which can have large impacts on plant growth and survival (Chazdon and Peary 1991). For instance, plants growing under dense shrub thickets similar to FM-Shade may have fewer sunflecks of lower intensity than those associated with populations growing under tree canopies with more diffuse cover. If this is the case, then removal of dense shrub canopies may be beneficial to populations of *L. melissifolia*, whereas,

thinning of mature tree canopies may not be effective, or could possibly be deleterious. Furthermore, natural plant populations are impacted by nearby competitors that can alter the spectral quality of light (i.e., red to far-red ratios), which have been shown to influence plant morphology (Morgan and Smith 1979, Stuefer and Huber 1998, Marcuvitz and Turkington 2000, Ammer 2003). Environmental factors not examined in this study, such as light quality, intensity and duration of sunflecks, or nutrient and moisture availability need further investigation and should be considered when assessing optimal habitat requirements and the development of management options for conservation of populations of *L. melissifolia.*

In conclusion, *L. melissifolia* is a facultative shade species with the ability to adjust to a range of light environments. Light saturated photosynthetic rates observed for this species were consistent with other shade-tolerant plants and were lower than typical rates for species adapted to high light environments. Even though plants can persist in full sun environments, these conditions do not appear to be optimal for growth. In addition, populations may be suppressed by competing vegetation that is favored in more open habitats (Wright 1990a). Our results indicate that canopy conditions at irradiance levels below 42% full sunlight are adequate for maintaining plant growth.

Table 2.1. Leaf-level photosynthetic parameters for *L. melissifolia* grown under three light treatments: light saturated rate of photosynthesis ($A_{max}$), apparent quantum yield ($A_{qe}$), rate of dark respiration ($R_d$), light compensation point (LCP), and light saturation point (LSP). Values (mean ± SE, N = 6) followed by different letters indicate significant differences (Tukey test, $P < 0.05$).

| Parameter | Treatment (% full sunlight) | | |
|---|---|---|---|
| | 19 | 42 | 100 |
| $A_{max}$ ($\mu$mol $CO_2 \cdot m^{-2} \cdot s^{-1}$) | 4.39 ± 0.20 $^{B}$ | 6.07 ± 0.65 $^{A}$ | 3.31 ± 0.31 $^{B}$ |
| $A_{qe}$ ($\mu$mol $CO_2 \cdot m^{-2} \cdot s^{-1}$) / ($\mu$mol$\cdot m^{-2} \cdot s^{-1}$) | 0.0087 ± 0.0003 $^{B}$ | 0.0091 ± 0.001 $^{AB}$ | 0.0142 ± 0.0023 $^{A}$ |
| $R_d$ ($\mu$mol $CO_2 \cdot m^{-2} \cdot s^{-1}$) | -1.03 ± 0.08 $^{A}$ | -1.79 ± 0.09 $^{B}$ | -2.51 ± 0.24 $^{C}$ |
| LCP ($\mu$mol$\cdot m^{-2} \cdot s^{-1}$) | 27.46 ± 2.30 $^{B}$ | 30.74 ± 1.97 $^{B}$ | 47.03 ± 6.14 $^{A}$ |
| LSP ($\mu$mol$\cdot m^{-2} \cdot s^{-1}$) | 861.29 ± 40.47 $^{AB}$ | 890.83 ± 100.85 $^{A}$ | 578.68 ± 74.38 $^{B}$ |

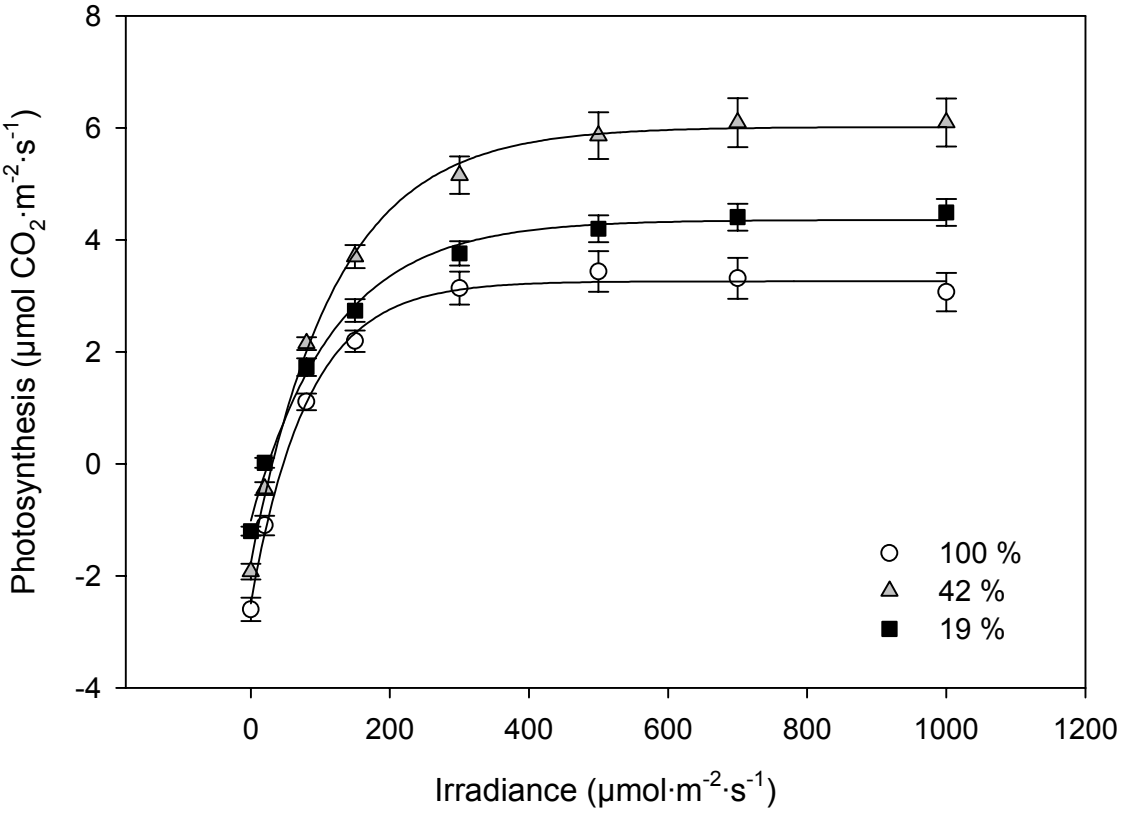



Figure 2.1. Light response curve of photosynthesis as a function of irradiance for *L. melissifolia* plants grown under three light treatments. Data points represent means ± standard errors (N = 6). Light curves were fitted by nonlinear regression using the Mitscherlich model equation.

Table 2.2. Biomass and growth variables for *L. melissifolia* grown under three light treatments. Values (mean ± SE, N = 6) followed by different letters indicate significant differences (Tukey test, $P < 0.05$). (Abbreviations: SLA = specific leaf area; LAR = leaf area ratio; LMR = leaf mass ratio; SMR = stem mass ratio; RMR = root mass ratio).

| | Treatment (% full sunlight) | | |
|---|---|---|---|
| Variable | 19 | 42 | 100 |
| Leaf biomass (g) | 11.21 ± 0.94 $^{A}$ | 12.04 ± 1.1 $^{A}$ | 8.43 ± 0.81 $^{A}$ |
| Stem biomass (g) | 10.03 ± 0.93 $^{A}$ | 9.03 ± 0.56 $^{A}$ | 6.74 ± 0.93 $^{A}$ |
| Root biomass (g) | 22.4 ± 2.23 $^{A}$ | 22.22 ± 1.42 $^{A}$ | 14.41 ± 1.2 $^{B}$ |
| Total biomass (g) | 43.64 ± 3.61 $^{A}$ | 43.29 ± 2.9 $^{A}$ | 29.59 ± 2.68 $^{B}$ |
| Total leaf area ($cm^2$) | 2200.38 ± 144.12 $^{A}$ | 1676.89 ± 143.67 $^{A}$ | 942.67 ± 99.08 $^{B}$ |
| SLA ($cm^2/g$) | 199.27 ± 6.49 $^{A}$ | 139.74 ± 4.28 $^{B}$ | 110.86 ± 1.53 $^{C}$ |
| LAR ($cm^2/g$) | 52.95 ± 1.30 $^{A}$ | 39.11 ± 1.69 $^{B}$ | 32.28 ± 1.81 $^{B}$ |
| LMR | 0.27 ± 0.01 $^{A}$ | 0.28 ± 0.01 $^{A}$ | 0.29 ± 0.01 $^{A}$ |
| SMR | 0.23 ± 0.01 $^{A}$ | 0.21 ± 0.01 $^{A}$ | 0.22 ± 0.01 $^{A}$ |
| RMR | 0.5 ± 0.01 $^{A}$ | 0.51 ± 0.01 $^{A}$ | 0.49 ± 0.02 $^{A}$ |
| Stomata # / 0.0625 $mm^2$ | 26.94 ± 1.14 $^{B}$ | 33.94 ± 1.30 $^{A}$ | 37.28 ± 1.98 $^{A}$ |
| Height (cm) | 62.44 ± 3.71 $^{A}$ | 53.88 ± 3.37 $^{AB}$ | 45.60 ± 3.97 $^{B}$ |
| Stem growth (cm) | 181.26 ± 15.78 $^{A}$ | 176.82 ± 13.86 $^{A}$ | 144.32 ± 30.38 $^{A}$ |
| Diameter growth (mm) | 2.28 ± 0.24 $^{A}$ | 2.09 ± 0.22 $^{AB}$ | 1.56 ± 0.29 $^{B}$ |

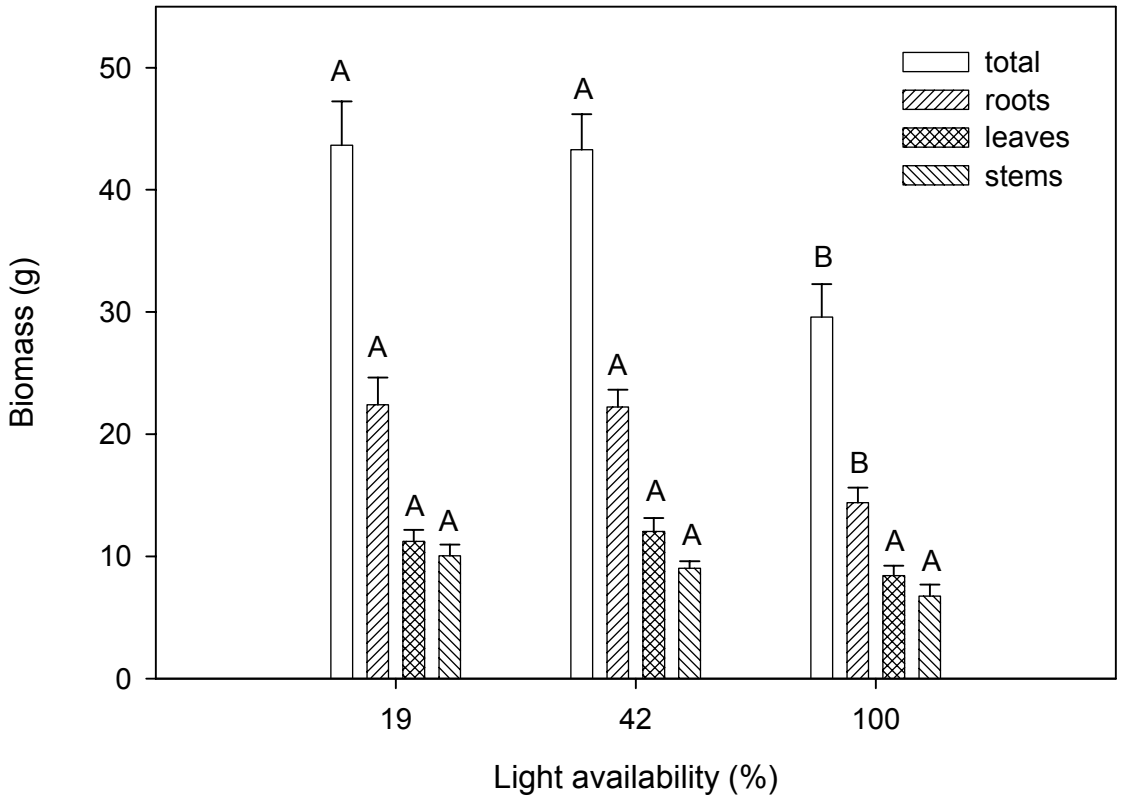



Figure 2.2. Biomass components for *L. melissifolia* plants grown under three light treatments. Data are means with standard error bars (N = 6). Means with different letters indicate significant differences (Tukey test, P > 0.05).

Table 2.3. (a) Leaf-level photosynthetic parameters (mean ± SE, N = 4) for *L. melissifolia* growing under natural light regimes in the Francis Marion National Forest, SC: light saturated rate of photosynthesis ($A_{max}$), apparent quantum yield ($A_{qe}$), rate of dark respiration ($R_d$), light compensation point (LCP), and light saturation point (LSP). Values followed by different letters indicate significant differences (Tukey test, $P < 0.05$).

| | Site | | |
|---|---|---|---|
| Parameter | FM-Shade | FM-Int | FM-Sun |
| $A_{max}$ (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) | 3.24 ± 0.28 $^{B}$ | 5.89 ± 0.45 $^{A}$ | 4.99 ± 0.582 $^{AB}$ |
| $A_{qe}$ (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) / (μmol $\cdot m^{-2} \cdot s^{-1}$) | 0.0214 ± 0.0009 $^{A}$ | 0.0130 ± 0.0009 $^{B}$ | 0.0106 ± 0.0018 $^{B}$ |
| $R_d$ (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) | -0.40 ± 0.03 $^{C}$ | -1.03 ± 0.04 $^{B}$ | -1.56 ± 0.18 $^{A}$ |
| LCP (μmol $\cdot m^{-2} \cdot s^{-1}$) | 5.70 ± 0.59 $^{C}$ | 12.69 ± 0.64 $^{B}$ | 27.58 ± 1.61 $^{A}$ |
| LSP (μmol $\cdot m^{-2} \cdot s^{-1}$) | 318.67 ± 12.89 $^{B}$ | 575.75 ± 40.74 $^{AB}$ | 757.07 ± 95.21 $^{A}$ |

(b) Light environment and morphological characteristics (mean ± SE, N = 20-33): percent light availability, leaf area index (LAI), stem height, basal stem diameter, specific leaf area (SLA) and stomatal density.

| | Site | | |
|---|---|---|---|
| Characteristic | FM-Shade | FM-Int | FM-Sun |
| Light availability (%) | 3 | 28 | 95 |
| LAI | 4.42 ± 0.13 | 2.19 ± 0.06 | 0.71 ± 0.03 |
| Height (cm) | 36.56 ± 3.19 $^{A}$ | 40.82 ± 2.13 $^{A}$ | 41.89 ± 1.46 $^{A}$ |
| Diameter (mm) | 2.13 ± 0.16 $^{B}$ | 4.09 ± 0.25 $^{A}$ | 4.07 ± 0.18 $^{A}$ |
| SLA ($cm^2/g$) | 382.83 ± 9.02 $^{A}$ | 288.56 ± 15.25 $^{B}$ | 178.10 ± 3.90 $^{C}$ |
| Stomata # / 0.0625 $mm^2$ | 14.20 ± 0.40 $^{C}$ | 25.43 ± 0.96 $^{B}$ | 32.73 ± 0.63 $^{A}$ |

Table 2.4. (a) Leaf-level photosynthetic parameters (mean ± SE, N = 4) for *L. melissifolia* growing under natural light regimes in three southwestern Georgia populations: light saturated rate of photosynthesis ($A_{max}$), apparent quantum yield ($A_{qe}$), rate of dark respiration ($R_d$), light compensation point (LCP), and light saturation point (LSP). Values followed by different letters indicate significant differences (Tukey test, $P < 0.05$).

| Parameter | Site | | |
|---|---|---|---|
| | I-Shade | A-Shade | A-Sun |
| $A_{max}$ ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$) | 5.99 ± 0.43 $^{A}$ | 4.11 ± 0.39 $^{AB}$ | 3.48 ± 1.17 $^{B}$ |
| $A_{qe}$ ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$) / ($\mu mol\ m^{-2} \cdot s^{-1}$) | 0.0126 ± 0.0021 $^{A}$ | 0.0158 ± 0.0009 $^{A}$ | 0.0138 ± 0.0016 $^{A}$ |
| $R_d$ ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$) | -0.63 ± 0.07 $^{B}$ | -0.43 ± 0.02 $^{B}$ | -1.58 ± 0.059 $^{A}$ |
| LCP ($\mu mol \cdot m^{-2} \cdot s^{-1}$) | 8.48 ± 1.25 $^{B}$ | 6.70 ± 1.22 $^{B}$ | 33.11 ± 6.97 $^{A}$ |
| LSP ($\mu mol \cdot m^{-2} \cdot s^{-1}$) | 667.63 ± 56.83 $^{A}$ | 454.54 ± 52.30 $^{A}$ | 490.71 ± 77.85 $^{A}$ |

(b) Light environment and morphological characteristics (mean ± SE, N = 22-33): percent light availability, leaf area index (LAI), stem height, basal stem diameter, specific leaf area (SLA) and stomatal density.

| Characteristic | Site | | |
|---|---|---|---|
| | I-Shade | A-Shade | A-Sun |
| Light availability (%) | 6 | 7 | 93 |
| LAI | 3.32 ± 0.26 | 2.93 ± 0.16 | 0.12 ± 0.02 |
| Height (cm) | 58.14 ± 2.8 $^{A}$ | 55.83 ± 5.76 $^{A}$ | 41.70 ± 2.47 $^{B}$ |
| Diameter (mm) | 4.20 ± 0.21 $^{A}$ | 4.28 ± 0.48 $^{A}$ | 4.01 ± 0.34 $^{A}$ |
| SLA ($cm^2/g$) | 408.30 ± 13.65 $^{A}$ | 397.13 ± 10.72 $^{A}$ | 130.33 ± 4.30 $^{B}$ |
| Stomata # / 0.0625 $mm^2$ | 19.35 ± 0.36 $^{B}$ | 15.24 ± 0.41 $^{C}$ | 36.72 ± 0.56 $^{A}$ |

# CHAPTER 3

# PROPAGATION AND EXPERIMENTAL REINTRODUCTION

# INTRODUCTION

Reintroduction of rare species has become an increasingly necessary management tool in plant conservation. Approximately one-fourth of the recovery plans for all plants listed under the Endangered Species Act include reintroduction as a recovery objective (Falk and Olwell 1992), and *Lindera melissifolia* (pondberry) is included among those plants (USFWS 1993). *Lindera melissifolia* occurs in seasonally flooded wetlands and is extant in only six states. The type of wetland habitat in which this species occurs varies regionally. In the southeastern Coastal Plain (GA, NC, SC), pondberry is found along the edges of limestone sinks, ponds or other depressional wetlands, whereas, populations in Arkansas, Missouri and Mississippi are associated with bottomland hardwood forests. *Lindera melissifolia* is believed to be extirpated from Alabama, Florida and Louisiana and has always been considered a rare species throughout its historical range (Steyermark 1949). Currently, the major threats to this species include the loss or alteration of its habitat through drainage modification, timber cutting, or the conversion of land for pine plantations, agriculture and urban development (USFWS 1993).

This species is a rhizomatous, clonal shrub, predominately reproducing asexually. Plants are dioecious with many populations consisting of one or two clones of a single sex, suggesting little potential for successful sexual reproduction or adaptation to environmental change (Wright 1994, Wright 1990, USFWS 1993, Godt and Hamrick 1996). Flower and fruit production is sporadic and seedling establishment is rarely observed in the wild (Morgan 1983, Wright 1990, USFWS 1993, Devall et al. 2001, Smith 2003). Therefore, increasing vigor of existing populations is critical for species persistence. Furthermore, infrequent sexual reproduction and seedling recruitment suggests that reintroduction of new populations will be necessary for species recovery (Godt and Hamrick 1996, Devall et al. 2001). Most rare species have small

population sizes and are more prone to localized extinction by genetic, demographic, environmental or catastrophic stochastic events (Shaffer 1981, Falk 1992). Consequently, establishment of several healthy populations of rare species safeguards against extinction.

The terminology used to describe the establishment of individuals of a rare species in a natural environment (e.g., reintroduction, introduction, augmentation, translocation, and outplanting) have been used inconsistently in the conservation literature. Falk et al. (1996) suggest the following clarification: (a) reintroduction typically refers to bringing a species back into a site where it occurred historically; (b) introduction involves establishing a population in an appropriate site where the species did not occur historically; (c) augmenting or enhancing a population is achieved by adding new individuals in order to increase genetic diversity or vigor; (d) translocation involves moving plants from *in situ* (on-site) to another location; and (e) outplanting refers to taking plants from an *ex situ* (off-site) location and planting them in the wild. Reintroduction and introduction are the two terms most commonly used interchangeably. For the purposes of this study, a broad definition of reintroduction will be used as the establishment of individuals within the historical range of a rare species.

Although rare plant reintroduction is somewhat controversial, and conserving natural populations should still remain the first objective, reintroduction may be necessary to ensure species survival under certain circumstances (Gordon 1994, Falk et al. 1996). Valuable information can be obtained from planting rare species in natural environments, such as learning more about species biology, habitat requirements, survivorship and establishment. Some concerns regarding reintroduction efforts include extending the historical range of species, confusing naturally occurring and planted populations for future research efforts, and the misuse of translocation as mitigation in place of conserving original habitat (Reinartz 1995). Often there

is a lack of proper monitoring and documentation, making it difficult to evaluate long-term successes of projects. In addition, increasing the opportunity for gene flow is disputable and often depends on the historical rarity of a species (Barret and Kohn 1991, Reinartz 1995). Mixing source populations can increase genetic diversity, which in turn increases the capacity of a species to adapt to changing environments. Conversely, mixing different ecotypes may lead to outbreeding depression, or the breaking up of coadapted gene combinations that may result in reduced fitness (Templeton 1986). An advantage to using a single source, or a particular genotype, would be to maintain gene combinations specifically adapted to a given environment. The decision to use single versus multiple sources for reintroduction efforts will be strongly case dependent (Barret and Kohn 1991, Guerrant 1996).

Species occurring in small populations are prone to genetic risks, such as genetic drift and inbreeding depression (Ellstrand and Elam 1993). However, reduction in fitness and vigor is expected to be less evident in populations that have undergone a long history of inbreeding (Barret and Kohn 1991, Reinartz 1995). Based on its historic rarity (Steyermark 1949), *L. melissifolia* may have experienced evolutionary genetic bottlenecks that have resulted in small population sizes and low genetic diversity of extant populations (Godt and Hamrick 1996). The dioecious and clonal nature of this species probably contributes to its low within-population diversity because populations are often dominated by one or two clones. Those species such as *L. melissifolia*, which historically occurred in restricted populations, and have been rare for many years, may have genetic systems adapted to inbreeding (Barret and Kohn 1991, Maunder 1992). These naturally rare species may be more likely to suffer from outbreeding depression than those populations that have become small due to more recent anthropogenic causes.

Little information is available to guide propagation of *L. melissifolia* for new population establishment. Previous experiments have used seedlings, seeds or stems dug from clones (Wright 1989, Devall et al. 2001, Smith 2003), but few attempts to propagate stem cuttings for use in reintroduction efforts have been reported. Although genetic homogeneity has obvious disadvantages, clonal material provides greater opportunity to control sex ratios, to evaluate growth responses to varied environments, and may have the least impact on small populations.

The optimal stage of stem growth for obtaining cuttings for propagation and the usefulness of rooting hormones varies by species (Dirr and Heuser 1987). For propagation purposes, three basic growth stages of woody stems are softwood, semi-hardwood (greenwood) and hardwood. Softwood cuttings are taken early in the growing season when tissue is young and tender. Semi-hardwood cuttings are obtained after leaves are mature, generally later in the summer, and hardwood cuttings are taken late fall or winter after leaf fall when plants are dormant (Dirr and Heuser 1987). Although, Bailey and Bailey (1976) recommend taking cuttings of *Lindera* spp. in the semi-hardwood (greenwood) stage, the optimal conditions and application of rooting hormones have not been reported in the vegetative propagation of *L. melissifolia*.

The objective of this study was to determine the most favorable conditions for propagation of *L. melissifolia* from stem cuttings and to evaluate the use of cuttings for future reintroduction efforts. In addition, to better understand habitat requirements, previously rooted pondberry cuttings were experimentally reintroduced into four wetlands with open and closed canopy conditions.

# MATERIALS AND METHODS

## *Propagation*

Terminal stem cuttings (8 - 15 cm) were taken at two stages of growth: (a) softwood, obtained early summer (June 4, 2002) and (b) semi-hardwood (greenwood), obtained late summer to early fall (September 17, 2003). Stems were cut from a natural population of *L. melissifolia* located in Baker County, GA at Ichauway, a reserve of the Joseph W. Jones Ecological Research Center. It is unknown what negative impacts, if any, stem cuttings will have on the vigor of a population, therefore we decided not to take dormant cuttings to prevent any further stress on the small, localized population.

Three treatments were applied to 422 softwood cuttings in 2002: (1) stems dipped in powder rooting hormone, Hormex Rooting Powder No. 3 (0.3% Indole-3-Butyric Acid); (2) liquid rooting hormone, Dip'n Grow (1% Indole-3-Butyric Acid and 0.5% Naphthalene Acetic Acid); and (3) no rooting hormone. In the fall of 2003, we repeated the experiment with some treatment modifications. Cuttings were taken from both previously rooted softwood cuttings (240 cuttings) and from the naturally-occurring population (120 cuttings). Three additional treatments were applied in 2003 where stems were scarred along each side with a pocket knife and then subjected to the same three treatments as above.

The Dip'n Grow concentration was 5 mL to 50 mL water in 2002 and was increased to 10 mL to 50 mL water in 2003. Stems were placed in the rooting hormone powder and any excess was tapped off. Soil media consisted of 6 parts ProMix 'PGX': 2 parts Bark mix: ½ part sand. Basal leaves were removed and remaining leaves were cut in half to prevent overcrowding and to minimize transpiration losses. Cuttings were misted every 10 minutes for 15 seconds until callus formation and then removed from the mist. At this time, cuttings were fertilized weekly

with Peters liquid fertilizer (20-20-20 N-P-K with micronutrients) at a rate of 2.5 mL per 4 L of water.

On October 17, 2002 softwood cuttings were transplanted into 10 cm pots in 1 part bark mix: 1 part Fafard 3B Mix: 1/10 part sand. Fertilization stopped at this point. After leaf development the following spring, plants were fertilized every two weeks with a dilute solution of Peters fertlizer. Softwood cuttings were repotted again in 15 cm pots on April 29, 2003 in the same soil mix used previously.

***Experimental reintroduction***

Cuttings from North Carolina, taken by Woodlanders Inc., Aiken, SC, were outplanted on June 5 - 6, 2002 under two contrasting canopy cover conditions in each of four depressional wetland sites at Ichauway. The natural pondberry population is located along the margin of a forested wetland (Goebel et al. 1997) dominated by swamp blackgum (*Nyssa sylvatica* var. *biflora*) and pond-cypress (*Taxodium ascendens*). Wetlands were selected for reintroduction sites based on similar characteristics (i.e., plant species and hydrology) as those found in the natural population that occurs at Ichauway. In each wetland, we identified two canopy conditions: (1) closed tree canopy, and (2) open canopy gap, approximately 10-15 m across. At some sites, it was necessary to further open sun treatment canopies by removing trees and other small shrubs. We planted 10 rooted cuttings along the margin of each wetland under gap and closed canopy treatments (five in gap, five under closed canopy). Plants in each treatment were enclosed in 2.25 $m^2$ of chicken-wire to prevent uprooting by armadillos or other small mammals. Plants were watered at the time of planting and approximately every two weeks during the summer drought of 2002. During winter and early spring, ponds were inundated due to increased precipitation due to exceptionally high precipitation (National Climate Data Center,

Asheville, North Carolina). Surface water levels were recorded on three separate occasions in the center of each treatment area. In May 2003, stem length as the total length of the main stem and all branches, and stem diameter at a marked point at the base of the stem were recorded at the beginning of the growing season. The objective was to obtain final stem length and diameter measurements in addition to photosynthetic measurements. However, at the end of the summer, only 10 plants were still alive. Therefore, due to the loss of plants, we were unable to continue with this experiment and perform any statistical analyses.

## RESULTS

### *Propagation*

Softwood cuttings developed calluses at the base of stems at approximately two months following hormone treatments and roots developed from the calluses one month later. For semi-hardwood cuttings, callus formation occurred approximately three months after cuttings were taken but no roots were observed by 2003. Of the 422 softwood cuttings, only 155 rooted (37%). Of those that rooted, there was no difference due to treatment [powder hormone (38%), liquid (37%), and no hormone (36%)]. Of the 155 rooted cuttings, only 59 survived the winter (38%). Therefore, only 14% of the original 422 cuttings survived throughout the course of this study.

### *Experimental Reintroduction*

Of the 40 outplanted cuttings, 37 transplants survived through the first growing season (approximately 3 months). The mean height (± SE) for all plants at the end of the summer was 21.33 (± 1.44) cm. Maximum water levels for treatments ranged from 8 to 35 cm during the months of December to April. As a result of prolonged submergence, only 10 transplants survived.

# DISCUSSION

Even though semi-hardwood cuttings could potentially develop roots in the future**,** cuttings taken earlier in the growing season appear to be preferable to those taken prior to dormancy because of the slower callus formation and lack of root development observed in cuttings taken late summer. Some species can be rooted regardless of time of year and others require a particular set of conditions where timing is crucial (Dirr and Heuser 1987). Growth may have already stopped at the time semi-hardwood cuttings were taken and we may have been more successful if they were taken earlier (e.g., mid-July to early September).

Rooting hormones did not improve softwood cutting success in this study. Roots developed primarily from the callus at the base of the stem where it was cut. Perhaps a stronger concentration of rooting hormone than used in this study would be more effective in promoting root formation and might result in more evenly-spaced root development along the stem. In addition, calluses were observed along sides of scarred semi-hardwood stems. This scarring method may promote greater root formation based on the observation that roots developed from the region of the stem that was cut.

The low percentage (14%) of softwood cuttings that survived suggests that stem cuttings may not be the most effective method to obtain plant material for reintroduction efforts. However, hormone concentration and/or timing in this study may not have been optimal for the propagation of pondberry by stem cuttings. Similarly, other investigations have had little success with direct seeding or transplanting seedlings for new population establishment (Wright 1994, Smith 2003). Devall et al. (2001) have demonstrated that translocation of young pondberry stems and rootstock from natural populations may be an alternative reintroduction method. However, the selection of a propagation method should also depend on the availability

of plant material, and the potential negative impact on existing populations by removal of source material. For example at Ichauway, plant material that can be used for reintroduction efforts is limited because the population consists of a single female clone with low fruit production. In this case, digging up existing stems and rootstock may negatively impact this relatively small population. Although long-term impacts of stem cuttings are unknown, cuttings probably will have minimal impact on this clonal species, primarily because stems are relatively short-lived and new stems are produced annually from the rootstock (pers. obs., Smith 2003).

For this species, it may be necessary to increase genotypic diversity in the future to enhance sexual reproduction and to increase effective population sizes (Godt and Hamrick 1996). Therefore, mixing of source populations in reintroduction efforts may be necessary to enhance sexual reproduction, but the possibility for outbreeding depression should be considered. The benefits of an experimentally reintroduced population at Ichauway would be the opportunity to cut material without impacting the natural population, as well as to provide insight into management needs for the natural population on site. Even though there is the risk of breaking up coadapted gene complexes through outbreeding, a focus on species survival for highly endangered species (Mehrhoff 1996) especially where sexual reproduction is scarce, may justify multiple source reintroductions. In such a situation, plant material from a mix of source populations with both sexes represented could be introduced to increase genetic diversity and to determine if outbreeding depression is a valid concern.

Although we were unable to evaluate outplanting responses to different light environments, we were able to make some observations about survivorship of young plants in inundated conditions. Our results indicate that young transplants of *L. melissifolia* can tolerate some degree of flooding, but cannot survive being submerged. This suggests that plants may

require dry down periods for establishment and need to obtain height growth adequate to survive inundation. Based on adult tolerance to prolonged inundation (USFWS 1993), cuttings with stem height exceeding that of standing water would have been more likely to survive the flooded conditions and larger cuttings should be considered for reintroduction efforts. Clearly, a better understanding of recruitment and establishment is still needed to devise a strategy that will meet the recovery needs of this species.

The surviving cuttings from our study will be outplanted experimentally in appropriate wetland habitats at Ichauway and monitored for establishment success. In addition, the newly established population will be used for future propagation source material, as opposed to continued harvest of the natural population and will serve as a genetic safeguard in the case of catastrophic loss of the natural population. The extant outplanted cuttings from North Carolina will be removed from the experimental sites and maintained in pots for use in education demonstrations of endangered species at the J.W. Jones Ecological Research Center or transferred to the Atlanta Botanical Garden, Atlanta, Georgia, the State Botanical Garden, Athens, Georgia, and the North Georgia College Botanical Garden, Statesboro, Georgia for use in native flora conservation gardens.

# CHAPTER 4

# GERMINATION STUDIES

# INTRODUCTION

*Lindera melissifolia* (Walt.) Blume, pondberry, is a federally endangered (U.S. Fish and Wildlife Service 1986) deciduous, aromatic shrub in the Lauraceae. It occurs in seasonally flooded wetlands and is extant in only six states. The type of wetland habitat in which this species occurs varies regionally. In the Southeastern Coastal Plain (GA, NC, SC), pondberry is found along the edges of limestone sinks, ponds or other depressional wetlands, whereas, populations in Arkansas, Missouri and Mississippi are associated with bottomland hardwood forests. Pondberry is believed to be extirpated from Alabama, Florida and Louisiana and has always been considered a rare species throughout its historical range (Steyermark 1949). The major threats to this species include the loss or alteration of appropriate habitat through drainage modification, timber cutting, or the conversion of land for pine plantations, agriculture and urban development (USFWS 1993).

This species is a rhizomatous, clonal shrub, predominately reproducing asexually. Plants are dioecious (separate female and male plants) and most populations are dominated by male plants (Wright 1994, Wright 1990a, USFWS 1993, Godt and Hamrick 1996). Many populations consist of one or two clones of a single sex, suggesting little potential for successful sexual reproduction or adaptation to environmental change (Godt and Hamrick 1996). Therefore, increasing vigor of existing populations is critical for species persistence. Furthermore, infrequent sexual reproduction and seedling recruitment suggests that reintroduction of new populations will be necessary for species recovery (Godt and Hamrick 1996, Devall et al. 2001).

*Lindera* is described as either dioecious or polygamodioecious (Radford et al. 1968). Plants that are polygamodioecious can have a few perfect flowers or a few flowers of the opposite sex on the same plant (Zomlefer 1994). This may explain why a few fruits are

occasionally produced in populations that appear to be all female (pers. obs.). Pale yellow flower clusters appear in late February to mid-March before leaf-break, and bright red drupes develop from August to early October (Patrick et al. 1995). Staminate flowers occur in dense clusters, whereas pistillate flowers are fewer and less conspicuous. Flowers are believed to be insect pollinated, but there is little opportunity for pollen transfer among isolated populations.

Flower and fruit production is sporadic and can vary greatly from year to year (Morgan 1983, Wright 1990a). Flowers are often damaged by late frosts resulting in reduced fruit set (Tucker 1984). Limited information is available on seed dispersers of *L. melissifolia*, but the bright red drupes suggest that they are dispersed by birds or mammals (USFWS 1993). Hermit thrushes (*Catharus guttatus*) have been observed gathering fruits in Mississippi (Devall pers. comm.). Germination of *Lindera benzoin* (spicebush; a widespread congener) seed was greatly reduced when fruit pulp was intact compared to seed with fruit pulp removed (Meyer and Witmer 1998). This suggests that frugivores may play a critical role by removing fruit pulp, aiding in germination as well as dispersal. In populations associated with floodplains, fruits may have been dispersed by floodwaters before riverflows were actively managed (Devall et al. 2001). Seeds may also be dispersed by simply falling from the parent plant. Some *L. melissifolia* populations have produced numerous viable seeds, but seedlings are rarely observed in the wild (Wright 1989, USFWS 1993, Devall et al. 2001, Smith 2003). Even though viable seeds are produced, it is unclear why germination is extremely limited in natural populations (USFWS 1993).

The absence of seedling establishment, and thus the limited ability to establish new colonies may be another factor contributing to the decline of this species (USFWS 1993, Morgan 1983). It is possible that lack of seedling establishment is due to the loss of suitable habitat

required for seed germination. Pondberry often occurs along margins of seasonally flooded wetlands, and it is unclear how the hydrologic cycle influences seed germination and seedling establishment. Little information is available on the germination requirements for this species, although seeds germinated quickly in petri dishes when the seed coat was ruptured and temperatures were elevated (Wright 1990a). In addition, germination occurred after seeds were pushed below the soil surface following disturbance of the forest floor by fruit harvesting (Wright 1989). It is possible that leaf litter inhibits seedling emergence (Werner 1975, Carson and Peterson 1990, Peterson and Facelli 1992), because a dense canopy and litter accumulation may prevent seeds from receiving light needed for germination. In the southeastern Coastal Plain, fire may have played a role in the past, providing suitable microsites for germination by removing leaf litter and decreasing the abundance of competing hardwoods.

The purpose of this study was to determine the effects of leaf litter, planting depth, pulp removal, seed scarification and flooding on seed germination. The information obtained from a combination of field and greenhouse germination studies will be useful in developing management strategies to promote sexual reproduction in natural populations and for future reintroduction efforts.

## MATERIALS AND METHODS

### *Field Studies*

Pondberry fruits (201 drupes) used in all germination studies were collected in the Delta National Forest, Sharkey Co., Mississippi on Nov. 4, 2002. Seeds were sown and treatments were applied 9 days following collection as recommended by Baskin and Baskin (1998) (i.e., within 7-10 days of collection). Three forested depressional wetlands (P-11, P-52, and P-64) located in Baker County, GA at Ichauway, a reserve of the Joseph W. Jones Ecological Research

Center, were selected for field experiments. These habitats occurred within a 3-6 mile radius of the naturally occurring population and had similar vegetation. These wetlands are dominated by swamp blackgum (*Nyssa sylvatica* var. *biflora*) and pond-cypress (*Taxodium ascendens*). Flagging was used to mark treatment locations and where individual seeds were sown. Germination was examined of seeds placed on the soil surface with litter and no litter, pulp and no pulp, and cage and no cage using a three-factor design (i.e., 2 x 2 x 2 factorial design); including two additional treatments with litter and no pulp or cage of different planting depths: (1) 2.5 cm, and (2) 5 cm.

A $10m^2$ plot was established along the margins of each wetland. Each treatment was randomly assigned to a $1m^2$ subplot with five seeds per treatment. A total of 150 seeds were either lightly pressed in the surface or buried at 2.5 or 5 cm below the soil surface. Litter was removed by raking or was left intact on the mineral soil surface. Additional accumulations of leaf litter were removed biweekly. The fruit pulp was either removed and the seed was then rinsed with water, or the drupe was left intact. For the bird and mammal exclusion treatment, a cage ($0.5 m^2$) of fine metal mesh attached to a wooden frame was placed on top of the plot. All seeds were watered at the time of treatment application. Seeds were monitored biweekly for presence, absence, or germination.

***Greenhouse Studies***

An additional 45 seeds were used in a greenhouse germination trial to examine the influence of soil depth and flood regime on germination. The seed was sown on November 10, 2002 at the Jones Center greenhouse. Fruit pulp was removed from each seed, and five seeds were sown in plastic containers and randomly assigned to one of the following treatments: (1) flooded for three months and then drained, planted at a depth of 2.5 cm; (2) kept moist, planted

at a depth of 2.5 cm; and (3) kept moist, planted at a depth of 5 cm. Each treatment was replicated three times (total of 45 seeds). The soil media used consisted of 10 parts Fafard 3B Mix: 3 parts bark mix: ½ part sand. The seeds used for field and greenhouse studies were not scarified. The six remaining seeds were used to examine seed scarification requirements. After the removal of fruit pulp, three seeds were scarified by carefully removing a portion of the seed coat with a dull blade and three were not scarified. These seeds were placed in a Petri dish with moist filter paper using reverse osmosis water. The Petri dish was placed inside on the lab windowsill with indirect sunlight, and monitored for germination from November 2002 to August 2003. Water was added as needed to keep the filter paper moist.

## RESULTS

### *Field Studies*

Of the three study sites, two were flooded during the winter due to exceptionally high precipitation levels (National Climate Data Center, Asheville, North Carolina). Therefore, statistical analysis of the data was not possible due to lack of treatment replication. Prior to inundation, most of the seeds missing from the surface were those with fruit pulp, no cage and no litter (Table 4.1). In the wetland where treatments were not flooded (P-64), germination began mid-April and no new seedlings were observed after the first week of May. Neither litter presence nor sowing depth influenced seed germination, but of the caged seeds, more seeds germinated when pulp was removed than with intact pulp (Table 4.2).

### *Greenhouse Studies*

Germination of seeds in the greenhouse began at the end of March and continued until mid-May (4-5 months after treatments were applied). There was no difference among the three treatment combinations. The flooded treatment at 2.5 cm had a total of 87% germination and the

non-flooded treatments at 2.5 cm and 5 cm both had 93% germination. Of the 45 seeds sown, a total of 41 germinated (Table 4.3). For scarification treatments, no seeds placed in the Petri dish germinated.

## DISCUSSION

Even though field data was inconclusive due to the flooded site conditions, litter and planting depth did not seem to influence seed germination in the site that was not inundated. The absence of the unprotected seeds with pulp suggests that fruits were removed by birds or small mammals. Although it is uncertain from this study what effect fruit pulp has on seed germination, removal of pulp prior to planting in the field appears to enhance germination.

Although our results indicate that scarification of the seed coat is not necessary for germination, scarification may promote quicker germination. Wright (1990) found that pondberry seeds germinated quickly when the seed coat was ruptured and temperatures were elevated, and also that seeds in Petri dishes with seed coats removed had greater germination than those with notched seed coats. Even though seeds did not germinate in the Petri dish in this experiment, field observations and the previous study demonstrate that seed burial is unnecessary for germination.

Because flooding and planting depth made no difference in seed germination in the greenhouse study, it is possible that seed germination in the field may still occur when flood waters recede, although seed viability under extended periods of inundation is unknown. Based on studies by Smith (2003), seed viability appears to be at least up to 7 years following planting in the field.

Despite the limited replication and number of seeds available for experimental purposes in this study, our observations about the germination requirements of this species may be useful

in seedling establishment efforts and assessment of natural populations. Our study confirms that a high percentage of germination can be achieved in containers. We also observed that seedlings do not have distinct above ground cotyledons and may be easily mistaken for new stems. Thus, the previous observation that seedlings are rarely observed in the wild (Wright 1989, USFWS 1993, Devall et al. 2001, Smith 2003) may be due partly to the difficulty in distinguishing new stems from seedlings.

Seedlings obtained from the germination trials will be maintained in pots for education demonstrations of endangered species at the J.W. Jones Ecological Research Center or transferred to the Atlanta Botanical Garden, Atlanta, Georgia, the State Botanical Garden, Athens, Georgia, and the North Georgia College Botanical Garden, Statesboro, Georgia for use in native flora conservation gardens.

Table 4.1. Treatments with missing seeds prior to inundation for each pond.

| Treatment | Pond | Missing seeds |
|---|---|---|
| surface + litter + pulp + no cage | P-11 | 4 |
| surface + no litter + pulp + no cage | P-11 | 5 |
| surface + no litter + no pulp + no cage | P-11 | 4 |
| surface + litter + pulp + no cage | P-52 | 3 |
| surface + litter + no pulp + no cage | P-52 | 1 |
| surface + no litter + pulp + no cage | P-52 | 5 |
| surface + no litter + no pulp + no cage | P-52 | 1 |
| surface + no litter + no pulp + cage | P-52 | 1 |
| surface + litter + pulp + no cage | P-64 | 5 |
| surface + no litter + pulp + no cage | P-64 | 5 |
| surface + no litter + no pulp + no cage | P-64 | 2 |

Table 4.2. Number of seeds that germinated in each treatment for P-64.

A total of 5 seeds were sown in each treatment.

| Treatment | Germination |
|---|---|
| surface + litter + pulp + cage | 2 |
| surface + litter + pulp + no cage | 0 |
| surface + litter + no pulp + cage | 5 |
| surface + litter + no pulp + no cage | 2 |
| surface + no litter + pulp + cage | 0 |
| surface + no litter + pulp + no cage | 0 |
| surface + no litter + no pulp + cage | 4 |
| surface + no litter + no pulp + no cage | 1 |
| 2.5 cm deep + litter + no pulp + no cage | 4 |
| 5 cm deep + litter + no pulp + no cage | 4 |

Table 4.3. Number of seeds that germinated in each treatment combination for the greenhouse study. A total of 5 seeds were sown in each treatment combination.

| Depth (cm) | Flooded | Replication | Germination |
|---|---|---|---|
| 2.5 | Yes | 1 | 4 |
| 2.5 | Yes | 2 | 4 |
| 2.5 | Yes | 3 | 5 |
| 2.5 | No | 1 | 5 |
| 2.5 | No | 2 | 5 |
| 2.5 | No | 3 | 4 |
| 5 | No | 1 | 4 |
| 5 | No | 2 | 5 |
| 5 | No | 3 | 5 |

# CHAPTER 5

# CONCLUSIONS

Infrequent sexual reproduction and seedling recruitment of the federally endangered shrub, *Lindera melissifolia*, suggests that reintroduction of new populations and increasing vigor of existing populations will be necessary for species recovery (USFWS 1993, Godt and Hamrick 1996, Devall et al. 2001). Developing active management strategies requires information about the ecological requirements and species biology necessary to ensure population growth and persistence. In this study, we examined several key questions regarding growth and establishment of this species including: (1) photosynthetic performance and morphological responses under natural and controlled light regimes; (2) growth of plants under controlled light regimes; (3) evaluation of stem cutting techniques for propagation and outplanting; and (4) factors influencing seed germination (Figure 5.1).

A significant finding of this study is that *L. melissifolia* has the ability to persist and adjust to a range of light environments. Plants exhibited typical sun-shade morphological changes including decreased stomatal density, and increased specific leaf area and leaf area ratio in response to decreasing light levels. Furthermore, photosynthetic capacity was consistent with that reported for other shade tolerant species. Even though plants can persist in full sun environments, our study indicates that high light conditions do not appear to be optimal for growth, based on photosynthetic measures as well as total biomass.

For purposes of propagation of plant material, we found that cuttings taken earlier in the growing season appear to be preferable to those taken prior to dormancy, and that rooting hormones did not improve cutting success. In fact, the low percentage of softwood cuttings that survived suggests that stem cuttings may not be the most effective method in obtaining plant material for reintroduction efforts. Our results indicate that young transplants of *L. melissifolia*

can tolerate some degree of flooding but cannot tolerate being submerged, suggesting that plants may require dry down periods for establishment.

Our seed germination study confirms that a high percentage of germination can be achieved in containers regardless of flooding and planting depth, even without scarification. However, scarification may promote quicker germination. Even though field data was inconclusive due to the flooded site conditions, litter and planting depth did not seem to influence seed germination in the site that was not inundated.

***Management opportunities and challenges***

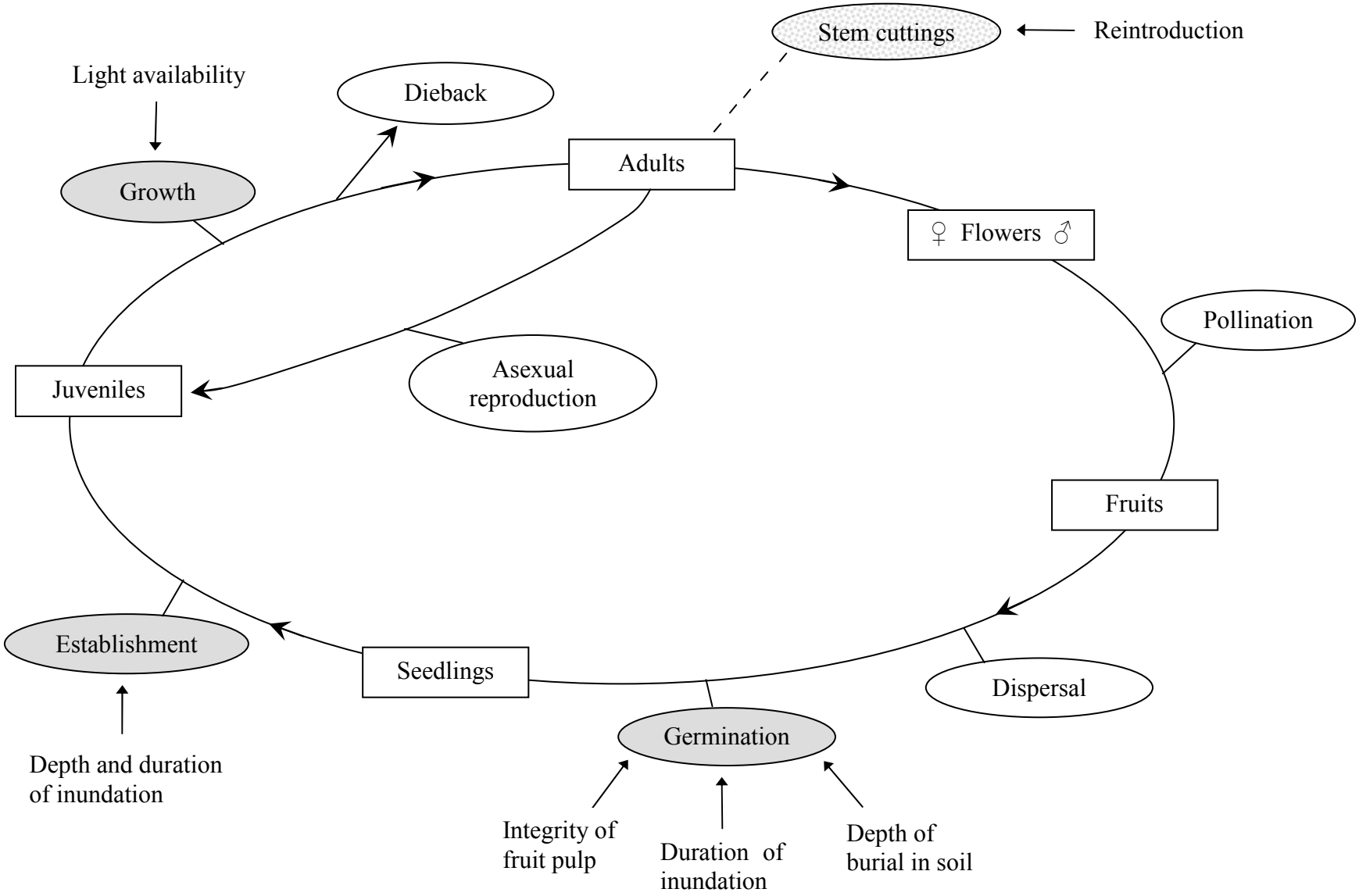
*Lindera melissifolia* is often found along the margins of depressional wetlands and may prefer intermediate light and hydrologic regimes associated with this ecotonal area. Furthermore, populations may be suppressed by competing vegetation that is favored in more open habitats. Canopy conditions at irradiance levels below 42% full sunlight appear to be adequate and perhaps optimal for maintaining plant growth, considering other factors are not limiting. Although controlled experiments offer information regarding a particular resource, a suite of environmental factors interact and influence plant establishment and growth in natural environments. Therefore, it is important to consider environmental factors not examined in this study, such as light quality, the intensity and duration of sunflecks, and nutrient and moisture availability when assessing management needs for populations of *L. melissifolia.*

Based on adult tolerance to prolonged inundation (USFWS 1993), cuttings with stem height exceeding inundation levels would have been more likely to survive the flooded conditions and such should be considered for reintroduction efforts. Previous studies suggest that digging up existing stems and rootstock may be advantageous to direct seeding or seedlings for new population establishment, because adult clones are robust and can be used to control sex

ratios (Devall et al. 2001, Smith 2003). Even though survival of propagated stem cuttings was low, hormone concentration and/or timing in this study may not have been optimal. Therefore, stem cuttings may prove to be an effective propagation method in future studies.

Although it is uncertain from this study what effect fruit pulp has on seed germination, removal of pulp prior to planting in the field appears to enhance germination. We also observed that seedlings do not have distinct above ground cotyledons and may be easily mistaken for new stems. Thus, the previous observation that seedlings are rarely observed in the wild (Wright 1989, USFWS 1993, Devall et al. 2001, Smith 2003) may be partly due to the difficulty in distinguishing new stems from seedlings. Further investigations are needed to understand factors limiting seedling survivorship and recruitment. Given the limited ability for new population establishment, efforts should focus on the protection of existing populations and suitable habitats, in addition to the creation of new populations.

Figure 5.1. Life cycle of *Lindera melissifolia*. Plant stages are represented in boxes and the developmental processes in circles. The shaded circles indicate the research focus of this study.


Stem cuttings
Reintroduction
Light availability
Dieback
Adults
Growth
♀ Flowers ♂
Pollination
Asexual reproduction
Juveniles
Fruits
Establishment
Seedlings
Dispersal
Germination
Depth and duration of inundation
Integrity of fruit pulp
Duration of inundation
Depth of burial in soil

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