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# Development and plasticity of endangered shrub *Lindera melissifolia* (Lauraceae) seedlings under contrasting light regimes

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## Abstract

***Lindera melissifolia* (Walt.) Blume seedlings were raised in a growth chamber to determine the effects of light availability on shoot growth pattern, and basic leaf and stem growth. *Lindera melissifolia* seedlings exhibited a sympodial shoot growth pattern for 3 months following emergence from the soil medium, but this pattern was characterized by a reduction in leaf blade area approximately 30 days after emergence, followed by increases in leaf blade area. Seedlings receiving low light were 76% taller than seedlings receiving high light. Seedlings receiving low light also had larger leaf blade dimensions, blade area, seedling leaf area, and greater mass. Seedlings raised in high light had a greater proportional distribution of biomass in the roots, suggesting possible water stress from greater vapor pressure deficits. Furthermore, these seedlings displayed sharp angles of blade inclination and blade folding – acclimation that reduces exposure to light and subsequent higher leaf temperatures in open environments. These differences in morphological response to light resulted in high phenotypic variability in *L. melissifolia* seedlings. *Lindera melissifolia* seedling development showed a brief period of phenotypic plasticity, followed by ontogenetic plasticity. The short period of phenotypic plasticity may, however, have profound ecological implications for the conservation and recovery of this federally endangered shrub. Further experimentation should take into account the development of ontogenetic standards for comparisons of plant traits in addition to temporal standards.**

*Keywords:* biomass distribution, light availability, ontogenetic plasticity, phenotypic plasticity, phenotypic variation.

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## Introduction

Advancing the recovery of imperiled plant species can be stymied by a complex snare of political interests, economic constraints and biological difficulties (Schemske *et al*. 1994; Boersma *et al*. 2001; Heywood & Iriondo 2003). Although political and economic factors can be substantial (Bowles & Whelan 1994), unsuccessful efforts aimed at the recovery of imperiled plant species have been largely attributed to inadequate biological data (Heywood & Iriondo 2003). Boersma *et al*. (2001), who reviewed 71 recovery plans written for endangered plants and animals endemic to the USA, illustrated that progress in species recovery was greatest when relevant biological information was linked to recovery plan goals.

Knowledge of the ecological interactions between imperiled plant species and their environment ranks highly among biological information central to developing a recovery approach (Schemske *et al*. 1994). However, the acquisition of ecological information can be challenged by knowledge gaps in basic species biology. Without fundamental information on a species' biology, implementation of scientifically sound and ecologically relevant experimentation can be compromised. Ontogeny and variation in phenotypic traits are key aspects of basic

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species biology that should be considered when conducting ecological experimentation on plants and their environment (Pigliucci 1996, 1998, 2005).

Ontogeny is the development of a plant through its life cycle, from embryo through to maturity (Gould 1977; Barthélémy & Caraglio 2007). Knowledge of a plant species' ontogeny, or developmental stages, is important because it influences functional processes, including photosynthesis and carbon allocation. For example, photosynthesis and carbon allocation patterns (source–sink relationships) change predictably in response to the development of new leaf blades or reproductive structures (Jeuffroy & Warembourg 1991; Dickson *et al*. 2000a, 2000b). Responses to abiotic or biotic stresses (e.g. elevated $CO_2$ levels) may accelerate ontogeny (Loehle 1995; Gunn *et al*. 1999; Lewis *et al*. 2002). Therefore, growth responses to changes in environmental conditions may be attributed to ontological development rather than variation in phenotypic traits (Coleman *et al*. 1994; McConnaughay & Coleman 1999; Wright & McConnaughay 2002). Coleman and McConnaughay's (1995) reinterpretation of earlier research showed that growth differences in response to environmental variation originally attributed to phenotypic variation disappeared when plants were compared relative to size. These findings have profound implications for research testing plant biomass partitioning theories, such as the plant strategy theory (Crick & Grime 1987; Campbell & Grime 1992), the resource-ratio hypothesis (Tilman 1988) and optimal partitioning models (Robinson 1986; Ägren & Ingestad 1987; Mooney & Winner 1991).

Variation in phenotypic traits of growth and biomass accumulation is also a significant aspect of a plant species' biology. Phenotypic variation in growth traits observed among plants has been attributed to ontogenetic drift, or to changes in plant growth and development as a result of changes in ontogeny (Evans 1972; Coleman *et al*. 1994). Ontogentic drift can be further subdivided into passive plasticity, ontogenetic plasticity or complex plasticity (Wright & McConnaughay 2002). Plants exhibit passive plasticity in traits where differences in phenotypes exist when compared at a common age, but no differences exist when compared at a common size (ontogeny). Plants exhibit ontogenetic plasticity when growth and development of the plant is altered by different environments, but the magnitude of change in a specific plant trait is maintained throughout ontogeny (Wright & McConnaughay 2002). Finally, plants exhibit complex plasticity when changes in a plant growth trait subject to different environments can be attributed to both changes in growth rate and ontogeny (Geng *et al*. 2007). Understanding the sources of phenotypic variation associated with environmental heterogeneity can lead to the development of more relevant experiments with improved interpretations of results (McConnaughay & Coleman 1999). Information derived from this type of research can also provide biological data needed to develop or improve endangered species recovery plans.

*Lindera melissifolia* (Walt.) Blume (pondberry), a federally listed endangered shrub endemic to the southeastern USA, grows on a variety of lowland sites, including the edges of limestone sinks in coniferous forests and depressions in bottomland hardwood forests (US Fish and Wildlife Service 1986; Schotz 2005). This deciduous, broadleaf shrub is dioecious, grows to approximately 2 m tall and is considered to be shade tolerant (Devall *et al*. 2001). Recent research on this species has revealed much about its photosynthetic light response (Aleric & Kirkman 2005), intraspecific competitive abilities (Hawkins *et al*. 2009b), genetics (Echt *et al*. 2006) and seed biochemistry (Connor *et al*. 2007), but basic information on *L. melissifolia* ontogeny and phenotypic variation is limited. We initiated research on seedling growth patterns in *L. melissifolia* to inform future research on the ecological interactions between this endangered shrub and prominent environmental factors in alluvial floodplain habitats. Our objectives for studying the ontogeny and phenotypic variation in this species were to: (i) describe shoot growth patterns in *L. melissifolia* seedlings under two levels of light availability; (ii) identify and quantify sources of phenotypic variation in shoot growth patterns in *L. melissifolia* seedlings relative to light availability; and (iii) summarize how our findings can be used to inform ecological experimentation on this endangered shrub species.

## Materials and methods

### *Plant material*

*Lindera melissifolia* drupes were collected from colonies located on the Delta National Forest, Sharkey County, MS, USA (32°58′N, 90°44′W, 30 m a.s.l.) on 2 October 2003. The climate at this locale is typical of the Lower Mississippi Riverine Forest Province in the Humid Temperate Domain and is marked by high humidity, particularly in the summer (Bailey 1995). The average daily temperature is 17.3°C, with a range from 27.3°C in July to 5.6°C in January (WorldClimate 2008), and precipitation averages 1350 mm per year (WorldClimate 2008). The dominant soils include Sharkey clay (very-fine, smectitic, thermic Chromic Epiaquerts), Alligator clay (very-fine, smectitic, thermic Chromic Dystraquerts) and Dowling clay (very-fine, smectitic, non-acid, thermic Vertic Endoaquepts), which are characterized by fine-textured shrink/swell clays deposited as Mississippi River alluvium (Pettry & Switzer 1996). These soils support typical bottomland hardwood forest cover including *Liquidambar styraciflua* L., *Celtis laevigata* Willd., *Acer rubrum* L., *Quercus nuttallii* Palmer and *Acer negundo* L. (Hawkins *et al*. 2009a), and

can experience 2–3 months of flooding in late winter and early spring in most years (Hawkins *et al*. 2010).

Harvested drupes were placed in plastic bags and stored in an ice chest for transport from the field. Drupes were processed in the laboratory by gently removing the exocarp and mesocarp with a razor blade. The clean seeds were then stratified for 8 months in moist sand at 2°C (Connor *et al*. 2007). On 1 May 2004, an individual seed was sown in each of 32, 7.5 L containers filled with a soil medium consisting of peat moss, sand, 0–46-0 and 10–10-10 (N-P-K) fertilizers, and Milorganite (Hawkins *et al*. 2009b). The seeds were sown 2 cm below the soil surface, and containers were randomly assigned to a light availability treatment in a growth chamber.

### *Germination and growing environment*

A Conviron PGR15 plant growth chamber (Pembine, ND, USA) located at the Center for Bottomland Hardwoods Research, Stoneville, MS, USA, was configured to produce two separate light regimes, high light availability and low light availability. Maximum light output of the growth chamber was maintained at 600 µmol/m$^2$/s photosynthetically active radiation (PAR) for the high light regime. Neutral density shade cloth (63%) was used to reduce the maximum light output of the growth chamber to 120 µmol/m$^2$/s (PAR) for the low light regime. Light levels for the respective regimes represent means of measurements taken near the soil medium surface prior to seedling emergence (107 cm from the light source). The diurnal photoperiod in the growth chamber was 16 h, including a 2 h step-up and 2 h step-down period during which light levels were increased or decreased by 25% each half hour. The growth chamber temperature was maintained at 26°C during the day and 20°C at night. The soil medium in the containers was watered three or four times per week to maintain the soil at field capacity. All containers were randomly relocated beneath their respective light regime once per week for the duration of the study.

### *Measurements*

Seedling height (mm), leaf blade length (mm) along the midrib, blade width (mm) at the widest point perpendicular to the midrib, and the subtending internode length (mm) were measured on each plant three times per week. After 3 months of growth, all plants were harvested and separated into leaf, stem and root tissues. The area (cm$^2$) of each leaf blade was determined by averaging two measurements collected with a LiCor LI-3100 leaf area meter (Lincoln, NE, USA). Roots were washed clean and then all seedling tissues were oven-dried at 50°C until completely desiccated.

### *Statistical analyses*

In addition to the directly measured variables listed above, other key indices of seedling development were generated for analysis. Proportional biomass accumulation by root, stem and leaf tissues was computed according to the following equations: root mass ratio (RMR) = root mass/total seedling mass, stem mass ratio (SMR) = stem mass/total seedling mass, and leaf mass ratio (LMR) = leaf mass/total seedling mass. The root/shoot ratio was calculated by dividing root dry mass by shoot (stem and leaves) dry mass. Phenotypic variation was calculated as the difference between the maximum and minimum values of a response variable divided by the maximum value (phenotypic plasticity *sensu* Valladares *et al*. 2002; Funk 2008).

An ANOVA using PROC GLM (SAS 9.2; Cary, NC, USA) in a completely randomized design (two levels of light availability) was conducted to test for light effects on: stem height, number of nodes, internode length, sum of internode lengths, number of leaves, blade length, blade width, blade area, specific blade area, plant leaf area, leaf mass, stem mass, root mass, total seedling mass, LWR, SWR, RWR, root/shoot ratio, and phenotypic variation for each response variable. Sixteen containers with sown seed were initially assigned to each light regime. One seed did not germinate under the low light regime ($n = 15$ seedlings) and three did not germinate under the high light regime. Two additional seedlings under the high light regime developed a deformed shoot soon after germination and were excluded from analysis ($n = 11$ seedlings). Significance for each response variable was set at $P = 0.05$.

Patterns of pondberry development were assessed by graphing individual seedling height and leaf area over time to illustrate trends. Repeated measures analyses of response variables were conducted using PROC GLM and a chronological standard (days after emergence [DAE]) as the independent variable. For this analysis, development of total plant leaf area was computed from periodic blade measurements and use of a linear regression equation developed by Lockhart *et al*. (2007).

Analyses and graphs of dependent variable response over seedling ontogeny were used to determine if phenotypic variation was attributable to passive plasticity, ontogenetic plasticity or complex plasticity. To address this question we needed to use an ontogenetic standard as an independent variable (Coleman *et al*. 1994; Wright & McConnaughay 2002). Periodic harvesting of plants to generate biomass variables is commonly used to develop ontogenetic standards. As plants were only harvested at the termination of the study, we chose to use individual leaf blades as our ontogenetic standard (Bonser & Aarssen 1996, 2003). A review of the data indicated that

**Table 1** *Lindera melissifolia* seedling characteristics at the time of harvest after growing under high and low light in a growth chamber

| Variable (unit) | Light availability | | Statistics |
|---|---|---|---|
| | High ($n = 11$) | Low ($n = 15$) | |
| Stem height (cm) | 343.9 (56.2) a | 606.6 (63.0) b | $F_{1,24} = 8.56$, $P = 0.007$ |
| No. nodes | 35.5 (2.4) a | 36.6 (2.2) a | $F_{1,24} = 0.10$, $P = 0.749$ |
| Internode length (mm) | 9.2 (1.0) a | 17.0 (1.1) b | $F_{1,24} = 19.59$, $P < 0.001$ |
| No. blades | 24.7 (2.6) a | 31.3 (2.2) a | $F_{1,24} = 3.75$, $P = 0.065$ |
| Blade length (mm) | 44.8 (6.2) a | 77.2 (5.8) b | $F_{1,24} = 12.32$, $P = 0.001$ |
| Blade width (mm) | 19.7 (2.6) a | 34.5 (3.4) b | $F_{1,24} = 10.75$, $P = 0.003$ |
| Blade area ($cm^2$) | 8.16 (1.97) a | 24.95 (4.32) b | $F_{1,24} = 9.88$, $P = 0.004$ |
| Specific blade area ($cm^2/g$) | 0.005 (0.0005) a | 0.003 (0.0001) b | $F_{1,24} = 24.02$, $P < 0.001$ |
| Plant leaf area ($cm^2$) | 242.7 (68.5) a | 891.9 (190.6) b | $F_{1,24} = 8.55$, $P = 0.010$ |
| Plant specific leaf area ($cm^2/g$) | 112.7 (20.4) a | 165.5 (12.4) b | $F_{1,24} = 5.42$, $P = 0.029$ |
| Root/shoot ratio (g/g) | 1.14 (0.14) a | 0.90 (0.22) a | $F_{1,24} = 0.36$, $P = 0.414$ |

Values in parentheses represent one standard error. Different lowercase letters within a row represent significantly different values at $P \leq 0.05$.

blade number 20 was the latest mature blade across all plants. A dataset of non-biomass plant response variables listed above was compiled through blade number 20 for each plant and analyzed using PROC GLM as previously described. Curvilinearity of the relationships between response variables and stage of seedling ontogeny (individual blades) indicated that polynomial regression was necessary to describe these responses (McConnaughay & Coleman 1999). Treatment effects were tested with log transformations of blade length, internode length, days to maturity for blade length and internode length, blade length and internode length relative growth rates, blade area and specific blade area (assuming no change in the dry weight of individual blades from maturity to harvest). Relative growth rates were calculated by dividing the final blade length or internode length measurement for a specific blade at blade maturity by the number of days to reach this value.

SigmaPlot 10.0 (Chicago, IL, USA) was used to determine relationships between the above variables and plant stage of ontogeny. A Kolmogorov–Smirnov test was used to assess the normality of the data and the Durbin–Watson statistic was used to assess autocorrelation between residual values. A $P^2$ statistic (coefficient of prediction) was hand calculated to determine model selection; $P^2$ is based on the PRESS statistic and is considered more accurate and precise than standard coefficients of determination (Mediavilla *et al*. 2008). A PROC MIXED analysis with dummy variables was used to determine if slopes of regression equations (high light regime and low light regime) for each variable were different. Repeated measures analysis using each blade as the repeated measure factor was used to compare measurements of individual blades (e.g. blade length, internode length) between light regimes.

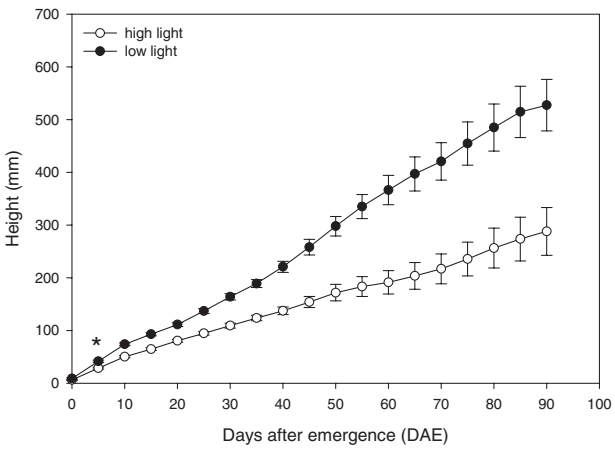


**Fig. 1** *Lindera melissifolia* seedling height development to 90 days after emergence (DAE). Individual points are at 5-day intervals. The asterisk at 5 DAE indicates the beginning of significant differences between light availability. Error bars represent one standard error. Coefficients of determination were 0.99 within respective treatments using simple linear regression.

## Results

### *Stem height and internodes*

*Lindera melissifolia* seedlings raised in low light were 76% taller than seedlings raised in high light (Table 1). This treatment difference in stem height surfaced by 5 DAE and continued through to 90 DAE (Fig. 1). Stem growth in both treatments was continuous, but exhibited differing rates, averaging 3.0 and 6.0 mm per day for high and low light, respectively (Fig. 1; $F_{18,432} = 10.73$, $P < 0.001$ for the time by treatment interaction).

Greater height under low light availability was associated with longer internode lengths, but not with the number of nodes (Table 1). Light availability did not

**Table 2** Internode length (mm) comparisons for *Lindera melissifolia* seedlings in high and low light

| Internode position | High light *n* | High light Length (mm) | Low light *n* | Low light Length (mm) | Statistics |
|---|---|---|---|---|---|
| 1 | 11 | 4.6 (0.9) a | 15 | 6.9 (1.4) a | $F_{1,24} = 1.53$, $P = 0.229$ |
| 2 | 11 | 3.6 (0.7) a | 15 | 7.9 (1.2) b | $F_{1,24} = 7.96$, $P = 0.010$ |
| 3 | 11 | 6.4 (0.9) a | 15 | 11.9 (0.9) b | $F_{1,24} = 17.32$, $P < 0.001$ |
| 4 | 11 | 5.2 (0.7) a | 15 | 9.5 (1.0) b | $F_{1,24} = 10.62$ $P = 0.003$ |
| 5 | 11 | 7.7 (0.6) a | 15 | 11.5 (1.1) b | $F_{1,24} = 7.61$, $P = 0.011$ |
| 6 | 11 | 7.6 (0.8) a | 15 | 10.7 (1.3) a | $F_{1,24} = 3.86$, $P = 0.061$ |
| 7 | 11 | 6.7 (0.8) a | 15 | 12.7 (1.0) b | $F_{1,24} = 20.88$, $P < 0.001$ |
| 8 | 11 | 7.6 (1.0) a | 15 | 10.6 (1.3) a | $F_{1,24} = 2.82$, $P = 0.106$ |
| 9 | 11 | 7.8 (1.3) a | 15 | 11.2 (1.4) a | $F_{1,24} = 2.96$, $P = 0.098$ |
| 10 | 11 | 8.4 (1.4) a | 15 | 16.5 (1.7) b | $F_{1,24} = 11.73$, $P = 0.002$ |
| 11 | 11 | 12.9 (1.0) a | 15 | 20.2 (1.9) b | $F_{1,24} = 9.73$, $P = 0.005$ |
| 12 | 11 | 10.6 (0.8) a | 15 | 19.5 (0.8) b | $F_{1,24} = 55.42$, $P < 0.001$ |
| 13 | 11 | 8.7 (0.5) a | 15 | 17.6 (1.1) b | $F_{1,24} = 43.17$, $P < 0.001$ |
| 14 | 11 | 9.8 (0.4) a | 15 | 16.4 (1.1) b | $F_{1,24} = 25.26$, $P < 0.001$ |
| 15 | 11 | 9.5 (0.7) a | 15 | 18.2 (1.7) b | $F_{1,24} = 17.36$, $P < 0.001$ |
| 16 | 11 | 8.6 (0.7) a | 15 | 21.9 (2.2) b | $F_{1,24} = 25.86$, $P < 0.001$ |
| 17 | 11 | 8.7 (1.2) a | 15 | 25.0 (2.2) b | $F_{1,24} = 33.77$, $P < 0.001$ |
| 18 | 11 | 8.9 (1.5) a | 15 | 26.1 (2.2) b | $F_{1,24} = 34.32$, $P < 0.001$ |
| 19 | 11 | 8.9 (2.0) a | 15 | 24.6 (2.4) b | $F_{1,24} = 23.05$, $P < 0.001$ |
| 20 | 11 | 8.9 (2.2) a | 15 | 22.9 (2.4) b | $F_{1,24} = 16.50$, $P < 0.001$ |
| 21 | 11 | 8.5 (2.3) a | 15 | 21.9 (2.4) b | $F_{1,24} = 15.01$, $P = 0.001$ |
| 22 | 11 | 8.7 (2.3) a | 15 | 21.3 (2.4) b | $F_{1,24} = 13.28$, $P = 0.001$ |
| 23 | 11 | 9.6 (2.3) a | 15 | 20.3 (2.2) b | $F_{1,24} = 10.80$, $P = 0.003$ |
| 24 | 11 | 9.3 (1.9) a | 15 | 20.5 (2.3) b | $F_{1,24} = 12.44$, $P = 0.002$ |
| 25 | 11 | 10.3 (2.1) a | 15 | 20.5 (2.7) b | $F_{1,24} = 7.95$, $P = 0.010$ |
| 26 | 11 | 11.7 (2.7) a | 15 | 18.5 (2.8) a | $F_{1,23} = 3.15$, $P = 0.099$ |
| 27 | 10 | 13.0 (3.1) a | 14 | 20.1 (3.3) a | $F_{1,22} = 2.29$, $P = 0.144$ |
| 28 | 10 | 14.0 (3.4) a | 12 | 22.2 (2.6) a | $F_{1,20} = 3.68$, $P = 0.070$ |
| 29 | 7 | 17.7 (2.6) a | 11 | 22.3 (2.2) a | $F_{1,16} = 1.74$, $P = 0.206$ |
| 30 | 7 | 16.1 (3.2) a | 11 | 20.6 (2.8) a | $F_{1,16} = 1.05$, $P = 0.320$ |
| 31 | 7 | 14.9 (3.5) a | 11 | 20.1 (3.4) a | $F_{1,16} = 1.05$, $P = 0.320$ |
| 32 | 6 | 15.0 (3.2) a | 10 | 21.6 (3.4) a | $F_{1,14} = 1.67$, $P = 0.217$ |

Values in parentheses represent one standard error. Different lowercase letters within a row represent significantly different values at $P \leq 0.05$.

influence length of the first internode for *L. melissifolia* (Table 2). Thereafter, internode lengths for seedlings receiving low light were usually longer than similar internodes for seedlings in high light (Table 2). For low light plants, internodes 10 through 25 were 125% longer than those from plants in high light. A length difference was not found beyond internode 25 as the number of plants with higher positioned internodes began to decrease and variability increased.

### *Blades, blade area and leaf area development*

*Lindera melissifolia* leaf blades that developed under low light were on average 72% longer and 75% wider than blades that developed under high light (Table 1). As a result, leaf blades receiving low light were 206% greater in area than blades receiving high light (Table 1). In contrast, specific blade area was greatest for leaves in high light (Table 1), averaging 66% higher than for blades developed under low light.

The greater blade area development by seedlings receiving the low light regime resulted in 267% more leaf area than that of seedlings receiving high light. A difference in leaf area between low and high light plants began at 15 DAE (Fig. 2), and this point marked the beginning of a rapid increase in leaf area for plants in low light ($F_{18,432} = 3.49$, $P < 0.001$ for the time by treatment interaction).

All 26 sample plants displayed an acropetal pattern of increasing blade area. Initial maximum blade area was attained at 52 and 59 DAE on blades 16 and 17 for plants in high and low light, respectively. Blade area then tapered through 76 and 78 DAE to blades 20 and 21 for plants in high and low light, respectively. For 24 of the 26 plants, blade area increased again to a greater blade area than the initial maximum (e.g. Fig. 3).

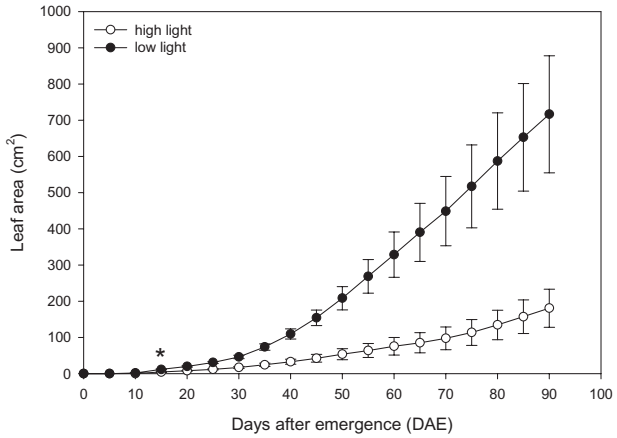


**Fig. 2** *Lindera melissifolia* seedling leaf area development to 90 days after emergence (DAE). Individual points are at 5-day intervals. The asterisk at 15 DAE indicates the beginning of significant differences between light availability. Error bars represent one standard error.

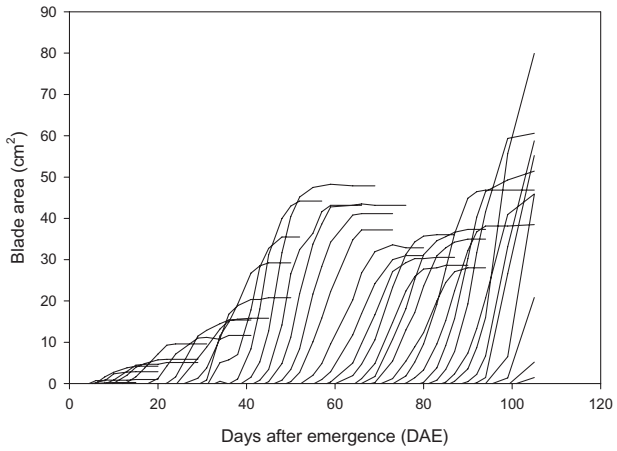


**Fig. 3** Blade area development in a representative *Lindera melissifolia* seedling in low light. Each line represents one leaf blade.

## *Height and leaf area development*

Two patterns of height and leaf area development emerged for *L. melissifolia*. For the first pattern, linear height development coincided with linear leaf area development (Fig. 4). For the second pattern, more rapid linear height development by plants receiving low light or slightly curvilinear height development for plants receiving high light coincided with strong curvilinear leaf area development (Fig. 5). The rapid increase in leaf area for plants receiving low light was initiated at approximately 30 DAE, whereas this increase was initiated between 30 and 60 DAE for plants receiving high light.

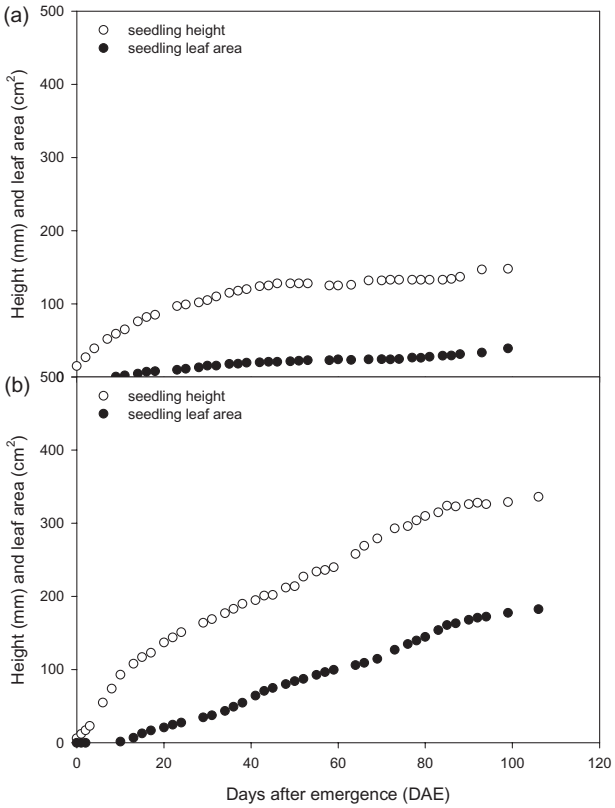


**Fig. 4** Linear height and leaf area development in a representative *Lindera melissifolia* seedling in (a) high light and (b) low light.

## *Biomass accumulation*

Biomass accumulation by *L. melissifolia* seedlings was determined by light availability. The greatest biomass accumulation was found for seedlings raised beneath the low light level; total mass was approximately 2.7-fold the mass of seedlings grown under high light ($F_{1,24} = 5.62$, $P = 0.027$). All biomass components accumulated the greatest mass under low light availability with root biomass gaining 113%, stem biomass gaining 280% and leaf biomass gaining 166% under low light availability. Biomass accumulation for plants receiving high light was greatest in roots and leaves, whereas accumulation for plants receiving low light was greatest in roots (Fig. 6).

## *Biomass distribution*

Root tissues accounted for the greatest proportion of fixed carbon in *L. melissifolia* seedlings. In addition, seedlings receiving high light had 11% more biomass distributed to root tissues compared with seedlings receiving low light (Fig. 7). Concurrently, seedlings receiving low light had 8% greater biomass distributed to stem tissues compared with seedlings receiving high light (Fig. 7). Biomass

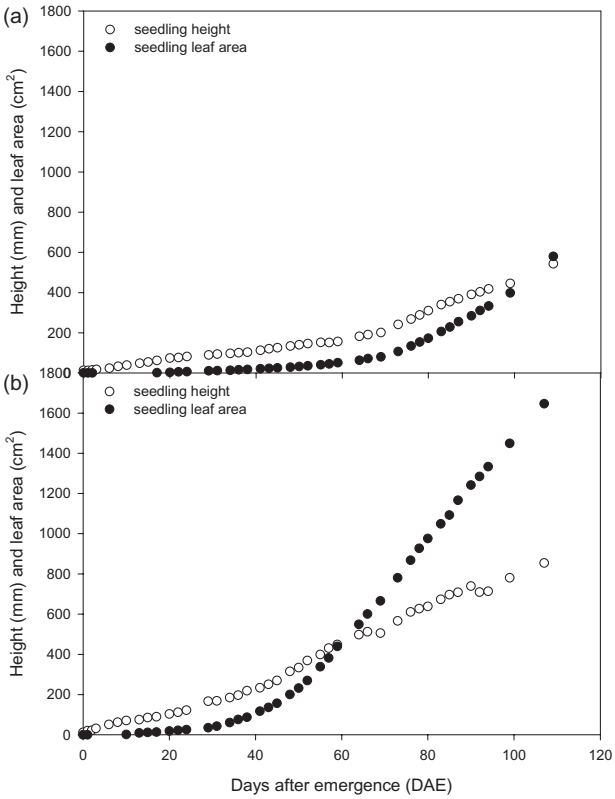


**Fig. 5** Rapid linear or slightly curvilinear height development and strong curvilinear leaf area development in representative *Lindera melissifolia* seedling in (a) high light and (b) low light.

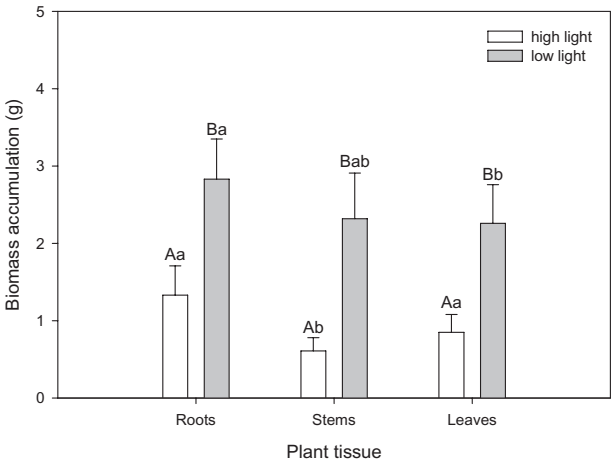


**Fig. 6** Biomass accumulation in *Lindera melissifolia* seedling tissues in high and low light. Error bars represent one standard error. Upper-case letters are comparisons between light availability within a plant tissue and lower-case letters are comparisons between plant tissues within a light level. Different letters are significantly different at $P < 0.05$.

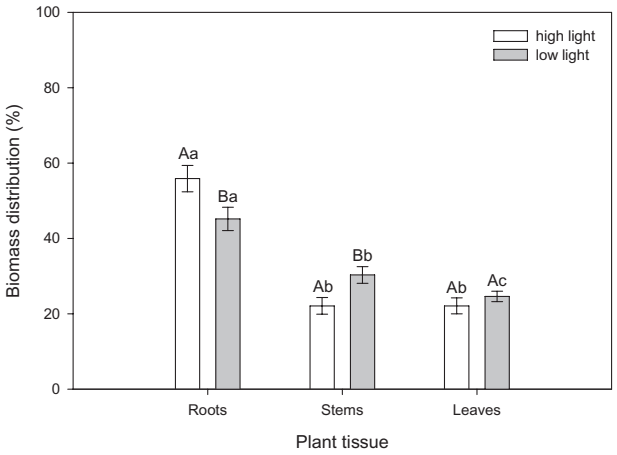


**Fig. 7** Biomass distribution in *Lindera melissifolia* seedling tissues in high and low light. Error bars represent one standard error. Upper-case letters are comparisons between light availability within a plant tissue and lower-case letters are comparisons between plant tissues within a light level. Different letters between each pair of bars are significantly different at $P < 0.05$.

distribution to leaf tissues was similar between light regimes. Stem tissues accumulated a greater proportion of biomass compared with leaf tissues for plants receiving low light, but we observed no difference between tissues for plants receiving high light.

### *Phenotypic variation*

Phenotypic variation among the 18 measured variables ranged from 0.49 for the number of nodes to 0.99 for plant leaf area, leaf dry weight and shoot dry weight (Table 3). Maximum phenotypic variation for 15 variables (83%) occurred under the low light regime, whereas minimum values for 14 variables (78%) occurred under the high light regime (Table 3). We noted that the minimum value for the number of nodes was observed under both light regimes, and maximum and minimum values observed for stem mass ratio were recorded under the low light regime.

### *Sources of phenotypic variation*

Treatment differences in morphological variables observed through leaf blade 20 (Table 4) changed little from those observed at the time of harvest (Table 1). Plants receiving low light showed greater internode length, blade length, blade width, blade area and dry mass compared with plants receiving high light (Table 4). Concurrently, plants receiving high light showed greater specific blade area and plant specific blade area.

Second-order polynomial regressions were significant across all dependent variables and light regimes, but differences between regression slopes for high light and low

**Table 3** Phenotypic variation in *Lindera melissifolia* seedling characteristics at the time of harvest after growing under high and low light in a growth chamber

| Variable | Phenotypic variation | Light availability treatment Maximum value | Minimum value |
|---|---|---|---|
| Stem height | 0.86 | Low | High |
| No. nodes | 0.49 | Low | Low and high |
| Internode length | 0.80 | Low | High |
| No. leaves | 0.81 | Low | High |
| Blade length | 0.80 | Low | High |
| Blade width | 0.84 | Low | High |
| Blade area | 0.97 | Low | High |
| Specific blade area | 0.71 | Low | High |
| Plant leaf area | 0.99 | Low | High |
| Shoot specific leaf area | 0.64 | High | Low |
| Root/shoot ratio | 0.93 | High | Low |
| Root dry mass | 0.96 | Low | High |
| Shoot dry mass | 0.99 | Low | High |
| Leaf dry mass | 0.99 | Low | High |
| Plant dry mass | 0.98 | Low | High |
| Root mass ratio | 0.72 | Low | High |
| Stem mass ratio | 0.78 | Low | Low |
| Leaf mass ratio | 0.74 | High | Low |

**Table 4** *Lindera melissifolia* seedling characteristics at mature leaf number 20 under high and low light in a growth chamber

| Variable (unit) | Light availability High ($n = 11$) | Low ($n = 15$) | Statistics |
|---|---|---|---|
| Internode length (mm) | 8.1 (0.4) a | 16.1 (0.9) b | $F_{1,24} = 57.35$, $P < 0.001$ |
| Blade length (mm) | 31.5 (2.5) a | 65.5 (4.0) b | $F_{1,24} = 42.56$, $P < 0.001$ |
| Blade width (mm) | 14.3 (1.1) a | 27.7 (2.0) b | $F_{1,24} = 29.22$, $P < 0.001$ |
| Blade area ($cm^2$) | 4.1 (0.9) a | 17.8 (2.8) b | $F_{1,24} = 19.02$, $P = 0.003$ |
| Blade dry mass (g) | 0.0174 (0.0028) a | 0.0456 (0.0068) b | $F_{1,24} = 11.34$, $P < 0.001$ |
| Specific blade area ($cm^2/g$) | 0.0052 (0.0004) a | 0.0029 (0.0001) b | $F_{1,24} = 48.62$, $P < 0.001$ |
| Plant leaf area ($cm^2$) | 47.6 (12.3) a | 300.9 (48.6) b | $F_{1,24} = 19.05$, $P < 0.001$ |
| Plant specific leaf area ($cm^2/g$) | 0.0049 (0.0003) a | 0.0027 (<0.0001) b | $F_{1,24} = 57.59$, $P < 0.001$ |
| Blade length/internode length index (mm/mm) | 4.4 (0.2) a | 4.4 (0.4) b | $F_{1,24} = 0.00$, $P = 0.976$ |
| Blade area/internode length index ($cm^2$/mm) | 0.3416 (0.0447) a | 0.8250 (0.1071) b | $F_{1,24} = 13.54$, $P < 0.001$ |

Values in parentheses represent one standard error. Different lowercase letters within a row represent significantly different values at $P \leq 0.05$.

light were detected only for blade length RGR, internode length RGR and specific blade area (Table 5). Comparison of individual blade lengths within the polynomial curves indicated few differences early in development (Blades 1–3; Fig. 8a). The non-linear shape of these curves at these blade numbers indicates ontogenetic drift in early *L. melissifolia* development. Beginning at Blade 4 and proceeding through to Blade 11 regression slopes differed between high and low light regimes (Fig. 8a). The significant treatment effect between most of these leaf blades and the associated slope differences are indicative of a true treatment response along an ontogeny gradient (blade numbers) or phenotypic plasticity (Fig. 8a). Beginning with Blade 12, blade lengths differed between light regimes, but regression slopes were similar (Fig. 8a). This response is consistent with ontogenetic plasticity.

A similar pattern of plasticity along a developmental gradient of leaf blades, ontogenetic drift followed by phenotypical plasticity then ontogenetic plasticity, occurred for blade length RGR (Fig. 8b) and blade area (Fig. 9a). Little to no ontogenetic drift occurred for specific blade area (Fig. 9b), internode length (Fig. 10a) and internode length RGR (Fig. 10b). In these latter cases, internode traits responded immediately to light, whereas the leaf blades were too small to obtain reliable dry mass data to determine specific blade area in plants receiving high light. No difference in slopes occurred between light regimes along individual blades for the number of days to

**Table 5** Summary statistics for polynomial regression (second order) relationships of *Lindera melissifolia* seedling traits in high and low light

| Dependent variable | High light | | | Low light | | | Interaction |
|---|---|---|---|---|---|---|---|
| | *F*-value | *P*-value | $P^2$ | *F*-value | *P*-value | $P^2$ | *P*-value |
| Blade length | $575.82_{(3,16)}$ | <0.001 | 0.978 | $1683.06_{(3,16)}$ | <0.001 | 0.995 | 0.317 |
| Blade length relative growth rate | $29.60_{(3,16)}$ | <0.001 | 0.683 | $55.29_{(3,16)}$ | <0.001 | 0.808 | 0.002 |
| Blade length – days to maturity | $162.95_{(3,16)}$ | <0.001 | 0.988 | $160.18_{(3,16)}$ | <0.001 | 0.993 | 0.503 |
| Internode length | $11.33_{(3,16)}$ | <0.001 | 0.521 | $46.53_{(3,16)}$ | <0.001 | 0.833 | 0.166 |
| Internode length relative growth rate | $16.09_{(3,16)}$ | <0.001 | 0.416 | $57.92_{(3,16)}$ | <0.001 | 0.879 | 0.004 |
| Internode length – days to maturity | $55.75_{(3,16)}$ | <0.001 | 0.770 | $75.17_{(3,16)}$ | <0.001 | 0.882 | 0.376 |
| Blade area | $243.91_{(3,16)}$ | <0.001 | 0.960 | $340.18_{(2,17)}$ | <0.001 | 0.956 | 0.376 |
| Specific blade area | $19.50_{(3,16)}$ | <0.001 | 0.697 | $3.62_{(3,16)}$ | 0.036 | 0.489 | 0.014 |

reach maturity in either blade length or internode length, although the number of days to maturity increased with increasing blade number (Figs 8c,10c).

## Discussion

### *Is* L. melissifolia *shoot growth monopodial or sympodial?*

A key question in basic woody plant growth and development research is, 'Does the plant exhibit monopodial or sympodial shoot growth?'. As a shade-tolerant forest shrub, we hypothesized that *L. melissifolia* would exhibit a monopodial shoot growth pattern. However, linear height growth (Fig. 1) and continuous production of leaf blades (Fig. 3) are indicative of sympodial shoot growth. Sympodial shoot growth is not uncommon in other forest shrub species. Deering and Vankat (1999) observed continuous shoot growth in *Lonicera manckii* (Rupr.) Herder during its pre-reproductive growth phase. Other iteroporous forest shrubs exhibit continuous shoot growth prior to reproductive maturity when photosynthates are not allocated to reproductive tissues (Deering & Vankat 1999). In *L. melissifolia*, sexual reproductive structures do not develop before the second or third growing season following shoot emergence from the soil (T. Hawkins, USDA Forest Service, Southern Research Station, pers. comm., 2010).

Although *L. melissifolia* displayed continuous growth under controlled conditions, blade area growth was not consistent (e.g. Fig. 3). Increases in blade size with each additional leaf are characteristic of young seedlings. As root development proceeds, resource availability to the shoot increases. Blade size then becomes relatively constant, particularly for sympodial plants, as roots and shoots reach a functional equilibrium in photosynthate production and distribution (Larson & Isebrands 1971). A reduction in blade size following earlier increases in blade size is characteristic of recurrent or flushing species, such as *Quercus* spp. seedlings, where hormones, water stress or carbohydrate concentrations have been implicated as reasons for the development of smaller blades near the shoot tip followed by a dormant, or resting, bud (Orchard *et al*. 1981; Abo-Hamed *et al*. 1983; Borchert 1991).

Our findings indicate that *L. melissifolia* was not able to maintain a functional equilibrium in carbon allocation between the root system and the shoot during pre-reproductive growth. As the growing environment was kept constant, and roots were not pot bound at harvest, it appears that the oscillation in blade size was not a function of resource availability. Rather, this observation may illustrate the inability of the root system to acquire adequate resources to maintain consistent blade growth. Although early development of *L. melissifolia* is characteristic of sympodial shoot growth, shifts in resource allocation occur as the plant grows. These cyclic changes appear similar to recurrent shoot growth without the 'resting' stage in which no apparent leaf or stem height growth occurs. Initial increases in blade size place great demand on the root system to acquire water and nutrients to sustain increased shoot growth. Eventually, the root system is unable to provide enough resources to meet increasing shoot demand, resulting in decreases in leaf blade size until more roots are produced. We hypothesize that with greater root growth and subsequent greater absorption area, *L. melissifolia* roots are able to acquire additional resources to meet shoot demand, resulting in increased blade size and area. Nutrient and water acquisition and distribution, controlled by hormone production, are likely mechanisms controlling leaf blade growth patterns in early *L. melissifolia* development.

Cyclic blade area growth in pre-reproductive *L. melissifolia* presents a difficulty for developing physiological measurement strategies based on morphological features. A common measurement strategy is the plastochron index, which is a numerical index of the developmental age, or morphological chronosequence, of plants where the plastochron is defined as the time interval between the initiation of successive leaf blade primorida on the shoot apex (Askenasy 1880 from Erickson & Michelini 1957).

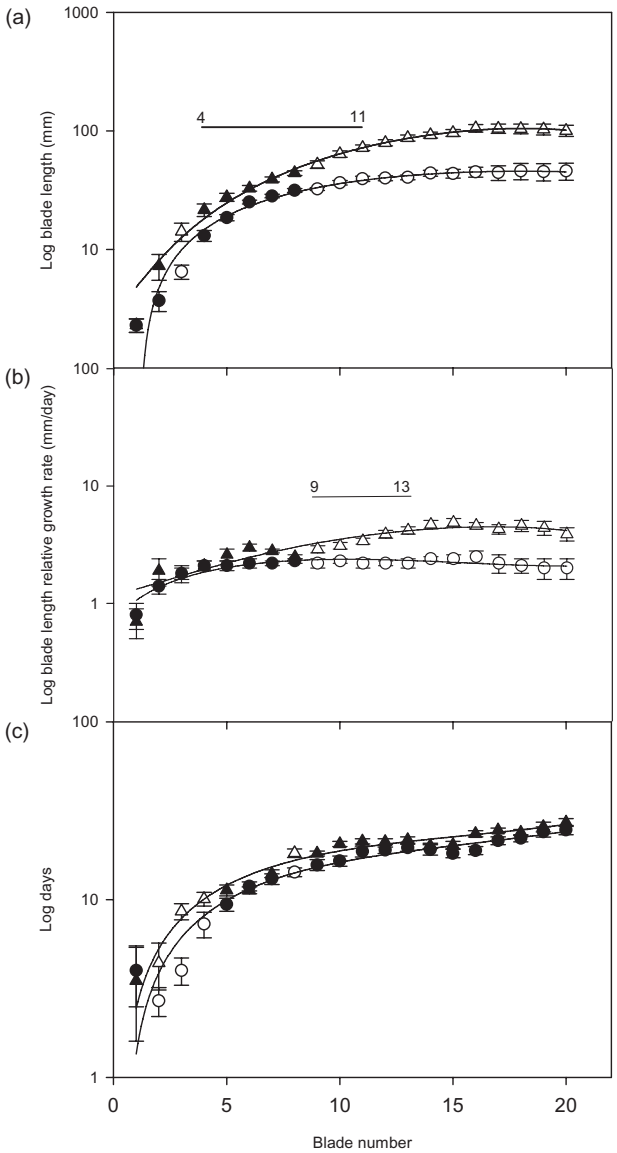


**Fig. 8** Ontongeny comparisons of blade (a) length, (b) relative growth rate and (c) days to maturity for *Lindera melissifolia* seedlings in response to high and low light regimes. Curves represent second-order polynomial regressions. Symbols are ○ (high light) and Δ (low light). Unshaded symbols represent differences between high and low light within blades ($P < 0.05$). Error bars represent one standard error. The horizontal line represents leaf blades where slopes between the regression lines are different ($P < 0.05$).

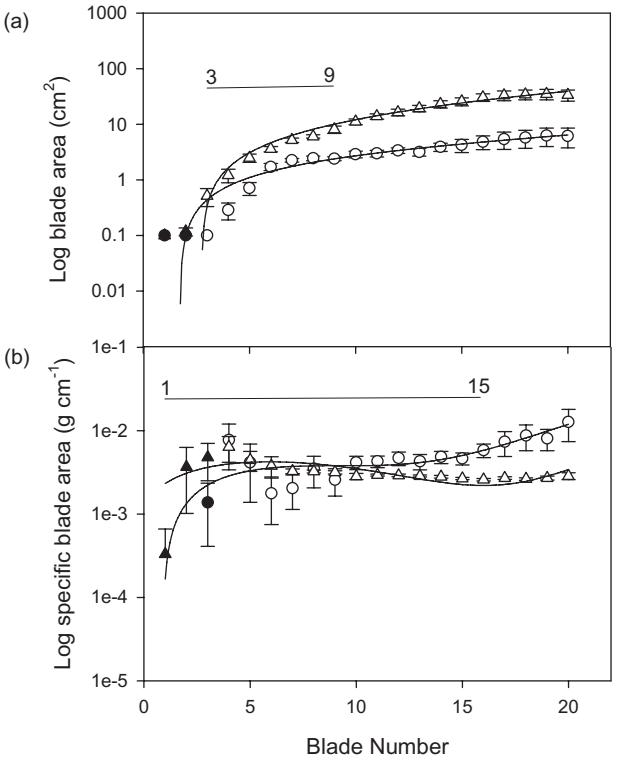


**Fig. 9** Ontongeny comparisons of (a) blade area and (b) specific blade area for *Lindera melissifolia* seedlings in response to high and low light regimes. Curves represent second-order polynomial regressions. Symbols are ○ (high light) and Δ (low light). Unshaded symbols represent differences between high and low light within blades ($P < 0.05$). Error bars represent one standard error. The horizontal line represents leaf blades where slopes between the regression lines are different ($P < 0.05$).

The primary advantage of the plastochron index is to quantify development of a plant for relating morphological measurements to physiological processes (Larson & Isebrands 1971). This uniformity reduces the variability often found in field measurements of physiological processes between treatments. Lamoreaux *et al*. (1978), in their review of the plastochron index, stated that once a reference blade length has been chosen, a plastochron can be conceived as the interval of time required for two successive leaf blades to pass through the reference length. How differences in growth between leaf blades in *L. melissifolia* affect the development of an index of plant growth is currently unknown. The cyclic pattern of blade area growth in *L. melissifolia* warrants further research to determine if this cyclic blade area growth pattern holds true across genotypes from different geographic areas. If true, then the mechanisms behind this cyclic blade growth pattern must be determined to make modifications to a morphological index of *L. melissifolia* growth and development.

### *Why does* L. melissifolia *exhibit differing height and leaf area development patterns?*

Two patterns of plant height and leaf area development were observed in *L. melissifolia* seedlings (Figs 4,5), regardless of light regime. In the first pattern, linear

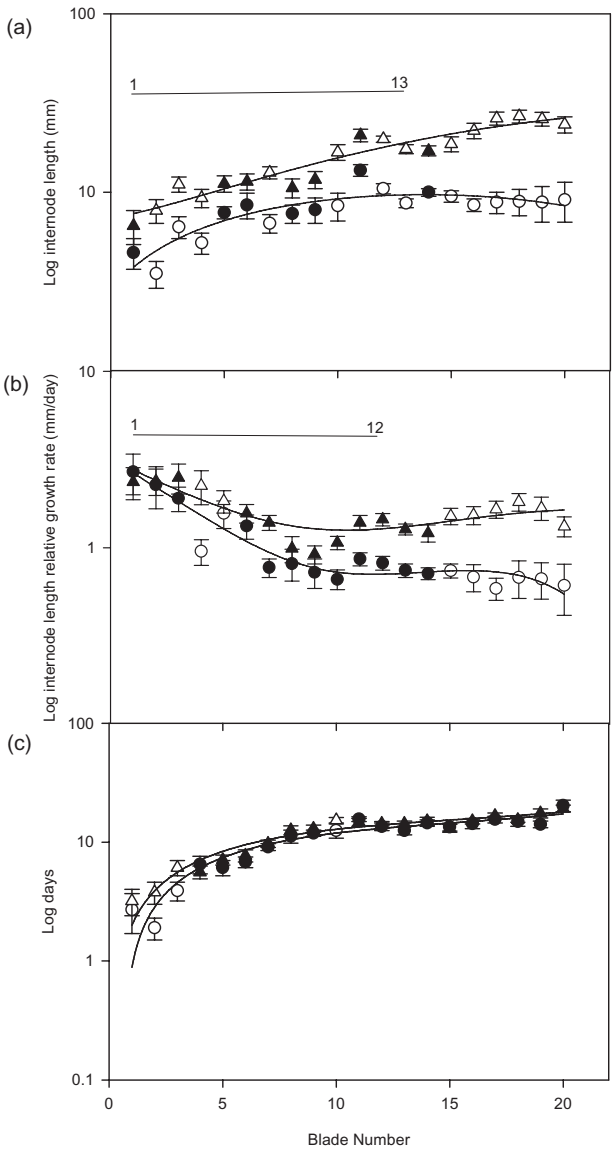


**Fig. 10** Ontongeny comparisons of internode (a) length, (b) relative growth rate and (c) days to maturity for *Lindera melissifolia* in response to high and low light regimes. Curves represent second-order polynomial regressions. Symbols are ○ (high light) and Δ (low light). Unshaded symbols represent differences between high and low light within blades ($P < 0.05$). Error bars represent one standard error. The horizontal line represents leaf blades where slopes between regression lines are different ($P < 0.05$).

height development following seedling emergence coincided with a linear pattern of leaf area development (Fig. 4). This development pattern was observed in six plants receiving high light and six plants receiving low light. In the second pattern, a more rapid linear pattern of height development for shaded plants or a slightly curvilinear pattern for unshaded plants coincided with a strong curvilinear pattern of leaf area development (Fig. 5). This development pattern was observed in five plants receiving high light and nine plants receiving low light.

One explanation for the two contrasting development patterns involves plant gender. In accordance with the cost of reproduction hypothesis (Obeso 2002), male *L. melissifolia* plants may grow more rapidly in height and leaf area because less energy is expended for gender-specific reproductive structures compared with female plants. As *L. melissifolia* plants in this study were in their pre-reproduction stage of development, we assumed gender would not affect early height and leaf area development, but Hawkins *et al*. (2009b) showed male *L. melissifolia* plants had greater allometric measures (height, diameter and leaf area) compared with female plants 21 weeks following transplanting of micropropogated stock. Unfortunately, we were not able to determine the gender of the plants used in the current study.

The effect of gender on growth and development in the widespread congener *Lindera benzoin* (L.) Blume (spicebush) showed dimorphism between male and female plants. Cipollini and Whigham (1994) showed that the shoot, leaves and lateral branches of female plants grew more slowly than male plants as a consequence of the greater reproductive costs (fruit production) borne by female plants. Likewise, Niesenbaum (1992) found sexual dimorphism in branch growth rates with female plants growing slower than male plants, particularly in shaded environments. Conversely, Primack (1985) found sexual isomorphism in plant height, number of stems per clump (ramets) and the diameter of the largest stem within two populations of *L. benzoin* in Massachusetts. Sexual dimorphism is common in other dioecious woody plants, including *Pistacia lentiscus* L. (Barradas & Correia 1999), *Simmondsia chinensis* (Link) Schneider (Kohorn 1995) and *Ilex aquifolium* L. (Obeso *et al*. 1998). Further study is needed to determine if sexual dimorphism is the cause for differing patterns of early height growth and leaf area development in pre-reproductive *L. melissifolia*.

### *Ecophysiological aspects of* L. melissifolia*: is it a plastic species?*

McAlpine and Jesson (2007) state that a species can be plastic if it exhibits differences in morphological or physiological traits under different environmental conditions. Furthermore, most species exhibit some level of plasticity to different growing environments, but a more plastic species is better able to utilize limiting resources in a wider range of environments compared with a less plastic species (Lantham 1992; Valladares *et al*. 2000; McAlpine & Jesson 2007). Therefore, a species with greater plasticity may be more successful competing against less plastic

species across an environmental gradient. *Lindera melissifolia* seedlings in our experiment exhibited high phenotypic variability in response to light availability. Seedlings raised in low light had greater height, internode lengths, blade size and area, and plant leaf area compared with seedlings raised in high light. Aleric and Kirkman (2005) also found greater height, height growth and diameter growth in eastern USA populations of *L. melissifolia* grown in shaded environments compared with plants grown in full sunlight.

*Lindera melissifolia* seedlings raised in high light had a lower accumulation of total biomass with lower accumulations in root, stem and leaf tissues compared with seedlings raised in low light. Aleric and Kirkman (2005) also found lower total plant biomass accumulation in *L. melissifolia* grown in full sunlight compared with plants grown in shaded environments. Lower biomass in *L. melissifolia* growing in high light environments may be the result of plants adjusting to greater levels of stress associated with high light, including increased leaf temperatures and vapor pressure deficits. Aleric and Kirkman (2005) showed reduced light-saturated rates of photosynthesis as plants adjusted to full sunlight. They attributed this response to plasticity in leaf morphology and physiology. Others have found similar patterns of biomass accumulation in shade-tolerant forest shrubs (Denslow *et al*. 1990; Valladares *et al*. 2000). Greater biomass accumulation among root, stem and leaf tissues in *L. melissifolia* in low light in our study indicates that this species has a preference for shaded environments and coincides with observations that most *L. melissifolia* populations are found in shaded environments (Wright 1990; Devall *et al*. 2001; Aleric & Kirkman 2005; Hawkins *et al*. 2009a). Conversely, Aleric and Kirkman (2005) found that the biomass distribution within leaves, stems and roots in *L. melissifolia* did not differ between contrasting light levels. Differences in biomass distribution between our study and Aleric and Kirkman (2005) may result from differences in plant material. We used young seedlings, whereas Aleric and Kirkman (2005) used rooted cuttings from mature plants.

Proportional biomass distribution in roots was greater for *L. melissifolia* seedlings raised in high light compared with seedlings raised in low light (Fig. 7). This carbon distribution pattern in high light reflects a plastic response in *L. melissifolia* seedling carbon distribution that is probably driven by water stress. High light environments are characterized by greater air temperatures and lower relative humidity than shaded environments (Hodges 1967). Air circulation through the growth chamber negated differences in relative humidity between light regimes, but *L. melissifolia* leaf surface temperatures under high light were probably greater than the surface temperatures for leaves in low light. Therefore, leaves in high light experienced greater vapor pressure deficits than leaves in low light (Fitter & Hay 1987). High vapor pressure deficits reduce stomatal opening (Cowan 1994; Monteith 1995; Yong *et al*. 1997; Oren *et al*. 1999, 2001; Addington *et al*. 2004), subsequently reducing photosynthetic rates and carbon fixation. Although the soil in our experiment was maintained at field capacity, *L. melissifolia* grown under high light probably experienced diurnal periods of water stress that triggered the observed carbon distribution response to roots. Support for this argument has been established by others who have examined root/shoot ratios in other species established under various light environments (Kolb *et al*. 1990; Welander & Ottosson 1998). We hypothesize that preferential root growth in high light, based on this carbon distribution pattern, is necessary to maintain a positive net photosynthesis as vapor pressure deficits increase diurnally.

Further evidence of stress in pondberry seedlings receiving high light include observations of sharp angles of blade inclination and leaf blade folding. These architectural features represent paraheliotropic mechanisms for a plant to avoid excessive irradiance (Muraoka *et al*. 1998; Pearcy *et al*. 2005; Chambelland *et al*. 2008). As in our study, Aleric and Kirkman (2005) also observed leaf blade rolling when *L. melissifolia* grew in full sunlight. Larger blade size and lower specific blade area were characteristics of plants raised in the low light regime. These features, along with a horizontal leaf display (B. Lockhart, pers. obs., 2004), are common characteristics of plants growing in low light environments (Boardman 1977; Givnish 1988). These differences in leaf morphology and display represent further phenotypic variability in *L. melissifolia* to contrasting light regimes.

*Lindera melissifolia* exhibited high phenotypic variation across a range of morphological variables under contrasting light regimes using a chronological standard (Table 3). The species also showed plasticity among leaf blades and internodes using mature blades as an ontogenetic standard. However, the presence of plasticity does not necessarily indicate trait variability in response to environmental heterogeneity. Wright and McConnaughay (2002) noted the importance of distinguishing treatment differences, or phenotypic variation, as a result of differences in plant size (ontogenetic plasticity) or to true treatment effects (phenotypic plasticity).

In our study, *L. melissifolia* exhibited three phases of development in early growth and development based on plasticity. First, soon after seedling emergence from the soil, plants showed increases in size of blades and internodes, but with no difference between treatments. This initial phase of development is characterized as ontogenetic drift. The second phase of development involved differences in growth trajectories between treatments (regression slopes). For example, blade length increased in size for plants receiving low light compared with plants

receiving high light from Blade 4 to Blade 11 (Fig. 8a). This phase of development involved differences based on treatment and is considered to be phenotypic plasticity (McConnaughay & Coleman 1999). The third phase of development contained differences in blade and internode traits between treatments, but trait development followed a similar trajectory (i.e. the slopes of these trajectories were similar). This phase is considered to be ontogenetic plasticity. Therefore, although *L. melissifolia* traits in low light were significantly greater than traits in high light, these differences through Blade 20 can be attributed primarily to differences in plant size rather than a true treatment effect.

Treatment effects that can be attributed to differences in ontogeny, or plant size, rather than to true treatment effects may result in erroneous interpretation of experimental results (Evans 1972; Wright & McConnaughay 2002). For example, Coleman and McConnaughay (1995) re-analyzed data reported by Mooney *et al*. (1988). They found that differences in cultivated radish (*Raphanus sativus* cv. Cherry Belle) leaf area production following exposure to $SO_2$ were actually a function of plant size rather than a response to different levels of $SO_2$. Furthermore, Gunn *et al*. (1999) found differences in plant responses to $CO_2$ levels were the result of accelerated development (plant size), and that $CO_2$ alone had little effect on biomass partitioning.

The results from the present study indicate that *L. melissifolia* exhibits changing plasticity in response to light availability. However, most of this plasticity may simply be the result of larger plants growing more rapidly (ontogenetic plasticity) rather than prolonged response to an increase in resource availability (phenotypic plasticity). Limited phenotypic plasticity in *L. melissifolia* may be the result of too short a period of study or the use of an improper ontogenetic standard. Furthermore, Wright and McConnaughay (2002) considered the pattern of ontogenetic drift early in development, followed by a short period of phenotypic plasticity then onset of ontogenetic plasticity as simply part of a plant's ontogenetic program, or an active pattern of adaptive phenotypic plasticity (Callahan *et al*. 1997)

Portsmuth and Niinemets (2007) state that shade-tolerant plant species are generally less plastic than shade-intolerant species among seedlings of tree species. However, the short period of phenotypic plasticity exhibited by *L. melissifolia* may have profound ecological consequences. The ability of plants to rapidly respond to increased resources, even for a brief period of time, and then maintain this gain through ontogenetic plasticity will determine their competitiveness. How other understory plant species associated with floodplains respond to increased resource availability based on types of plasticity, and ultimately compete with *L. melissifolia*, is presently unknown. Phenotypic variation (sensu plasticity) to light availability in other shade-tolerant understory ferns, herbs and shrubs has been documented (Veres & Pickett 1982; Nicola & Pickett 1983; Luken *et al*. 1995; Luken 1997; Muraoka *et al*. 1998; Callaway *et al*. 2003; Runk & Zobel 2007). In essence, plasticity may allow understory plants to function under relatively low light availability, but respond to the short periods of increased irradiance that are commonly found in canopy gaps (Canham *et al*. 1990). Understory plant species with greater plasticity to resources, such as light availability, may be more competitive than plant species with lower plasticity, with consequences for understory plant community composition. Greater emphasis on the types of plasticity, particularly the duration of phenotypic plasticity, among understory plant species is needed to better understand species responses to environmental heterogeneity and its role in community dynamics.

### *Conclusions*

Findings from the present study can be used to inform future ecological experimentation in *L. melissifolia* seedling growth and development. Using a leaf plastochron index of morphological development based on *L. melissifolia* early indeterminate growth, patterns of carbon distribution within the plant and carbon allocation to different chemical fractions within plant tissues can be determined based on changes in the stage of plant and leaf blade development. A basic model of plant growth and development, similar to ECOPHYS (Host *et al*. 1990), can be developed to further test *L. melissifolia* responses to environmental stressors such as increasing $CO_2$ concentrations, temperature, drought or soil saturation.

We found plant plasticity important in *L. melissifolia* seedling growth and development. Experiments are needed to take advantage of the brief period of phenotypic plasticity, in which *L. melissifolia* responds to increased resource availability, before the onset of ontogenetic plasticity. For example, knowledge gained from controlled experiments involving increased light availability or fertilization applied to plants in concert with specific stages of plant development may increase plant growth, and lead to field experiments with natural and planted plants.

*Lindera melissifolia* is a rhizomatous plant. Field observations indicate that asexual reproduction through rhizomes may be the primary form of plant regeneration (Devall *et al*. 2001). The environmental conditions necessary to trigger rhizome production need to be determined. Then, experiments on the comparative development of plants produced by seed to those produced by rhizomes can be conducted so that management practices can be developed to favor one or the other to increase *L. melissifolia* growth and survival in the field. Experiments such as

those listed above will provide necessary information to resource managers in their efforts to conserve this endangered shrub.

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