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Seasonal patterns of light availability and light use of broadleaf evergreens in a deciduous forest understory: Potential mechanisms for expansion

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ABSTRACT

In recent years, expansion of native and exotic evergreen shrubs into forest understories has been documented worldwide. Dense shrub thickets may interfere with tree establishment, suppress herbaceous cover, and contribute substantially to total standing crop of leaf biomass. Expansion may occur because evergreen shrubs exploit seasonal variations in irradiance and temperature that are characteristic of temperate understory environments. We quantified leaf-level light environment and photosynthetic activity of three sympatric broadleaf evergreens (*Ilex opaca*, *Kalmia latifolia*, and *Myrica cerifera*) in a deciduous forest understory in Charles City County, Virginia, USA in order to understand seasonal intra- and interspecific ranges of broadleaf evergreen physiology. Two species (*K. latifolia* and *M. cerifera*) represent a diverse taxonomic range within broadleaf evergreens, and often form expansive thickets. We measured parameters related to canopy structure (e.g., bifurcation ratio, leaf angle) and photosynthetic performance (e.g., electron transport rate or ETR, chlorophyll content), to identify potential mechanisms facilitating expansion. ETR varied both seasonally and among species. In summer, *M. cerifera* ETR was nearly double that of *I. opaca* or *K. latifolia*. Additionally, leaf temperature enhanced photosynthetic capacity of expansive species. Evergreen species, though capable of fixing carbon throughout the year, often exhibit slow growth rates and low physiological activity. Yet, we observed that the range of evergreen physiological activity may be broader than previously recognized. Furthermore, our results indicate potential for changes in composition and expansion of the evergreen shrub layer by species that exhibit structural and physiological mechanisms advantageous for future rises in temperature.

Keywords: Chlorophyll Fluorescence; Evergreenness; Temperate Forest; Photosynthetic Capacity; Seasonal Irradiance

1. INTRODUCTION

Expansion of evergreen shrubs, both native and exotic, (e.g., *Ligustrum robustum*, *Ligustrum sinense*, *Myrica faya*, *Rosa multiflora*) into forest understories worldwide has been documented [1-5]. Dense shrub thickets may interfere with tree establishment, suppress herbaceous cover, and contribute a substantial amount to total standing crop of leaf biomass [6,7]. Over the past several decades, expansion of the evergreen understory layer of deciduous forests has also been documented [8]. Within the Southern Appalachian Mountains of North America, 2.5 million ha are covered by thickets of *Kalmia latifolia* and *Rhodedendron maximum* [6,9]. Recently *Myrica cerifera*, a nitrogen-fixing, evergreen shrub, which occurs in open environments and forest understories, has also received attention for forming dense, monospecific thickets that encroach into nearby communities [10-12]. Species that form dense thickets may provide insight for understanding what suites of characteristics and physiological responses lead to occurrence and expansion within the understory.

Species that display the evergreen leaf habit may remain active throughout the year and invest less annually in carbon and nutrients necessary to maintain foliage [6, 13-15].This leads to longer leaf life spans than deciduous species, but lower rates of photosynthesis [15-18], growth, nutrient loss, and litter decomposition [6,13,14]. Evergreen trees and shrubs are favored in nutrient poor environments because the consequences of lower photosynthetic capacity can be mitigated by assimilating carbon over a longer growing season [19-21]. Typical growth characteristics of evergreens are linked to low responsiveness to environmental change and consequently, climate change may lead to shifts in evergreen distribution [15].

In temperate climates, broadleaved evergreen species in the deciduous forest understory are exposed to large fluctuations in irradiance and temperature throughout the year [22-24]. The light environment in the forest understory is highly dynamic and photosynthetically active radiation (PAR) reaching leaves may increase or decrease over two orders of magnitude within seconds [25-27]. Large changes in PAR occur in the understory due to leaf-out in the spring and autumn canopy leaf fall. Understory leaf temperature exhibits large fluctuations due to seasonality, and intensity and duration of irradiance [22,24]. Some understory evergreens, such as *Ilex opaca*, are dependent on the relatively high irradiance when the overstory is leafless [28,29]. Though previous studies quantified variations in understory light, few studies have examined seasonal physiology of understory woody plants.

The objective of our study was to characterize and compare leaf-level light environment and photosynthetic capacity among broadleaf evergreen species to identify mechanisms related to expansion. We investigated the response of evergreen understory woody species to seasonal differences in light environment and temperature by 1) quantifying seasonal variations in leaf pigments, as related to light availability, 2) identifying structural traits associated with light capture (e.g., branch bifurcation, leaf angle) and that were related to photosynthetic capacity, 3) comparing photosynthetic capacity across seasons, and 4) determining if expansive, thicket-forming species (*K. latifolia* and *M. cerifera*) display physiology reflective of expansive potential in the understory. We hypothesized that species would display traits enabling maximum light capture due to a heterogeneous seasonal light environment and that species considered as expansive in some environments would display higher photosynthetic capacity than the non-expansive *I. opaca* throughout the growing season.

2. MATERIALS AND METHODS

2.1. Site Description

Field work was conducted at the Inger and Walter Rice Center for Environmental Studies, located in Charles City County, VA (37˚19'N, 77˚12'W), from March 2010 to November 2011. Species were sampled within a mature (80 - 150 years old) hardwood forest understory. The deciduous forest canopy was primarily composed of a mixture of *Quercus* spp., and *Acer rubrum*. Physiological measurements were conducted from March 2010 to November 2011 on *Ilex opaca* Aiton (Aquifoliaceae)*, Kalmia latifolia* L. (Ericaceae), and *Myrica cerifera* L. (Myricaceae), broadleaf evergreen species of varying leaf longevity which represent a wide geographic range in eastern North America (**Table 1**).

Ilex opaca, which grows in either the tree or shrub growth form, is frequently observed in forest under stories as a small tree ~10 m tall. *Kalmia latifolia*, which typically occurs on forested slopes, can grow between 3 - 9 m tall and is capable of forming dense thickets. *Myrica cerifera*, also capable of forming dense thickets, fixes nitrogen symbiotically, can reach heights of 5 - 6 m, and typically occurs in the southern United States.

2.2. Measurements

Early spring, late spring, summer and autumn measurements were performed in March, May, July and

Table 1. General life history, physiology, and habitat characteristics of study species: *I. opaca*, *K. latifolia*, and *M. cerifera*. Maximum photosynthetic rates have not been published for *I. opaca* and therefore, values presented are from closely related *Ilex aquifolium*, which is typically observed in European oak and beech forests.

Species	N-Fixer	Leaf life span (yrs)	Maximum photosynthetic rate ($\mu mol \cdot m^{-2} \cdot sec^{-1}$)	Preferred habitat	Distribution
I. opaca	N	~2.4[a]	4 - 5[b] *Ilex aquifolium*	an understory tree, but may grow in full sun: grows best in mesic, well-drained, slightly acidic soils	native to eastern and southeastern United States from coastal Massachusetts south to central Florida, and west to southeastern Missouri and eastern Texas
K. latifolia	N	~3[c]	4 - 8[d]	an understory shrub, but may grow in full sun: grows best in mesic, well-drained, acidic soils	native to eastern United States, occurring from southern Maine south to northern Florida, and west to Indiana and Louisiana
M. cerifera	Y	<1[e]	24 - 28[f]	frequently occurs in open areas, but also along forest edges and in understories: grows best in mesic soils	native to eastern and southeastern United States from coastal New Jersey south to central Florida, and west to Texas

[a][30], [b][31], [c][32], [d][33], [e,f][34].

September, respectively. For each species, leaf angle to the horizontal $(\theta, n = 100)$ was quantified to the nearest 5° using a clinometer. Leaf angle was measured in early spring and again in summer to track seasonal changes. Plant bifurcation ratios were measured for ten stems exhibiting terminal shoots exposed to partial to full sunlight. The following equation was used to approximate branch bifurcation ratio (R_b):

$$R_b = \frac{N-1}{N-N_1} \tag{2}$$

where N is the total number of branches of all branch orders on the stem and N_1 is the total number of first order branches [35]. Additionally, the number of leaves per primary branch was quantified and leaf area $(n = 25)$ was measured using an area meter (LI-3100C). In order to assess leaf pigments, chlorophyll concentrations were quantified using standard spectrophotometric methods following acetone extraction [36].

Chlorophyll fluorescence analysis, including quantification of electron transport rate (ETR), is an increasingly popular alternative to gas exchange measurements for determination of photosynthetic performance of plants [37] and making comparisons among species [38]. Changes in fluorescence emissions correlate with the light use efficiency of O_2 evolution [39,40] and CO_2 assimilation [41-43]. Further, maximum apparent ETR represents a measure for the capacity of photosynthetic activity [44,45]. We utilized chlorophyll fluorescence analysis in order to relate physiology to photosynthetic capacity [45,46].

Measurements were collected on sunny days between 1000 h and 1400 h. To estimate photosynthetic capacity, light-adapted leaf photosynthetic electron transport rate (ETR) was measured on fully expanded leaves $(n = 50)$ using a pulse amplitude modulated leaf fluorometer (PAM-2000, Walz, Effeltrich, Germany). Leaves were selected from partially sunlit plants to capture natural variation in incident light and to ensure that some samples were acclimated to full sun. Concomitant incident photosynthetically active radiation (PAR) and leaf temperature were determined using the mini-PAM leaf clip (2030-B) quantum sensor and thermocouple. Apparent ETR was calculated as:

$$ETR = \Delta F / F'_m \times PAR \times 0.84 \times 0.5 \tag{1}$$

where $\Delta F / F'_m$ represents quantum yield, 0.84 is the assumed light absorbance of the sample, and 0.5 corrects for 2 quanta of light required for the transport of 1 electron [41,44].

2.3. Data Analysis

The program R was used for all statistical tests. One-way analysis of variance (ANOVA) was conducted to compare leaf angles, leaf PAR, parameters relating to leaf pigments and ETR among species. Post-hoc testing was performed using Tukey's honestly significant difference (HSD). Linear regression analysis was used to determine relationships between PAR and leaf temperature for each species and significance of slopes was determined. The slopes of significant relationships were compared using a Student's t-test. Linear regression was also used to quantify relationships between PAR and ETR for determination of photosynthetic efficiency (PE) among species [47]. This was achieved by utilizing the linear portion of the relationship between PAR and ETR, which corresponded to PAR values ≤600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$ and resulting ETR. Steeper slope in the linear portion of the relationship indicates higher PE (*i.e.*, higher capacity for light processing). Slopes were compared to each other using analysis of covariance methods (ANCOVA) and when appropriate, pairwise contrasts were performed.

3. RESULTS

3.1. Canopy Architecture and Leaf Anatomy

Frequency distributions of θ among species in early spring were all different from one another (**Figure 1**; $F_{2,297} = 37.87, P < 0.001$). Within species, frequency distributions of θ were similar between early spring and summer for *I. opaca* and *M. cerifera* ($P = 0.41$, 0.18, respectively) and differed for *K. latifolia* $(P < 0.001)$. In early spring for both *I. opaca* and *M. cerifera*, the largest proportion of leaves was angled horizontally between 0° - 15°. The largest proportion of leaves of *K. latifolia* displayed a 30° - 45° leaf angle. Frequency distributions θ in summer varied among species ($F_{2,297}$ = 10.26, P < 0.001; **Figure 1**). Leaf angle distribution of *I. opaca* differed from both *K. latifolia* and *M. cerifera* (P = 0.016 < 0.001, respectively). In summer the largest proportion of leaves for all species occurred between 0 - 15°, though a similarly large proportion of leaves of *M. cerifera* occurred between 15° - 30° (**Figure 1**).

Bifurcation ratios (R_b) were also significantly different among species ($F_{2,27} = 4.31, P = 0.02$; **Table 2**). Mean R_b of *M. cerifera* was approximately one and a half times the value of *I. opaca* or *K. latifolia*, but values were not significantly different between *I. opaca* and *M. cerifera*. Leaf area differed among species ($F_{2,72}$ = 63.0, P < 0.001) such that leaves of *M. cerifera* were smaller than leaves of *I. opaca* or *K. latifolia* (**Table 2**). *Myrica cerifera* also produced nearly twice as many leaves per primary branch as *I. opaca* or *K. latifolia* ($F_{2,12}$ = 16.31, P < 0.001). Yet, when expressed as leaf area per primary branch, *I. opaca* produced the most $(151 \pm 0.4\ \text{cm}^2)$, followed by *K. latifolia* $(121 \pm 0.4\ \text{cm}^2)$, and *M. cerifera* $(100 \pm 0.8\ \text{cm}^2)$.

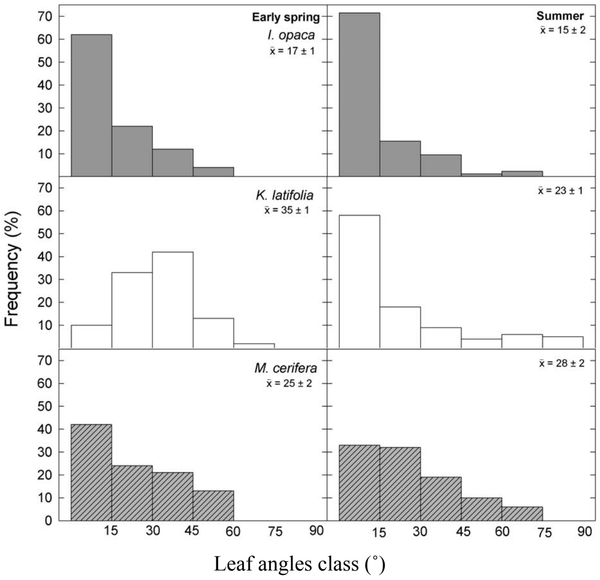

Figure 1. Frequency distributions of leaf angles relative to horizontal of *I. opaca*, *K. latifolia*, and *M. cerifera* during early spring and summer. Distributions are divided into 15° classes. Mean leaf angle ±1 standard errors (SE) are presented for each species during a given season.

Table 2. Anatomical and structural characteristics of *I. opaca*, *K. latifolia*, and *M. cerifera.* Significant differences ($P < 0.05$) among species for a given characteristic are represented by letters.Mean values are presented with ± one standard error (SE).

Characteristic	N	*I. opaca*	*K. latifolia*	*M. cerifera*
Leaf area (cm^2)	25	16.7 ± 0.7^a	15.7 ± 0.9^a	6.5 ± 0.5^b
No. of leaves per 1° branch	15	9.1 ± 0.6^a	7.7 ± 0.4^a	15.3 ± 1.6^b
Bifurcation ratio	10	2.6 ± 0.2^{ab}	2.4 ± 0.1^a	4.0 ± 0.7^b

Chlorophyll *a*:*b* ratios varied among species during early spring $\left(F_{2,27} = 12.8, P < 0.001\right)$ and summer ($F_{2,27} = 12.2$, $P < 0.001$; **Figure 2**). *Ilex opaca* had the largest chlorophyll *a*:*b* ratio, while there were no significant differences between *K. latifolia* and *M. cerifera*. All species showed significantly reduced chlorophyll *a*:*b* ratios in late spring and autumn compared to early spring and summer (**Figure 2**). Carotenoid content was highest in all species during early spring and summer (**Figure 2**). Carotenoid content of *I. opaca* was significantly lower than *K. latifolia* and *M. cerifera* during all seasons, and was similar between *K. latifolia* and *M. cerifera.*

3.2. Light Environment

Significant variation in incident leaf PAR on frequency distributions among species occurred in both early spring $\left(F_{2,148} = 3.67, P = 0.028\right)$ and summer

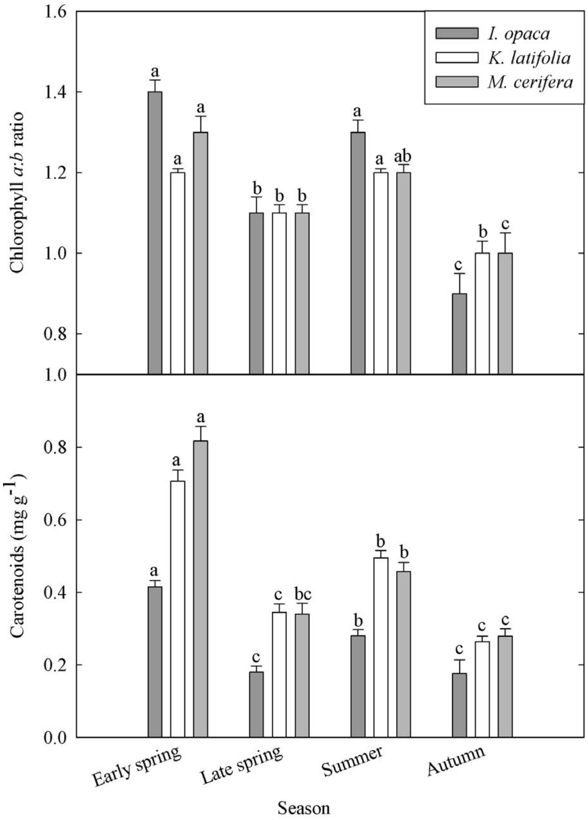

Figure 2. Seasonal chlorophyll *a*:*b* ratio and carotenoid content ($mg \cdot g^{-1}$) of *I. opaca*, *K. latifolia*, and *M. cerifera*. Intraspecific seasonal significant differences ($P < 0.05$) are represented by letters. Error bars represent ± 1 SE of the mean.

($F_{2,147} = 7.01, P < 0.001$; **Figure 3**). In early spring the distribution of leaf PAR was significantly lower in *M. cerifera* compared to *K. latifolia*, yet the leaf PAR of *I. opaca* did not differ from the other two species. In summer leaf PAR of *I. opaca* was significantly lower than *K. latifolia*, yet the leaf PAR of each was similar to *M. cerifera*. Throughout the growing season, leaves of each species were exposed to PAR ranging from <25 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ to ≥1600 $\mu mol \cdot m^{-2} \cdot sec^{-1}$.

3.3. Electron Transport Rate

ETR varied both among species and seasonally (**Figure 4**). Throughout the growing season, both ETR and PE were similar for *I. opaca* and *K. latifolia*; yet, during all seasons except early spring, PE of *M. cerifera* was significantly higher than *I. opaca* and *K. latifolia* (**Table 3**). During most of the growing season,the slope of PAR and ETR for *M. cerifera* was steeper than that of *I. opaca* or *K. latifolia* (**Table 3**). To further investigate this relationship, light levels were classified as "low," "intermediate," and "high" intensities. "Low" intensity was considered light values ranging from 0 - 400 $\mu mol \cdot m^{-2} \cdot sec^{-1}$, "intermediate" intensity was values ranging from 600 –

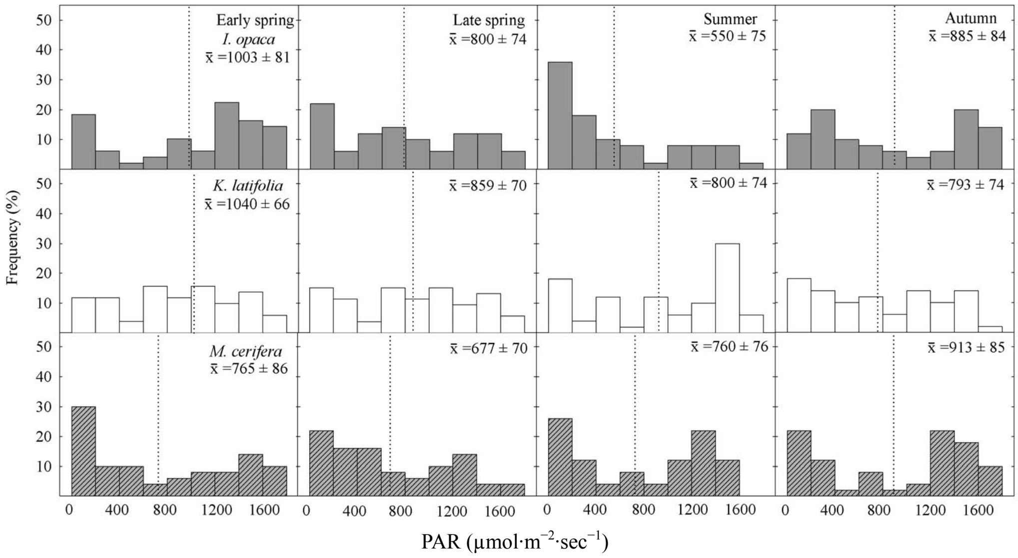

Figure 3. Seasonal frequency distributions of leaf photosynthetically active radiation (PAR μmol·m^{-2}·sec^{-1}), obtained from the mini-PAM quantum sensor, for *I. opaca*, *K. latifolia*, and *M. cerifera*. Mean PAR ± 1 SE are presented for each species during a given season and mean values are represented by dotted lines.

Table 3. Slopes of seasonal relationships between incident photosynthetically active radiation (PAR) ≤ 600 μmol·m^{-2}·sec^{-1} and electron transport rate (ETR) for *I. opaca* (I), *K. latifolia* (K), and *M. cerifera* (M). Higher slopes correspond to greater photosynthetic efficiency (PE). ANCOVA model significance and *P*-values for pairwise contrasts are presented. ES = early spring; LS = late spring; S = summer; and A = autumn.

	Slope			Model Significance		Contrasts		
Season	I	K	M	*F*	*P*	I vs K	I vs M	K vs M
ES	0.07	0.06	0.09	36.44	<0.0001	0.21	0.52	0.40
LS	0.11	0.12	0.21	97.27	<0.0001	0.93	<0.0001	0.0002
S	0.08	0.10	0.19	30.23	<0.0001	0.66	0.0002	0.005
A	0.06	0.09	0.23	33.76	<0.0001	0.68	<0.0001	<0.0001

1000 μmol·m^{-2}·sec^{-1} and "high" intensity was values ranging from 1200 - 1600 μmol·m^{-2}·sec^{-1}. The only difference between ETR of *I. opaca* and *K. latifolia* was observed at high light during autumn $(P < 0.001)$. From late spring to autumn, ETR of *M. cerifera* was greater than *I. opaca* and *K. latifolia* at all light levels ($P < 0.05$ in low light, and $P < 0.005$ in intermediate and high light during this time period), with the largest differences occurring at intermediate and high light levels. Among species, highest observed ETR occurred for *M. cerifera* during summer.

At all light levels, ETR of *M. cerifera* increased with increasing leaf temperature (e.g., $r^2 = 0.85$ at low light, $r^2 = 0.89$ at intermediate light and $r^2 = 0.99$ in high light, $P < 0.01$ in all cases; **Figure 5**). At high light, ETR of *K. latifolia* also increased linearly with increasing leaf temperature ($r^2 = 0.69$; **Figure 5**). At low and intermediate light levels, no significant relationship existed between leaf temperature and ETR for *K. latifolia* ($r^2 = 0.27, P = 0.29$ and $r^2 = 0.34, P = 0.23$, respectively, data not shown). No relationship existed between temperature and ETR for *I. opaca* at all light levels ($r^2 \le 0.5$ and $P > 0.1$ in all cases, data not shown). Additionally, the slope of the relationship between temperature and ETR was substantially steeper for *M. cerifera* than *I. opaca* or *K. latifolia* ($P < 0.001$; **Figure 5**).

4. DISCUSSION

Evergreen species are often noted for the ability to fix carbon throughout the year at the expense of low physiological activity and slow growth rates [9,13,16,19]. Yet, the range of physiological activity that broad-leaved evergreens display may be more extensive than previously recognized. We quantified a wide range of responses in parameters related to light capture and photosynthetic potential in understory evergreens in a deciduous forest demonstrating that there are several strategies for sur- vival in the understory among broadleaf ever greens.

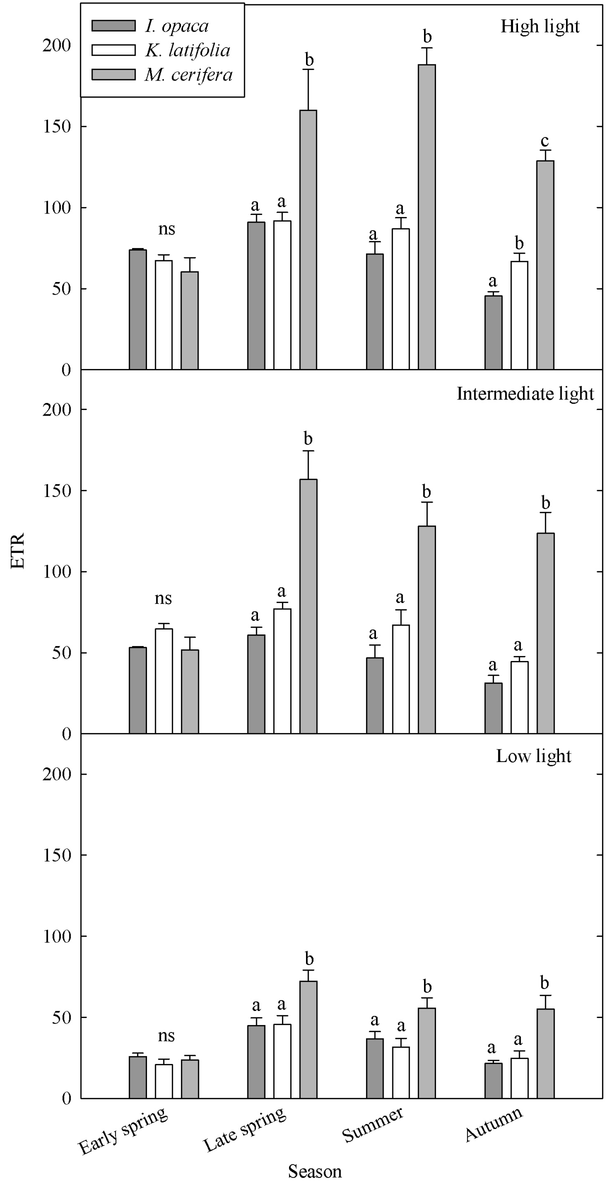

Figure 4. Seasonal ETR of three evergreen understory shrub species at low (0 - 400 μmol·m^{-2}·sec^{-1}), intermediate (600 - 1000 μmol·m^{-2}·sec^{-1}), and high (1200 - 1600 μmol·m^{-2}·sec^{-1}) light intensities. Significant differences ($P < 0.05$) among species during a given month are represented by letters. A notation of ns indicates no significant differences were observed. Error bars represent ±1 SE of the mean.

Plant traits associated with light capture were not necessarily related to photosynthetic capacity, but all species showed traits aimed at maximizing light capture rather than light avoidance. Seasonal variations in leaf chlorophyll *a*:*b* ratios and accessory pigments mirrored changes in light availability. Photosynthetic performance varied substantially throughout the growing season both intra- and interspecifically. In addition, physiology of thicket-forming species (*K. latifolia* and *M. cerifera*) reflected potential for expansion in the understory.

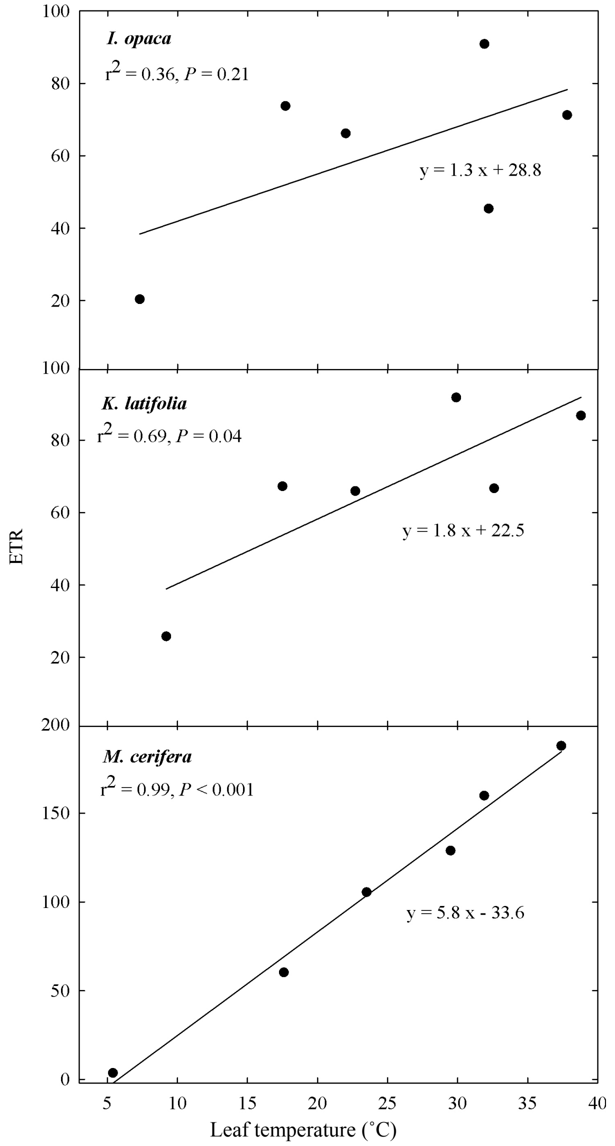

Figure 5. Relationship between leaf temperature (˚C), obtained from the mini-PAM during seasonal leaf chlorophyll fluorescence readings, and ETR at "high" light (*i.e.*, PAR ranging from 1200 - 1600 μmol·m^{-2}·sec^{-1}) for *I. opaca*, *K. latifolia*, and *M. cerifera*.

Maximizing light capture through horizontal leaf orientation [48] was evident in all three species. Self-shading may be prevented at the leaf level by effective foliage orientation, a mechanism which enhances daily whole-canopy radiation capture through optimal light intercepttion earlier and later in the day when radiation is reduced [49-51]. The difference in leaf angle of *K. latifolia* between early spring and summer corresponded to closure of the overstory canopy; more leaves were oriented horizontally. In summer, the majority of leaves were closer to the horizontal plane, suggesting maximimum light capture for all three species. Instantaneous measurements of incident PAR showed that during any given season, leaves of each species were exposed to PAR values ranging from full shade (< 25 μmol·m^{-2}·sec^{-1}) to nearly full sun (≥1600 μmol·m^{-2}·sec^{-1}), which also emphasizes the importance of foliage orientation for optimal light interception.

Canopy structure is a key factor influencing light interception by a forest stand with a given leaf area index (LAI) [52] and may influence branching strategies that optimize light capture [53-55]. Trees and shrubs growing in low light tend to exhibit lower bifurcation ratios and are associated with a nonrandom monolayer canopy, as compared to those exposed to high light and within a multilayered canopy [53,56]. Bifurcation ratio was highest for *M. cerifera*, yet similar to other values reported for forest understories [29,35,54]. *Myrica cerifera* also produced the most and smallest leaves per primary branch, minimizing self-shading [35]. Coupling these traits with consistent seasonal horizontal leaf angles suggests that *M. cerifera* displays architecture that promotes maximum light capture more so than *I. opaca* or *K. latifolia*.

Changes in carotenoids, which contribute to either light collection or photoprotection [57], tracked seasonal light availability. All species showed increased carotenoids when seasonal light availability was highest in early spring. Chlorophyll *a*:*b* ratio, which increases with available light [58-60], also mirrored patterns in seasonal light availability. Interestingly, instantaneous leaf-level light did not vary at midday throughout the seasons for *K. latifolia* and *M. cerifera*, though it was lower in late spring and summer than early spring or autumn for *I. opaca*. Leaf pigments such as carotenoids and chlorophyll *a*:*b* ratios provide metrics for understanding light availability that integrates seasonal variation.

Photosynthetic capacity varied substantially throughout the seasons for all species. ETRs observed during our study were proportional to previously reported photosynthetic rates for these, or closely related, species (**Table 1**). Most striking were the increases in ETR from late spring to autumn for *M. cerifera*. In cooler months, ETR of *M. cerifera* was the same as *I. opaca* and *K. latifolia*, but from late spring to early fall, ETR of *M. cerifera* was more than double that of the sympatric evergreen species. An increase in leaf temperature enabled *M. cerifera* to exploit higher light levels during summer. Young (1992) observed that field-measured net CO_2 assimilation rate of *M. cerifera* peaked around 30˚C, and at 40˚C, net photosynthesis remained within 50% of maximum values [34]. These results suggest that temperature is a key driver of seasonal changes in photosynthetic performance of *M. cerifera*. Muller *et al.* (2005) also found that within a given light regime (*i.e.*, that of deciduous forest understory, evergreen forest understory, or a gap in a mixed forest), the photosynthetic apparatus of *Aucuba japonica*, a temperate understory evergreen shrub, acclimated mainly to changes in temperature and to a lesser extent PAR [22].

There was wide interspecific variation in chlorophyll fluorescence emissions in response to variation in PAR. Steeper slopes in the relationship between PAR and ETR are indicative of greater PE for *M. cerifera* than for *I. opaca* or *K. latifolia*. Also, *M. cerifera* displayed similar ETR at intermediate (600 - 1000 μmol·m^{-2}·sec^{-1}) and high (1200 - 1600 μmol·m^{-2}·sec^{-1}) light levels throughout the growing season. Net CO_2 assimilation rate of *M. cerifera* approached an asymptote at intermediate light levels, also suggesting that photosynthetic performance of *M. cerifera* saturates at intermediate light intensity [34]. The aforementioned results indicate a potential interaction between temperature and PAR. *Myrica cerifera* may be equally efficient at assimilating carbon at intermediate light intensity as at high light intensity; however, high light (~1600 μmol·m^{-2}·sec^{-1}) and high temperature (~30˚C - 38˚C) may facilitate increased assimilation rate.

5. CONCLUSION

We found that expansive species were more responsive to changes in seasonal leaf temperature. *Myrica cerifera* and *K. latifolia*, which are both thicket-forming and show enhanced physiological response to rising temperature, are expected to show future range expansion in response to warming climates. Conversely, *I. opaca*, which is slow-growing and shows a low responsiveness to changes in temperature, is likely to occur in a diminished range in the future [15]. While species physiologically similar to *I. opaca* represent the vast majority of evergreens, studies of expanding species, including some that form thickets, have shown a dramatic increase in range on the order of decades [8,12]. Over the past century, southern Switzerland forest understories have shifted from an indigenous deciduous to an exotic broadleaved shrub layer that appears to benefit from milder winter conditions [61]. From a physiological perspective, our study provides support for the hypothesis that climate change may lead to adjustments in the distribution of evergreens.

The three broadleaf evergreen species of our study demonstrate a wide range of physiological activity. *Ilex opaca* characteristics and physiology exemplify a typical evergreen species [15]. It has relatively long-lived leaves, slow growth rates [62], and low ETR. *Kalmia latifolia* also exhibits characteristics and physiology representtative of a typical evergreen species; however, it also displays some traits and physiology that reflect expansive potential. *Kalmia latifolia*, which has equally long-lived leaves as *I. opaca*, similar seasonal PE and ETR, adjusted leaf angle seasonally, and responded to changes in ambient temperature, while *I. opaca* did not. In contrast to *I. opaca*, *M. cerifera* departed from characteristics generally associated with evergreenness and is physiologically adapted for a high light environment [34]. It has high growth rates [34,63], PE, ETR across light levels, and leaf nitrogen content [64]. Thus, multiple strategies related to photosynthetic performance and carbon assimilation occur among sympatric evergreen species in deciduous forest understories. Furthermore, our results indicate potential for changes in composition and expansion of the evergreen shrub layer by species that exhibit structural and physiological mechanisms advantageous for future rises in temperature.

6. ACKNOWLEDGEMENTS

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