Comparative Ecophysiology of Four Invasive Temperate Lianas: Responses to Light Quality and Quantity

Thesis

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By

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Abstract

Invasive temperate lianas are ecologically impactful and increasing in abundance in North America, but information regarding their ecophysiology is relatively scarce. I selected four introduced species representing potentially contrasting shade strategies, "light-demanding" *Ampelopsis brevipedunculata* and *Celastrus orbiculatus* and shade-tolerant *Hedera helix* and *Euonymus fortunei,* to compare their responses to either neutral shade or shade with a reduced R:FR ratio of 0.88 from the ambient 1.3, with the reduced-R:FR shade intended to more closely resemble canopy shade and induce a phytochrome-mediated shade avoidance response. I tested whether responses differed by species and by light quality, measuring five morphological and physical traits in all species and four photosynthetic traits in the shade-tolerant species. Mortality in shaded *Ampelopsis* was high along with *Celastrus* in all conditions, while no mortality was observed in shade-tolerant species. Differential responses to light quality were detected in three morphological traits and one photosynthetic parameter. Relative to neutral shade, leaf mass as a proportion of total aboveground biomass increased in *Ampelopsis* and *Celastrus* in reduced R:FR shade while increasing in both treatments for shade-adapted species. Internode length was only greater in R:FR-reduced shade than neutral shade for *Celastrus,* with no difference in elongation detected between shade treatments in any other species. These changes in allocation patterns and gross morphology were limited to the light-demanding species. While internode length was greatest for all species in control conditions, a subsequent analysis of biomass-adjusted internode length indicated that internodes were longest in the shade treatments, and nonsignificantly longer in R:FR-reduced shade relative to neutral shade. *Hedera* biomass was greater in R:FR-reduced shade, increasing nonsignificantly in all other species. Quantum yield (ϕ) was greatest in *Hedera* but unaffected by treatment, while A_{max} and light compensation points were highest in control conditions but did not vary by species or spectral quality. Though there was no accompanying drop in ϕ, A_{max}, or light compensation point, the convexity (θ) of the light response curves was responsive to spectral quality, decreasing in reduced R:FR shade in addition to being lower overall in *Hedera*. Responses in other traits varied only by species or irradiance and not by spectral quality, largely reflecting light-demanding and shade-tolerant divisions. Specific leaf area was highest in the light-demanding species and increased most in the shade in *Ampelopsis*, but the second-highest increase in the shade occurred in shade-tolerant *Hedera*. Estimated chlorophyll content was lower in both *Ampelopsis* and *Celastrus* than in either shade-tolerant species and was not affected by treatment. The four liana species appear to demonstrate different strategies for coping with shade, and their behavior may align with broad shade-tolerant and shade-avoidant archetypes. The apparent interspecific differences in responses to spectral quality suggest that spectrally neutral shading, such as shade cast by standard black shade cloth, may be insufficient for ecologically relevant studies of the response of lianas to their light environment. Additional research is needed to further quantify the extent to which these differences might dictate the suitability of uninvaded forests for a given liana species or determine its invasive potential.

Dedication

To my partner, Kylen Franz, and my parents Daniel and Laura Finley, for their patience and unwavering support throughout my circuitous academic journey. To my grandmother, Bridget Moran, and my grandfather, Thomas Moran, for always making sure I knew that they were proud.

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Introduction

Woody vines, known collectively as lianas, are ecologically impactful across their native ranges and abroad thanks in part to their relatively rapid growth and phenotypic plasticity (Wyka et al. 2013). They are often aggressive colonizers of light gaps with substantial influence over both canopy and understory architecture (Schnitzer and Carson 2010; Toledo-Aceves 2015), capable of dramatic morphological and physiological responses to light cues that aid in canopy navigation. Many of these adjustments, such as leaf shape, leaf area, and internode length, are governed by the shade avoidance response (SAR). Though its strength varies considerably across all plant taxa, the SAR is a cluster of phytochrome-mediated responses to spectral shifts caused by competitor shade. It serves to modify light capture and, in lianas, modify climbing mechanisms or mounding habits. Research has been focused primarily on liana-rich tropical latitudes, within which liana frequency is increasing (Schnitzer and Bongers 2011). Globally the overall success of lianas has been attributed to various ecophysiological mechanisms including high photosynthetic rates and growth rates (Schnitzer 2005; Wyka et al. 2013; Santiago et al. 2015; Rounsaville et al. 2018). They are fierce competitors in light-limited forest ecosystems, and their response to their light environment underpins every aspect of their ecology. At temperate North American latitudes there are comparatively few native liana species, yet liana frequency may be increasing in these communities as well (Ladwig and Meiners 2010b). The relatively small number of native taxa in North American systems may facilitate successful invasions by non-native liana species (Leicht-Young and Pavlovic 2014). Though they often escape the gaze of liana biologists, invaded and species-poor temperate ecosystems lend themselves well to ecophysiological research at the species level.

Both native and invasive liana species tend to excel at colonizing forest edges and sites of disturbance (Campbell et al. 2018), but subsequent strategies for contending with their light environments span a spectrum between shade tolerance and shade avoidance (Valladares et al. 2011). The relatively high light availability at forest edges tends to support rapid liana growth upon their arrival. Following establishment, light availability continues to direct the rate and extent of liana growth as vines generally seek areas of high light and languish in deep canopy shade. In the absence of disturbance the growth and spread of native temperate lianas is therefore typically limited by the surrounding vegetation (Pavlovic and Leicht-Young 2011). This principle stands at odds with the rampant growth observed among invasive liana taxa in North America (Leicht-Young and Pavlovic 2014). In light-limited forest environments the invasive potential of a liana species may correspond to its ability to tolerate or escape canopy shade (Gianoli et al. 2012).

Shade responses across plant taxa vary along a spectrum from shade tolerance to shade avoidance (Gommers et al. 2013), with the responses in the former group typically resulting in increased light-harvesting efficiency in the shade while morphological responses in the latter “light-demanding” group typically reflect an effort to escape low-light environments. Shade-tolerating and shade-avoiding behaviors are directed by plant detection of both light quantity and light quality, or the overall amount of light paired with the relative abundance of all relevant wavelengths. Light filtered through canopy leaves is far-red enriched, and this reduced red:far-red (R:FR) ratio promotes the phytochrome-mediated shade avoidance response (SAR). The SAR drives a suite of photomorphogenic effects including reduced leaf area, reduced branching, increased petiole length, and internode elongation (Pierik and de Wit 2014).

Given the heterogeneity of light conditions in the forest edges, understories, and canopies they inhabit, identifying diversity in the strength or nature of liana responses to shade is critical to our understanding of liana ecology. Shade-avoidant morphological shifts such as internode elongation may correspond to an increased capacity for horizontal and vertical movement, and for twining or tendril-forming climbers could expand the pool of suitable hosts (Ladwig and Meiners 2010a). Interspecific differences in light-induced morphological and physiological changes have the potential to dramatically alter a given species' trajectory at all scales. For example *Celastrus orbiculatus*, an invasive liana in North America that has largely displaced its native congener *C. scandens*, increases its height and aboveground biomass when grown in shade with reduced R:FR while *C. scandens* increases its leaf area and stem diameter (Leicht and Silander 2006). The changes observed in the invasive *Celastrus orbiculatus* suggested a higher capacity for light-foraging, and notably these traits did not differ when the two species were subjected to 'neutral' shade with unaltered spectral ratios, underscoring the potential ecological importance of interspecific variation in lianas' responses to canopy shade and the selection of an appropriate shade-simulating methodology. Both light quantity and quality must therefore be considered in experimental designs to ensure applicability to natural environments.

Light is already regarded as an important regulator of liana community structure and a salient environmental quality driving their establishment and spread in North America (Robertson et al. 1994). For some temperate taxa such as North American native *Vitis sp.* and *Toxicodendron radicans* growth is concentrated around or even limited to forest edges (Londré and Schnitzer 2006); *T. radicans* has been found to be capable of germinating in dark forest interiors, but it struggles to escape herbivory when its growth stalls at low irradiances (Dickinson et al. 2021). Other species, by contrast and including many invaders of temperate ecosystems, have proven capable of penetrating mature forest canopies and surviving their deep shade (*e.g.* Pavlovic and Leicht-Young 2011). Some species introduced to North America such as English ivy (*Hedera helix*) are highly tolerant of shade overall and thrive in the understory. *Hedera helix* was found to be driving an overall increase in liana frequency under closed canopies even in its native European range (Perring et al. 2020). Other species such as Oriental bittersweet (*Celastrus orbiculatus*) are proficient colonizers of disturbed sites that have mastered a “sit and wait” strategy that allows the propagation and persistence of small individuals in low light; rapid growth occurs when light availability increases (Greenberg et al. 2001). In all cases the plasticity of the lianescent growth form contributes to successful navigation of heterogeneous light conditions. Interspecific diversity in navigation strategies suggests important diversity in the nature and magnitude of plastic responses to light environments (Lee 1988). An improved understanding of this diversity will aid our understanding of the differing invasive potentials of temperate liana taxa as well as our understanding of temperate liana community structures moving forward.

I aimed to improve our understanding of invasive temperate lianas through comparative study and address the need for information regarding their ecophysiology and basic biology. I was primarily interested in their responses to reduced irradiance relative to a brighter forest edge-like light environment, as this could help to identify and explain patterns in their spread and establishment. Furthermore, I sought to identify interspecific differences in shade-avoidance behaviors that could be adaptive in the understory or, in future research, could be tested for their association with invasiveness in lianas. Their responses would be measured in both neutral and spectrally altered shade to compare their growth in response to a reduced R:FR ratio. Differential responses to the two shade treatments, if they occurred, would suggest that simulation of canopy shade will be important in future research of lianas' response to their light environments. I selected four non-native lianas that are currently spreading across temperate North American forests, chosen on the basis of the habitats that they are known or thought to occupy, with the aim of contrasting two groups with different strategies for coping with shade. Each group included a species that could be more confidently ascribed to a strategic archetype along with a purportedly similar species lacking empirical tests of its response to shade. These tests for differential shade-coping strategies among temperate lianas would therefore have the potential to simultaneously contextualize the responses of lesser-studied species. I selected English ivy (*Hedera helix*), a highly shade-tolerant species that frequently forms clonal mounds in the understory (Metcalfe 2005, Strelau et al. 2018), as the primary representative of the shade-tolerant pair. Wintercreeper (*Euonymus fortunei*) is a species with similar mound-forming habits that appears to have an affinity for deep shade, but claims regarding its shade tolerance are sparse and contradictory (Zouhar 2009); *Euonymus* has been reported to be capable of growth in full sun as well as deep shade (Bray et al. 2017). These species were selected to represent taxa in which strong shade avoidance responses are less likely to be observed. I selected Oriental bittersweet (*Celastrus orbiculatus*) as the primary representative of the light-demanding species pair. Although it has proven capable of survival and persistence in the dark understory, it is highly responsive to increases in light availability and exhibits rapid growth after escaping from shade (Leicht and Silander 2006; Ladwig et al.

2012; Gudžinskas et al. 2020). Survey efforts in its introduced range have previously suggested that porcelain berry (*Ampelopsis brevipedunculata*) is shade-intolerant (Yost et al. 1991), with an affinity for fields and riparian areas (Robertson et al. 1994), but the nature of its relationship with its light environment has yet to be described through empirical study; in both *Celastrus* and *Ampelopsis* I anticipated stronger shade-avoidance responses. In this manuscript all references to these species take the form of their generic name, but observations in this study are not intended to be applied to congeners. All four of the selected species have escaped cultivation in the US and are present in Ohio forests. Individuals collected from two Ohio regions were collected and cloned to grow in either a control light environment, neutral shade, or reduced R:FR shade in order to distinguish general low-light responses from those produced by shifts in spectral composition. Because of its association with light foraging I expected that the species in the “light-demanding” group would demonstrate pronounced shade responses, particularly in the form of stem elongation, while avoidance responses in the shade-tolerant species would be weaker or nonexistent.

Methods

Stock plant collection

In the summer of 2021, I collected between six and ten stock plants per species from wooded areas in each of two Ohio regions corresponding roughly to the regions surrounding Columbus and Cincinnati. Because many of these liana species are capable of vegetative propagation, collection sites were divided into two regions in order to limit the likelihood of collecting genetically identical samples. Collection was limited to young plants with branches 0.5m-1m in length and with minimal branching. Samples collected from a single site were separated by at least 5m to further reduce chances of clonal duplicates. Plants were planted in 1-gallon trade nursery pots (2.5 L) in an all-purpose growing medium and then grown in a shaded, naturally lit greenhouse at the Ohio State University for one year with no supplemental lighting. This shading served to prevent damage from excess light in the understory-acclimated stock plants and to more closely match the light levels planned for the experimental treatments, described below. Irradiance in the shaded greenhouse was approximately 13% of the outdoor ambient irradiance, permitting a photosynthetic photon flux density (PPFD) on clear days of up to 230-270 μmol m^{-2} s^{-1} at midday in June. Temperature was allowed to fluctuate seasonally, with heating and evaporative cooling systems keeping greenhouse temperatures between 22-32°C in the summer and 11-19°C in the winter. Within the year the leaves of all deciduous species, but not evergreen *Euonymus* and *Hedera*, completed one cycle of seasonal senescence and reemergence.

Experimental plant propagation

In July of 2022 I randomly selected five stock plants per species to represent each region. Three cuttings were taken from each of the selected stock plants. Each cutting consisted of two nodes and two internodes taken in sequence from the most terminal portions of stock plant branches containing mature leaves. The lower node of each cutting was defoliated so that only one leaf or pair of leaves remained on the more terminal node. The cuttings were placed in water for propagation and potted upon root formation, approximately one month later. Time to root formation varied by species, with adventitious root-forming *Hedera* and *Euonymus* rooting in less than 2 weeks, *Ampelopsis* in 2 to 3 weeks, and *Celastrus* in 3 to 4 weeks. For two weeks they were grown in a greenhouse environment matching that of the stock plants with an additional shade cloth to reduce ambient light by a further 50% to an average of 90 µmol m^{-2} s^{-1}, a value typical of forest edge environments at a depth of 1-2m into the canopy (L. Finley, unpublished data; see also Table 1). This irradiance level provided a suitable control environment that would later be sufficiently high to support plants growing under additional shade frames for shade treatments (at least 10-20 µmol m^{-2} s^{-1}).

From September 12-November 8, 2023 (8 weeks), potted cuttings were grown in greenhouse conditions under randomly-assigned custom shade fames to manipulate light quality and quantity as detailed in the following section. However, an unforeseen experiment in a neighboring greenhouse bay introduced artificial lighting that threatened to interfere with the established light treatments and photoperiod. I therefore re-randomized the plants and moved them into a growth chamber where they remained for the duration of the 34-week experiment (Figure 1).

Growth chamber environment and treatments

On November 8, 2022, all cuttings were moved into a temperature-controlled walk-in growth chamber (Thermolinear, Cincinnati, Ohio, USA). Growth from the previous greenhouse environment was cut back on December 26, 2022, leaving just 4 intact nodes and ensuring that measurements would only be conducted on growth from the new environment. Because root tissue was persistent and could not be pruned in this way and its age would ultimately be unverifiable, belowground growth and development spanned multiple light environments and was not analyzed. Plants were grown until July 10, 2023 under their assigned light treatments. Climbing was discouraged through manual separation of branches from both climbing surfaces and other branches due to its potential to differentially alter biomass allocation between species (Wyka et al. 2019a). The chamber was set to a day length of 12 hours and a temperature of 22°C, but this temperature was only reached at night as the lighting system heated the chamber to 27-28°C during the day. Fungicide was periodically applied to control powdery mildew (Eagle 20EW specialty fungicide, Dow AgroSciences, Zionsville, Indiana), and commercial cultures of predatory *Amblyseius cucumeris* mites were used to control thrips and mites (AMBLYforce, Beneficial Insectary, Redding, CA).

I tested and configured to match the irradiance and spectral quality of the greenhouse environment as closely as possible to minimize changes associated with relocation and to maintain a forest edge-like ambient irradiance. Measurements of the light environment were conducted with a handheld spectroradiometer (LI-180, LI-COR Biosciences, Lincoln, NE). Eight color-adjustable LED arrays (SolarSystem550, California Lightworks Canoga Park, CA) supplied most of the photosynthetically active radiation (PAR) in the chamber. Their red-rich and far red-poor output produced a R:FR ratio that was much higher (R:FR > 8) than that of natural sunlight (R:FR ca. 1.3; Table 1, Figure 2A). To attenuate red wavelengths, green vinyl window tint films (Green-silver static vinyl window film, KESPEN Window Films) were suspended from the LED arrays to filter out red light while preserving the chamber's blue:green ratio. Supplemental incandescent light bulbs (150 Soft White, Sylvania Lighting) were then introduced to slightly increase PAR while also increasing the relative amount of far-red light in the chamber, bringing the chamber's ambient R:FR ratio to approximately 1.3 (Table 1, Figure 3A).

Cuttings were subjected to one of three light treatments: control, neutral shade, and R:FR-reduced shade. The control treatment consisted of growth in ambient growth chamber light at the highest irradiance, approximately 89 µmol m^{-2} s^{-1}. Plants in the neutral treatment were grown in neutral shade – a reduced irradiance of approximately 12 µmol m^{-2} s^{-1} with no spectral change – provided by black shade cloth stretched over a 1x0.5x1m wooden frame (Inslat black HDPE shade cloth 70%). The reduced R:FR treatment (referred to as R:FR hereafter) was produced in the same manner but with green shade cloth (SEJCYXO green polyethylene shade cloth, 80%) that served to reduce the amount of red light penetrating the frame while matching light availability in the neutral treatment as closely as possible. The green cloth reduced the R:FR ratio from 1.31 to 0.88 with an irradiance of approximately 15 µmol m^{-2} s^{-1}, a slightly higher light availability than that of the neutral treatment (Table 1).

Each treatment was divided into 5 blocks containing 8 plants each. The 15 blocks were arranged randomly in the growth chamber. The three clones from each stock plant were assigned to blocks at random such that 1) each stock plant was represented once in each treatment and 2) each block contained one individual from each region for every species.

Light response curves

Photosynthetic measurements were conducted after 3 months of growth in the growth chamber using a portable photosynthesis system (LI-6800 with 6800-01A flash fluorometer, LI-COR Biosciences, Lincoln, NE). Due to mortality and sensitivity to photoinhibition in *Ampelopsis* and Celastrus, only *Hedera* and *Euonymus* were measured. A subset of five individuals per treatment was measured for each species, except in the case of Neutral and R:FR *Euonymus* where 4 and 6 individuals were measured respectively. The youngest fully-expanded leaf was selected from each plant and allowed to acclimate in the fluorometer chamber for 30 minutes at 400 µmol m^{-2} s^{-1}, with leaf temperature held at 28C to match ambient temperature. Following acclimation, gas exchange parameters were logged at 10 decreasing irradiance levels at 120s intervals. An initial saturating irradiance of 1000 µmol m^{-2} s^{-1} to 0 µmol m^{-2} s^{-1} was used after tests determined that photoinhibition began to occur in some species at higher irradiances. The acclimation period and irradiance as well as the saturating irradiance used here ultimately aligned with those used by Cai et al. (2005) in their study of light acclimation in shade-adapted woody species. Assimilation rate (µmol CO_2 $m^{-2}s^{-1}$) over the range of irradiances was used to estimate photosynthetic parameters from light response curves constructed according to the methods described in the statistical methods section; these included maximum photosynthetic rate A_{max}, light compensation point (LCP), quantum yield (ϕ), and curve convexity (θ).

Estimated chlorophyll and morphological measurements

Estimated chlorophyll measurements along with leaf mass and area measurements were taken from June 27-July 5, 2023. Plants with no living leaves were considered dead and excluded from these measurements, as well as from all subsequent destructive morphological measurements. The five youngest mature leaves per living plant were selected and measured with a handheld chlorophyll meter (MC-100, Apogee Instruments, Logan, Utah); these data are reported using the unitless Chlorophyll Content Index (CCI) as measures of relative chlorophyll content. These five leaves were then harvested and their areas were measured from scanned images (WinFOLIA 2015a, Regent Instruments, Québec City, Quebec, Canada) before they were dried in a drying oven (SHEL LAB SMO28-2, Sheldon Manufacturing, Cornelius, OR) for one week at 60°C and weighed. Chlorophyll content and SLA (cm^2 g^{-1}) were calculated as averages for the five-leaf samples.

Destructive harvests to measure branch length, branch count, and average internode length measurements for living plants took place from July 10-17, 2023. Average internode length was calculated as the average length of the five most terminal, consecutive internodes intermediate between fully-expanded leaves. Branches were counted only if their length was greater than 0.5cm and they contained more than a single node, even if the nodes were immature. The harvested branch material was dried for one week at 60°C and separated into stem mass and leaf mass before weighing from July 16-19, 2023; tendrils were included in stem mass and petioles were included in leaf mass. Total aboveground biomass (g) consisted of dry stem and leaf mass, including the masses of the 5-leaf samples collected for leaf mass and leaf area measurements. Leaf mass fraction of total aboveground biomass (g g^{-1}) was calculated using total leaf mass and total aboveground biomass and differs from traditional leaf mass fraction which typically accounts for root biomass.

Statistical Methods

A correlation matrix was used to identify traits that were too highly correlated to warrant separate analyses, with this determination made for morphological traits with absolute correlation values >0.8. Total aboveground mass was selected as the best representative of a suite of strongly positively correlated traits that included total branch length, internode count, and branch count.

Analyses were conducted in R v.4.3.0 (R Core Team 2023). For each continuously-varying trait a mixed linear effects model was constructed with the R package lme4 (Bates et al. 2015) using the treatment × species interaction and both main effects as predictors, with a random effect of collection site nested within collection region. For traits in which outlier groups caused skewedness at the extremes of plotted residuals vs. estimates, a log-transformed model was selected after confirming its suitability relative to the original model on the basis of AIC comparisons. Inferences about predictor significance were based on Type III sums of squares using the chi-squared test statistic from the package car (Fox and Weisberg 2019). For cases in which the treatment × species interaction was not significant, a simpler model excluding the interaction term was used instead (in all cases this was justified on the basis of AIC comparisons). An α of 0.05 was selected as the threshold for determining significance. Reported means in this text and all figures are shown with their standard error.

To determine where pairwise differences occurred following a significant effect of species, treatment, or treatment:species, I used the contrast() statement from the emmeans package (Lenth 2023) to compare estimated means conditioned by species for intraspecific comparisons or by treatment for interspecific comparisons. Because my hypotheses centered around responses to the different light treatments and whether those responses varied by species, I interpreted significant interactions by assessing treatment differences by species. I used Tukey corrections to the critical alpha level for all pairwise comparisons. P-values from these comparisons were sorted into significantly differing groups using the cld() function in the multcomp package (Hothorn et al. 2008) in order to visualize comparison results. Light response or AQ curve parameters were estimated with a non-rectangular hyperbolic model (Mashall and Biscoe 1980) using the photosynthesis package (Stinziano et al. 2021); these parameters included light-saturated photosynthetic rate (A_{max}), light compensation point (LCP), quantum yield of CO_2 assimilation (ϕ), and curve convexity (θ).

Results

Mortality

While no mortality was observed in *Hedera* or *Euonymus*, both *Ampelopsis* (Control survival = 100%, Neutral = 40%, R:FR = 60%) and *Celastrus* (Control survival = 40%, Neutral = 40%, R:FR = 50%) experienced high rates of mortality in the shade treatments, and *Celastrus* experienced high mortality even in the control treatment.

Morphological traits

Total aboveground biomass. Biomass was greatest overall in the control treatment followed by the R:FR treatment (all *treatment* Tukey $p < 0.01$, Table 2), but the influence of the treatments on plant biomass varied by species (*treatment* × *species* $p = 0.049$). A contrast analysis by species indicated that all control groups were significantly larger than either of their corresponding shade groups (control mean = 31.64 ± SE 5.80; neutral mean = 0.64 ± SE 0.089; R:FR mean 1.27 = ± SE 0.27; *treatment* p = < 0.01; all Control – Shade Tukey $p < 0.01$, Figure 4A). The difference in biomass between the Control treatment and the mean of the two shade treatments was greatest in *Celastrus* which increased by a factor of 59.0, followed by *Ampelopsis* by a factor of 41.6, *Euonymus* by 40.3, and *Hedera* by 18.3. Biomass in all species was nonsignificantly higher in the R:FR treatment than the Neutral treatment with the exception of *Hedera* which saw a significantly higher R:FR biomass (*Hedera* neutral mean = 1.06g ± 0.17g, R:FR mean = 2.05g ± 0.23g, Tukey $p = 0.027$). However, *Hedera's* significant 1.9-fold increase in biomass from the neutral treatment to the R:FR treatment was only slightly greater than the nonsignificant 1.8-fold increase in *Celastrus* (Tukey $p = 0.422$).

Leaf mass fraction of aboveground biomass. Leaf mass represented a higher proportion of total biomass in R:FR shade overall than in control conditions and in R:FR shade compared to neutral shade, while the neutral treatment did not produce an effect that differed from the control group (Control – R:FR and Neutral - R:FR Tukey $p < 0.01$; Control – Neutral Tukey $p = 0.609$, Figure 4B). However, within-species patterns varied (*treatment* × *species* $p=0.002$, Table 2) and did not match this ranking by treatment. While *Hedera* and *Euonymus* means showed comparable increases in both shade treatments relative to their control groups (*Hedera* and *Euonymus* Control – Neutral and Control – R:FR Tukey $p <0.034$; Neutral – R:FR Tukey $p > 0.9$) no corresponding significant difference from control to shade was detected in *Ampelopsis* and *Celastrus*. Instead, their leaf mass fraction was lower in neutral shade than in R:FR-reduced shade (*Ampelopsis* Neutral – R:FR Tukey $p < 0.01$; *Celastrus* Neutral – R:FR Tukey $p = 0.049$, Figure 4B), with leaf mass fraction intermediate for these species under control conditions.

Specific leaf area. Specific leaf area (SLA) increased overall in shade (*treatment* $p<0.01$; all Control – Shade Tukey $p < 0.01$, Neutral – R:FR Tukey $p = 0.737$, Figure 4C, Table 2) and was greatest in *Ampelopsis* and *Celastrus*, which did not differ from each other but had a larger SLA than each shade-tolerant species (*species* $p<0.01$, *Ampelopsis* – *Celastrus* Tukey $p = 0.478$; all other Tukey $p < 0.01$). The magnitude of the SLA increase in shade varied by species (*treatment* × *species* $p <0.01$). Tukey comparisons of mean SLA in *Ampelopsis, Euonymus,* and *Hedera* showed significant within-species differences between control and shade treatments (Control – Shade Tukey $p < 0.012$) while no change was detected in *Celastrus* SLA across treatments. No significant difference in response to Neutral vs. R:FR treatments was detected within any species. In spite of the low sample size for *Ampelopsis* SLA resulting from a lack of mature leaves among surviving plants (Neutral n=1, R:FR n=2) its 169% increase in Neutral shade and 157% increase in R:FR shade proved to be statistically detectable, dwarfing *Hedera*'s next-highest SLA increases of 59% and 49%. *Euonymus* SLA did not differ significantly across treatments, though it did increase from control SLA by 28% in the Neutral treatment and 27% in the R:FR treatment.

Measured average internode length. Average internode length differed on the basis of treatment (*treatment* $p < 0.01$, Table 2) and species (*species* $p < 0.01$). The longest internodes were produced by *Ampelopsis* and *Hedera* and the shortest by *Celastrus* and *Euonymus*, with internode lengths in the latter two species not differing from each other (*Celastrus* – *Euonymus* Tukey $p = 0.830$; all other Tukey $p < 0.017$, Figure 5A). Treatment responses varied by species (*treatment* × *species* $p = 0.043$). Contrary to expectations, in a contrast analysis by species *Ampelopsis* and *Euonymus* had significantly longer internodes in their control groups than in either of their shade groups (All *Ampelopsis* Control – Shade Tukey $p < 0.030$; All *Euonymus* Control – Shade Tukey $p < 0.01$). Internode length in these species did not differ across shade treatments (*Ampelopsis/Euonymus* Neutral – R:FR Tukey $p > 0.3$). Internode length in *Hedera* did not differ significantly between any treatments, decreasing nonsignificantly in length by only 15% in neutral shade and increasing nonsignificantly by 2% in R:FR. In *Celastrus* the Control group had longer internodes than the Neutral group (Tukey $p < 0.01$) but not the R:FR group. Although all species had at least nonsignificantly higher internode lengths in R:FR shade than in Neutral shade, this difference was only significant in *Celastrus* (*Celastrus* Neutral – R:FR Tukey $p < 0.01$). *Celastrus* internode length was higher in R:FR shade than neutral shade by a factor of 2.0, with all other species increasing nonsignificantly by a factor of approximately 1.2.

The apparent decrease in internode length observed in shade-treated plants prompted a second analysis of internode length incorporating biomass as a covariate given the large size difference between control and shade plants. The biomass-adjusted model determined treatment ($p < 0.01$, Table 2) and biomass ($p < 0.01$) to be significant drivers of length differences along with species, with overall internode length still greatest in *Ampelopsis* but no longer differing between any other species (all *Ampelopsis* – Other species Tukey $p < 0.01$; all other species pairs Tukey $p > 0.16$; *species* $p < 0.01$, Figure 5B). Following this adjustment, the estimated marginal mean lengths of all control groups were significantly lower than both of their respective shade groups while shade group means did not differ, with R:FR internode lengths only nonsignificantly higher than neutral lengths (Control – Neutral Tukey $p = 0.021$; Control – R:FR Tukey $p < 0.01$; Neutral – R:FR Tukey $p = 0.345$, Figure 5B).

Leaf chlorophyll. Estimated leaf chlorophyll (CCI) varied only by species and was highest in *Euonymus* (*Ampelopsis* mean = 13.08 ± 5.66; *Euonymus* mean = 87.85 ± 1.76; *Celastrus* mean = 14.70 ± 3.34; *Hedera* mean = 32.56 ± 1.63; *species* $p<0.01$, Figure 4D, Table 2). Contrast analysis indicated that mean chlorophyll content did not differ significantly between *Ampelopsis* and *Celastrus* (Tukey $p = 0.718$), with significant differences between all other species pairs (Tukey $p < 0.01$) resulting in the formation of three groups.

Photosynthetic traits

Light-saturated photosynthetic rate (A_{max}). Assimilation rate at light saturation differed by treatment but not between species, with saturated photosynthetic rates being highest for plants in the control group and statistically indistinguishable between shade groups (Control mean = 9.90 µmol CO_2 m^{-2} s^{-1} ± 0.58; Neutral mean = 6.00 ± 0.40; R:FR mean = 6.31 ± 0.28; *treatment* $p < 0.01$; all Control – Shade Tukey $p < 0.01$, Figure 6A, Table 3).

Light compensation point (LCP). Light compensation point varied only on the basis of treatment and was greater overall among Control groups, decreasing by an average of 43% in the shade (Control mean = 11.96 µmol m^{-2} s^{-1} ± 1.40; Neutral mean = 6.88 ± 0.95; R:FR mean = 6.65 ± 0.67; *treatment* $p < 0.01$, Figure 6B, Table 3). Tukey comparisons by treatment showed no light quality effect, with means only significantly different in the control group (Control – Neutral Tukey $p = 0.026$, Control – R:FR $p = 0.016$, Neutral – R:FR $p = 0.999$).

Quantum yield (ϕ). Quantum yield was highest overall in *Hedera* (*Hedera* mean = 0.075 mol CO_2 mol^{-1} quanta ± 0.001; *Euonymus* = 0.67 ± 0.002; *species* $p < 0.01$, Figure 6C, Table 3). No significant changes in quantum yield were detected between treatments in either species, with ϕ decreasing nonsignificantly in *Euonymus* by an average of 12% in the shade and *Hedera* decreasing nonsignificantly by 3%.

Curve convexity (θ). Light response curve convexity varied by treatment and was highest in the control treatments and lowest in R:FR while being only marginally higher in neutral shade than R:FR (Control mean = 0.78 ± 0.02; Neutral mean = 0.75 ± 0.03; R:FR mean = 0.68 ± 0.03; *treatment* $p < 0.01$; Control – R:FR Tukey $p = 0.012$; Neutral – R:FR Tukey $p = 0.075$; Control – Neutral Tukey $p = 0.733$, Figure 6D, Table 3). Convexity was greatest overall in *Euonymus* (*Euonymus* mean = 0.77 ± 0.02; *Hedera* mean = 0.71 ± 0.03; *species* $p < 0.01$).

Discussion

I selected the four species in this study with the expectation that they would represent at least two broad light acquisition strategies that might be observed in a forest understory: light-demanding species that demonstrate limited growth until released from shade, *Ampelopsis* and *Celastrus*, and shade-adapted or shade-tolerant species that thrive even when light availability is limited, *Hedera* and *Euonymus*. As expected, several measured traits and their responses to reduced irradiance differed in accordance with these pairings.

Light-demanding species

A stark contrast emerged between the mortality rates of the shade-tolerant and light-demanding species. While no mortality was observed in shade-tolerant *Hedera* or *Euonymus*, roughly half of all light-demanding individuals in the shade treatments died before the final measurements and harvest. In *Ampelopsis,* death occurred following the spontaneous and gradual loss of healthy-appearing leaves from new growth in established cuttings. *Celastrus* cuttings rooted slowly and were slow to put on new growth, and in most cases individuals died after a period of a month or more with no visible leaf or stem growth. Apart from mild outbreaks of powdery mildew in *Ampelopsis* which were promptly treated, no pathogen was identified as a probable cause of death in either species. It is therefore possible that the low light availability in the shaded treatments at a PPFD < 20 μmol m^{-2} s^{-1} was insufficient to reliably support these species. This does not necessarily explain the mortality observed in *Celastrus* in control conditions, but even the control environment could be considered to be relatively dark;

while comparable to values in the light canopy cover at a forest edge its PPFD of 90 µmol m^{-2} s^{-1} was substantially lower than that of full sunlight, which can exceed 1800 µmol m^{-2} s^{-1}.

For those individuals that survived, total aboveground biomass of the light-demanding species did not differ from that of the other species. While higher net assimilation rates in high light might be expected in light-demanding species (Kitajima 1994; Murchie and Horton 1997), the comparable biomass found between species groups may indicate that the control irradiance was too low to reveal any separation between the groups at just 90 µmol m^{-2} s^{-1}. The effect of shade on biomass in the light-demanding species did align with expectations; both species saw the greatest decreases in biomass when shaded, each falling by an average of 65% compared to their control groups while *Euonymus* and *Hedera* decreased by just 41% and 20%, respectively.

Celastrus and *Ampelopsis* had the highest and second-highest SLA overall. A high SLA is frequently associated with shade-acclimated leaves or shade-adapted plants, but it is not necessarily unusual in plants that thrive in high-light environments due to its association with a high relative growth rate (Lambers et al. 2008). The difference in magnitude between the SLA responses of the two light-demanding species appears to be substantial. In control conditions *Celastrus* saw the highest SLA of any species followed by *Ampelopsis*, but its smaller average increase of 29% in shade caused its rank to fall below that of *Ampelopsis* which increased its SLA by an average of 163% in shade. Estimated chlorophyll content was lowest in these species with no significant differences across treatments, and unlike the shade-tolerant species the mean chlorophyll content did not differ between them. The use of a handheld chlorophyll meter introduces challenges in the interpretation of interspecific chlorophyll data given the potential for differences in the physical and optical qualities of leaves combined with potentially non-uniform distributions of chlorophyll (Parry et al. 2014). While no intraspecific differences were significant, it is possible that the nonsignificant decrease in relative chlorophyll seen in shaded *Ampelopsis* is related to its substantial shift in SLA. When measured over a fixed area chlorophyll concentration would appear to decrease if shade-induced increases in leaf area and decreases in chloroplast density outpaced increases in chloroplast size. In both light-demanding and shade-tolerant groups the magnitude of nonsignificant differences in within-species chlorophyll content appear to correlate with the magnitude of within-species differences in SLA.

Shade-tolerant species

Both shade-tolerant species saw smaller reductions in total aboveground biomass in shaded conditions than the light-demanding species, with the smallest decreases observed in *Hedera.* At the tested irradiances there is no indication that this diminished decrease in carbon gain in low light is accompanied by compromised performance at higher light levels, given that biomass did not differ significantly between any species in control conditions

SLA was lowest in the shade tolerant species in all treatments, and this is largely explained by the visibly smaller and thicker leaves found in both species relative to the light-demanding species across all treatments. While *Euonymus* SLA increased nonsignificantly in shade, the increase observed in *Hedera* was the second highest of any species after that of light-demanding *Ampelopsis*. This result aligns with the observations of Sack and Grubb (2002) that *Hedera* seedlings exhibit a high degree of SLA plasticity relative to other shade-tolerant woody species, and that this is not significantly impacted by reductions in R:FR. Although high SLA is sometimes associated with a high relative growth rate, substantial increases in SLA are generally expected in shade-tolerant plants that aim to maximize light interception through broader, thinner leaves when shaded (Evans and Poorter 2001). The less dramatic response observed in *Euonymus* SLA appears to detract from this generalization, but it should be considered that *Euonymus* is the only species in this study with opposite leaves. As such, all changes in leaf morphology (or any leaf-linked trait) could be considered to multiply on a per-node basis, an effect further magnified by its relatively short internodes which are discussed in later sections.

Light response curves

The effects of the light treatments on light-saturated rates of photosynthesis and light compensation point aligned with expectations in that control plants achieved higher maximum rates than shade plants, and that efficient light-harvesting and a potentially lower respiration rate allowed positive net assimilation rates at lower irradiances for shaded individuals. Light quality did not appear to impact these traits and responses by *Hedera* and *Euonymus* did not differ across treatments, with other studies similarly concluding that photosynthetic capacity may not be directly impacted by spectral quality (Kitajima 1994; Pons and de Jong-Van Berkel 2004).

Quantum yield (ϕ), while not differing between treatments, tended to be higher in *Hedera* in all conditions. Its relative stability across treatments contrasts the reduction in convexity (θ) observed in both species' R:FR groups. In shade-adapted species and shade-acclimated leaves a high or unreduced ϕ along with high θ are typically seen, reflecting efficient light capture at low irradiances and abrupt and early light saturation at increasing irradiance (Ögren 1993). Possible causes of the decrease in θ, or "flattened" light response curves, produced by the R:FR treatment are discussed further in the following section.

Responses to reduced R:FR

Differential responses to neutral vs. reduced R:FR shade were observed in four measured traits: reducing R:FR induced an increase in leaf mass fraction of aboveground mass in *Ampelopsis* and *Celastrus*, lengthened internodes in *Celastrus*, increased total aboveground biomass in *Hedera*, and decreased the convexity (θ) of light response curves in both measured species, *Hedera* and *Euonymus*. In these cases the observed responses may reflect a phytochrome-mediated shade avoidance response that is distinct from, or modifies, a generalized response to low irradiance. Light-demanding species were expected to show a relatively strong shade avoidance response given their reliance on light foraging to sustain optimal growth under a canopy, while diminished or nonexistent shade avoidance responses were expected in shade-tolerant species.

Internode length did not prove to be a clear or conclusive indicator of strong shade avoidance responses, though in accordance with expectations for light-demanding plants (Lee 1988; Lambers et al. 2008; Morelli et al. 2021) it did respond to spectral quality in *Celastrus*, increasing in low R:FR, prior to the adjustment of internode length for biomass. Unadjusted internode lengths for all other species were reduced by one or both shade treatments, or unchanged in the case of *Hedera*. The apparent decrease or lack of change in internode length from the control groups in all species was unexpected, and it is likely that the overall disparity in size between control plants and shaded plants exceeded and masked any internode elongation in the shade plants. This gave the initial impression of internode elongation in the control groups, which prompted a follow-up analysis of internode length adjusted for biomass. The adjusted model suggested instead that internode length was greatest in shaded individuals, and nonsignificantly greater in R:FR-reduced shade than in neutral shade. Model adjustment precluded the assessment of any interactive effects of treatments and species, but its estimates align better with the expected influence of low irradiance on stem elongation.

In both *Ampelopsis* and *Celastrus*, the leaf mass fraction of total aboveground biomass was greater in R:FR-reduced shade than in neutral shade though it did not differ significantly from that of the control groups. These results contradict a meta-analysis by Poorter et al. (2012) that suggests a positive correlation between leaf mass fraction and R:FR ratio along with a negative correlation with stem mass fraction, though the authors do not separate lianoid taxa out in their analysis. It is tempting to attribute this reversal to the lianescent growth form as it has been proposed that structural parasitism spares lianas some of the costs associated with self-support (Schnitzer and Bongers 2002; Toledo-Aceves 2015), but Wyka et al. (2019b) succinctly summarize some of the contradictory conclusions reached in both earlier and subsequent research of this topic; tropical vs. growth chamber studies of lianas have separately identified greater and lower relative investment in leaf tissue compared to stem tissue, and few studies account for the influence of age and size on patterns of biomass allocation. Some of these contradictions may also arise from an overall responsiveness of liana allometry to their light environments that is difficult to generalize in terms of higher and lower leaf:stem investment, although putatively similar allometric patterns among disparate liana taxa continue to invite generalization (French et al. 2017). Kazda et al. (2009) found that the terminal twigs of lianas and trees did not differ in leaf area per supporting biomass in either the understory or canopy, but the relationship among liana samples was less variable in both environments as though they were more capable of converging on allometric optima.

The increase in *Hedera's* aboveground biomass in R:FR-reduced shade relative to neutral shade is mirrored by increases (although nonsignificant) in all other species with all R:FR means falling between Control and Neutral means. As *Hedera* did not give any other morphological indications of a strong shade avoidance, the attribution of this effect on biomass to spectral quality is more questionable than the other putative R:FR-induced effects. While light availability was matched between the two shade treatments as closely as possible, individuals in the R:FR treatment saw a slightly greater PPFD than those in the neutral treatment (Table 1). Although only *Euonymus* is available as a comparison, *Hedera's* higher quantum yield across all treatments suggests efficient light use that may have maximized the impact of the additional light energy on carbon gain. This could exacerbate the otherwise nonsignificant increases observed in all other R:FR groups if the effect was produced solely by a difference in light availability.

In the absence of changes in quantum yield (ϕ) between treatments, the decrease in convexity (θ) observed in the light response curves of both measured species' R:FR groups is difficult to interpret. Decreases in θ correspond to lower photosynthetic efficiency at intermediate irradiances, delaying the onset of the curve’s asymptote that indicates the light-saturated photosynthetic rate (Ögren 1993; Herrmann et al. 2020). Lower-convexity curves are generally expected in sun-acclimated leaves, which are occasionally paired with a reduction in the curve’s initial slope, ϕ; structural changes and photoprotective pigments guard against photoinhibition in high light, permitting high maximum photosynthetic rates at the expense of efficiency in low and moderate light (Lambers et al. 2008). The light-saturated photosynthetic rate of R:FR-treated plants was not found to differ from that of the neutral group in either species. Significant interspecific differences in the light response curves were limited to a higher ϕ in *Hedera* while *Hedera* was found to have a lower θ overall. Without a treatment effect in ϕ or A_{max}, causes of the decreased convexity are perhaps limited to transient decreases in efficiency that accumulated during the 30 minutes of acclimation for each leaf or occurred at the onset of the highest measurement irradiance, resolving rapidly over the course of the 10 to 12 minute response curve as irradiance decreased in intensity. Given the high irradiances experienced by the previously shaded leaves during measurement, transient, “dynamic” photoinhibition or other photoprotective processes stand out as possible causes of reduced convexity. It must be the case that the causative factor was transient, because severe or chronic damage to the photosynthetic apparatus would generally be accompanied by reductions in ϕ and potentially a depression of light-saturated photosynthetic rate (Leverenz et al. 1990; Akhkha et al. 2001), which was not observed. It is not clear why this would be more pronounced in plants that were acclimated to spectrally altered shade. Relatively strong chloroplast movement responses have been observed in response to irradiance shade-tolerant species, and this fairly rapid response along with anatomical changes in the palisade mesophyll can alter the optical qualities of leaves (Brugnoli and Björkman 1992; Davis et al. 2011; Howard et al. 2019). However, R:FR has not yet been conclusively shown to augment or magnify this effect (Buisson and Lee 1993).

The overall magnitude and prevalence of responses to changes in light quality in this study were likely diminished by limitations of the experimental treatments. The reduced R:FR ratio achieved in the R:FR treatment was relatively modest. Measured at approximately 0.88 (Table 1), the ratio was lower than that of full sun (approximately 1.2 to 1.3) and the shade produced by a single tree (0.95 to 0.91) (L. Finley, unpublished data), but significantly greater than the ratios produced by moderate to deep forest canopy shade (0.3 to 0.15). Furthermore, the proportion of blue light in this study (Figure 3), while consistent between all treatments, may have been effectively greater than that found in typical, blue-depleted canopy shade (Figure 2) due to its clustering within a narrower and photosynthetically active band of blue wavelengths – a feature that would typically be desirable in LED arrays designed for efficient plant growth. Depletion of blue light prompts cryptochrome-mediated morphogenic changes that parallel those regulated by phytochrome in response to R:FR, and both have been found to contribute to shade avoidance responses (Keuskamp et al. 2012; Pierik and de Wit 2014; Ballaré and Pierik 2017; Sessa et al. 2018). Given that blue light depletion is concomitant with red depletion in natural canopy shade, the blue light present in this study may have detracted from the fully realized shade avoidance responses that might be observed under real or simulated canopies. The irradiances explored in this study are ecologically germane to those supporting lianas in natural systems, but treatments yielding blue light attenuation and further reduction of the R:FR ratio will be necessary to more faithfully replicate the spectral composition of canopy shade. The differential responses observed in some species between neutral and altered shade observed here suggest that this would be a worthwhile endeavor in future research concerning sub-canopy ecophysiology. It is unlikely that incandescent light sources will be widely available to future researchers as sources of far-red enrichment, but LED lighting systems can be assembled to produce virtually any spectrum using a fraction of the energy of tungsten filament bulbs. Additionally, groups such as Petrella et al. (2022) have very effectively combined commercial lighting gels to replicate canopy shade using natural sunlight.

Conclusion

In this study I have characterized the responses of four non-native liana species to reduced irradiance and altered spectral quality. At least two of these species, *Ampelopsis brevipedunculata* and *Euonymus fortunei*, have thus far lacked empirical studies assessing their tolerance of shade. The parallels identified between these species and the better-studied *Celastrus orbiculatus* and *Hedera helix* identified here may serve as a preliminary empirical basis for considering *Ampelopsis* to be shade-intolerant and *Euonymus* to be generally shade-tolerant, offering insight into their interactions with native communities and the trajectory of their expanding introduced ranges. Intrinsic morphological differences between the species are visible, with these results also suggesting that they vary in their capacity to change in accordance with irradiance. The morphological consequences of either a strong shade avoidance response, particularly stem and internode elongation, or a strong response to low irradiance, have additional potential to drive ecological patterns in lianas through their direct contribution to light-foraging and climbing ability. The lianescent growth form recontextualizes many commonly measured ecophysiological traits. For example, the responsiveness of *Ampelopsis* SLA to reduced irradiance observed in this study likely reflects, in part, an optimization of light capture. However, when considered alongside its increased leaf mass ratio in R:FR-reduced shade, its relatively long internodes in all light environments, and its tendril-climbing habit, a picture emerges of a species capable of rapidly and inexpensively suppressing sessile hosts and competitors via shading. Only one species in this study, *Celastrus*, demonstrated an increase in unadjusted internode length when subjected to R:FR-reduced shade, the trait that was expected to most reliably indicate shade-avoiding species. As the only twining climber of the four species, the consequences of internode elongation for *Celastrus* may include an increased climbing rate or improve its ability to climb hosts with a greater stem diameter, in addition to an overall contribution to its light foraging ability (Ladwig and Meiners 2010b). In their study of temperate lianas in Chilean rainforests, Gianoli et al. (2012) concluded that the most abundant liana taxa in the system were those that, according to their ecophysiological characteristics, balanced resource exploitation with metabolic cost. By improving our understanding of the comparative biology of introduced temperate liana species in North America I have attempted to lay a foundation that supports comparison of the relatively less studied and more recently established North American liana assemblages to better-studied tropical communities.

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Appendix - Tables and Figures

Table 1: Spectral composition and irradiance of experimental light environments (µmol photons m^{-1} s^{-2}) and their ratios of red to far-red and blue to green light. Means for each light treatment are shown as well as representative values from single-block samples corresponding to Figure 3 and representative values from outdoor samples corresponding to Figure 2. Sample sizes for mean spectral measurements are indicated for each treatment.

Environment	PPFD	PFD-UV (380-400nm)	PFD-B (400-500nm)	PFD-G (500-600nm)	PFD-R (600-700nm)	PFD-FR (700-780nm)	R:FR	B:G
Control mean (n=5)	89.40	0.22	18.72	37.10	33.57	25.62	1.31	0.50
Neutral mean (n=7)	11.50	0.04	2.45	4.82	4.22	3.15	1.34	0.51
R:FR mean (n=9)	15.23	0.05	3.17	7.51	4.55	5.19	0.88	0.42
Control (block C3)	86.06	0.22	18.23	36.06	31.77	23.92	1.33	0.51
Neutral (block N1)	13.77	0.05	3.01	5.74	5.03	3.70	1.36	0.52
R:FR (block R3)	14.16	0.05	3.03	6.99	4.14	4.65	0.89	0.43
Full sun	1957.00	41.50	514.20	698.60	743.80	569.90	1.31	0.74
Forest edge	74.66	2.10	20.75	29.76	24.14	82.80	0.29	0.70
Forest interior	17.80	0.43	4.20	7.73	5.88	41.66	0.14	0.54

Table 2: Results of chi-squared tests of linear mixed effects models based on Type III sums of squares. For all traits except leaf mass fraction models were constructed using log-transformed data as indicated by AIC comparisons, which also served as the basis for the exclusion of the treatment × species interaction term where no significant interaction was found.

Response	Predictor	χ^2	Df	P
Total aboveground biomass	(Intercept)	306.6246	1	<0.001
	Treatment	185.9967	2	<0.001
	Species	5.3838	3	0.146
	Treatment:Species	12.6326	6	0.049
Leaf mass frac. of total aboveground biomass	(Intercept)	186.7528	1	<0.001
	Treatment	7.7859	2	0.02
	Species	4.7977	3	0.187
	Treatment:Species	20.7822	6	0.002
Specific leaf area	(Intercept)	9706.425	1	<0.001
	Treatment	71.367	2	<0.001
	Species	118.12	3	<0.001
	Treatment:Species	32.477	6	<0.001
Measured internode length	(Intercept)	1214.283	1	<0.001
	Treatment	11.957	2	0.003
	Species	33.482	3	<0.001
	Treatment:Species	12.983	6	0.043
Biomass-adjusted internode length	(Intercept)	194.977	1	<0.001
	Treatment	18.606	2	<0.001
	Species	62.63	3	<0.001
	Total aboveground mass	34.794	1	<0.001
Leaf chlorophyll	(Intercept)	982.8624	1	<0.001
	Treatment	0.6666	2	0.717
	Species	504.8594	3	<0.001

Table 3: Results of linear mixed effect models for photosynthetic traits, measured only in *Hedera* and *Euonymus*. All were simplified by the removal of the treatment × species interaction after no significant interactions were found and the simplified models were indicated by AIC comparisons. Log-transformed data were modeled for A_{max}, LCP, and θ on the basis of AIC comparisons.

Response	Predictor	χ^2	Df	P
A_{max}	(Intercept)	912.4593	1	<0.001
	Treatment	49.5661	2	<0.001
	Species	0.6027	1	0.438
Light compensation point	(Intercept)	273.7567	1	<0.001
	Treatment	13.7001	2	0.001
	Species	0.3654	1	0.546
Quantum yield (ϕ)	(Intercept)	924.1799	1	<0.001
	Treatment	3.1085	2	0.211
	Species	11.1848	1	<0.001
Convexity (θ)	(Intercept)	878.5833	1	<0.001
	Treatment	11.4733	2	0.003
	Species	8.2496	1	0.004

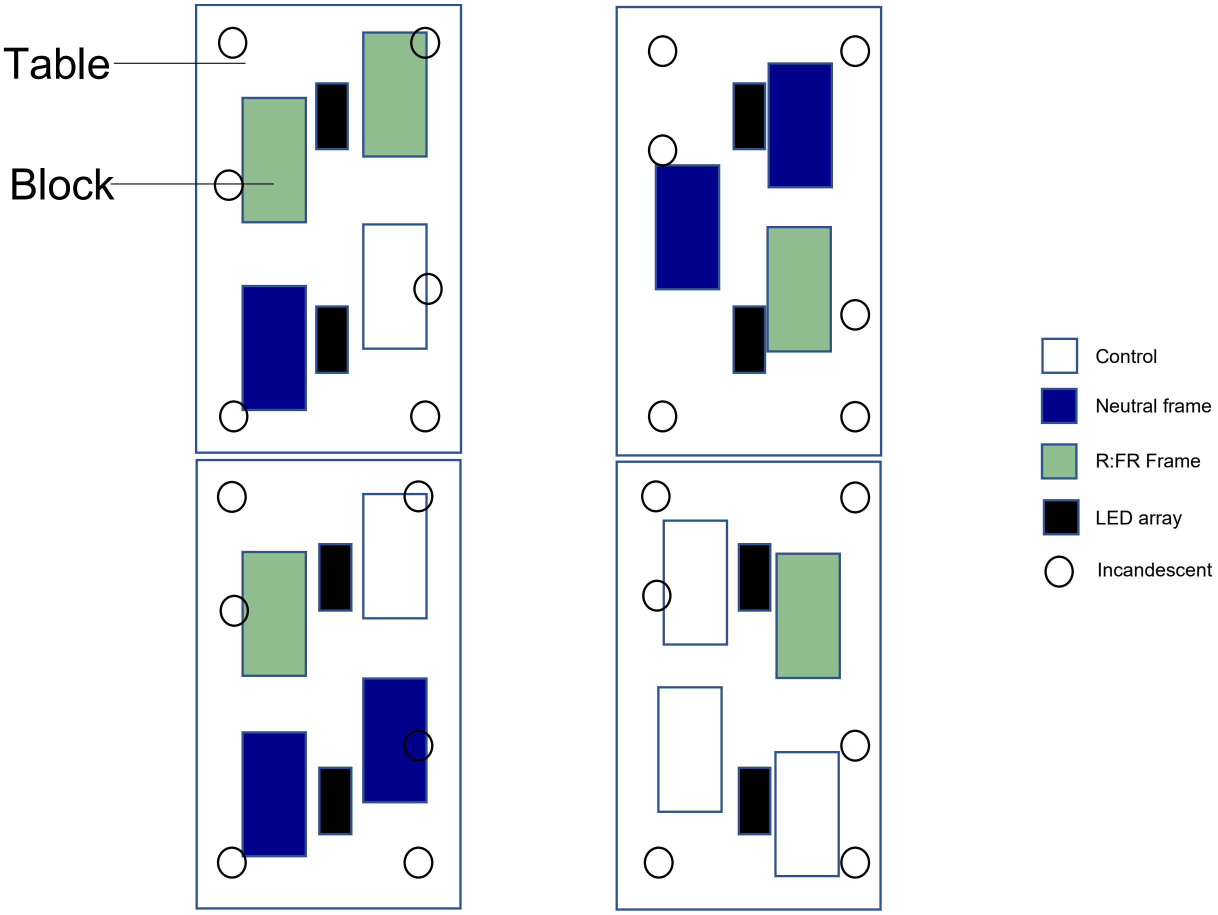

Figure 1: Final arrangement of experimental blocks within the growth chamber along with lighting configuration. Five blocks per treatment housed eight plants each (one per species per region). Each shade treatment consisted of a black (neutral) or green (R:FR-reducing) shade cloth stretched over a custom wooden frame. No frames were built for control blocks.

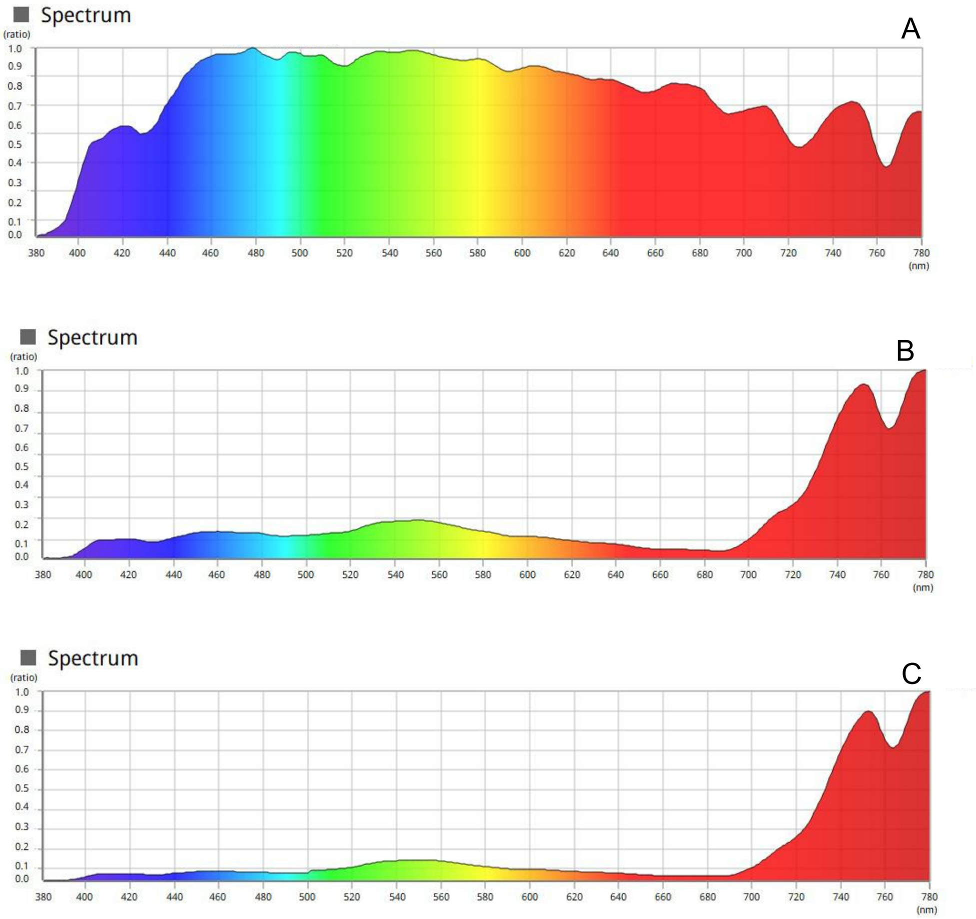

Figure 2: Representative spectral distributions from a mesic deciduous forest on The Ohio State University's Columbus campus: Full sun (panel A, PPFD = 1957.0 µmol m^{-2} s^{-1}, R:FR = 1.31, no canopy cover), forest edge (panel B, PPFD = 74.66, R:FR = 0.29, approx. 2m into forest interior), and forest interior (panel C, PPFD = 17.80, R:FR = 0.14, approx. 10m into forest interior). All measurements were taken between noon and 12:30pm on May 31, 2022. The spectral compositions of each sample are detailed in Table 1.

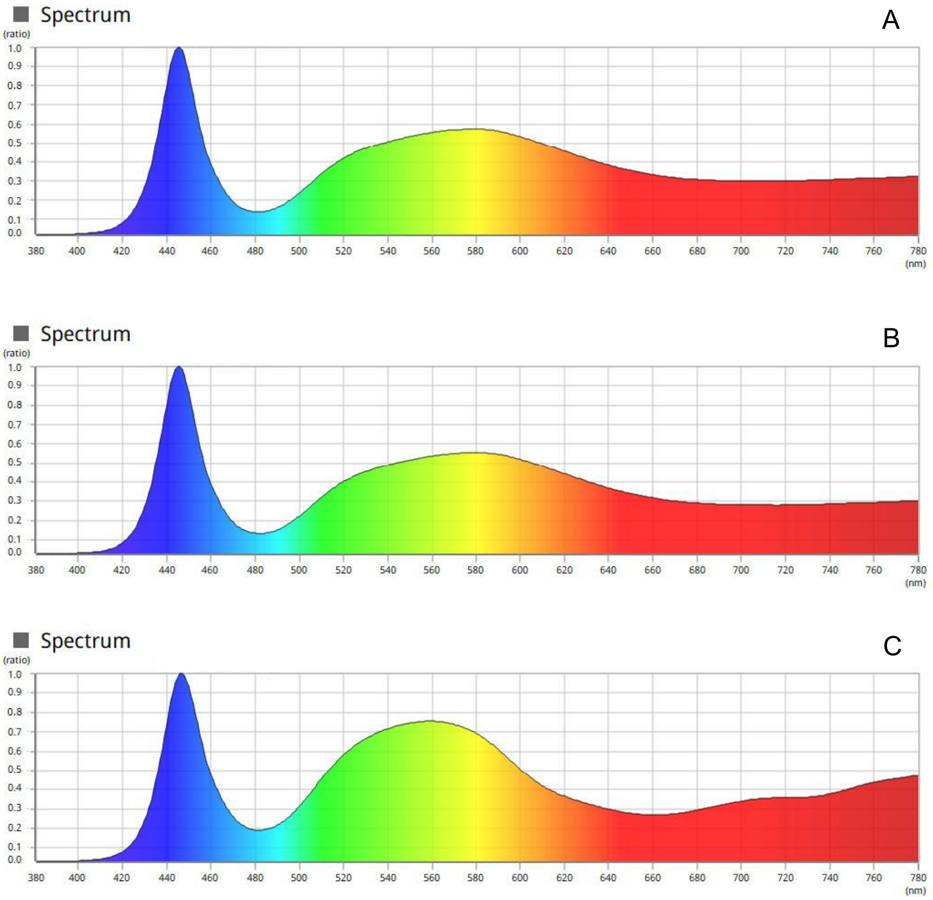

Figure 3: Representative spectral distributions from single-block samples within each growth chamber light treatment. Panel A shows control block C3 (PPFD = 86.1 μmol m^{-2} s^{-1}, R:FR = 1.33), panel B shows neutral block N1 (PPFD = 13.9, R:FR = 1.36), and panel C shows R:FR block R3 (PPFD = 14.2, R:FR = 0.89). The spectral compositions of each sample are detailed in Table 1.

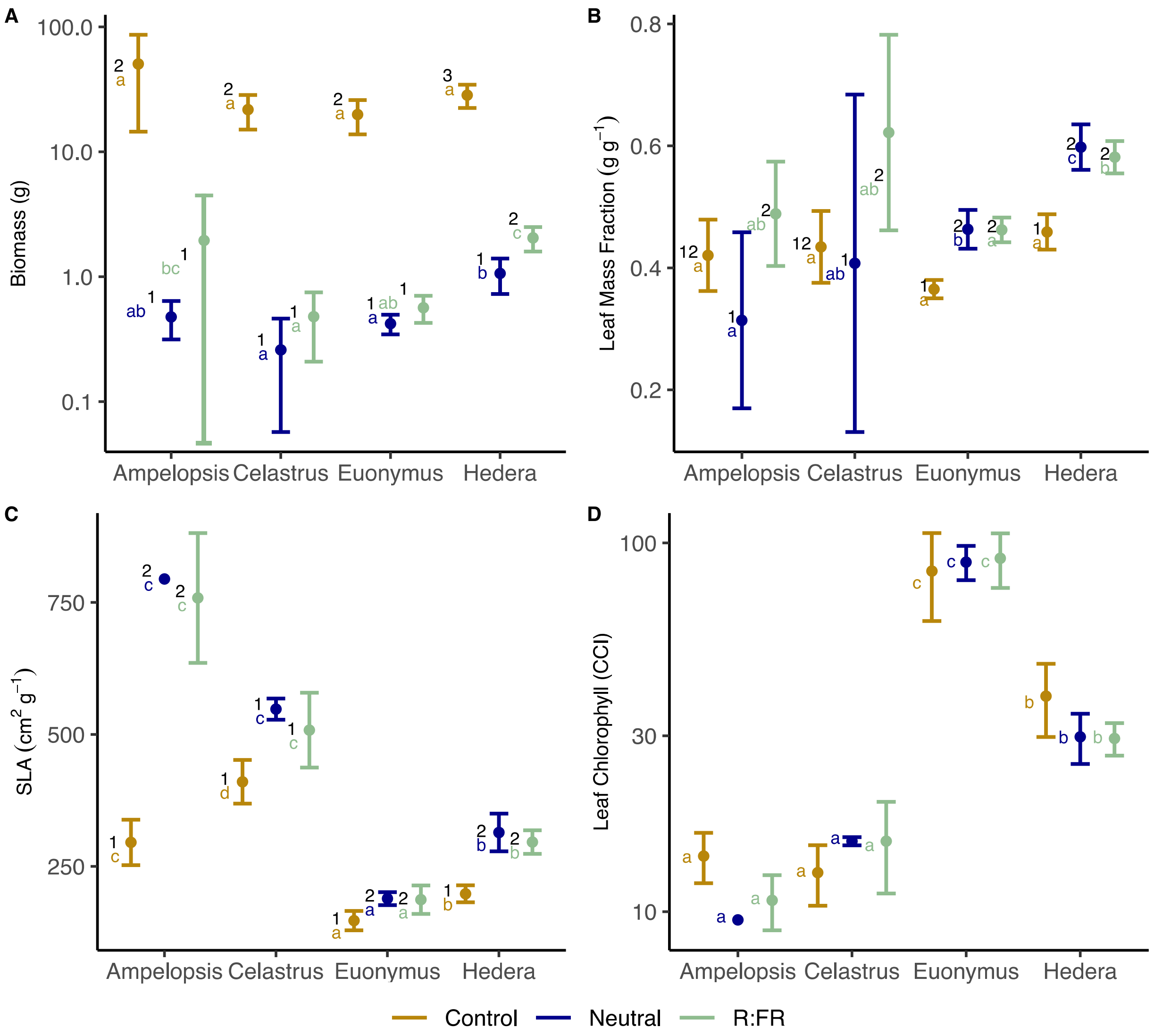

Figure 4: Mean morphological trait values (A-C) and estimated chlorophyll content (D) in control conditions, neutral shade, and reduced R:FR shade by species. Square error bars represent 95% confidence intervals for the means, not shown for *Ampelopsis* leaf traits (panels C and D) due to its sample size of n=1. Due to the figure's log-transformed Y-axis and the restriction of possible biomass values to values ≥ 0, the lower boundary of the interval for biomass (panel A) in R:FR *Ampelopsis* (-0.57g) is adjusted to zero. Number groups represent significantly differing intraspecific treatment means as indicated by Tukey comparisons. Letter groups represent significantly different treatment means between species and are colored according to treatment.

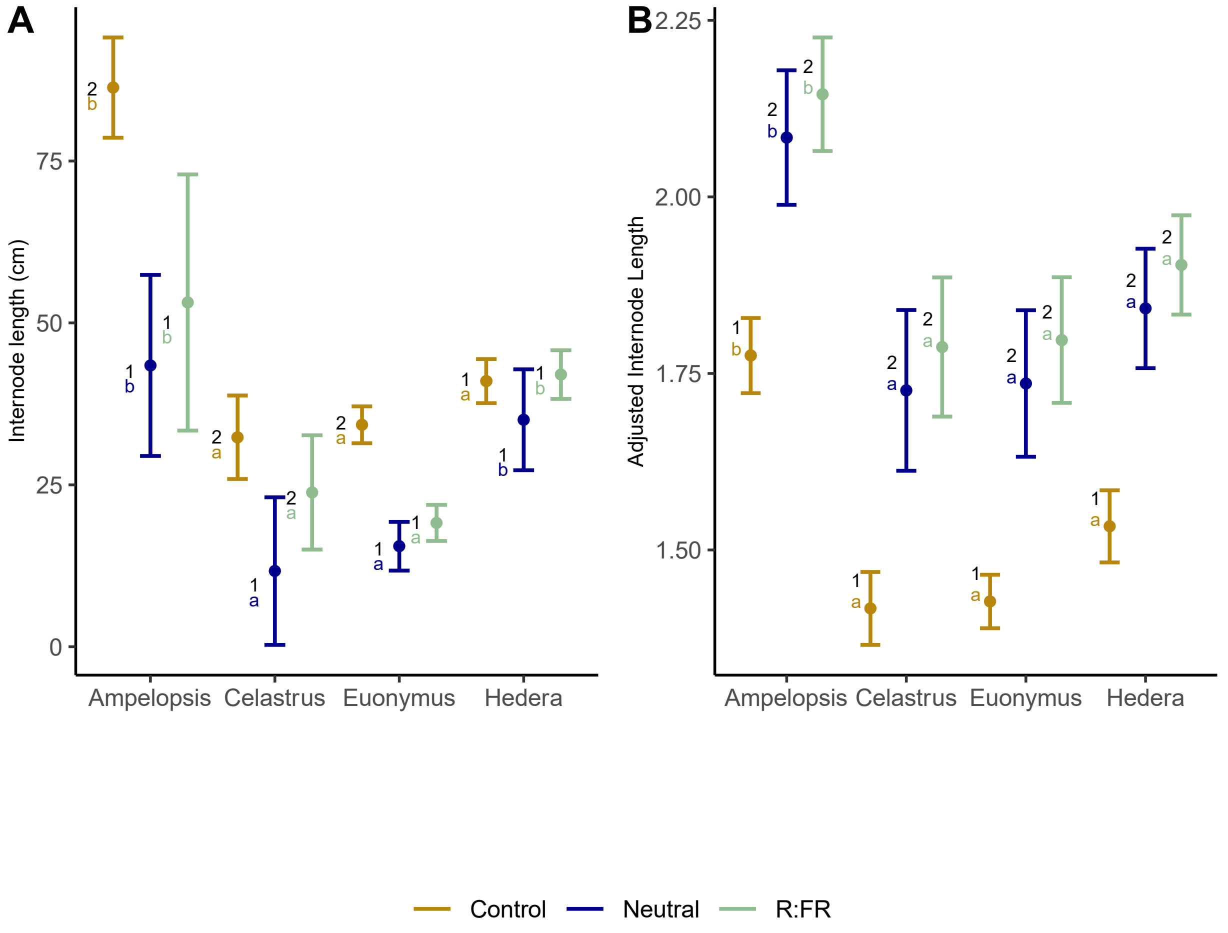

Figure 5: Mean internode lengths as measured (A) and estimated log-transformed mean internode lengths following adjustment for biomass (B) in control conditions, neutral shade, and reduced R:FR shade by species. Square error bars represent 95% confidence intervals for the means. Number groups represent significantly differing intraspecific treatment means as indicated by Tukey comparisons. Letter groups represent significantly different treatment means between species and are colored according to treatment. The biomass-adjusted model (panel B) lacks an interaction term (See Table 2), and the relationships among intraspecific treatment means are therefore consistent.

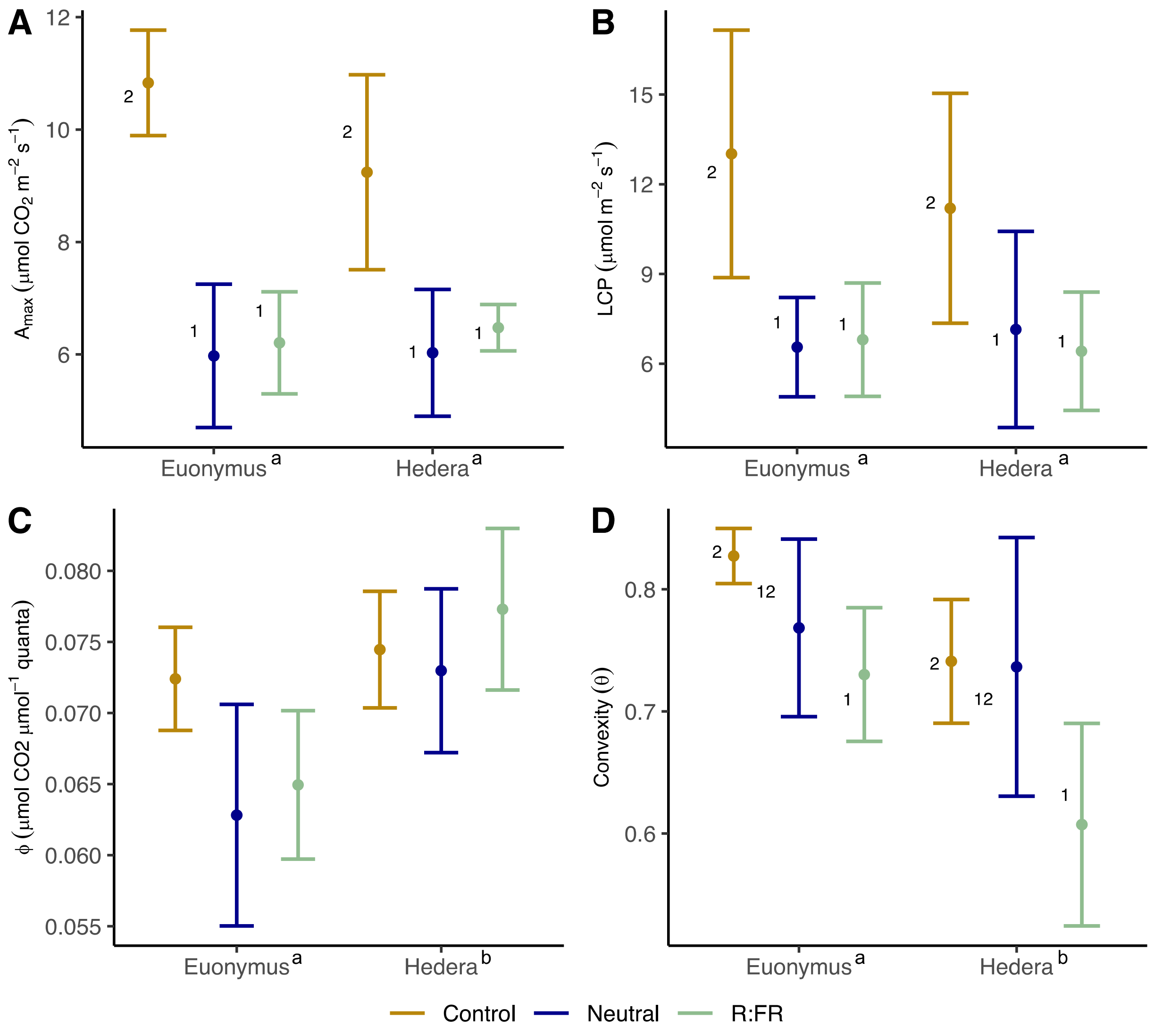

Figure 6: Light-saturated photosynthetic rate (A), light compensation point (B), quantum yield (C), and convexity (D) in control conditions, neutral shade, and reduced R:FR shade by species as estimated from light response curve models, measured only in *Euonymus* and *Hedera*. Square error bars represent 95% confidence intervals for the means. Number groups represent significantly differing intraspecific treatment means as indicated by Tukey comparisons. Letter groups on the X-axis represent significantly different species means. All photosynthetic traits were limited to a single main effect of treatment or species (see Table 3) with the exception of θ (panel D) in which the effects of both treatment and species were significant.