

THESIS

USING FAR-RED LIGHT TO PROMOTE LEAF EXPANSION FOR YOUNG PLANT
PRODUCTION

Submitted by

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ABSTRACT

USING FAR-RED LIGHT TO PROMOTE LEAF EXPANSION FOR YOUNG PLANT PRODUCTION

At northern latitudes, a reduction in the natural light quantity during the winter production of young annual bedding plants (plugs) often necessitates the use of supplemental lighting to reach a target daily light integral (DLI) to ensure high plug quality. However, the low leaf area index (LAI) of plugs during the initial stages of production suggests that a portion of applied light is not intercepted by leaves. Because electric lighting represents a significant percentage of total production costs for greenhouses utilizing supplemental lighting, minimizing wasted light (photons not absorbed by the plant) is critical. Some species have shown an increase in leaf area in response to growth under light with a low ratio of red to far-red light (R:FR); this is generally considered as a shade avoidance response to improve light capture, but there is considerable variation across species. An early increase in leaf area would allow for more effective light capture by seedlings and a reduction in wasted light, but other shade avoidance responses such as elongation of stems and petioles are undesirable for plug production and could outweigh benefits of leaf expansion. Far-red mediated shade avoidance responses may also depend on background photosynthetic photon flux density, DLI, and temperature. The objective of this research was to investigate the effects of far-red radiation on leaf expansion and other shade avoidance responses for the popular annual bedding plant, *Petunia ×hybrida* (petunia), and to examine potential influences of other environmental variables.

Reducing the R:FR in a greenhouse environment with supplemental lighting is challenging due to the relatively high proportion of natural light, so an end-of-day far-red (EOD-FR) lighting strategy was utilized to investigate the promotion of leaf expansion by far-red light for seedlings of petunia ‘Wave Purple’, and ‘Dreams Midnight’. Seedlings were grown in 128-cell trays in a common greenhouse environment under an ambient DLI of $5.26 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ to simulate a winter light environment. Seedlings received no EOD-FR, supplemental lighting for the duration of the experiment, or one of the following EOD-FR treatments that varied in far-red intensity, R:FR ratio, and treatment duration: $10 \text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of far-red light (R:FR ~ 0.8) for 30 minutes, 10 or $20 \text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of far-red light (R:FR ~ 0.15) for 30 minutes, or $20 \text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of far-red light (R:FR ~ 0.15) for 240 minutes. In addition to end-of-day (EOD) treatments, some seedlings under EOD-FR were moved under supplemental lighting after 2 or 3 weeks of EOD lighting. Destructive data was collected 2, 3, and 4 weeks after treatment initiation. Seedlings that received EOD-FR lighting showed stem elongation responses, and seedlings under the lower R:FR or longer EOD duration resulted in greater elongation, but no EOD treatment resulted in an increase in leaf area compared to control (no supplemental lighting or EOD lighting) or supplemental lighting treatments. Results of this study indicate that under low DLIs, EOD-FR light applied in the first three weeks of seedling production does not promote early leaf area expansion and reduces seedling quality under these experimental conditions.

To further examine leaf expansion as a response to far-red radiation, seedlings of petunia ‘Dreams Midnight’ were grown for 28 days under the recommended target DLI of $\sim 10 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ using a 17.25-h photoperiod with either a high (~ 10.8) or low R:FR (~ 0.50). The effects of EOD-FR were also examined by subjecting seedlings grown under the high R:FR to a 1-hour low intensity (target total photon flux density of $46 \text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) EOD lighting

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CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1 Young Annual Bedding Plant Production and Seedling Light Interception

The floriculture industry in the United States is substantial with the wholesale value of floriculture crops sold in the United States estimated to be approximately \$4.42 and \$4.77 billion dollars in 2018 and 2019, respectively; the number of people that work in the industry is likewise large with the average peak number of workers reported as 21.4 among the 4,007 producers in 2019 that reported having hired workers. Additionally, in 2019, more than 30% (\$1.41 billion dollars) of the said wholesale value was attributed to the sale of annual bedding plants. (U.S. Department of Agriculture National Agriculture Statistics Service, 2020).

To be ready for sale in spring and summer, the production of young annual bedding plants (plugs) typically begins in winter and early spring in greenhouse settings (Styer, 2003); the timing of this practice presents challenges to growers at northern latitudes due to the seasonal drop in natural light available to plants for normal growth and development. To be specific, the daily light integral (DLI; $\text{mol} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$), a metric of light quantity used to describe the photosynthetic photon flux density (PPFD; measured in $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; the number of photons able to drive photosynthesis that hit a square meter area over the course of one second), integrated over a single day (or photoperiod) in many parts of the U.S. drops significantly during this time, with lower DLIs observed at more northern latitudes (Faust and Logan, 2018). The DLI within greenhouse environments is reduced further due to greenhouse material transmissivity as well as the angle at which radiant energy strikes the greenhouse (Both and Faust, 2017).

In general, DLI has been shown to influence a wide variety of plant traits including anatomical and morphological features such as the thickness and density of leaves, chemical traits such as the nitrogen content in leaves, physiological traits such as stomatal conductance, and traits related to general growth and reproduction such as the relative growth rate (Poorter et al., 2019). In the production of annual bedding plants, higher DLIs have been shown to generally improve traits considered important in production such as biomass accumulation and decreased time to flower (Faust et al., 2005). For young annual bedding plants specifically, a general minimum DLI of $10\text{-}12\text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ has been recommended to produce plants with a high shoot and root biomass as well as a generally compact growth form as to better facilitate transplant to larger containers or to better tolerate shipping conditions (Pramuk and Runkle, 2005; Lopez and Runkle, 2008).

To compensate for low natural light levels in greenhouse environments, growers may install electrical lighting systems to supplement the natural DLI (Faust and Logan, 2018; Oh et al., 2010; Ouzounis et al. 2015). However, while the capital costs and electrical efficiency of different electric light sources such as high-pressure sodium (HPS) and light emitting diode (LED) fixtures may differ, the cost of supplemental lighting as it is currently deployed in commercial greenhouses can be ~30% of total greenhouse operating costs (Nelson and Bugbee, 2014; van Iersel and Gianino, 2017; Watson, Boudreau, and van Iersel, 2018). As this is a significant cost, facilitating efficient light capture by crop plants where supplemental lighting is deployed is important. However, young plants have little leaf area with which to intercept light (Oh et al., 2010), and a low leaf area index (LAI) may lead to “wasted light” as photons supplied from supplemental lighting strike greenhouse bench space, trays, or substrate. Thus, promoting

an early increase in leaf area may be advantageous in reducing wasted light and increasing the cost-effectiveness of electric lighting.

In some species, far-red radiation has been found to promote leaf expansion as a response to vegetational shade which could improve light capture in young plants, but far-red radiation also promotes enhanced elongation of stems and petioles which are negative plug quality attributes. If applicable, it would be advantageous to utilize far-red radiation to promote leaf expansion in seedlings early in the production cycle to improve light capture for seedling production and improve supplemental lighting efficacy.

1.2 Far-red Radiation

Far-red radiation has been defined as those wavelengths of the electromagnetic spectrum from 700-800nm, but other measures of what constitutes far-red radiation such as 700-750 nm, 700-780 nm, or wavelengths greater than 680 nm are also utilized (Kami et al., 2010; Kubota et al., 2012; Park and Runkle, 2016; Zhen et al., 2019). Far-red radiation is abundant during the day, and the red to far-red radiation ratio (R:FR) measured parallel to the ground outside on a clear day is roughly 1.2 (Franklin, 2008). Under vegetational shade, light intensity decreases significantly, but the relative abundance of far-red radiation increases compared to wavelengths below 700nm (Casal, 2013; Franklin, 2008, Gommers et al., 2013). For example, Franklin (2008) showed that the light intensity and R:FR ratio of sunlight at noon in Leicester, UK decreased from $1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and 1.2, respectively, to $120 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and 0.2, respectively, underneath a single *Arabidopsis* (*Arabidopsis thaliana*) leaf.

The change in the light environment beneath plant canopies is due to the selective absorption, reflection, and transmission by leaves of different portions of the electromagnetic spectrum; green leaves transmit or reflect greater proportions of green (500-600 nm) and far-red

light and absorb greater proportions of red (600-700 nm), and blue (400-500 nm) light (Casal, 2013; Franklin, 2008). For example, Kasperbauer (1971) showed the percent light penetration of several peak wavelengths ranging from 391-791 nm through single tobacco (*Nicotiana tabacum*) leaves; the percent penetration through a single leaf for wavelengths 391-483 (violet-blue-teal), 511-601 (green-yellow-orange), 601-686 (orange-red), and 725 and 791 (far-red) were 0.9-1.7%, 3.3-22.7%, 6.1-10.8%, and 27.5-49.5%, respectively. Similarly, the absorption, reflectance, and transmission of light by leaves has been demonstrated for several plants such as tomato (Casal, 2013; *Solanum* sp.), Arabidopsis (Franklin, 2008), soybean (Kasperbauer, 1987; *Glycine max*), chrysanthemum (Ouzounis et al., 2015; *Chrysanthemum morifolium*), *Pueraria lobata* (Kami et al., 2010), lettuce (*Lactuca sativa*), corn (*Zea mays*), and spinach (*Spinacia oleracea*) (Zhen and Bugbee, 2020a).

Prior to direct shading of leaves by neighboring foliage (i.e. when leaves are still receiving direct sunlight), the light environment within a plant canopy may be significantly altered due to the reflection of far-red radiation by the leaves of said neighbors (Casal, 2013, Franklin, 2008). Ballaré et. al (1987) evaluated light quality prior direct mutual shading of leaves in canopies of young *Datura ferox* (in this case, before the LAI of the canopy was greater than 0.3) and found that the R:FR ratio of light measured perpendicular to plant stems (i.e., facing nearby plants) was reduced compared to that measured at the same location parallel to the ground (i.e., facing up). Specifically, the R:FR measured parallel to the ground decreased from ~0.8 to 0.6 as the LAI increased from 0.01 to 0.1 while the R:FR measured facing up varied little from ~1.2. Similarly, Casal (2013) showed that at tomato planting densities of <20, 90, 120, and 600 plants·m⁻², the R:FR ratio measured at leaf level facing up remained at 1.1 for all densities sans 600 plants·m⁻² where it decreased to 0.7; in contrast the R:FR ratio measured perpendicular

to the stem was 1.1, 0.9, 0.8, and 0.5 at planting densities of <20, 90, 120, and 600 plants·m⁻², respectively. As the distance from nearby plant canopies or stands decreases, the R:FR ratio measured facing said canopies decrease significantly and the effect is greater if facing away from the primary light source (i.e., the sun) (Smith et al., 1990).

In the absence of vegetation, the R:FR ratio of sunlight striking the earth does not radically fluctuate during the day because of changes in weather, season, or other environmental conditions making it an excellent indication of vegetational shade (Casal, 2013; Franklin, 2008). Two notable exceptions to the consistent R:FR ratio occur at the beginning and end of each day due to the increased distance through the earth's atmosphere that solar radiation must pass through to reach the surface; this results in the increased scattering and absorption of short-wave relative to long-wave radiation (Franklin, 2008). For example, Kasperbauer (1987) measured the far-red radiation to red radiation ratio (FR:R; the inverse of the R:FR ratio now seldom used as a metric) for the last 2 hours of a sunny day in Florence, South Carolina. From 2 hours prior to sundown, the FR:R increased from <1 to a peak of ~3 between 12-0 minutes before nightfall, before decreasing to >1.5 at nightfall; during the measurement period photosynthetically active radiation (PAR) decreased from 1150 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ to less than 1 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Kasperbauer, 1987).

For plants growing under canopy shade or in high density plant habitats the reduction in overall light quantity or increased competition for access to light presents a serious threat to survival (Franklin, 2008; Smith and Whitelam, 1997). However, plants are unable to move to more favorable conditions and thus have evolved two general strategies in responses to shade: tolerance and avoidance; overlapping responses between the two strategies are not uncommon,

but the next section of this review focuses generally on the latter (Franklin, 2008; Gommers et al., 2013).

1.3 The Shade Avoidance Syndrome and Sensing Shade

While there is variability between and within species in specific responses to shade, shade avoiding species tend to undergo a series of common developmental, morphological, anatomical, and physiological changes which have been collectively referred to as the shade avoidance syndrome (SAS); the name was chosen to characterize the variety of “symptoms” that arise from growing under a low R:FR ratio (Franklin, 2008; Smith and Whitelam, 1997). The common SAS responses detailed by Smith and Whitelam (1997) included elongation internodes, hypocotyls, and leaves, leaf hyponasty, a decrease in leaf thickness and variable effects on leaf area, a decrease in chlorophyll content and shift in the chlorophyll a:b ratio, increased apical dominance and shoot to root ratio, and hastened flowering. For example, wild type *Arabidopsis* plants grown under white light supplemented with far-red light with R:FR ratios ranging from 0.32-0.035 showed longer hypocotyls compared to plants grown under white light (R:FR ~2.5) (Martínez-García et al., 2014). At less extreme R:FR ratios, the main stems of petunia (*Petunia ×hybrida*) ‘Countdown Burgandy’ grown in a greenhouse under a R:FR of 0.67 were 17% longer compared to control plants grown under a R:FR of 1.05 (Illias and Rajapakse, 2005).

Importantly, overhead canopy shade or the direct shading by leaves of nearby plants is not required to induce SAS responses, rather the presence of nearby vegetation per se serves as warning of potential competitors for light by altering the R:FR ratio within the plant canopy (Franklin, 2008). Ballaré et. al (1987) tested the hypothesis that plants can detect the presence of nearby competitors by way of the change in light quality of sunlight reflected by plants; these authors found that internode length of *Datura ferox* seedlings increased significantly from 9.4 to

10.5 mm as planting density increased from 32 to 100 plants·m⁻² prior to significant mutual shading by leaves. To better understand plant detection of nearby competitors with no influence of overhead shading, the authors positioned individually potted *Sinapis alba* 6 cm north (in the southern hemisphere) of healthy (green) or paraquat bleached (yellow) “fences” of wheat and rye grass; the seedlings grown adjacent to green fences showed a 54% increase in internode length compared to yellow fence adjacent seedlings (Ballaré et al., 1987). Ballaré et al. (1991) found that localized (around a single internode) perception of neutral shade (a decrease in intensity without significant spectrum alterations), neutral shade with a low R:FR, and a reduction in blue light can also promote internode elongation in *Datura ferox* and *Sinapis alba*.

Planting density or plant spacing is also known to affect field- and greenhouse-grown crops. In barley (*Hordeum vulgare*), greater internode elongation, longer leaf sheaths, reduced number of leaves, and an earlier transition to reproductive growth were found when barley rows bordered other dense rows of barley that reduced the R:FR compared to non-bordered rows (Davis and Simmons, 1994). Similarly, when greenhouse- and field-grown tomatoes were grown at plant spacings ranging from 23 to 60 cm, both overall plant height and internode length increased as plant spacing decreased (Papadopoulos and Ormrod, 1991).

The sensation of red and far-red radiation and the mediation of responses to the shade induced changes in the R:FR ratio is one function of the photoreceptor family, phytochrome. In angiosperms three phytochromes (phyA-C) are present in monocots while five (phyA-E) are present in the dicot, *Arabidopsis*; diverse phytochromes are also found in all other land plants and most green algae (Casal, 2013; Franklin, 2008; Ouzounis et al., 2015; Pham et al., 2018; Zheng et al., 2019). Phytochrome molecules display photoreversibility and exist within plant tissue in two forms; the biologically inactive red absorbing form (Pr) is constructed in the cytosol

of cells and is converted to the biologically active far-red absorbing form (Pfr) upon the absorption of red radiation wherein it moves to the nucleus to regulate photomorphogenesis through interaction with transcription factors (Ballaré and Pierik, 2017; Casal, 2013; Fernández-Milmanda and Ballaré, 2021; Galvão and Fankhauser, 2015). The Pfr form will shift back to the Pr form if far-red light is absorbed, but regular thermal reversion of Pfr to Pr occurs independently of the light environment (Ballaré and Pierik, 2017, Casal 2013; Fernández-Milmanda and Ballaré, 2021; Galvão and Fankhauser, 2015).

Phytochrome B (phyB) has been identified as the most important family member in regulating SAS responses to changes in the R:FR ratio with phytochromes D (phyD) and E (phyE) serving somewhat functionally redundant roles that can be seen when comparing phyB with phyBDE Arabidopsis mutants (Casal, 2013; Franklin and Quail, 2010). Phytochrome A (phyA) functions in the inhibition of elongation responses when the R:FR ratio is excessively low, and otherwise shows generally consistent activity under R:FR ratios from 0.3-1.1 (Casal, 2013); Martínez-García et al. (2014) found that phyA Arabidopsis mutants showed similar and relatively similar (compared to phyB mutants) hypocotyl elongation to wild type plants under white light or intermediate shade (R:FR~0.3) light, respectively, but as the R:FR decrease further (0.148-0.035) phyA mutant hypocotyl length progressively increased compared to wild type and phyB mutants.

The relative amount of Pfr to total phytochrome ($Pfr/Pfr+Pr$) is referred to as the phytochrome photoequilibrium [PPE; or phytochrome photostationary state (PSS)]; the estimated PPE decreases with the R:FR ratio and both serve as important metrics showing an inverse relationship to the intensity of SAS elongation responses (Ballaré and Pierik, 2017; Casal 2013; Kusuma and Bugbee, 2021). For example, Kalaitzoglou et al. (2019) showed that as the PPE of

light from LED and solar sources decreased from ~0.87 to 0.70, tomato height increased from ~90 to ~120 cm. Similarly, Casal (2013) showed that as the R:FR perceived by the stem of tomato plants decreased from 1.1 to 0.5, the mean stem growth rate per day increased from 1.4-4.0 mm·d⁻¹. While it won't be discussed in detail, it's important to note that both the R:FR ratio and the PPE are subject to valid criticisms such as a lack of standardization of wavelengths used to calculate the R:FR and the thermal reversion of phyB that affects PPE (Kusuma and Bugbee, 2021).

1.4 Vegetational Shade and Far-red Light Manipulation in Controlled Environments

While an overhead canopy of trees creating a far-red rich light environment would be a rare sight in a greenhouse production setting, vegetational shade and artificial alterations in far-red light can occur in greenhouses and plants retain their ability to perceive and respond to shade or the threats thereof. The use of hanging baskets above bench tops with actively growing plants is a common strategy used by growers to increase production and maximize the use of space, and extensive hanging basket canopies have been reported to result in low quality stretched bedding plants with fewer branches (Faust et al., 2014; Llewellyn et al., 2013; Mah et al., 2018).

Llewellyn et al. (2013) observed that the presence of a hanging basket canopy in a one-tier arrangement reduced the broad R:FR (600-700 nm and 700-800 nm) by 6.89-21.43%, resulting an average R:FR of 0.94 and 1.1 below and at hanging basket level, respectively; the DLI was reduced by 42.5% from 17.4 to 9.9 mols·m⁻²·d⁻¹ under the same tier system. Similarly, Faust et al. (2014) found that compared to control treatments with no hanging baskets, containers with poinsettia (*Euphorbia pulcherrima*) 'Freedom Red' hung 10 ft above the ground at a density of 2.57 containers·yd⁻² intercepted between 40-50% and >20% of the PPF in green and white containers, respectively, measured 1ft above the greenhouse floor; the R:FR did not drop below 1

in this study, and the authors concluded that the DLI reduction would occur first and affect benchtop plant growth prior reductions in the R:FR due to vegetational shade. Mah et al. (2018) investigated the morphological and flowering responses of petunia ‘Duvet Red’, marigold (*Tagetes erecta*) ‘Antigua Orange’, calibrachoa (*Calibrachoa ×hybrida*) ‘Kabloom Deep Blue’, and geranium (*Pelargonium ×hortorum*) ‘Pinto Premium Salmon’ grown under R:FR 0.7-1.1 (broad and narrow R:FR measurements were very similar) using LEDs in growth chambers, simulating the range of the R:FR found by Llewellyn et al. (2013). Calibrachoa, petunia, and marigold showed a 32%, 22%, and 11% increase in height growing under a R:FR of 0.7 compared to the 1.1 control, flower bud emergence occurred 2 days earlier in marigold under R:FR 0.7 compared to 1.1, and geranium showed 10% longer petioles under R:FR 0.7 compared to 1.1; the summarized results from this study includes only one of two experiments with D/N temperatures of 25.1/21.4°C while the other experiment was run at 20.4/18.3°C where effects were less pronounced (Mah et al., 2018).

As discussed previously, the proximity of nearby plants is sufficient to alter the R:FR perceived by plant stems and within a plant canopy, and this is likewise true for plant spacing in greenhouse-grown crops (Carvalho and Heuvelink, 2001; Hauser and Steinbacher, 2000; Papadopoulos and Pararajasingham, 1997). For example, Hauser and Steinbacher (2000) found that the R:FR ratio measured within the canopy of poinsettia ‘Peterstar’ was 0.88 at a plant density of 8 plants·m⁻² compared to 0.29 at 16 plants plants·m⁻² 10 days after potting; the R:FR of the above treatments decreased to 0.46 and 0.06, respectively, at 16 weeks post-potting. Similarly, Papadopoulos and Ormrod (1988) found that the narrowband R:FR (R:654-666 nm; FR:724-736 nm) measured at pot level for greenhouse-grown tomato cultivars ‘Jumbo’ and ‘CR-6’ increased from <0.4 to >0.6 as plant spacing increased from 38 to 60 cm, while plants spaced

closer than 38 cm showed higher R:FR ratios due to leaf senescence at lower canopy levels. At the same planting densities, Papadopoulos and Ormrod (1991) found a significant increase in plant height and internode length as plant spacing decreased from 60 to 23 cm, although the magnitude of increase depended on the growing season and the cultivar.

Outside of the effects of vegetation proximity and overhang, the use of artificial light sources as well as screens and films that selectively absorb regions of the electromagnetic spectrum have been shown to alter the light environment in controlled environment settings (Craig and Runkle, 2013; Craver et al., 2018; Khattak et al., 2004; Mattson and Erwin, 2005; Park and Runkle, 2019; Runkle and Heins, 2003). In greenhouses, relatively high natural light levels can limit spectral manipulation using supplemental lighting, but flexibility exists and can influence plant growth depending on species, the timing of artificial lighting, and the relative contribution of supplemental lighting and natural light to the DLI (Hernandez and Kubota, 2017). End-of-day (EOD) lighting is one commonly used strategy in horticultural production where light quality manipulation is more feasible; when applied with a low R:FR, EOD lighting has been shown to promote shade avoidance responses such as enhanced hypocotyl elongation in tomato and squash (Chia and Kubota, 2010; Yang et al., 2012). In SSL environments, no interference from an outside light source and the advent of LEDs with a variety of peak wavebands theoretically allows for extensive light quality manipulation including the incorporation of far-red radiation, but few peak wavebands are generally used in research, and grower choice is understandably limited in part by what is produced by manufacturers (Craver et al., 2018; Park and Runkle 2019; Wu et al., 2020).

1.5 Stem elongation and leaf expansion in response to far-red radiation

The most visible response of shade avoiding species growing under vegetational shade or under an artificial low R:FR ratio is an enhanced elongation of plant stems and petioles; in many cases the elongation of these structures occurs in tandem with inhibited leaf development (Franklin, 2008; Smith and Whitelam, 1997). In seedlings, the inactivation of phyB by low R:FR light quality results in increased phytochrome interacting factor (PIF) activity and subsequent synthesis of indole-3-acetic acid (IAA; biologically active auxin) in cotyledons; IAA is then transported to the hypocotyl where the auxin-induced elongation response occurs (Küpers et al., 2020; Ma and Li, 2019). There is significant auxin-promoted cell elongation that occurs in elongating plant structures under shade conditions including in hypocotyls, petioles, and internodes (Ma and Li, 2019). For example, Beall et al. (1996) grew *Phaseolus vulgaris* ‘Kentucky Wonder’ under a R:FR of 2.73 and 0.50 and found significantly longer internode cell lengths under the latter. Similarly, Bachman and McMahon (2006) found that far-red intercepting filters significantly decrease internode cell length compared to neutral filters in petunia ‘Celebrity White’.

When grown under a consistent low R:FR ratio during the entirety of the day or when treated with extended shade events in the afternoon prior to the dark period, elongation responses are clearly visible in several accessions of the model plant, *Arabidopsis*, wherein hypocotyl and petiole elongation are significantly enhanced relative to high R:FR treatments (Patel et al., 2013; Sellaro et al., 2012). For example, the Cape Verde Islands accession of *Arabidopsis* grown at 22°C had hypocotyls with average lengths of ~8 and ~4.6 mm under a low (0.1) and high R:FR, respectively (Patel et al., 2013). End-of-day light with a low R:FR has been shown to promote similar responses to continuous growth under a low R:FR (Franklin, 2008). However, EOD

treatments may less effectively promote elongation responses compared to continuous growth under a low R:FR, but the duration and timing of far-red treatments also play a role in responses (Demotes-Mainard et al., 2016; Morgan and Smith, 1978; Sellaro et al., 2012). For example, Sellaro et al. (2012) showed that a 2-hour shade event for the last two hours of a 10-hour photoperiod with a R:FR of 0.1 significantly increased hypocotyl length in *Arabidopsis* compared to a 10-minute EOD treatment with the same R:FR; these authors also show that 2-hour shade treatments applied at the beginning or middle of the photoperiod (i.e, returned to sunlight prior to night) did not effectively promote elongation responses compared to plants without a shade treatment; lastly, neither 2-hour shade treatments nor EOD treatments with a R:FR of 0.1 promoted hypocotyl elongation to the extent of growing under a constant R:FR of 0.1.

These responses to low R:FR light can occur quickly, and wild type *Arabidopsis* plants have been shown to increase hypocotyl elongation rate from 0.1 to 0.45 $\mu\text{m}\cdot\text{min}^{-1}$ in under an hour after supplementary far-red light reduced the R:FR from 2.37 to 0.23 (Cole et al., 2011). Work with *Sinapis alba* has shown that the site of perception of far-red influences the lag time prior to changes in elongation rates; applying far-red light directly to the first internode of the stem or two primary leaves leaf resulted in an observable increase in the stem extension rate after 10-15 minutes and 3-4 hours, respectively (Casal, 2013; Morgan et al., 1980).

As mentioned previously, the elongation responses promoted by changes in the R:FR are inversely correlated with the R:FR (Casal, 2013); several important floriculture crops such as petunia (Kim et al., 2002), *Zinnia elegans* (Cerny et al., 2003), chrysanthemum (Lund et al., 2007), and viola (*Viola xwittrockiana*) (Runkle and Heins, 2002) display this response as measured by overall stem length, plant height, and petiole length. For example, Lund et al. (2007) found that

plant height of chrysanthemum growing under EOD treatments ranging from 2.4-0.4 increased as the R:FR of EOD treatments decreased. Interestingly, R:FR ratios above ~1.2 can further inhibit elongation responses; Kim et al. (2002) found that for petunia plant height was significantly reduced when grown under a R:FR of 1.69 compared to 1.14.

Leaf expansion in response to far-red radiation varies by species, but EOD treatments and growth under artificial shade with a low R:FR have been shown to promote or inhibit leaf area expansion for multiple taxa (Casal et al., 1987; Demotes-Mainard et al., 2016; Kwesiga and Grace, 1986). For example, Casal et al. (1987) found that for *Petunia axilaris*, EOD-FR treatments with a low R:FR promoted leaf expansion compared to EOD-R treatments; EOD-R treatments were found to have a higher epidermal cell count than EOD-FR treatments, and the authors attributed leaf expansion under the latter in part due to larger epidermal cells. Similarly, Patel et al. (2013) found that low R:FR treatments that promoted leaf expansion in the *Arabidopsis Ler* accession also showed an associated increase in pavement cell area and general cell expansion relative to high R:FR grown plants. In contrast, Kwesiga and Grace (1986) found that for shade tolerant *Khaya senegalensis*, growth at low relative to high R:FR at multiple PPFDs resulted in lower leaf area measurements; the opposite was true for the shade intolerant *Terminalia ivorensis*. In *Arabidopsis* seedlings, a decrease in leaf area under a low R:FR was found to be due to reduced cell number caused by reduced cell proliferation in developing leaf primordia (Carabelli, 2007).

The effects of far-red light on leaf area expansion are thought to be in-part dependent on a sufficient quantity of PAR as a shortage results in the prioritization of stem elongation at the expense of leaf development (Demotes-Mainard et al., 2016; Heraut-Bron et al., 1999; Park and Runkle, 2017; Park and Runkle, 2018; Smith and Whitelam, 1997); this in-part aligns with Smith

and Whitelam's (1997) observation that under sufficient PAR, growth under a low R:FR leads to exaggerated shade avoidance responses. For example, *Terminalia ivorensis* grown under a PPFD of $250 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and a R:FR of 0.59 had an average leaf area of 115.9 cm^2 , over twice that of plants grown under $125 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and a R:FR of 0.28 (Kwesiga and Grace, 1986); in this study, leaf number also may have played a role in addition to individual leaf expansion as there was a difference of 3 leaves between the former and latter treatments (15.1 versus 12.1, respectively). Focusing on individual developing leaves, Heraut-Bron et al. (1999) grew clover (*Trifolium repens*) under either 320 or $110 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, while developing leaves were also exposed to a R:FR of 2.4 or 0.4 at each PPFD using far-red LEDs; leaves grown at $110 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ had similar (albeit larger compared to $320 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) leaf area regardless of the R:FR, but a R:FR of 0.4 at $320 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ increased leaf area compared to leaves treated with a R:FR of 2.4.

The method for applying far-red radiation may also differentially affect leaf expansion. Zou et al. (2019) found that for lettuce, growth under constant supplemental far-red radiation ($200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD; $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of far-red light) led to a greater increase in leaf area compared to 1-hour EOD-FR treatments with the same far-red intensity without PAR. For tomato, the addition of $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of far-red light to $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ provided by red-blue LEDs significantly increased leaf area (individual leaves and total leaf area) compared to treatments with no far-red light, while a 15-minute EOD-FR treatment (R:FR 0.1) did not promote a significant increase in leaf area (Kalaitzoglou et al., 2019); the lack of leaf expansion in the latter treatment could have also been influenced by the short duration of the EOD treatment. Importantly, in both experiments, leaf expansion in response to far-red light

contributed to greater light interception relative to treatments without far-red light (Kalaitzoglou et al., 2019; Zou et al., 2019).

1.6 Flowering and Far-red Radiation

The transition from vegetative to reproductive growth in a variety of plant taxa is regulated by environmental signals such as temperature and photoperiod as well as the integration of multiple signals (Franklin, 2009; Song et al., 2015); inter- and intraspecific endogenous cues such as the required transition from the juvenile to adult phase prior to the promotion of flowering by environmental signaling also play a large role in timing of flowering, and DLI can significantly influence the time required for flowering for several ornamental plants (Amasino and Michaels, 2010; Faust et al., 2005; Jackson, 2009). Light quality also plays an important role in flowering and Smith and Whitelam (1997) highlighted that the acceleration of flowering under low R:FR growing conditions was an important piece of the SAS; this early flowering response is a form of temporal shade avoidance wherein plants at risk of being overtaken and shaded by competitors may complete their lifecycle (Casal, 2013). Interestingly, until the 1960s phytochrome was generally accepted as the “photoperiodic timer” with the Pfr form serving to repress flowering; night length determined the amount of Pfr reverted to Pr and thus under shorter nights flowering was inhibited due to increased Pfr, but this is no longer the accepted explanation concerning flowering responses (Song et al., 2015). In nature, the ability to synchronize reproductive growth to key seasonal environmental signals is adaptive in ensuring reproductive conditions are favorable (Jackson, 2009), and understanding these responses allows for the successful manipulation of environmental signals to promote reproductive responses in horticultural crops (Runkle et al., 2017).

In flowering plants, taxa are often classified based on photoperiodic flowering responses that depend on the length of the night, but are labeled with regard to day length; day-neutral plants show an indifferent flowering response to day length, facultative long-day (LDP) and short-day (SDP) plants will flower earlier under short and long nights, respectively, while obligate LDP and SDP require short and long nights, respectively, to flower; what defines a “short” or “long” night varies inter- and intraspecifically (Jackson, 2009; Runkle et al., 2017). To be more specific, Erwin and Warner (2002) classified facultative SDP and LDP as those that have fewer leaves at flowering under inductive versus non-inductive photoperiods. As an example, Mattson and Erwin (2005) used Erwin and Warner’s (2002) classification to examine 41 herbaceous species grown under 8-hour short days or 18-hour long days using day-extension lighting or night interruption strategies; examples of facultative LDP, facultative SDP, obligate LDP, obligate SDP, and DNP included were *Collinsia heterophylla*, *Cosmos bipinnatus* ‘Sensation White’, *Ipomopsis rubra* ‘Hummingbird Mix’, *Dolichos lablab*, and *Amaranthus hybridus* ‘Pygmy Torch’, respectively. For growers, the timing of flowering crop production may not synchronize well with natural day lengths due to seasonality and latitude, so day length must be manipulated with artificial lighting to ensure promotion or inhibition of flowering depending on the crop. Day-extension and night-interruption lighting strategies are both effective means to manipulate flowering, with the consensus being that 4 hours of continuous night-interruption lighting in the middle of the dark period or slightly longer periods of day extension lighting at an intensity of at least $2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ effectively promotes and inhibits flowering in LDP and SDP plants, respectively (Runkle et al., 2017).

At a molecular level, photoperiodic flowering is complex, but central to photoperiodic flowering in LDP is the Flowering Locus T (FT) protein that is synthesized in leaves and travels

to the shoot apical meristem wherein it causes the shift from vegetative to reproductive growth (Demotes-Mainard et al., 2016; Song et al., 2015). The expression of the FT gene is promoted at the end of long days most importantly by CONSTANS (CO), a transcription activator with highly regulated expression levels and protein abundance by extensive circadian rhythm and photoreceptor action (Song et al., 2015). Especially important in the context of light quality used in the horticultural production of flowering crops are the action of the photoreceptors FKF1, cryptochromes, phyA, and phyB; the CO protein is stabilized by the action of these blue light (FKF1 and cryptochromes) and far-red light (phyA) activated photoreceptors while red light activated phyB works to destabilize the CO protein (Song et al., 2015). Photoperiodic flowering has also been studied in the SDP rice where flowering is regulated by phyA and red light that inhibit flowering in long days (Demotes-Mainard, 2016; Song et al., 2015).

For several species, growth under low R:FR ratios results in hastened flowering (Demotes-Mainard, 2016), and the effects of far-red light on flowering of several horticulturally important crops in controlled environments have been investigated through the use of spectrum excluding screens in greenhouses during the regular photoperiod (Cerny et al., 2003; Ilias and Rajapakse, 2005; Runkle and Heins, 2003; Runkle and Heins, 2002; Runkle and Heins, 2001), supplemental far-red in SSL and greenhouse environments (Craver et al., 2018; Kalaitzoglou et al., 2019; Kohler and Lopez, 2021; Mah et al., 2018; Park and Runkle, 2018) as well as through light quality manipulations of night-interruption, day-extension lighting, or EOD lighting (Craig and Runkle, 2012; Craig and Runkle, 2013; Craig and Runkle 2016; Kalaitzoglou et al., 2019; Garret Owen et al., 2018; Ilias and Rajapakse, 2005). However, species-specific responses are apparent, and the effects of far-red radiation on flowering can be affected by the duration and timing of far-red light, the length of the photoperiod, DLI, and the R:FR ratio of electric lighting

(Cerny et al., 2003; Craig and Runkle, 2016; Garrett et al., 2018; Kohler and Lopez, 2021). For example, using photoselective films that screened out red light (R:FR ~0.77), far-red light (R:FR ~1.51), and a neutral shade film (R:FR ~1.05), Cerny et al. (2003) showed the effects on flowering of six species including three SDP: zinnia (*Zinnia elegans*) ‘Cherry Ruffles’ (zinnia), cosmos (*Cosmos bipinnatus*) ‘Sonata White’; two LDP: snapdragon (*Antirrhinum majus*) ‘Ribbon White’ and petunia ‘Supercascade Burgundy’; and one DNP: rose (*Rosa ×hybrida*) ‘Cherry Cupido’. In their study, SDP plants under inductive and non-inductive photoperiods showed minimal or no difference in days to anthesis under far-red excluding films compared to control or red-light excluding films, and rose showed no difference regardless of film; in contrast, LDP showed an increase of 7 and 13 days to anthesis under non-inductive photoperiods for snapdragon and petunia, respectively, but when petunia was grown under an inductive photoperiod no difference in time to anthesis was found between films.

1.7 Photosynthetic responses to far-red radiation

Far-red photons remain excluded from the definition of PAR due to the relative ineffectiveness of monochromatic far-red light in driving photosynthesis compared to shorter wavelengths (Zhen et al., 2021). For example, Emerson and Lewis (1943) showed that for the green algae, *Chlorella*, wavelengths of light longer than 685 nm led to a severely reduced quantum yield. McCree (1972) and Inada (1976) later showed the action spectra and quantum yield of a diverse group of species which also showed drastic decreases in photosynthetic activity in the far-red region (Zhen and Bugbee, 2020a). However, Emerson et al. (1957) showed that when applied concurrently, wavelengths shorter and longer than 680 nm resulted in an increase in photosynthesis relative to the summed rates of longer and shorter wavelengths applied separately; this is due to the balanced excitation of reaction centers of photosystems I (PSI) and

II (PSII) that absorb longer and shorter wavelengths, respectively (St. Onge, 2018, Zhen et al., 2021; Zhen and van Iersel, 2017). For example, short-term leaf-level photosynthesis measurements on tomato at a total photon flux density (TPFD; 400-800 nm) of $160 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ showed higher rates of photosynthesis under red-blue LEDs with added far-red (PSS ~0.80) compared to solely red-blue light (PSS~0.87) (Kalitzoglou et al., 2019).

Recent research incorporating far-red wavelengths into backgrounds of white or red-blue LEDs for plant production has further confirmed that far-red photons ineffectively drive photosynthesis when alone but do enhance photosynthesis when applied concurrently with PAR; there is additional evidence that PAR should be expanded to include far-red radiation (700-750 nm) (Zhen et al., 2021). Zhen and Bugbee (2020a) showed that canopy gross photosynthetic rate for several crops including lettuce, corn, and wheat was significantly reduced under far-red light ($\text{PFD} = 70 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) alone compared with white light at the same intensity. Additionally, when far-red light ranging in PFD from 40-140 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was added to a background of 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of white light, gross canopy photosynthesis rate of lettuce increased by 6.7-20%. In the same study, the addition of equal intensities of white light resulted in similar increases in the photosynthetic rate, but the significantly lower absorption of far-red relative to white photons suggested that added far-red light improved photosynthesis by balancing photosystem excitation. Zhen and Bugbee (2020b) also showed that the quantum yield of lettuce canopies under red-blue LEDs and white LEDs with or without 15% of the TPFD comprising the 700-750 nm range was similar. Zhen et al. (2021) argue that PAR should be expanded to include 700-750 nm; these authors include important caveats for this proposed change including the exclusion of wavelengths >750 nm due to minimal effects on photosynthetic enhancement, a far-red photon

limit of 30% of TPDF due to a lack of further photosynthetic rate enhancement beyond this percentage, and a practical limit of 20% of total TPDF to limit excessive elongation effects.

1.8 Ambient Temperature, DIF, and Far-red-temperature interactions

Temperature significantly influences the rate of plant growth and development, and the minimum, maximum, and optimum temperature range preferences of plants are species-specific; conditions outside of this temperature range can severely inhibit growth and development (Bahunga and Jagadish, 2015; Hatfield and Prueger, 2015). Additionally, ambient temperature as well as the difference (DIF) in alternating day (DT) and night (NT) temperatures can promote or inhibit specific morphological changes in plants resulting in a change in final plant form (Casal and Balasubramanian 2019). In controlled environment production, temperature manipulation can be relatively precise and is used to achieve several production goals including the control of plant height in lieu of plant growth regulators (Myster and Moe, 1995). Additionally, some studies have examined interactive and/or additive effects of far-red radiation and diurnal temperature alterations as well as the effects of ambient temperature on plant responses to shade or a low R:FR (Bachman and McMahon, 2006, Blom and Kerec, 2003; Patel et al., 2013; Qaderi et al., 2015; Slauenwhite and Qaderi, 2013; Thingnaes et al., 2008; Xiong et al., 2002).

Myster and Moe (1995) conducted a review on the effects of diurnal temperature changes on greenhouse crop morphology, $DT > NT$ (+DIF) promotes greater stem or internode elongation, plant height, and petiole length while $DT < NT$ (-DIF) inhibits these parameters. Several species generally follow this trend such as viola (Niu et al., 2000), Arabidopsis (Thingnaes et al., 2003; Thingnaes et al., 2008), petunia (Bachman and McMahon, 2006), cucumber (*Cucumis sativus*) (Grimstad and Frimanslund, 1993; Xiong et al., 2002), *Lilium longiflorum* (Erwin et al., 1994; *L. longiflorum*), *Lilium* spp. (Blom and Kerec, 2003; *L. spp.*),

and *Fuchsia ×hybrida* (Patil et al., 2001). In both petunia and *L. longiflorum*, +DIF also promoted increases in cell length in stems compared to -DIF while cell number was similar between treatments indicating that longer stems in response to DIF are due to increased cell length rather than a greater number of cells (Bachman and McMahon, 2006; Erwin et al., 1994). Significantly decreasing temperature for 2-4 hours during the 24-hour growth cycle (DIP) has also been shown to be an effective strategy in reducing stem elongation and plant height in some species (Myser and Moe, 1995). For example, Shibaeva et al. (2018) grew cucumber seedlings at an air temperature of 23°C and exposed seedlings to six, 2-hour 10°C DIP treatments that began during the dark period at 2100, 0200, or 0700-0900; or during the day at 0900, 1400, and 1900; these authors found that all DIP treatments reduced plant height similarly compared to control plants (no DIP), but DIP treatments during the day also generally reduced leaf area and total plant dry mass.

The effects of EOD-light quality on DIF responses have been studied in multiple species, and EOD-FR treatments have been shown to enhance and inhibit responses of +DIF and -DIF, respectively (Blom and Kerec, 2003; Moe et al., 1991; Thingnaes et al., 2008; Xiong et al., 2002). Xiong et al. (2002) conducted an experiment with cucumber wherein cucumber seedlings were grown under +DIF or -DIF (25/19°C and 19/25°C, respectively) and either an EOD-R or EOD-FR (30 minutes) light treatment; petiole, internode, and hypocotyl responses to DIF followed the normal trends, but EOD-FR enhanced +DIF responses and inhibited -DIF responses resulting in longer stems under EOD-FR regardless of DIF treatment. Similarly, Thingnaes et al. (2008) grew wild-type *Arabidopsis* under +DIF or -DIF (25/17°C and 17/25°C, respectively) with either EOD-R or EOD-FR treatments; under EOD-R, +DIF promoted increased petiole

length compared to -DIF once again resembling the conventional DIF responses, but EOD-FR slightly increased petiole length under +DIF and drastically increased petiole length under -DIF.

Importantly, Thingnaes et al. (2008) and Xiong et al. (2002) utilized phyB and long-hypocotyl mutants of *Arabidopsis* and cucumber, respectively, and showed a significant role for phyB in thermoperiodic elongation as mutants showed inhibited responses to DIF. Additionally, plant phenotypic responses to higher temperatures have been compared to typical shade avoidance responses (Franklin, 2009; Patel and Franklin, 2009), and phyB has been shown to function as a temperature sensor wherein warmer temperatures promote a faster reversion of the active to inactive forms of phyB independent of light (Casal and Balasubramanian, 2019; Legris et al., 2016). Patel et al. (2013) noted that few studies examining shade avoidance responses incorporated ambient temperature as a potential interacting factor but cited findings of Franklin et al. (2003); the latter authors observed that *Arabidopsis* grown under a low R:FR (~0.09) at 16°C, showed increased leaf area, which contrasted with previous findings potentially due to growth temperatures.

Patel et al. (2013) further investigated *Arabidopsis* responses to low R:FR (0.1) at 16°C and 22°C; for the temperate accession *Ler*, growth at 22°C under a low R:FR resulted in elongated petioles and thinner leaves compared to high R:FR control plants, but under 16°C petiole length between high and low R:FR treatments was similar while 16°C plants had greater leaf area and thickness compared to all other treatments; light and scanning electron microscopy revealed leaf responses were likely due to increased cell expansion. In contrast, the tropical Cape Verdes Island accession showed phenotypically similar low R:FR responses regardless of temperature that were characteristic of typical shade avoidance responses. This showed variability in responses within a species accustomed to different climates, and one conclusion of

the authors was that the temperature-dependent shade avoidance responses observed may be morphologically advantageous in ensuring light capture in tandem with reducing the risk of succumbing to heat or cold stress (Patel et al., 2013).

1.9 Further Research

Using far-red radiation to promote leaf expansion for young plant production may reduce wasted light from artificial lighting, while improved light interception might also improve plant quality or reduce production time. However, quality-reducing responses such as the excessive elongation of stems and leaves may negate the benefits of improved light interception. We first investigate the use of EOD-FR radiation in a greenhouse under low DLI conditions, and then shift to examining the relationship between far-red radiation and non-limiting PPFD and DLI, as well how shade avoidance responses in petunia may be affected by growth under low temperature.

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CHAPTER 2. END-OF-DAY FAR-RED LIGHTING WITH A LOW DAILY LIGHT INTEGRAL INCREASES STEM LENGTH BUT DOES NOT PROMOTE EARLY LEAF EXPANSION FOR *PETUNIA* × *HYBRIDA* SEEDLINGS

2.1 Summary

Young annual bedding plant (plug) production in the northern hemisphere typically begins in midwinter to early spring when the daily light integral (DLI) in greenhouse environments is low. While supplemental lighting provides a means to compensate for low DLIs, the electricity for this input contributes significantly to operating costs. Additionally, supplemental lighting may be applied as a static input at all stages of seedling development, resulting in “wasted” light due to a low leaf area index (LAI). An early increase in leaf area may allow for more effective radiation capture by seedlings and a reduction in wasted light. Therefore, the objective of this study was to examine the effects of end-of-day far-red (EOD-FR) lighting varying in intensity, red to far-red ratio (R:FR), and duration on *Petunia* × *hybrida* ‘Wave Purple’ and ‘Dreams Midnight’ to determine the effects on early leaf expansion and seedling quality.

Seedlings were grown in 128-cell trays in a common greenhouse environment. Four end-of-day (EOD) treatments were examined in this study, with seedlings receiving one of the following treatments for 2 or 3 weeks: 10 $\mu\text{mol} \cdot \text{m}^2 \cdot \text{s}^{-1}$ of far-red light (R:FR ~0.8) for 30 minutes, 10 or 20 $\mu\text{mol} \cdot \text{m}^2 \cdot \text{s}^{-1}$ of far-red light (R:FR ~0.15) for 30 minutes, or 20 $\mu\text{mol} \cdot \text{m}^2 \cdot \text{s}^{-1}$ of far-red light (R:FR ~0.15) for 240 minutes. In addition to EOD treatments, seedlings were grown under a supplemental lighting treatment (target photosynthetic photon flux density of 70 $\mu\text{mol} \cdot \text{m}^2 \cdot \text{s}^{-1}$) or moved under supplemental lighting after 2 or 3 weeks of EOD lighting. Destructive data was collected 2, 3, and 4 weeks after treatment initiation.

Seedlings that received EOD-FR lighting did not show any increase in leaf area compared to control (no supplemental or EOD lighting) or supplemental lighting treatment. Stem length generally increased under EOD-FR lighting treatments compared to the supplemental lighting treatment and the control, with greater increases under treatments with lower R:FR ratios and increased duration of the EOD treatment. Results of this study indicate that under low DLIs, EOD-FR radiation applied in the first three weeks of seedling production does not promote early leaf area expansion, nor increase overall seedling quality. As responses to artificial shade may vary based on study taxa, incident radiation, and DLI, future research examining EOD-FR induced morphological changes are warranted and may lead to strategies growers can use to reduce wasted supplemental lighting or improve production through enhanced light capture.

2.2 Introduction

The recommended daily light integral (DLI) to produce high quality annual bedding plant plugs is 10 to 12 mol·m⁻²·d⁻¹ (Lopez and Runkle, 2008; Pramuk and Runkle, 2005). However, as the production of young plants often begins in mid-winter to early spring, northern latitude greenhouses experience a low angle of insolation in tandem with decreased day length resulting in DLIs as low as 1 to 5 mol·m⁻²·d⁻¹, with further decreases as latitude increases or with the presence of cloud cover (Both and Faust, 2017; Faust and Logan, 2018; Lopez and Runkle, 2008; Pramuk and Runkle, 2005; Styer, 2003).

As DLI decreases, many metrics of plug quality, such as average shoot dry mass per internode (Pramuk and Runkle, 2005), stem caliper (Craver et al. 2019), and root (RDM) and shoot dry mass (Oh et al., 2010; Poel and Runkle, 2017a), also decrease. Increasing the DLI when natural light is low requires the use of supplemental lighting, but the costs of supplemental lighting can be extensive (van Iersel and Gianino, 2017). Additionally, Oh et al. (2010) showed

that supplemental lighting does not provide equal benefit at all stages of seedling development; in this study, supplemental lighting provided to seedlings of *Petunia ×hybrida* (petunia) ‘Madness Red’ and *Viola ×wittrockiana* (pansy) ‘Delta Premium Yellow’ during the 4-6 and 3-4 leaf stage, respectively, led to greater or similar dry mass at transplant compared to when supplemental lighting was applied while cotyledons were developing and during the 1-3 or 1-2 leaf stage. Thus, it may be advantageous to promote early leaf expansion in young plants to facilitate increased light capture when DLI is low or lighting costs are limiting to growers, and to increase the efficacy of supplemental lighting by reducing “wasted light” that may strike substrate, plug trays, or bench space.

A decreased ratio of red relative to far-red radiation (R:FR) perceived by the photoreceptor family phytochrome is one important signal for plants of current or future shade; the change in the R:FR under forest canopies or in dense plantings is due to the relative decrease in transmission of red light and increased transmission and reflectance of far-red light, and shade avoiding plants exposed to decreased R:FR undergo a variety of shade avoidance responses such as hypocotyl, internode, and petiole elongation to improve light capture (Ballaré and Pierik, 2017; Casal, 2013; Franklin, 2008; Smith and Whitelam, 1997). Increases, decreases, and minimal effects of shade light quality or supplemental far-red light on leaf area expansion have also been reported and vary interspecifically and with leaf age or photosynthate availability (Casal et al., 1987; Casal and Smith, 1989; Demotes-Mainard et al., 2016; Park and Runkle, 2017).

The addition of far-red light in sole source lighting (SSL) environments has been shown to increase leaf area in *Arabidopsis thaliana* (Arabidopsis) (Franklin et al., 2003; Patel et al. 2013), *Antirrhinum majus* (snapdragon), *Pelargonium ×hortorum* (geranium), petunia (Park and

Runkle, 2016; Park and Runkle 2017; Park and Runkle, 2018), and *Solanum lycopersicum* (tomato) (Kalaitzoglou et al. 2019). For example, Kalaitzoglou et al. (2019) found that an addition of $54 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of far-red to a background photosynthetic photon flux density (PPFD) of $149 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ increased leaf area of tomato “Komeett” by 3%, facilitating an increase in whole plant light absorption by 10% compared to plants with no added far-red light. Similarly, Park and Runkle (2018) found that leaf area of petunia ‘Wave Blue’ seedlings increased as the R:FR ratio decreased. In the above-mentioned experiments, significant increases in plant height often accompanied any leaf area expansion and is an undesirable response for annual bedding plant seedlings.

An SSL research environment allows for the relative customization of light quality to promote plant morphological change but using supplemental lighting to achieve the same within greenhouses is more difficult, especially when the relative contribution of the supplemental lighting to total light is low (Craver et al., 2019; Hernandez and Kubota, 2014; Poel and Runkle, 2017a; Poel and Runkle, 2017b,). Thus, the use of end-of-day (EOD) treatments with a low R:FR ratio may be more effective in eliciting desirable responses in a greenhouse environment. End-of-day far-red (EOD-FR) treatments have been found to promote shade avoidance responses such as hypocotyl elongation (Chia and Kubota, 2010; Mizuno et al. 2015; Yang et al., 2012), leaf expansion (Casal et al., 1987; Casal and Sadras, 1987) and internode elongation (Kalaitzoglou et al. 2019). Additionally, lower R:FR ratios applied as EOD-FR or as simulated shade during the day often increase the intensity of elongation responses (Chia and Kubota, 2010; Kalitzoglou et al. 2019; Lund et al., 2007), while other responses such as leaf area expansion may in part depend on resource availability or age of the leaves (Casal et al., 1987; Casal and Sadras, 1987; Park and Runkle, 2018). Some responses to far-red light such as

hypocotyl elongation have been shown to be dose dependent (Chia and Kubota, 2010; Yang et al., 2012).

Due to the relatively high costs of lighting and the importance of timing supplemental light application, it is worth examining whether EOD-FR can elicit increases in the leaf area index (LAI) of young annual bedding plants to improve radiation capture and more efficiently utilize supplemental lighting. Additionally, as prolonged EOD-FR treatments may lead to intense elongation responses, it is necessary to examine leaf area expansion at multiple time points. Thus, the objectives of this study were to 1) investigate the effects of EOD-FR radiation (R:FR ratio, far-red intensity, and duration) on early leaf expansion under low DLI; 2) investigate if increasing DLI through supplemental lighting increases plug quality post-cessation of EOD-FR treatments if early leaf expansion occurs; and 3) quantify detrimental shade avoidance responses such as excessive elongation, attributable to EOD-FR treatments. To improve the applicability of this study to greenhouse production, commercially available flowering lamps in addition to programable light emitting diode (LED) fixtures were utilized to examine the effects of differing R:FR ratios. As past research has shown that leaf expansion and stem elongation in *Petunia* spp. have occurred in response to supplemental far-red light and EOD-FR, we selected two petunia cultivars, ‘Wave Purple’ (‘WP’) and ‘Dreams Midnight’ (‘DM’) to examine treatment responses.

2.3 Materials and Methods

2.3.1 Plant Material and Greenhouse Environment

Seeds of petunia ‘WP’ and ‘DM’ were sown between September 12th and October 7th, 2020, in 128-cell trays (15mL individual cell volume) using a commercial soilless germination medium (Berger BM2 Seed Germination; Berger, Saint-Modeste, QC). Trays were germinated under a translucent plastic tarp in a common greenhouse environment at the Colorado State University Horticulture Center (Fort Collins, CO). Upon germination, trays were transferred

underneath shade cloth covered polyvinyl chloride (PVC) pipe structures to simulate wintertime light conditions.

Greenhouse air temperature was controlled using a Veristep integrated environmental control system (Wadsworth Control Systems, Arvada, CO) with a target air temperature of 21/19 °C (D/N). Seedlings were irrigated as needed with water-soluble fertilizer (Jack's 13-2-13 Plug LX, J.R. Peters, Inc., Allentown, PA) providing (in $\text{mg}\cdot\text{L}^{-1}$) 150 nitrogen (N), 23 phosphorus (P), 150 potassium (K), 69 calcium (Ca), 34 magnesium (Mg), 0.15 boron (B), 0.07 copper (Cu), 0.75 iron (Fe), 0.37 manganese (Mn), 0.07 molybdenum (Mo), and 0.37 zinc (Zn). Appropriate pH and electrical conductivity of the water-soluble fertilizer was confirmed using a handheld meter (Growline H19814, Hanna Instruments, Woonsocket, RI).

2.3.2 Greenhouse Supplemental Lighting and EOD-FR Treatments

Shade structures described above were used to simulate the light environment commonly reported in northern hemisphere greenhouse production facilities during the winter season. Two shade structures were deployed per experimental replication with one serving as a wintertime control (WTC) and the other serving as wintertime control with supplemental lighting (WSL). The WSL environment was created by hanging a supplemental lighting fixture (Phillips GreenPower LED Toplighting Linear 2.1, Koninklijke Philips N.V., Amsterdam, Netherlands) 1.15 m above the greenhouse bench (6 cm above shade structures) for the duration of the study. The fixture provided a target total photon flux density (TPFD) of $70\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with a 14-hour photoperiod (0600-2000). Light intensity and spectrum were measured using a spectrometer (LI-180; LI-COR Biosciences, Lincoln, NE), and mean TPFD, photon flux density (PFD), and R:FR for the WSL environment are reported in Table 1. For the duration of the study, one plug tray per replication per cultivar was placed underneath the WTC to serve as a low DLI control (CN) from 0800-1830 (moved under blackout at 1830 for a 10.5-hr photoperiod), and one plug tray per

replication per cultivar was placed underneath the WSL to serve as a supplemental lighting control (SR). All trays receiving EOD-FR were placed under the WTC shade structure during the day. Tray position was randomized every day to minimize any effects of uneven radiation distribution.

Two PVC structures covered with blackout cloth to prevent outside light interference were used to apply EOD-FR treatments. Under the first blackout structure, three flowering lamps (FL; Philips GreenPower LED DR/W/FR, Koninklijke Philips N.V.) were mounted 0.68 m above the greenhouse bench to provide a target far-red PFD of $10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at canopy height (Table 1, Fig. 1B). Under the second blackout structure, a programmable LED fixture (Elixia; Heliospectra, Fiskhamngatan, Sweden) was mounted 0.71 m above the greenhouse bench to provide two EOD-FR light environments based on proximity to the fixture with a target far-red PFD of 10 and $20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at canopy height. Light intensity and spectrum were measured using a spectrometer (LI-180; LI-COR Inc.) and a summary of mean TPDF, PFD, and R:FR for each EOD-FR environment is reported in Table 1, and spectral quality of WSL and EOD-FR environments are reported in Figure 1.

Using the EOD-FR environments described previously, four treatments were established varying in far-red PFD (10 or $20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), R:FR ratio (0.15 or 0.80), and duration of EOD-FR treatment (30 or 240 min). EOD-FR treatments are designated as “EOD” with subscripts describing the light source as with FL (EOD_{FL}), or in the case of Elixia fixtures, describing the intensity of far-red light and the duration of the EOD light treatment. Thus, EOD treatments included EOD_{FL} (30 minutes of EOD-FR under FL), EOD_{10:30} (30 minutes of EOD-FR with far-red PFD $10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), EOD_{20:30} (30 minutes of EOD-FR with far-red PFD of $20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

¹), and EOD_{20:240} (240 minutes of EOD-FR with far-red PFD of 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Treatment summaries including light source and duration of EOD application are reported in Table 2.

At 1800 HR every evening, all EOD-FR treatments were removed from the WTC and placed under their respective light sources. Trays were positioned randomly every evening to minimize any effect of uneven radiation distribution. At the end of the EOD period, treatments were placed under blackout cloth until 0800 HR the following day at which time they were returned to the WTC. EOD-FR treatments ceased after 21 days due to no observable effect on leaf area compared to control seedlings.

Two dynamic lighting treatments were used to examine if early leaf expansion promoted by EOD-FR treatments during the first 14 and 21 days could benefit from supplemental lighting due to a potential increased radiation capture capacity. Specifically, after 14 days, one tray from EOD_{20:240} was moved to the WSL structure and received no further EOD-FR for the duration of the experiment (EOD₂SR₁; subscripts describe the number of weeks under EOD-FR and supplemental lighting conditions). Additionally, after 21 days one tray from EOD_{FL} was moved to the WSL structure and received no further EOD-FR for the remainder of the experiment (EOD₃SR₁). Dynamic lighting treatment summaries including EOD-FR light source and days under EOD-FR and WSL conditions are reported in Table 3.

Air temperature and PPFD were measured every 15 s using precision thermistors [fan-aspirated solar radiation shields (ST-110; Apogee Instruments, Inc., Logan, UT)] and quantum sensors (LI-190R; LI-COR Biosciences, Lincoln, NE), respectively, and the average was logged every 15 min by a data logger (CR1000X; Campbell Scientific, Inc., Logan, UT). The mean $\pm$ SD canopy air temperature (D/N), WTC DLI, and WSL DLI averaged across four replications

was 21.18 ± 0.86 °C/ 18.90 ± 0.39 °C, $5.26 \text{ mol} \cdot \text{m}^2 \cdot \text{d}^{-1} \pm 2.32$, and $8.98 \text{ mol} \cdot \text{m}^2 \cdot \text{d}^{-1} \pm 1.00$, respectively.

2.3.3 Seedling Data Collection.

Seedling data was collected 14, 21, and 28 days after treatment initiation, and five seedlings from each treatment per cultivar were randomly selected for measurement and analysis. Roots of selected seedlings were thoroughly washed and measurements were taken including stem length (cm; measured from the base of the hypocotyl to the shoot apical meristem), stem caliper [mm; measured directly under and perpendicular to cotyledons using a digital caliper (Fisherbrand™ Traceable™, Thermo Fisher Scientific, Waltham, WA)], and relative chlorophyll content [RCC; measured on the youngest fully expanded leaf using a SPAD chlorophyll meter (Chlorophyll Meter SPAD-502Plus, Konica Minolta, Chiyoda City, Tokyo, Japan)]. Leaves were removed from seedlings at the node to be counted and leaf area (cm²) was determined using a leaf area meter (LI-3100; LI-COR Biosciences). Leaves, stems, and roots of each measured seedling were separated and dried at 70 °C to determine the dry mass of each using an analytical microbalance (Analytical Balance ME54E, Mettler Toledo Ltd, Columbus, OH). Leaf area index was calculated by dividing individual seedling leaf area by the area of a tray cell (9 cm²), and leaf mass area (LMA; g·m⁻²) was calculated by dividing individual seedling leaf area by leaf dry mass (LDM).

2.3.4 Statistical Analysis.

This experiment was a randomized complete block design with EOD-FR, CN, SR (7 levels) as treatment factors and replication (4 levels) as a blocking variable; cultivars were evaluated separately. Four experimental replications were conducted from early September to late-November 2020 with replications one/two and three/four occurring over the same period in separate locations in the greenhouse bay with similar light and temperature conditions. Analyses

on days 14 and 21 includes all treatments sans EOD₃SR₁, while day 28 only includes treatments CN, SR, and EOD₃SR₁ due to a lack of leaf area expansion observed in any EOD-FR treatment. For replication three at day 21, treatments EOD_{20:30} and EOD_{20:240} for ‘DM’ were found to have pest presence and were excluded from analysis. At day 21, analysis of root dry mass (RDM) for ‘WP’ included only 19 measurements for EOD_{10:30}, EOD_{20:30} and EOD_{FL} due to zero values displayed by the microbalance; for the same reason, ‘WP’ analysis included only 19, 17, 10, 14, and 18 measurements for CN, EOD_{10:30}, EOD_{20:240}, EOD_{20:30}, and EOD_{FL}, respectively. The effects of EOD-FR and dynamic lighting treatments on the parameters described above were compared by two-way anova using R statistical software and pairwise comparison of estimated marginal means using Tukey’s HSD at $p < 0.05$ (Lenth, 2021; R Core Team, 2021).

2.4 Results

2.4.1 Stem Length and Caliper

Generally, EOD-FR radiation treatments promoted stem elongation in both cultivars (Fig. 2C-D). Specifically, at day 14 and 21, seedlings under EOD_{20:240}, EOD_{20:30}, and EOD_{10:30} had longer stems compared to control seedlings. Additionally, stem length in EOD_{20:240} was longer than all other treatments sans EOD₂SR₁. For example, stem length of petunia ‘DM’ was 25%, 26%, 37%, 64%, and 105% longer under EOD_{20:240} compared to EOD_{10:30}, EOD_{20:30}, EOD_{FL}, CN, and SR, respectively, at day 21. Similarly, petunia ‘WP’ EOD₂SR₁ had stems that were 60%, 72%, 115%, 132%, and 201% longer than EOD_{10:30}, EOD_{20:30}, EOD_{FL}, CN, and SR respectively, at day 21. In contrast to the other EOD-FR treatments, seedlings of petunia ‘DM’ grown under EOD_{FL} did not display longer stems compared to CN for any harvest week. For petunia ‘WP’, EOD₃SR₁ was 18% and 24% longer than CN and SR seedlings, respectively, at day 28.

Stem caliper for both cultivars was not significantly impacted by the duration, far-red PFD, or R:FR of EOD-FR treatments compared to CN (Fig. 2A-B). Differences in stem caliper between EOD-FR treatments and CN were only observed at day 28, and the magnitude of these differences varied by cultivar. (Table 5.). For example, at day 28, stem caliper of petunia ‘WP’ was 18% greater under EOD₃SR₁ compared to CN. Stem caliper of petunia ‘DM’ under EOD₃SR₁ was 25% greater compared CN.

Supplemental lighting generally promoted increased stem caliper in both cultivars compared to all other treatments, with larger values observed under SR at days 14 and 21 (Fig. 2A-B). For example, stem caliper for petunia ‘WP’ under SR at day 21 was 20%, 19%, 18%, 13%, 12%, and 12% greater compared to EOD_{FL}, EOD_{20:30}, CN, EOD_{10:30}, EOD₂SR₁, and EOD_{20:30}, respectively. Similarly, stem caliper for petunia ‘DM’ at day 21 was 27%, 26%, 22%, 18%, 15%, and 13% greater under SR than EOD_{20:240}, CN, EOD_{20:30}, EOD_{FL}, EOD₂SR₁, and EOD_{10:30}, respectively. At day 28, petunia ‘WP’ stem caliper under SR was significantly larger than all other treatments (Table 5). Specifically, stem caliper for petunia ‘WP’ was 15%, and 36% greater under SR than EOD₃SR₁ and CN, respectively. In contrast, stem caliper for petunia ‘DM’ under SR was not significantly different from EOD₃SR₁, but 35% larger compared to CN.

2.4.2 Leaf Area, Leaf Number, and Relative Chlorophyll Content

End-of-day far-red radiation did not significantly promote leaf area expansion for any treatment compared to CN in either cultivar (data not shown, see Fig. 3 E-F and section 2.4.4 for LAI). Seedlings that received supplemental lighting for the duration of the study had greater leaf area than all other treatments at each measurement day.

Leaf number was generally not impacted by EOD-FR radiation compared to CN, but supplemental lighting resulted in seedlings under SR having significantly more leaves than all

other treatments at day 21 and 28 for both cultivars (data not shown). Additionally, at day 28, petunia ‘DM’ grown under EOD₃SR₁ had 20% more leaves compared to CN (data not shown).

No difference in RCC was observed between any EOD-FR treatment and CN at day 14 and 21 for petunia ‘DM’ (Table 4, Fig. 3.). However, petunia ‘WP’ grown under CN was found to have 13% greater RCC than EOD_{20:240} at day 21. Additionally, RCC was greater under EOD_{FL} compared to EOD_{20:240} at day 14 for petunia ‘DM’ and at day 21 for petunia ‘WP’. Relative chlorophyll content was promoted in seedlings that received supplemental lighting in both cultivars for all weeks, with the highest values observed under supplemental lighting for both cultivars (Table 4, Fig. 3.). At day 21, petunia ‘DM’ under EOD₂SR₁ had significantly greater RCC than all other treatments sans SR. Specifically, petunia ‘DM’ under EOD₂SR₁ had 19%, 20%, 21%, 26%, and 30% greater RCC than CN, EOD_{20:30}, EOD_{FL}, EOD_{10:30}, and EOD_{20:240} respectively. For petunia ‘WP’, no difference was observed for RCC between CN and EOD₂SR₁, but seedlings under EOD₂SR₁ had greater RCC than all other EOD-FR treatments.

At day 28, supplemental lighting in EOD₃SR₁ promoted an increase in RCC compared to CN for both cultivars (Table 4.). Specifically, seedlings under EOD₃SR₁ had 21% and 16% greater RCC compared to CN for petunia ‘DM’ and ‘WP’, respectively. Additionally, no difference in RCC was found between seedlings under SR and EOD₃SR₁ for petunia ‘WP’ at day 28.

2.4.3 Dry Mass

Stem dry mass (SDM) was not statistically analyzed for days 14 or 21 and RDM was not statistically analyzed for day 14 as a preponderance of seedlings in each treatment aside from SR had a mass lower than the microbalance could detect. For SDM, it is of note that at day 21 only EOD₂SR₁ and SR had $n > 12$ and 13 (non-zero values) for ‘DM’ and ‘WP’, respectively, indicating that supplemental lighting positively influenced stem dry mass, but this data was not

analyzed. EOD-FR did not significantly reduce or increase RDM for either cultivar compared to CN (Table 4, Fig. 4). Supplemental lighting applied for the duration of the study promoted a significantly greater dry mass in seedlings compared to all other treatments at days 21 and 28 for both cultivars. Additionally, one week of supplemental lighting for EOD₃SR₁ increased RDM by 100% and 48% compared to CN for petunia ‘DM’ and ‘WP’, respectively.

End-of-day far-red treatments did not significantly affect leaf dry mass (LDM) compared to CN for either cultivar, while supplemental lighting increased LDM for both cultivars (Table 4). In both cultivars, seedlings under SR had significantly greater LDM compared to all other treatments at day 14, 21, and 28. EOD₂SR₁ had greater LDM compared to CN at day 21, and EOD₃SR₁ had greater LDM compared to CN at day 28. For example, at day 28, seedlings under EOD₃SR₁ had 48% and 100% greater LDM compared to CN for petunia ‘WP’ and ‘DM’, respectively.

2.4.4 Leaf Mass Per Unit Area and Leaf Area Index

Leaf mass per unit area was only reduced under EOD-FR compared to CN at day 21 for petunia ‘WP’ (Table 4, Fig 3D). Specifically, petunia ‘WP’ under CN had 16%, 19%, and 24% greater LMA than EOD_{20:240}, EOD_{10:30}, and EOD_{FL}, respectively, at day 21. Supplemental lighting generally promoted an increase in LMA. At day 28, LMA in petunia ‘WP’ under SR and EOD₃SR₁ was 33% and 30% greater than CN, respectively. Similarly, LMA for petunia ‘DM’ at day 28 under SR and EOD₃SR₁ was 50% and 20% greater compared to CN, respectively.

No significant difference in LAI was found between any EOD-FR group and CN at day 14 and 21 in either cultivar. In contrast, SR seedlings of both cultivars had significantly greater LAI compared to all other treatments at day 14, 21, and 28 (Table 4). For example, LAI for petunia DM under SR was 168%, 167%, 164%, 153%, 150%, and 117% greater than EOD_{10:30}, EOD_{20:240}, EOD_{20:30}, CN, EOD₂SR₁, and EOD₃SR₁, respectively, on day 21. Similarly, LAI for

petunia ‘WP’ under SR on day 21 was 122%, 122%, 118%, 108%, 103%, and 100% greater than CN, EOD_{20:30}, EOD_{20:240}, EOD_{FL}, EOD_{10:30}, and EOD_{2SR₁}, respectively. Additionally, EOD_{3SR₁} was found to be similar to CN at day 28 in petunia ‘WP’, while in petunia ‘DM’, EOD_{3SR₁} had a LAI 60% greater than CN.

2.5 Discussion

In the production of young annual bedding plants, low ambient DLI at northern latitudes necessitates supplemental lighting use in the production of high-quality compact plugs for transplant and shipping, but the low LAI early in production reduces light interception and thus the efficacy of supplemental light. In this study, no EOD-FR treatment resulted in an increase in the LAI for petunia ‘WP’ and ‘DM’ (Fig. 3E-F). Dry mass of seedlings of both cultivars was similar or less under EOD-FR compared to CN plants (Table 4.), and hypocotyl elongation was generally promoted by EOD-FR resulting in stretched and fragile seedlings (Fig. 2C-D). Shade avoidance responses to low a R:FR ratio are primarily regulated by the phytochrome family of photoreceptors with phytochrome B (phyB) shown to generally be the most important (Casal, 2013; Franklin, 2008). A reduced R:FR ratio under shade is sensed by phyB and results in a shift in the amount of biologically active (Pfr) form to the biologically inactive (Pr) form (Casal, 2013; Küpers et al. 2020). When phyB is inactivated by low R:FR due to direct shading or by an increased reflectance of far-red by nearby neighbors, phytochrome interacting factor (PIF) transcription factor activity is increased leading to auxin synthesis and subsequently enhanced elongation responses (Casal 2013; Fernández-Milmanda and Ballaré, 2021; Küpers et al. 2020).

All EOD-FR treatments showed increased stem length compared to SR seedlings for both cultivars, but cultivar-specific responses were apparent between EOD-FR treatments (Fig.2C-D). For example, seedlings of both cultivars under EOD_{10:30} were found to have greater stem length

than CN seedlings, but compared to EOD_{FL} (R:FR~0.8) only ‘WP’ EOD_{10:30} had statistically significantly longer stems. Previous studies indicate that petunia has elongation responses to far-red light (Illias and Rajapakse, 2005; Park and Runkle, 2017), but to our knowledge no study has specifically compared the magnitude of EOD-FR induced stem elongation between the two petunia cultivars ‘WP’ and ‘DM’.

Neither petunia cultivar showed differential stem elongation responses to increased intensity of far-red radiation when the R:FR and duration of treatment were ~0.15 and 30 minutes, respectively; this lack of further elongation under higher intensities may have been the result of an EOD-FR dose (far-red PFD × duration of treatment) response that has been examined in horticulturally important species such as tomato and the squash hybrid, *Cucurbita maxima* × *Cucurbita moschata* ‘Tetsukabuto’ (Chia and Kubota, 2010; Yang et al., 2012). Specifically, Chia and Kubota (2010) examined hypocotyl elongation responses in two tomato rootstock cultivars, ‘Aloha’ and ‘Maxifort’ in response to different doses of far-red light (R:FR ~0.05) achieved by varying intensity of far-red light and the duration of the EOD-FR treatment; longer hypocotyls were found in young plants that received a far-red dose of 2 mmol·m⁻²·d⁻¹ compared to 1 mmol·m⁻²·d⁻¹ regardless of whether the higher dose was achieved by increasing far-red intensity or EOD-FR treatment duration showing the relative importance of the far-red dose compared to far-red PFD and treatment duration by themselves. Chia and Kubota (2010) also utilized a Michaelis-Menten-type model to estimate far-red light dose required to achieve 90% maximum hypocotyl elongation for tomato rootstock cultivars ‘Aloha’ and ‘Maxifort’ to be 5 to 14 mmol·m⁻²·d⁻¹ and 8 to 15 mmol·m⁻²·d⁻¹, respectively, while the “practical near saturation dose” resulting in similar hypocotyl elongation for both cultivars was only 2 to 4 mmol·m⁻²·d⁻¹; the near saturating dose of squash ‘Tetsukabuto’ was found to be 4 mmol·m⁻²·d⁻¹ (Chia and

Kubota, 2010; Yang et al., 2012). The approximate far-red dose received by ‘DM’ and ‘WP’ under EOD_{10:30} and EOD_{20:30} was 20 mmol·m⁻²·d⁻¹ and 40 mmol·m⁻²·d⁻¹, respectively. Thus, the saturating far-red dose for petunia stem elongation in the present study was likely achieved at or below 20 mmol·m⁻²·d⁻¹, resulting in no further significant increase at 40 mmol·m⁻²·d⁻¹. However, a saturating dose response does not explain the further elongation observed for both cultivars under EOD_{20:240} (estimated dose ~290 mmol·m⁻²·d⁻¹) compared to EOD_{10:30} and EOD_{20:30}. One possibility is that there may be a higher dose ceiling for the hypocotyl elongation response. Chia and Kubota (2010) calculated the far-red dose required for near saturation (90%) of hypocotyl elongation in the aforementioned cultivars and the EOD-FR dose response curves showed that increasing the dose beyond the “practical near saturation dose” would yield diminishing hypocotyl elongation returns; this could explain why EOD_{20:240} showed increased elongation compared to EOD_{10:30} and EOD_{20:30}, but far less of an increase than if the EOD-FR dose response were strictly linear.

Alternatively, the increased elongation under EOD_{20:240} may be due to the timing and duration of the EOD-FR treatment during the photoperiod. Sellaro et al. (2012) showed that in wild-type *Arabidopsis* seedlings grown under sunlight for an 8-hr photoperiod, a 2-hr “afternoon shade event” at the end of the photoperiod at a R:FR ratio of 0.1 was far more effective in promoting hypocotyl elongation compared to a 10-minute pulse of red and far-red light with the same R:FR ratio; only a 10 minute pulse of pure far-red (no red) showed comparable hypocotyl elongation to the afternoon shade event. An EOD-FR pulse can promote SAS responses because the pulse decreases the Pfr:Pr ratio prior to night (Franklin, 2008). However, Sellaro et al. (2012) showed for a 10-minute EOD pulse, a R:FR ratio of 0.1 was not effective in reducing Pfr to levels required to promote *Arabidopsis* hypocotyl elongation responses comparable to “afternoon

shade events”; this makes sense as a reduced sensitivity to shorter EOD-FR treatments has been in part attributed to the evening complex that prevents the confusion between shade and night through reduced expression of PIF4 and PIF5 during the beginning of the night (Casal, 2013). This would explain the increased hypocotyl elongation in our four-hour EODFR treatment of EOD_{20:240} as it effectively increased the photoperiod from 10.5hrs (as experienced by all other treatments sans SR) to 14 hours making this EOD-FR treatment more analogous to an afternoon shade event than a far-red pulse at the end of a photoperiod. Additionally, Mizuno et al. (2015) found that compared to PIF4 and PIF5, PIF7 is the primary transcription factor associated with EOD-FR induced hypocotyl elongation in *Arabidopsis*, and Jiang et al. (2019) reported that part of the evening complex, ELF3, inactivates PIF7 further explaining why shorter EOD-FR periods at the beginning of night were less effective in promoting hypocotyl elongation compared to the “afternoon shade event”. While not especially relevant to produce young annual bedding plants, if increased hypocotyl elongation is desired and the strategy is cost effective, afternoon shade events could be a beneficial approach to production, although further research would be required on different R:FR ratios, intensities, and duration requirement of the shade event for relevant taxa.

Lastly, similarity in stem length between EOD_{FL} and CN seedlings for both cultivars after two and three weeks of EODFR treatments may be due to the lower R:FR ratio that naturally occurs at dusk (Franklin, 2008). While dusk light quality for CN seedlings was not measured in this study, Lund et al (2007) showed that chrysanthemum internode length under a simulated twilight (R:FR ~0.7, 30-minute duration) was increased compared to a treatment with a R:FR~2.4. In our study, the EOD_{FL} treatment had a R:FR of ~0.8 perhaps explaining the similar hypocotyl length between the two treatments.

In this study, leaf number between EOD-FR and CN seedlings did not significantly differ after 2 and 3 weeks (data not shown), thus individual leaf expansion determined total leaf area. The LAI did not differ between CN and any EOD-FR treatment at the end of week 2 and 3 (Fig. 3E-F), and this may have been due to multiple factors. In shade avoiding plants, leaf area responses to lower R:FR ratios vary by species, but in many cases a reduction in leaf development occurs in tandem with elongation growth (Franklin, 2008; Smith and Whitelam, 1997). In the course of normal leaf ontogeny, two cellular processes that control leaf size are cell division and cell expansion that primarily occur earlier and later in development, respectively, and both processes have extensive regulatory mechanisms (Gonzales et al., 2012). Carabelli et al. (2007) found that under a low R:FR ratio, new leaf primordia in young *Arabidopsis* plants showed inhibited cell proliferation and that the resulting smaller leaves were due to decreased cell number rather than smaller cells; this may have been the case in young *Helianthus annuus* L. ‘Contifolor’ and ‘P75’ (sunflower) leaves where EOD-FR inhibited increases in leaf length and width in younger leaves compared to end-of-day red (EOD-R) treatments while the opposite was observed in older leaves (Casal and Sadras, 1987). Similarly, for *Petunia axilaris* EOD-FR had a promotive effect on leaf area of individual leaves at the late development stage whereas younger leaves were less affected by treatments (Casal et al., 1987). Casal et al. (1987) also found that leaf area expansion of petunia was due in part to larger epidermal cells. Patel et al. (2013) similarly found that for *Arabidopsis* plants under a constant R:FR of 0.1, expanded leaves had increased cell size compared to high R:FR controls. Thus, the EOD-FR applications or the twilight R:FR in CN seedlings may have reduced cell proliferation in developing leaves of both ‘WP’ and ‘DM’ contributing to the lack of leaf expansion at the end of week 2 and 3, as EOD-FR treatments began upon seedling germination rather than later in leaf development stages. Of

note for future studies may be to examine the effects of EOD light quality on leaf area in young plants at different leaf development stages as enhancing cell proliferation earlier in development and elongation later in development could be an effective strategy to increase light capture in young plants.

It has also been suggested that expansion of leaves under lower R:FR ratios may be related to light availability or competition with the plant stem; leaf development may be inhibited in tandem with promoted stem elongation when PPFD is low, while added far-red light under a sufficient PPFD for normal growth may promote leaf expansion (Casal et al., 1987; Demotes-Mainard et al., 2016; Park and Runkle, 2017). Park and Runkle (2018) found that the addition of far-red light in tandem with non-limiting PPFD in an SSL environment promoted leaf area expansion in petunia ‘Wave Blue’, and Kalaitzoglou et al. (2019) found similar results for tomato ‘Komeett’ when far-red light was included in the normal photoperiod, but not under EOD-FR. For *Lactuca sativa* L. ‘Tiberius’ (lettuce), far-red included in the normal photoperiod and applied as EOD-FR led to an increase in leaf area (Zou et al., 2019). The low DLI experienced by CN and EOD-FR seedlings in our study may have influenced resource investment in the elongation of hypocotyls, further limiting leaf area expansion. However, the lack of an increase in leaf area for tomato (Kalaitzoglou et al., 2019) in EOD-FR treated plants compared to plants with far-red included in the normal spectrum may indicate a lack of leaf area expansion under EOD-FR even when PPFD is not limiting for some species. The promotion of leaf area expansion under both EOD-FR and far-red included in the spectrum in lettuce (Zou et al., 2019) supports interspecific responses to low R:FR light quality applied differentially during production.

Compared to CN seedlings, EOD-FR treatments did not significantly increase or decrease stem caliper, LDM, or RDM; all three parameters increased under higher relative to lower DLI (Fig. 2A-B, Table 4.). The lack of a differential stem caliper response in either cultivar to EODFR treatments compared to CN is similar to the aforementioned far-red dose response experiments for tomato and squash as well as the EOD-FR response of chrysanthemum (Chia and Kubota, 2010; Lund et al., 2007; Yang et al., 2012). For example, no significant difference in stem diameter was reported in tomato ‘Aloha’ seedlings under EOD-FR treatments with a R:FR ratio of 0.47 compared to 0.05 (Chia and Kubota, 2010). The lower DLI experienced by CN and EOD-FR seedlings during the first 3 weeks of the experiment is the likely cause for the reduced stem caliper in all treatments compared to SR, as studies have demonstrated that stem caliper and average stem dry weight per internode of annual bedding seedlings increases under higher DLIs, contributing to higher seedling quality (Craver et al. 2019; Craver et al., 2018; Pramuk and Runkle, 2005). As mentioned in the previous section, if hypocotyl elongation production goal, caution should be taken in utilizing EOD-FR alone as it is unlikely to promote an increase in stem caliper if DLI is limiting to growth.

In bedding plants, it has been shown that overall plant dry mass generally increases as DLI increases and multiple studies have shown that annual bedding seedlings grown under a higher DLI develop higher shoot and root dry mass compared to those grown under a lower DLI for a variety of species (Craver et al., 2019; Faust et al., 2005; Oh et al., 2010; Poel et al., 2017a.). In the present study, increased LDM and RDM under SR compared to all other treatments at the end of week 2 and 3 for LDM and week 3 for RDM indicate that DLI was the main factor in determining biomass gain (Table 4.). Additionally, no significant difference in leaf number at the end of week 2 (data not shown) between any treatment indicates that individual

leaves under SR had developed more biomass, while significant increases in leaf number in SR compared to all other treatments at the end of week 3 indicates that increased leaf dry mass may have been in part the result of a greater number of leaves.

A decrease in leaf chlorophyll content is a common response often visible to the eye in shade avoiding species grown under a relatively low R:FR ratio (Franklin, 2008; Smith and Whitelam, 1997); this decrease in chlorophyll content is very apparent in SSL environments, especially when compared with plants receiving no far-red radiation (Kalaitzoglou et al., 2019; Park and Runkle, 2017; Park and Runkle, 2019; Patel et al., 2013). For example, Park and Runkle (2017) found that a decrease in the phytochrome photoequilibrium (PPE) also showed a decrease in SPAD values measured for seedlings of the sun-loving geranium, petunia, and snapdragon, while this metric was not affected in the shade tolerant *Impatiens walleriana*. Phytochrome interacting factors are known to negatively regulate chlorophyll biosynthesis (Liu et al., 2017); EOD-FR treatments or natural twilight in our study may have reduced active phyB prior to night and thus allowed for increased PIF activity and a subsequent reduction in overall chlorophyll content compared to treatments receiving SR. EOD-FR has also notably been found to reduce leaf chlorophyll content in plants such as *Petunia axillaris* and tomato (Casal et al., 1987; Kalaitzoglou et al., 2019). However, Kalaitzoglou et al. (2019) observed a clear reduction in chlorophyll content from EOD-FR treatment (15 minutes, PPE = 0.1) compared to a control (90:10 RB LEDs) in a SSL experiment that was not apparent in a second experiment where approximately 25% of total light received by plants was solar in origin (including $\sim 11 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of far-red); this may indicate that short EOD-FR periods may not severely reduce total leaf chlorophyll content in some shade avoiding plants when grown with small amounts of far-red light. Alternatively, a high R:FR ratio applied at the end of the day may reduce chlorophyll

biosynthesis inhibition by increasing active phyB levels prior to night; this may in-part explain the consistent high SPAD levels seen in SR plants, as SR used in this study extended the natural photoperiod and created a light environment with a R:FR ratio of ~69 (Table 1.). The blue light included in this spectrum (approximately 10% of total PPFD) may also have led to increased leaf chlorophyll content (Bantis et al., 2018).

Both shade avoiding and shade tolerant species have been found to exhibit an increase in specific leaf area (SLA; the inverse of LMA) in response to shade as a strategy of maximizing light interception; in nature this response may be advantageous in regard to light capture in shaded environments but decreased biomass per unit leaf area can also make leaves more vulnerable to mechanical stressors (Gommers et al., 2013; Poorter et al., 2009). In a horticultural context, a reduced LMA is a negative attribute for young plants as they may be more easily damaged during shipping and transplant. Regarding radiation, LMA seems to be positively related to the daily photon irradiance (DPI; analogous to DLI) (Poorter et al., 2009), and a decrease in LMA due to decreased PPFD has been shown in petunia (Park and Runkle, 2018). In SSL experiments, a reduction in LMA is also observed when far-red light is added to the spectrum without a reduction in PPFD (Kalaitzoglou et al., 2019; Zou et al., 2019); Kalaitzoglou et al. (2019) showed that while total leaf dry weight was generally similar in tomato leaves between SSL treatments with and without included far-red, LMA was reduced under treatments including far-red. In our study, the lower DLI in CN and EOD-FR treated seedlings compared to SR plants was likely the cause of the reduced LMA.

Dynamic lighting strategies for plant production include those in which light intensity, quality, and the timing of both may be altered throughout the production process; some examples include Oh et al. (2010) restricting supplemental lighting use to different periods during seedling

development, Hurt et al. (2019) using instantaneous threshold lighting to only use supplemental lighting when natural light drops below a certain level, or the use of EOD-FR or night interruption lighting with a low R:FR to promote flowering in long day plants (Demotes-Mainard et al., 2016; Craig and Runkle 2012). Dynamic lighting treatments in this study were designed to test if or when in young petunia seedling development EOD-FR might promote early leaf expansion under a low DLI, and whether supplemental lighting provided post cessation of EOD-FR treatments would enhance plug quality if EOD-FR treatments increased LAI. However, no EOD-FR treatment promoted leaf area expansion and thus the effects on measured parameters post cessation of EOD-FR treatments are likely the result of a shift from lower to higher DLI after two (EOD₂SR₁) or three (EOD₃SR₁) weeks in tandem with any residual effects on seedlings from EOD-FR treatments. Similar to the findings of Oh et al. (2010), seedlings that received one (EOD₃SL₁) or two (EOD₂SL₁) weeks of supplemental lighting after periods of overall low DLI showed a significant increase in overall quality, such as increased dry mass and stem caliper, compared to CN plants. However, detrimental aspects of shade avoidance, such as elongated stems, still reduced overall seedling quality which is most evident under EOD₂SL₁ (Fig. 2D, Table 5.)

2.6 Conclusion

Under greenhouse conditions, lighting at the end of the natural photoperiod is one way that light quality can be manipulated to elicit desired plant responses. While the addition of far-red light in SSL environments and the use of EOD-FR has been shown to induce leaf expansion in some species, including *Petunia* spp., the use of EOD-FR treatments in the present study were ineffective in promoting early leaf area expansion under a low DLI. Additionally, characteristics deemed negative in regard to seedling quality were common across EOD-FR treatments

compared to plants receiving supplemental lighting. However, the effect of the “afternoon shade effect” seen here on hypocotyl elongation compared to shorter EOD-FR periods may be of use in rootstock production for crops such as tomato and squash; if this response can be promoted with commercially available lamps with relatively low R:FR output then this strategy may be more easily implemented. Further research into responses of seedlings to different EOD light quality at different seedling development stages may yet yield beneficial applications in the production of annual bedding plants under greenhouse conditions. Additionally, examination of the effects of EOD lighting applications under varying DLI may further elucidate how resource availability interacts with photomorphological responses for seedling production.

Table 1. Total (TPFD), blue (BPFD), green (GPFD), red (RPFD), and far-red photon flux densities (FRPFD; $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), and R:FR provided by supplemental or end-of-day (EOD) lighting treatments. Subscripts “a” and “b” represent spectral scans taken 1m away from and directly under Elixia fixtures, respectively.

Light source	TPFD	RPFD	GPFD	BPFD	FRPFD	R:FR
Supplemental	73.49 ± 1.63	61.16 ± 1.99	3.96 ± 0.1	7.215 ± 0.15	<1	69 ± 3.62
Flowering lamps	21.59 ± 0.19	7.69 ± 0.03	2.85 ± 0.04	1.35 ± 0.03	9.69 ± 0.17	0.79 ± 0.01
Elixia _a	11.29 ± 0.26	1.56 ± 0.13	<0.1	<0.1	9.71 ± 0.21	$0.16 \pm <0.01$
Elixia _b	23.32 ± 0.23	3.07 ± 0.05	<0.1	<0.1	20.39 ± 0.12	$0.15 \pm <0.01$

Table 2. End-of-day (EOD) treatments, light sources for respective treatments, and the duration of the end-of-day lighting period in minutes. Subscripts “a” and “b” represent spectral scans taken 1m away from and directly under Elixia fixtures, respectively.

Treatment	EOD Light Source	Duration (minutes)
EOD _{FL}	Flowering Lamps	30
EOD _{10:30}	Elixia _a	30
EOD _{20:30}	Elixia _b	30
EOD _{20:240}	Elixia _b	240

Table 3. Dynamic lighting treatments, the end-of-day (EOD) far-red treatment utilized, the number of days under the EOD treatment, and the number of days under supplemental lighting.

Dynamic Treatment	EOD Treatment	EOD Treatment Duration (d)	Supplemental Light Treatment (d)
EOD ₃ SR ₁	EOD _{FL}	21	7
EOD ₂ SR ₁	EOD _{20:240}	14	7

Table 4. Leaf dry mass (LDM) and root dry mass (RDM), for *Petunia ×hybrida* ‘Dreams Midnight’ (DM) and ‘Wave Purple’ (WP) 14 and 21 days post germination under end-of-day far-red (EOD-FR) treatments including control (CN; no EOD-FR or supplemental lighting), EOD_{FL} [30 minutes of EOD-FR under flowering lamps (FL) with a far-red PFD of 10 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, R:FR~0.8], EOD_{10:30} (30 minutes of EOD-FR under a far-red PFD of 10 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, R:FR~0.15), EOD_{20:30} (30 minutes of EOD-FR under a far-red PFD of 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, R:FR~0.15), and EOD_{20:240} (240 minutes of EOD-FR under a far-red PFD of 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, R:FR~0.15). Lighting treatments also included seedlings under supplemental lighting with no EOD-FR (SR) and seedlings subjected to two weeks of EOD_{20:240} and one week of supplemental lighting (EOD₂SR₁). Means within a cultivar sharing a letter are not statistically different by Tukey’s honest significant difference (HSD) test at $P \leq 0.05$.

Day	Cultivar	Treatment	LDM (mg)	RDM (mg)
14	DM	CN	0.57 a	-
		EOD ₂ SR ₁	0.75 a	-
		EOD _{10:30}	0.63 a	-
		EOD _{20:30}	0.58 a	-
		EOD _{20:240}	0.60 a	-
		EOD _{FL}	0.70 a	-
		SR	2.48 b	-
	WP	CN	0.81 a	-
		EOD ₂ SR ₁	0.75 a	-
		EOD _{10:30}	0.84 a	-
		EOD _{20:30}	0.84 a	-
		EOD _{20:240}	0.79 a	-
		EOD _{FL}	0.71 a	-
		SR	2.17 b	-
21	DM	CN	4.05 a	0.56 a
		EOD ₂ SR ₁	5.56 b	1.01 b
		EOD _{10:30}	3.59 a	0.52 a
		EOD _{20:30}	3.73 a	0.37 a
		EOD _{20:240}	3.42 a	0.44 a
		EOD _{FL}	4.05 a	0.74 ab
		SR	13.7 c	2.79 c
	WP	CN	4.78 a	0.87 ab
		EOD ₂ SR ₁	6.33 b	1.09 b
		EOD _{10:30}	4.34 a	0.75 ab
		EOD _{20:30}	4.22 a	0.81 ab
		EOD _{20:240}	3.90 a	0.71 a
		EOD _{FL}	4.34 a	0.74 a
		SR	13.17 c	2.80 c

Table 5. Leaf dry mass (LDM), root dry mass (RDM), stem dry mass (SDM), leaf area index (LAI), relative chlorophyll content (SPAD), leaf mass per unit area (LMA), stem length (SL), and stem caliper (SC) for *Petunia ×hybrida* ‘Dreams Midnight’ (DM) and ‘Wave Purple’ (WP) seedlings 28 days after germination under supplemental lighting (SR), control (CN; no supplemental lighting), or end-of-day far-red (EOD-FR) treatments for three (EOD₃SR₁) weeks before being moved under supplemental lighting for the remainder of the experiment. Specifically, EOD₃SR₁ received 30 minutes of EOD-FR under a far-red PFD of 10 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and R:FR~0.8. Means within a cultivar sharing a letter are not statistically different by Tukey’s honest significant difference (HSD) test at $P \leq 0.05$.

Cultivar	Treatment	LDM (mg)	RDM (mg)	SDM (mg)	LAI	SPAD	LMA ($\text{g}\cdot\text{m}^{-2}$)	SL (cm)	SC (mm)
DM	CN	8.4a	1.4 a	0.59 a	0.52 a	25.8 a	18.9 a	0.50 a	0.96 a
	EOD ₃ SR ₁	16.8 b	2.8 b	1.2 b	0.83 b	31.1 b	22.7 b	0.51 a	1.2 b
	SR	32.4 c	6.2 c	2.9 c	1.3 c	34.4 c	28.4 c	0.47 a	1.3 b
WP	CN	13.1 a	2.3 a	0.63 a	0.82 a	27 a	18.2 a	0.44 a	1.1 a
	EOD ₃ SR ₁	19.4 b	3.4 b	1.1 b	0.93 a	31.3 b	23.7 b	0.52 b	1.3 b
	SR	42.2 c	9.1 c	2.2 c	1.9 b	31.9 b	24.2 b	0.42 a	1.5 c

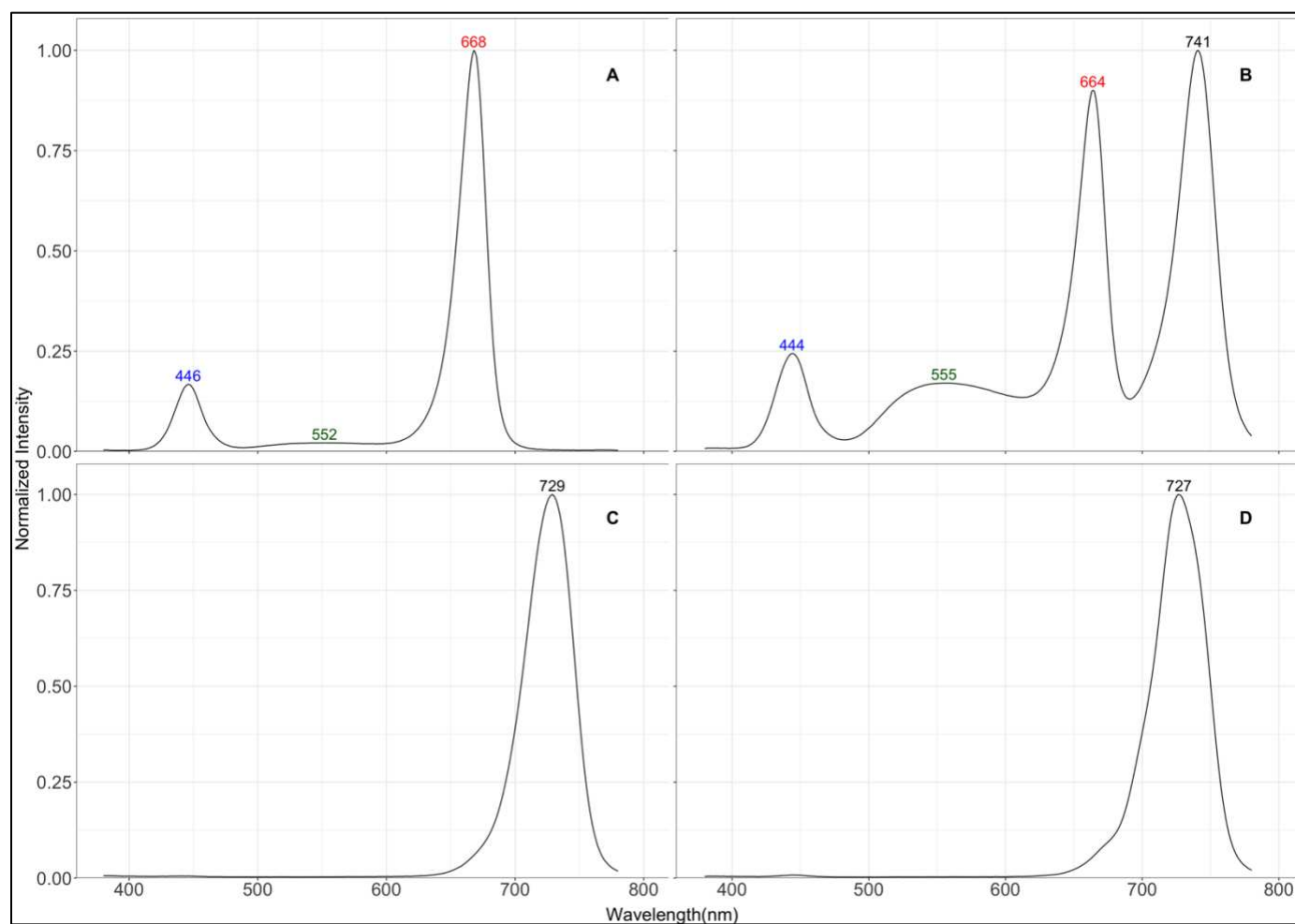


Fig. 1. Normalized spectral distribution from 400-800 nm measured at canopy height for supplemental lighting fixtures (Phillips GreenPower LED Toplighting) (A), photoperiodic flowering lamps (Phillips GreenPower LED Flowering Lamps) (B), Heliospectra Elixia fixtures providing a far-red PFD of $10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (C), Heliospectra Elixia fixtures providing a far-red PFD of $20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (D). Data were averaged across four experimental replications with peak wavelengths in the blue, green, red, and far-red regions shown above their respective peaks.

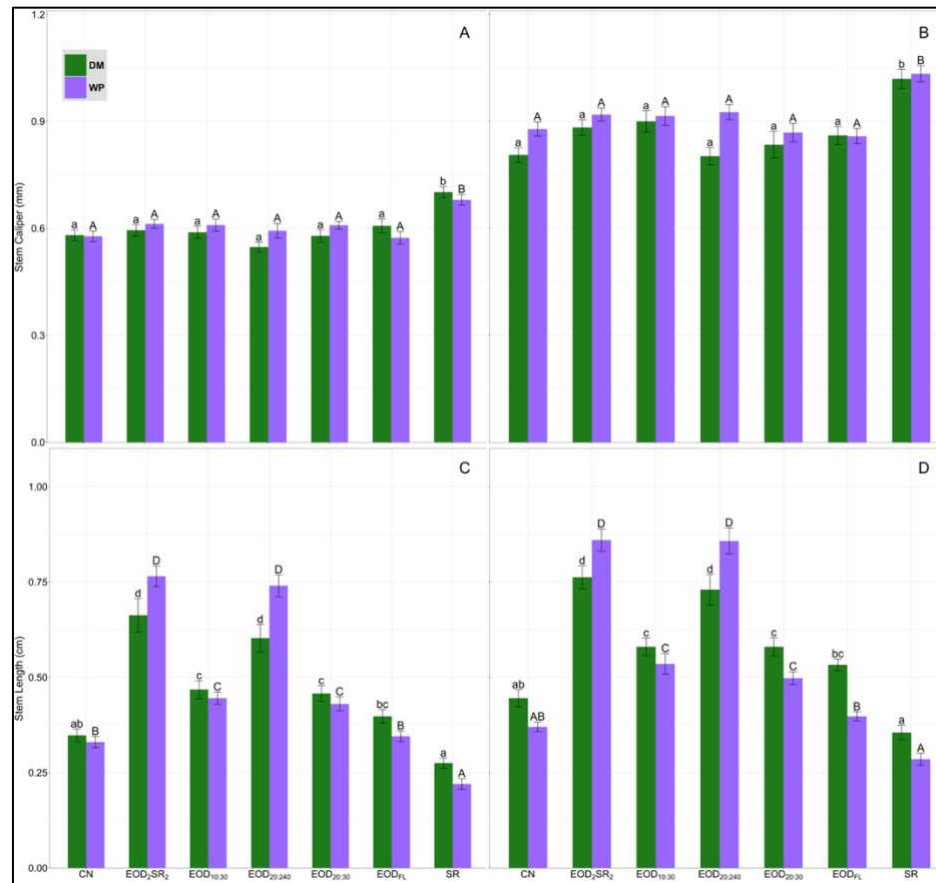


Fig. 2. Stem length and stem caliper of *Petunia xhybrida* 'Wave Purple' (WP) and 'Dreams Midnight' (DM) seedlings 14 (A and C) and 21 (B and D) d after germination under end-of-day far-red (EOD-FR) treatments including control (CN; no EOD-FR), EOD_{FL} [30 minutes of EOD-FR under flowering lamps (FL) with a far-red PFD of 10 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, R:FR~0.8], EOD_{10:30} (30 minutes of EOD-FR under a far-red PFD of 10 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, R:FR~0.15), EOD_{20:30} (30 minutes of EOD-FR under a far-red PFD of 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, R:FR~0.15), and EOD_{20:240} (240 minutes of EOD-FR under a far-red PFD of 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, R:FR~0.15). Lighting treatments also included seedlings under supplemental lighting with no EOD-FR (SR) and seedlings subjected to two weeks of EOD_{20:240} and one week of supplemental lighting (EOD₂SR₁). Means within a cultivar sharing a letter are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$.

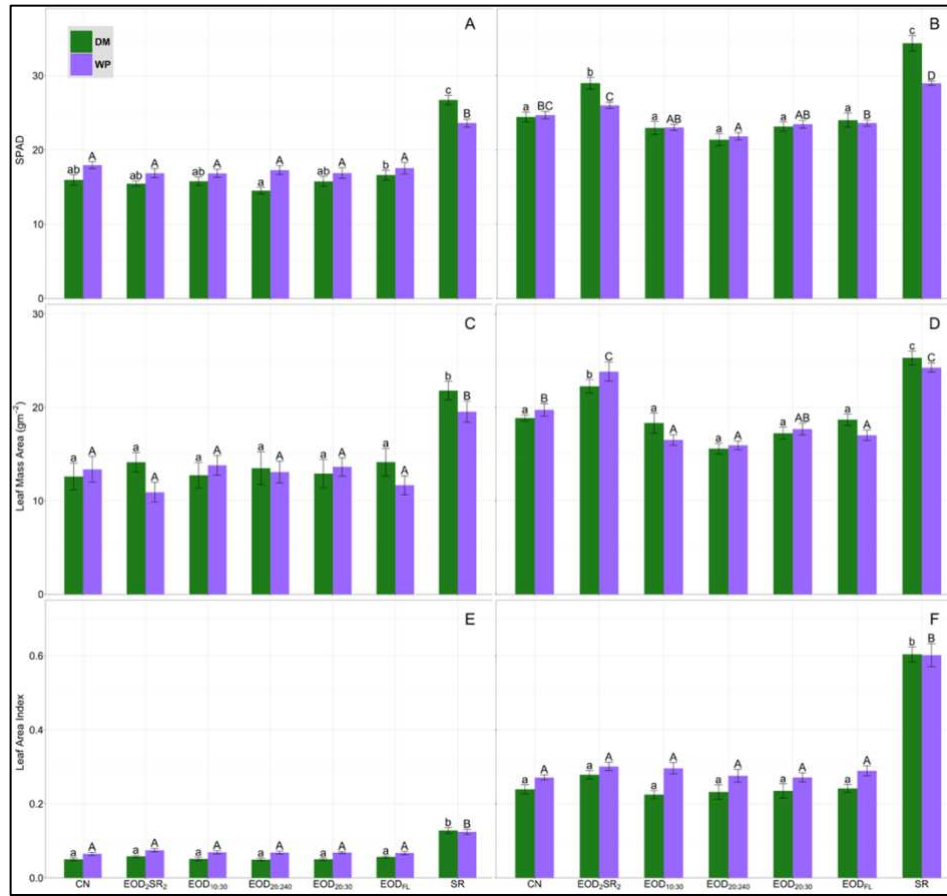


Fig. 3. Chlorophyll Content (SPAD), leaf mass per unit area, and leaf area index of *Petunia xhybrida* ‘Wave Purple’ (WP) and ‘Dreams Midnight’ (DM) seedlings 14 (A, C, and E) and 21 (B, D and F) d after germination under end-of-day far-red (EOD-FR) treatments including control (CN; no EOD-FR or supplemental lighting), EOD_{FL} [30 minutes of EOD-FR under flowering lamps (FL) with a far-red PFD of 10 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, R:FR~0.8], EOD_{10:30} (30 minutes of EOD-FR under a far-red PFD of 10 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, R:FR~0.15), EOD_{20:30} (30 minutes of EOD-FR under a far-red PFD of 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, R:FR~0.15), and EOD_{20:240} (240 minutes of EOD-FR under a far-red PFD of 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, R:FR~0.15). Lighting treatments also included seedlings under supplemental lighting with no EOD-FR (SR) and seedlings subjected to two weeks of EOD_{20:240} and one weeks of supplemental lighting (EOD₂SR₁). Means within a cultivar sharing a letter are not statistically different by Tukey’s honest significant difference (HSD) test at $P \leq 0.05$.

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CHAPTER 3. THE IMPACTS OF FAR-RED LIGHT AND TEMPERATURE ON SHADE AVOIDANCE RESPONSES FOR *PETUNIA* ×*HYBRIDA*

3.1 Summary

Supplemental lighting is often necessary to reach the target daily light integral to ensure the production of high-quality young annual bedding plants (plugs) in commercial greenhouses, but the low leaf area index of said plugs limits light interception and likely results in wasted applied light. Far-red radiation has been shown to promote leaf expansion in some species and cultivars, and the increase in leaf area in response to far-red radiation may allow for increased light interception and reduce wasted light. However, leaf expansion in response to far-red radiation may depend on other factors such as the daily light integral, the method of far-red application, and ambient temperature. Thus, the objectives of this work were to examine the effects of far-red radiation applied throughout the normal photoperiod and as an end-of-day lighting strategy on leaf area of petunia (*Petunia* ×*hybrida*) ‘Dreams Midnight’ seedlings grown under the recommended minimum daily light integral, as well as to investigate the effects of different ambient temperature on responses to far-red radiation during seedling growth.

To investigate the effects of far-red radiation of leaf expansion, seeds of petunia ‘Dreams Midnight’ were sown in 128-cell trays and moved to one of two growth chambers upon the unfolding of cotyledons. Growth chambers were divided in half using white vinyl to allow for two light environments per chamber provided by programmable LED fixtures. All seedlings received a DLI of $10.2 \text{ mol} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ over a 17.25-hour photoperiod, and either a high R:FR (~10.8; RL) or a low R:FR (~0.5; SHD) for the duration of the photoperiod. Additionally, all RL treated seedling received 1-hour low intensity end-of-day treatments with either a high R:FR (~10.8; EOD_W) or a very low R:FR (~0.15; EOD_{FR}). Each chamber also had an ambient

temperature setpoint of either 21 or 16 °C to allow for the examination of the effects of temperature on responses to far-red light. Specifically, seedlings received one of the following treatments: RL + EOD_W at 21 °C (control; CN), RL + EOD_{FR} at 21 °C (EOD₂₁), RL + EOD_{FR} at 16 °C (EOD₁₆), RL at 21 °C + EOD_{FR} + dark period at 16 °C (CN₂₁EOD₁₆), SHD at 21 °C (SH₂₁), SHD at 16 °C (SH₁₆), RL + EOD_W at 21 °C for 14 days followed by SHD at 21 °C (DSH₂₁), RL + EOD_W at 21 °C for 14 days followed by SHD at 16 °C (DSH₁₆). Destructive data was collected three and four weeks after treatment initiation.

When grown at 21 °C, end-of-day far-red (EOD-FR) treatments did not promote leaf expansion compared to control plants, and a similar number of leaves between CN and EOD₂₁ plugs suggests that the similar leaf area found was due to the size of individual leaves rather than the number of leaves. Additionally, the excessive stem elongation in response to EOD-FR seen in the previous experiment (chapter 2) when plants were grown under a low DLI was not seen in this experiment which may have been due to the higher DLI and/or longer photoperiod. In contrast to EOD₂₁, SH₂₁ seedlings were found to have significantly greater leaf area at week four relative to CN plants as well as greatly elongated stems and leaves; the fewer number of leaves under SH₂₁ also suggests that the greater leaf area was due to the size of individual leaves rather than the number of leaves. Regardless of lighting treatment, stunted growth was observed in plants growing at 16 °C. The results of this study indicate that leaf expansion in response to EOD-FR may not be limited by DLI for some species, and that a high DLI or long photoperiod may limit stretching and elongation seen in response to EOD-FR under a low DLI or short photoperiod. Further research is warranted to examine the influence of photoperiod on responses to EOD-FR as well as how lower temperatures may affect far-red mediated responses in species or cultivars that are tolerant of or thrive under lower temperatures.

3.2 Introduction

A low natural daily light integral (DLI) in winter at northern latitudes often necessitates the use of supplemental lighting from artificial light sources to produce high-quality young annual bedding plants (plugs) in commercial greenhouses (Poel and Runkle, 2017; Pramuk and Runkle, 2005). Alternatively, the reduced emission of radiant heat from light emitting diodes (LEDs) as well as the small size, short crop cycle, and high value of plugs makes high-density multilayer indoor production using sole-source lighting (SSL) an option to produce highly uniform seedlings (Craver et al., 2018; Park and Runkle, 2017; Wu et al., 2020). However, the small size of plugs means they often have little leaf area with which to intercept photons and a low leaf area index (LAI) likely results in wasted light striking substrate or bench space. Considering all light within SSL environments is artificial and thus requires electricity, minimizing wasted light in these production environments is especially important. One strategy to reduce wasted light could be to increase light capture for plugs by promoting an early increase in leaf area using far-red light.

Under vegetational shade, light quantity (intensity) is significantly reduced, and there is a distinct decrease in the ratio of red to far-red radiation (R:FR); under full sunlight the R:FR is ~1.2 while deep shade can reduce this value to 0.1 (Ballaré and Pierik, 2017). In response to a decreasing R:FR, shade intolerant species often undergo morphological shade avoidance responses that can increase access to light, such as the elongation of stems and petioles (Ballaré and Pierik, 2017; Smith and Whitelam, 1997). In some species, a low R:FR can promote increases in leaf area while in others leaf area decreases or shows little change, but responses are often species-specific (Casal et al., 1987; Demotes-Mainard et al., 2016). For example, Kwesiga and Grace (1987) grew seedlings of the shade tolerant *Khaya senegalensis* and the shade

intolerant *Terminalia ivorensis* under high or low R:FRs at multiple photosynthetic photon flux densities (PPFDs); *Khaya senegalensis* showed reduced leaf area under a low R:FR while, *Terminalia ivorensis* showed a drastic increase in leaf area under a low R:FR. The R:FR is sensed primarily by the photoreceptor phytochrome B (phyB) which photoconverts between a biologically active far-red absorbing form (Pfr) and inactive red absorbing (Pr) form upon absorption of red and far-red light, respectively. The Pfr form of phyB regulates shade avoidance responses through the inhibition of phytochrome interacting factors (PIFs) that function in the promotion of auxin biosynthesis (Ballaré and Pierik, 2017; Fernández-Milmanda and Ballaré, 2021).

End-of-day (EOD) lighting with a low R:FR (EOD-FR) can promote shade avoidance responses, but some responses to EOD-FR strategies may be reduced relative to growth under a constant artificial low R:FR (Franklin, 2008; Sellaro et al., 2012). Additionally, responses have been found to depend on the R:FR ratio and duration of the EOD treatment (Chia and Kubota, 2010; Sellaro et al., 2012). For example, lettuce (*Lactuca sativa*) grown under a PPFD of 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ followed by a 1-hour EOD-FR treatment was sufficient to promote a 27% increase in leaf area relative to control plants that did not receive an EOD-FR, but lettuce that received an additional 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of far-red radiation during the regular photoperiod showed a 49% increase in leaf area compared to control plants (Zou et al., 2019). However, for tomato (*Solanum lycopersicum*), a 15-minute EOD-FR treatment did not promote leaf expansion compared to plants that received no EOD-FR treatment (Kalaitzoglou et al., 2019). For *Arabidopsis* (*Arabidopsis thaliana*), enhanced hypocotyl elongation was readily promoted by growth under a constant R:FR of 0.1, while plants treated with a 2-hour shade event (R:FR = 0.1) during the last 2 hours of the photoperiod showed less than 40% of the constant shade induced

elongation responses; a 10-minute EOD-FR (R:FR = 0.1) showed similar hypocotyl length compared to control plants with no low R:FR treatment (Sellaro et al., 2012).

In previous work (unpublished data), we found that EOD-FR treatments led to significant stem elongation with little effect on leaf area for seedlings of petunia (*Petunia ×hybrida*) ‘Wave Purple’ and ‘Dreams Midnight’ grown in a common greenhouse under a simulated winter light environment (DLI~5.26, 10-hour photoperiod). However, previous research has shown that petunia seedlings show a slight increase in leaf area in SSL experiments with supplemental far-red radiation, while EOD-FR has been shown to promote an increase in leaf area compared to end-of-day red (EOD-R) treatments for *Petunia axillaris* (Casal et al., 1987; Park and Runkle, 2018). An increase in leaf area in response to far-red radiation in multiple species has been attributed to adequate resources (e.g., increased carbon assimilation under sufficient PPFD) to allow for leaf development (Casal et al., 1987; Demotes-Mainard, 2016; Heraut-Bron et al., 1999; Park and Runkle, 2018). For petunia, individual leaf expansion under supplemental far-red radiation was attributed at least in-part to a sufficient PPFD (96 or $288 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; 18-hour photoperiod) that allowed for both significant stem elongation as well as leaf expansion (Park and Runkle, 2018). For *Petunia axillaris* grown under $180 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for a 10-hour photoperiod ($\text{DLI} \sim 6.5 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$) prior to EOD-R and EOD-FR treatments, it was discussed that *Petunia axillaris* did not show significant stem elongation under EOD-FR compared to EOD-R, suggesting resources for leaf development were not severely limited (Casal et al., 1987).

Phytochrome mediated shade avoidance responses may also be dependent on or influenced by ambient temperature as well as the difference (DIF) between day (DT) and night (NT) air temperature; the manipulation of DIF is generally an effective technique in controlling plant height wherein plants grown under a positive (+) DIF ($\text{DT} > \text{NT}$) and negative (-) DIF (DT

< NT) show enhancements or reductions in height, respectively (Blom and Kerec, 2003; Myster and Moe, 1995; Patel et al., 2013; Thingnaes et al., 2008; Xiong et al., 2002). End-of-day far-red treatments have also been shown to enhance responses to +DIF and inhibit responses to -DIF for multiple species. Xiong et al. (2002) and Thingnaes et al. (2008) showed that phyB plays a significant role in DIF responses as long-hypocotyl and phyB mutants in *Cucumis sativus* (cucumber) and *Arabidopsis*, respectively, showed severely inhibited DIF responses. Growth under a constant low R:FR under different ambient temperature conditions (constant temperature or DIF=0) have also been examined in at least a few species including *Arabidopsis* (Patel et al., 2013), *Oenothera biennis* (Qaderi et al., 2015), and *Brassica napus* (Slauenwhite and Qaderi, 2013).

Patel et al. (2013) specifically examined the effects of reduced ambient temperature on shade avoidance responses in *Arabidopsis* growing under a low (0.1) or high (cool white fluorescence lamps, R:FR > 1.2) R:FR ratio with an equal intensity of photosynthetically active radiation (PAR) at either 16 or 22 °C. *Ler*, a temperate accession of *Arabidopsis*, was found to display the more typical elongated petioles common in this species under a low R:FR when grown at 22 °C, but at 16 °C showed highly reduced petiole elongation, as well as increased leaf area and thickness. One conclusion of this study was that these temperature-dependent growth forms may be advantageous in light capture in climates where heat or freezing stress are common issues. In contrast to *Ler*, the subtropical Cape Verde islands accession of *Arabidopsis* showed intense petiole elongation under a low R:FR regardless of air temperature showing genetic variability in temperature-dependent shade avoidance responses within a species (Patel et al., 2013).

Leaf expansion in response to a low R:FR may depend on some or all the factors described above, thus the objective for our study was to investigate the growth and development of petunia ‘Dreams Midnight’ seedlings in response to far-red radiation applied as an EOD treatment when DLI is not limiting, and as “shade light” for the full photoperiod under two different growth temperatures, 21 or 16 °C. For this research, the following questions were proposed: 1) Does leaf expansion for petunia occur in response to EOD-FR when grown under the recommended target DLI for production?; 2) Does EOD-FR differentially promote leaf expansion compared to “shade light” when plants are grown at the same total photon flux density (TPFD; 400-780 nm)?; and 3) Does petunia display a temperature-dependent shade-avoidance response when grown under 16 versus 21 °C? We grew petunia ‘Dreams Midnight’ in two growth chambers using multiple programmable LED fixtures with tunable red, blue, far-red, and white channels to allow for air temperature and light quality manipulation to address these questions.

3.3 Materials and Methods

3.3.1 Plant Material and Germination Environment.

Seeds of petunia ‘Dreams Midnight’ were sown in 128-cell trays (14-mL individual cell volume) using a commercial soilless germination medium (Berger BM2 Seed Germination; Berger, Saint-Modeste, QC), and placed in a reach-in growth chamber (PG2500; Conviron, Winnipeg, Canada) after sowing; seedlings were misted multiple times daily with tap water to ensure germination. Air temperature and day/night relative humidity in the chamber were set at 21 °C and 55%/65%, respectively. An average TPFD at canopy height of $164 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with 10% blue (400-500 nm), 15% green (500-600 nm), 69% red (600-700 nm), and 6% far-red (700-780 nm) was provided by programmable LED fixtures (Elixia; Heliospectra, Fiskhamnsgratan, Sweden) for a 16-hour photoperiod (0600-2000 HR). Upon cotyledon unfolding (8 days in

replication one; 9 days in replications 2 and 3), trays were immediately moved to treatment conditions and grown for 28 days. Trays were thinned to one seedling per cell 3-4 d after treatment initiation, and watered as needed with water-soluble fertilizer (Jack's 13-2-13 Plug LX, J.R. Peters, Inc., Allentown, PA) providing (in $\text{mg}\cdot\text{L}^{-1}$) 150 nitrogen (N), 23 phosphorus (P), 150 potassium (K), 69 calcium (Ca), 34 magnesium (Mg), 0.15 boron (B), 0.07 copper (Cu), 0.75 iron (Fe), 0.37 manganese (Mn), 0.07 molybdenum (Mo), and 0.37 zinc (Zn). Appropriate pH and electrical conductivity of the water-soluble fertilizer was confirmed using a handheld meter (Growline H19814, Hanna Instruments, Woonsocket, RI).

3.3.2 Growth Chamber and Light Conditions

Two reach-in growth chambers described above were utilized to create two temperature conditions. For each experiment replication, one chamber had a temperature setpoint of 21 °C, and the second had a temperature setpoint of 16 °C; the day/night relative humidity was set at 55%/65% in both chambers. Chambers were divided using white vinyl fabric to allow for two concurrent light treatments. Air temperature was measured using two precision thermistors (ST-100; Apogee Instruments Inc., Logan, Utah) per chamber with one thermistor on each side of the vinyl cloth. Leaf temperature of at least one treatment per chamber was measured with a fixed mounted infrared thermocouple with ABS plastic housing (OS36-01-T-80F; Apogee Instruments Inc., Logan, Utah). The recorded mean air temperature $\pm$ SD averaged across three replications in the 21 and 16 °C chambers were 20.91 ± 0.12 °C and 15.97 ± 0.11 °C, respectively, and the recorded mean leaf temperature $\pm$ SD averaged across three replications in the 21 and 16 °C chambers were 20.35 ± 0.03 °C and 15.74 ± 0.07 °C, respectively.

Two concurrent light treatments were provided by Elixia fixtures hung ~0.9 m above canopy level during the normal photoperiod (17.25 hours; 16:45-1000 hr) and EOD photoperiod (1 hour; 1000-1100). Light conditions during the normal photoperiod differed in red photon flux

density (PFD), far-red PFD, and R:FR; seedlings received either a high R:FR (RL) or a low R:FR “shade light” (SHD) light treatment, and both light conditions had the same mean TPDF, blue PFD, green PFD, and DLI (calculated from TPDF; Table 6.). Total photon flux density is not typically used to calculate DLI due to the low photosynthetic activity of far-red photons when applied alone. However, recent research has indicated that the addition of far-red photons to shorter wavelengths (white or red-blue LEDs) increase photosynthesis similarly to the same quantity of added white photons; the reader is directed to the following papers for a detailed explanation as there are important caveats to these findings: Zhen et al., 2021; Zhen and Bugbee, 2020a; Zhen and Bugbee, 2020b. Additionally, all RL treated seedlings received either an end-of-day white (EOD_w) or end-of-day far-red (EOD_{FR}) treatment that provided the same mean $\pm$ SD TPDF of $46.5 \pm 0.4 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with different red, blue, green, and far-red PFD and a different R:FR; SHD treated seedlings did not receive an EOD treatment and were instead placed under small blackout structures within the chambers. End-of-day far-red treatments were included in both 21 and 16 °C chambers while the EOD_w treatment was only present in the 21 °C chamber. Light intensity and spectrum of all treatments at canopy level were measured prior to the start of each replication using a spectrometer with no less than 9 scans per condition (LI-180; LI-COR Biosciences, Lincoln, NE.). The DLI, as well as blue, green, red, and far-red PFDs, and R:FR ratio of RL, SHD, EOD_w, and EOD_{FR} are summarized in Table 6 while normalized light spectrums are shown in Figure 4.

3.3.3 Treatments

Seedlings received either RL or SHD during the normal 17.25hr photoperiod, a one-hour EOD treatment if grown under RL, and no EOD treatment if grown under SHD. Every day for the duration of the experiment, trays were moved under normal photoperiod conditions at 1645 hr, and then under respective EOD or blackout conditions at 1000 hr the following morning. All

treatments are summarized in Table 7. Seedlings received one of the following treatments: RL + EOD_W at 21 °C (control; CN), RL + EOD_{FR} at 21 °C (EOD₂₁), RL + EOD_{FR} at 16 °C (EOD₁₆), RL at 21 °C + EOD_{FR} + dark period at 16 °C (CN₂₁EOD₁₆), SHD at 21 °C (SH₂₁), SHD at 16 °C (SH₁₆), RL + EOD_W at 21 °C for 14 days followed by SHD at 21 °C (DSH₂₁), RL + EOD_W at 21 °C for 14 days followed by SHD at 16 °C (DSH₁₆).

3.3.4 Data Collection and Statistical Analysis

Seedling data was collected 21 and 28 days after treatment initiation, and five seedlings from each treatment were randomly selected for measurement and analysis. Roots of selected seedlings were thoroughly washed and measurements were taken including stem length (cm; measured from the base of the hypocotyl to the shoot apical meristem), stem caliper [mm; measured directly under and perpendicular to cotyledons using a digital caliper (Fisherbrand™ Traceable™, Thermo Fisher Scientific, Waltham, WA)], and relative chlorophyll content [RCC; measured on the youngest fully expanded leaf using a SPAD chlorophyll meter (Chlorophyll Meter SPAD-502Plus, Konica Minolta, Chiyoda City, Tokyo, Japan)].

Leaves were removed from seedlings at the node to be counted. Leaf area (cm²) was determined using a leaf area meter (LI-3100; LI-COR Biosciences), and the length of the youngest fully expanded leaf was measured (leaf length; cm). Leaves, stems, and roots of each measured seedling were separated and dried at 70 °C for at least 5 days to determine the dry mass of each using an analytical microbalance (Analytical Balance ME54E, Mettler Toledo Ltd, Columbus, OH). Leaf mass per unit area (LMA; g·m⁻²) was calculated by dividing individual seedling leaf area by leaf dry mass, and stem dry mass per unit stem length (mg·mm⁻¹) was calculated by dividing individual seedling stem length by stem dry mass.

This experiment was a randomized complete block design with treatment (8 levels) as treatment factors and replication (3 levels) as a blocking variable. Three experimental

replications were conducted from January through June 2021, and chamber temperature conditions were switched between each replication. The effects of EOD treatments, shade light treatments, temperature, and timing of shade light treatments on the parameters described above were compared by two-way ANOVA using R statistical software and pairwise comparison of estimated marginal means using Tukey's HSD at $p < 0.05$ (Lenth, 2021; R Core Team, 2021).

3.4 Results and Discussion

The early expansion of leaves in the plug production cycle may allow for increased light capture and a reduction in wasted light, and the incorporation of far-red wavelengths into artificial lighting could be one way to promote leaf expansion. However, leaf expansion as a response to shade may be accompanied by excessive elongation of stems, petioles, and leaves which are undesirable traits for plugs. In our study, including far-red light as EOD or shade light led to different results. At the same constant temperature, the one-hour EOD-FR treatment resulted in similar seedlings compared to CN plants for the majority of measured variables, while shade light led to drastically altered plant morphology somewhat characteristic of Smith and Whitelam's (1997) description of exaggerated shade avoidance responses that occur when growth under a low R:FR is coupled with sufficient PPFD. Plant growth was consistently stunted under cooler temperatures regardless of lighting treatment.

3.4.1 The effects of EOD-FR on petunia 'Dreams Midnight'

Petunia seedlings were grown under the recommended DLI ($10\text{-}12 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$) for quality plug production with a 1-hour EOD-FR treatment to investigate whether DLI was a limiting factor in far-red mediated leaf expansion. Under a constant temperature, the majority of measured variables including leaf area, stem length and caliper, RCC, and all dry mass parameters were statistically similar under CN and EOD₂₁ treatments at day 21 and day 28 (Fig 5-7., Table 8). For example, at day 28 the leaf area and stem length of EOD₂₁ and CN plants

were 34.6 and 34.0 cm², and 0.86 and 0.77 cm, respectively (Fig 5B, Table 8.). There were two exceptions to the similarities between CN and EOD₂₁ treatments, LMA and leaf length at day 21 (Fig. 2C, Table 8); leaf length was 13% greater under EOD₂₁ compared to CN while LMA was 11% greater under CN compared to EOD₂₁. The former may in-part explain the latter as slightly longer leaves with a similar leaf dry mass (Table 8) would result in a reduced LMA.

The lack of a significant effect on leaf area in response to EOD-FR contrasts with the responses of some species, such as lettuce and *Petunia axilaris*, but is similar to others, such as tomato and poinsettia (*Euphorbia pulcherrima*) (Casal et al., 1987; Kalaitzoglou et al., 2019; Islam et al., 2014; Zou et al., 2019). For example, for poinsettia cultivars ‘Christmas Eve’ and ‘Christmas Spirit’, 30-minute EOD-FR treatments resulted in plants with similar leaf area compared to EOD-R treatments, while in *Petunia axillaris* EOD-FR treated plants had greater leaf area compared to EOD-R treated plants (Casal et al., 1987; Islam et al., 2014). In both studies, EOD-R and EOD-FR treated plants had a similar leaf number, indicating leaf area differences, and similarities were due to the size of individual leaves (Casal et al., 1987; Islam et al., 2014.); this is similar to our study as CN and EOD₂₁ plants had a similar leaf number at day 21 and 28 (Table 8). Interestingly, for both poinsettia and tomato a lack of a leaf area increase in responses to EOD-FR was still accompanied by a significant increase in shoot length and plant height, respectively, while in our study stem length between CN and EOD₂₁ plants were similar (Fig 6A-B; Islam et al., 2014; Kalaitzoglou et al., 2019.) The similar measured values for leaf area and all other variables between CN and EOD₂₁ plants may thus be species specific, but a lack of leaf expansion under EOD-FR with a recommended DLI suggests that, at least under our experimental conditions, leaf expansion specifically in response to EOD-FR does not depend on adequate PPFD or DLI for petunia. However, other factors such as the R:FR of the EOD-FR

treatments as well as the R:FR and length of the regular photoperiod may have affected plant responses.

One potential reason for the lack of shade responses could be due to the action of both phyB and phytochrome A (phyA). Phytochrome B is considered the most important phytochrome in inhibiting shade avoidance responses when the R:FR is high, but as the R:FR decreases, phyB Pfr is photoconverted to Pr which does not inhibit shade avoidance responses (Ballaré and Pierik, 2017). However, at very low R:FRs (<0.3) characteristic of canopy shade, phyA shows enhanced regulatory activity of hypocotyl elongation (Casal, 2013; Martínez-García et al., 2014). Martínez-García et al. (2014) found that *Arabidopsis* phyA mutant seedlings had relatively similar or slightly greater hypocotyl length compared control plants at R:FR > 0.3 while phyB mutants showed a much greater increase in hypocotyl length; in contrast, when the R:FR was decreased to ~0.15, phyA mutants showed a much greater increase in hypocotyl length relative to control plants while phyB mutants were more similar to control plants. In our study, the R:FR of the normal photoperiod and EOD-FR treatments were ~10.9 and ~0.15, respectively (Table 6), and past research shows that R:FR > 1.2 (sunlight) can further inhibit elongation responses of petunia compared to plants grown at R:FR ~ 1.2 (Ilias and Rajapakse, 2005; Kim et al., 2002). Kim et al. (2002) found that for petunia cultivars ‘Priscilla’, ‘Purple Sunspot’, ‘Blue’ and ‘Blue Vein’, growth under a far-red deficient environment (R:FR ~1.69) led to significantly shorter plants relative to control plants (R:FR~1.09) and plants under a neutral filter (R:FR~1.14). Similarly, Ilias and Rajapakse (2005) found that petunia ‘Countdown Burgandy’ was ~50% shorter under far-red deficient (R:FR~1.51) growth environments compared to control plants (R:FR~1.05); under the far-red deficient environment, EOD-FR treatments still promoted increased plant height compared to plants receiving no EOD treatment or EOD-R treatments.

While it was not directly examined in the present study, it is possible that the extraordinarily high R:FR (~10.9) of the regular photoperiod significantly limited shade avoidance responses beyond what would be seen if plants were grown at a R:FR of 1.2, predominately due to the action of phyB. Additionally, the very low (0.15) R:FR of the EOD-FR treatment may have increased phyA activity resulting in an inhibition of shade avoidance responses under EOD-FR.

The length of the normal photoperiod (17.25 hours) may have also reduced the effectiveness of the EOD-FR treatment (Lund et al., 2007; Lund et al., 2008). Lund et al. (2007) found that for chrysanthemum (*Chrysanthemum morifolium*) ‘Coral Charm’ grown under a 9-hour photoperiod, 30-minute EOD-FR treatments with R:FRs of 0.4 or 0.7 increased plant height compared to an EOD-R (R:FR = 2.4) treatment; these authors cited earlier work by Mortenson and Moe (1992) showing that natural EOD light quality did not affect chrysanthemum grown under longer photoperiods (12-18.5 hours), as well as work by Downs et al. (1957) showing that the effect of EOD-FR on elongation was reduced for bean (*Phaseolus vulgaris*) when grown under longer relative to shorter photoperiods. Lund et al. (2008) conducted further work using the same EOD-FR treatments and study taxa as Lund et al. (2007), but with photoperiod durations of 9, 14, and 19 hours; they found EOD-FR was more effective in promoting an increase in plant height when grown under the 9-hour treatment compared to the 14- and 19-hour treatments. Thus, another possible explanation for the lack of EOD-FR induced shade avoidance responses in our study may have been due to the photoperiod duration.

3.4.2 Shade light promotes exaggerated shade avoidance responses in petunia

In contrast to EOD-FR treatments, growth under shade light at 21 °C resulted in drastically altered growth and development that was characteristic of shade avoidance responses including an increase in leaf area as well as stem and leaf length, reduced chlorophyll content, and a reduction in shoot and root biomass (Franklin, 2008; Poorter, 2012; Smith and Whitelam,

1997). Smith and Whitelam (1997) enthusiastically noted that when PPFD remained adequate for continuous growth, reducing the R:FR would elicit exaggerated responses in shade-intolerant species such as sunflower (*Helianthus annuus*). Similarly, exaggerated shade avoidance responses were observed for petunia in the present study under SH₂₁. For simplicity, we only discuss comparisons between SH₂₁ and EOD₂₁ in this section as EOD₂₁ and CN plants were generally similar across all metrics as described in the prior section (Fig. 5-8; Table 8).

Petunia ‘Dreams Midnight’ under SH₂₁ displayed clearly visible shade avoidance responses including increased length of stems and leaves, as well as a drastic lightening of leaf color indicative of reduced chlorophyll that was confirmed by SPAD readings (Fig. 6A-B; Table 8). For example, stem length under SH₂₁ at day 21 and day 28 was found to be more than two- and four-fold greater than under EOD₂₁, respectively (Fig. 6A-B). However, these stem elongation responses were not accompanied by proportional increases in biomass. Specifically, while stem dry mass was similar to and significantly greater under SH₂₁ compared to EOD₂₁ at day 21 and 28, respectively (Table 8), calculating the dry mass per unit stem length revealed EOD₂₁ seedlings to have nearly triple and double the dry mass per unit stem length at day 21 and 28, respectively, compared to those under SH₂₁ (Fig. 6C-D). These results are in line with previous research wherein growth under a low R:FR, shade, or in crowded canopies results in an increase in specific stem length (stem length per unit dry mass) (Poorter et al., 2012). Working with foxglove (*Digitalis purpurea*) grown under different supplemental far-red treatments, Elkins and van Iersel (2020) utilized a similar metric, compactness (shoot dry mass per unit plant height), to differentiate between shade induced elongation without an associated increase in biomass, and faster growth wherein if plant height were increased there would be an associated increase in biomass. These authors found that compactness was unaffected by supplemental far-

red light, but the highest percentage of far-red utilized in their lighting treatments was 26.9% compared to ~50% in the present study (Elkin and van Iersel, 2020). As mentioned previously, supplemental far-red radiation incorporated into background red-blue or white light from LEDs results in an enhancement of photosynthesis similar to an addition of an equal number of white photons, with the caveat that far-red photons do not exceed approximately 30% of TPDF (Zhen et al., 2021; Zhen and Bugbee, 2020a; Zhen and Bugbee, 2020b.). Thus, it is likely that a portion of stem dry mass accumulation under SH₂₁ was due to an enhancement of photosynthesis by far-red light, but the low R:FR still reduced stem dry mass per unit length due to excessive stem elongation.

Leaf responses were similar to stem responses. Specifically, while the size (area and length) of the leaf increased under SH₂₁ compared to EOD₂₁, the proportional investment of dry mass was reduced (Fig 5, Table 8). For example, at day 21 leaf area was similar between SH₂₁ and EOD₂₁ while at day 28 leaf area under SH₂₁ was 18% greater than EOD₂₁ (Fig 5A-B). However, on day 21 and 28, seedlings grown under SH₂₁ had fewer leaves than EOD₂₁ suggesting larger individual leaves under the former; the magnitude of this difference was greater at day 28 compared to day 21 (Table 8). The use of supplemental far-red radiation has been shown to increase total and individual leaf area in lettuce (Zou et al., 2019), petunia (Park and Runkle, 2018), and tomato (Kalaitzoglou et al., 2019), with the increase attributed to an adequate background PPFD that would not occur under vegetational shade (Ballare and Pierik, 2017; Franklin, 2008). In *Arabidopsis* leaves, decreased leaf area has been attributed to a reduction in the number of cells but not cell size when grown under a low PPFD and low R:FR, whereas growth under a higher PPFD and a low R:FR leads to an increase in leaf area due to cell expansion (Carabelli et. al., 2007; Patel et al., 2013); the increase in leaf area under SH₂₁ was

likely due to a similar increase in cell expansion. The LMA of petunia was reduced under SH₂₁ compared to EOD₂₁. Specifically, under EOD₂₁ plants had a LMA that was 65% and 58% greater than under SH₂₁ at day 21 and 28, respectively (Fig. 5C-D). While a reduced LMA (or an increased specific leaf area) has been attributed to growth under a low R:FR, a strong correlation with light quantity has also been observed with decreasing DLI resulting in reduced LMA (Ballare and Pierk, 2017; Poorter et al., 2012; Poorter et al., 2009).

While the increase in leaf area under SH₂₁ compared to EOD₂₁ may have facilitated an increase in light interception, the decrease in LMA and stem dry mass per unit length likely reduced the ability of leaves and stems to resist mechanical stress (Ballare et al., 2017); plants with these characteristics would be more likely to be damaged during production and shipping. The significantly reduced root dry mass of SH₂₁ plants compared to EOD₂₁ may also have contributed to the reduction in mechanical stability (Fig. 7C-D). The SH₂₁ treatment was not designed to evaluate a specific plug production strategy, but the reduced structural stability and exaggerated elongation demonstrate that the deleterious shade avoidance responses outweigh potential benefits of leaf expansion and potential increased light interception under these conditions.

3.4.3 Growth under 16 °C inhibits growth in petunia

Growth outside of a plant's optimal temperature range can inhibit plant growth and development (Bahunga and Jagadish, 2015; Hatfield and Prueger, 2015), and a constant air temperature of 16°C generally stunted growth of petunia 'Dreams Midnight' regardless of lighting treatment. Specifically, relative to their 21 °C counterparts (EOD₂₁ and SH₂₁), EOD₁₆ and SH₁₆ showed significantly lower leaf area and length, leaf number, stem caliper, and all dry mass parameters sans LMA; LMA was greater under EOD₁₆ and SH₁₆ compared to 21 °C counterparts (Figure 5-7, Table 8.). For example, shoot dry weight under EOD₂₁ was 230%

greater than under EOD₁₆ (Fig 7.), and SH₁₆ and EOD₁₆ had ~4 and ~9 fewer leaves on average, respectively, compared to their 21 °C counterparts at day 28 (Table 8.). Additionally, stem length was reduced under EOD₁₆ and SH₁₆ relative to their 21 °C counterparts, but this relationship was only statistically significant for SH₁₆ (Table 8). These responses aren't necessarily surprising; growth and development of petunia under different temperature conditions has been investigated and the optimal temperature for flowering of *Petunia* spp. seems to be around or above 25-26 °C (Kaczperski et al. 1991; Warner, 2010).

The temperate *Arabidopsis* accession *Ler* was characterized as displaying temperature-dependent shade avoidance responses when grown under a low R:FR at 16 °C that included increased leaf area and thickness, a reduction in petiole angle and length, and increased shoot biomass relative to high R:FR controls; these temperature-dependent responses were speculated to be advantageous in maximizing light capture without severe risk of cold stress (Patel et al., 2013). In contrast, the Cape Verdes islands accession of *Arabidopsis* showed typical shade avoidance responses at both temperatures (Patel et al., 2013). Visually, petunia grown under SH₁₆ resembled a “smaller” SH₂₁, and this visual observation is supported by a reduction of all measured morphological and dry mass variables aside from LMA (Fig 5-7, Table 8). However, increased LMA is a common response of plants growing under low temperature, thus increased LMA in SH₁₆ is likely a more general temperature response (Poorter et al., 2009); this relationship was also true for EOD₁₆ compared EOD₂₁.

As growth at a constant 16 °C was somewhat expected to result in highly stunted growth, we implemented one +DIF treatment (CN₂₁EOD₁₆) wherein plants were grown under a high R:FR during the day at 21 °C, and were moved to the 16 °C chamber for the EOD-FR and dark period (Table 7). Restricting the period of reduced temperature to the dark period reduced leaf

area and leaf number, as well as root and shoot dry mass of CN₂₁EOD₁₆ seedlings compared to EOD₂₁, but these reductions were less extreme compared to EOD₁₆ (Fig 5-7, Table 8). For example, at day 28, leaf area under EOD₂₁ was 18% greater than CN₂₁EOD₁₆ and 295% greater than under EOD₁₆ (Fig. 5B). Similar to the relationship between SH₂₁ and SH₁₆ as well as EOD₂₁ and EOD₁₆, CN₁₆EOD₂₁ resembled a slightly stunted version of EOD₂₁. Thus, a shift to cooler temperatures for the EOD-FR and dark period doesn't elicit a different temperature-dependent shade avoidance response under these experimental conditions.

3.4.4 Dynamic Treatments

Dynamic treatments (DSH₂₁ and DSH₁₆) were included in this study to investigate whether a shift from a constant high R:FR at 21 °C to shade light at 16 °C would avoid early stunting by constant growth at 16 °C; DSH₂₁ was used as a 21 °C temperature control. Similar to SH₂₁, while the number of leaves under DSH₁₆ was reduced, a similar leaf number to CN plants suggests larger individual leaves for both day 21 and day 28 (Fig 5A-B, Table 8). However, as leaf length and LMA were significantly higher and lower, respectively, under DSH₁₆ compared to CN plants, leaves were likely more vulnerable to mechanical stress (Fig 5C-D, Table 8; Ballere and Pierk, 2017). Reductions in all other dry mass parameters compared to control plants suggests inhibited growth due to colder temperatures (Fig 6-7; Table 8).

3.5 Conclusions and Future Work

Leaf expansion in response to growth under a low R:FR is often species specific and can depend on the PPFD. Additionally, while some shade avoidance responses including an increase in leaf area can be promoted by EOD-FR, these may be species-specific as well as dependent on R:FR or length of the photoperiod. In the present study, one-hour EOD-FR treatments with a R:FR of 0.15 resulted in very similar petunia seedlings to those under the one-hour EOD_w treatment. Thus, EOD-FR applications do not appear to be an effective strategy for promoting

early leaf expansion in petunia seedlings. However, additional research may be useful in elucidating the effects of photoperiod length as well as the R:FR during the normal photoperiod on shade avoidance responses elicited by EOD-FR treatments.

Shade light providing a less extreme R:FR (0.50) for the duration of the photoperiod at 21 °C resulted in highly exaggerated shade avoidance responses including significant increase in leaf area and stem elongation, but also a large reduction in dry mass investment into each organ. This resulted in fragile plants that are highly undesirable in commercial plug production, while growth at a constant 16 °C generally inhibited plant growth and development. Examining shade avoidance responses at different temperatures in cool season or cold tolerant crops may lead to further understanding of temperature-dependent shade avoidance responses and could open opportunities to improve plug production for certain crops.

Table 6. The average $\pm$ standard deviation of blue, green, red, and far-red photon flux densities (PFD; $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), the red:far-red ratio (R:FR), and daily light integral (DLI) of the high R:FR (RL), shade light (SHD), end-of-day white (EOD_w) and end-of-day far-red (EOD_{FR}) light conditions provided by Elixia fixtures in growth chambers. The total photon flux density (TPFD) is equal between the SHL and RL, and the EOD_w and EOD_{FR} treatments, respectively.

Light Condition	DLI	Blue PFD	Green PFD	Red PFD	Far-Red PFD	R:FR
RL	10.2 $\pm$ 0.05	16.4 $\pm$ 0.1	24.8 $\pm$ 0.2	112.7 $\pm$ 0.1	10.4 $\pm$ 0.3	10.91 $\pm$ 0.3
SHD	10.2 $\pm$ 0.06	16.4 $\pm$ 0.1	24.8 $\pm$ 0.2	41.1 $\pm$ 0.7	81.9 $\pm$ 0.6	0.50 $\pm$ 0.01
EOD _w	<0.2	4.3 $\pm$ 0.04	6.8 $\pm$ 0.1	32.4 $\pm$ 0.6	3.0 $\pm$ 0.08	10.8 $\pm$ 0.5
EOD _{FR}	<0.2	<1	<1	5.9 $\pm$ 0.3	40.6 $\pm$ 0.5	0.15 $\pm$ <0.01

Table 7. Light and temperature conditions for each of the eight lighting treatments. Treatments consisted of either a high red to far-red (R:FR) ratio light environment (RL) for 17.25 hours followed by a 1-hour end-of-day (EOD) treatment with either a high (EOD_w) or low (EOD_{FR}) R:FR; or a low R:FR shade light (SHD) light environment for 17.25 hours with no EOD treatment. Dynamic treatments DSH₂₁ and DSH₁₆ were grown under control (CN) conditions for 14 days post treatment initiation, and moved under SH₂₁ and SH₁₆, respectively, for the last 14 days prior to harvest.

Treatment	Normal Photoperiod	EOD
CN	RL at 21°C	EOD _w at 21°C
EOD ₂₁	RL at 21°C	EOD _{FR} at 21°C
EOD ₁₆	RL at 16°C	EOD _{FR} at 16°C
CN ₂₁ EOD ₁₆	RL at 21°C	EOD _{FR} at 16°C
SH ₂₁	SHD at 21°C	Blackout at 21°C
SH ₁₆	SHD at 16°C	Blackout at 16°C

Treatment	Day 1-14 Treatment	Day 15-28 Treatment
DSH ₂₁	CN	SH ₂₁
DSH ₁₆	CN	SH ₁₆

Table 8. Leaf length, leaf number, relative chlorophyll content (SPAD), leaf dry mass, stem dry mass and stem caliper for *Petunia ×hybrida* ‘Dreams Midnight’ 21 and 28 days post treatment initiation under a high red to far-red (R:FR) ratio during for 17.25-hours and a one-hour end-of-day white treatment at 21 °C (control; CN), end-of-day far-red treatment at 21 °C (EOD₂₁) or 16 °C; a high R:FR for 17.25-hours at 21 °C and then end-of-day far-red at 16 °C (CN₂₁EOD₁₆), or a low R:FR shade light treatment for 17.25 hours at 21 °C (SH₂₁) or 16 °C (SH₁₆) with no end-of-day treatment. Dynamic treatments DSH₂₁ and DSH₁₆ were grown under CN conditions for 14 days post treatment initiation, and moved under SH₂₁ and SH₁₆, respectively, for the last 14 days prior to harvest. Means sharing a letter are not statistically different by Tukey’s honestly significant difference (HSD) test at $P \leq 0.05$.

Day	Treatment	Leaf Length (cm)	Leaf Number	SPAD	Leaf Dry Mass (g)	Stem Dry Mass (g)	Stem Caliper (mm)
21	CN	2.72 ^z c ^y	7.7 e	32.6 d	33.1 d	1.7 d	1.46 c
	EOD ₂₁	3.08 d	7.7 e	30.4 cd	32.6 d	1.79 d	1.49 c
	SH ₂₁	4.04 f	6.7 bc	20.6 b	18.3 b	1.65 cd	1.27 b
	SH ₁₆	2.17 b	4.0 a	17.8 a	5.5 a	0.25 a	0.97 a
	CN ₂₁ EOD ₁₆	2.65 c	7.2 cd	32.5 d	25.9 c	1.23 bc	1.27 b
	EOD ₁₆	1.69 a	4.1 a	29.8 c	8.3 a	2.60 a	0.97 a
	DSH ₂₁	4.28 f	7.4 de	20.8 b	21.1 b	1.49 cd	1.19 b
	DSH ₁₆	3.59 e	6.6 b	21.3 b	18.2 b	1.03 b	1.23 b
28	CN	4.25 c	16.1 e	34.5 d	94.1 d	5.10 b	1.88 bc
	EOD ₂₁	4.42 c	15.4 e	32.9 cd	100.0 d	6.01 b	1.97 cd
	SH ₂₁	6.67 e	10.3 c	23.9 b	74.4 bc	12.27 c	1.76 b
	SH ₁₆	3.61 b	5.9 a	20.0 a	21.5 a	1.12 a	1.35 a
	CN ₂₁ EOD ₁₆	4.45 c	13.1 d	33.0 cd	80.9 c	4.59 b	1.92 bcd
	EOD ₁₆	2.54 a	6.3 a	30.6 c	30.8 a	1.28 a	1.39 a
	DSH ₂₁	5.98 d	12.2 d	22.2 ab	79.8 c	18.8 d	2.10 d
	DSH ₁₆	6.19 de	8.9 b	22.0 ab	67.5 b	5.69 b	1.86 bc

^zMean values are based on 5 samples from each treatment across three experimental replications.

^yMeans sharing a letter within a harvest day are not statistically different by Tukey's honest significant difference (HSD) test at $p < 0.05$.

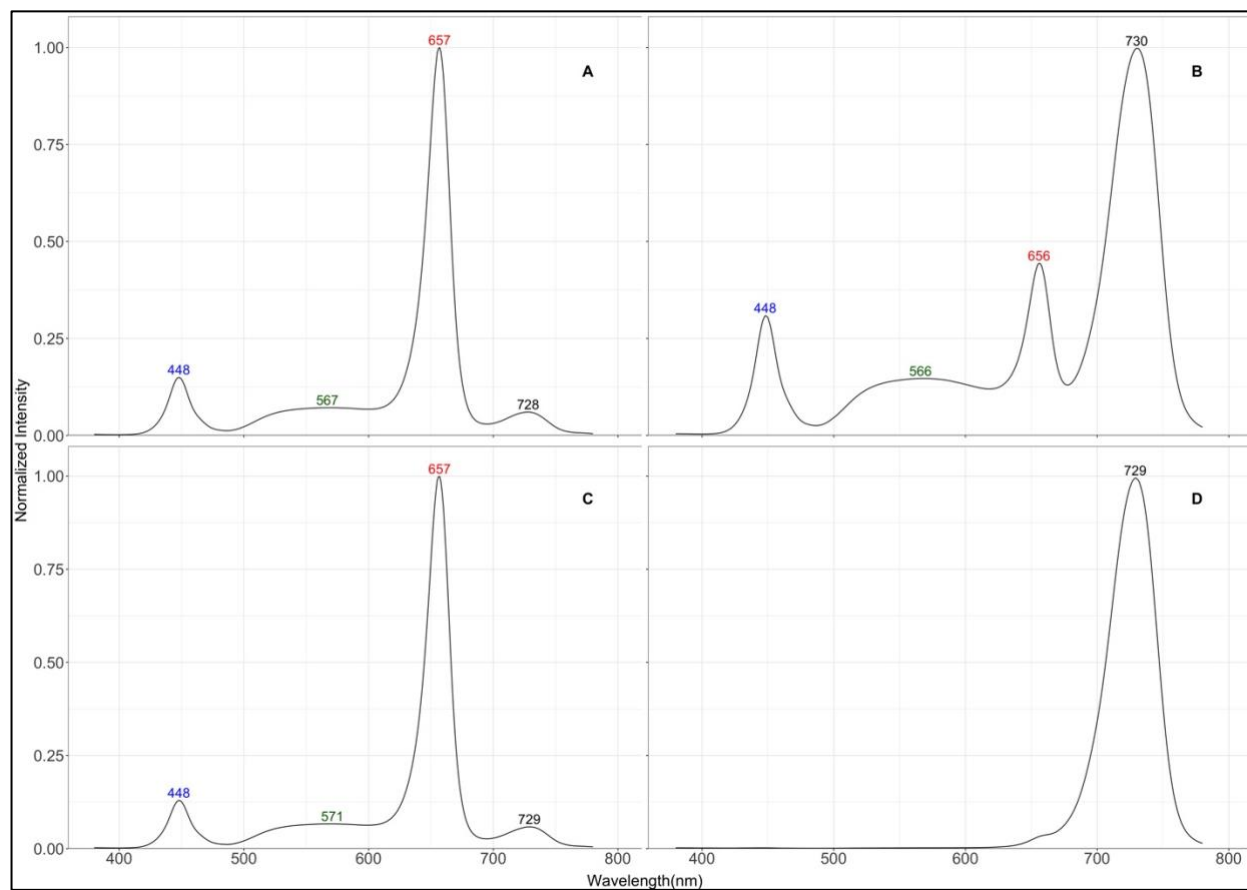


Fig. 4. Normalized spectral distribution from 400-800 nm measured at canopy height for the high R:FR (A; RL), shade light (B; SHD) end-of-day white (C; EOD_w), and end-of-day far-red (D; EOD_{FR}) light environments. Data were averaged across three experimental replications with peak wavelengths in the blue, green, red, and far-red regions shown above their respective peaks.

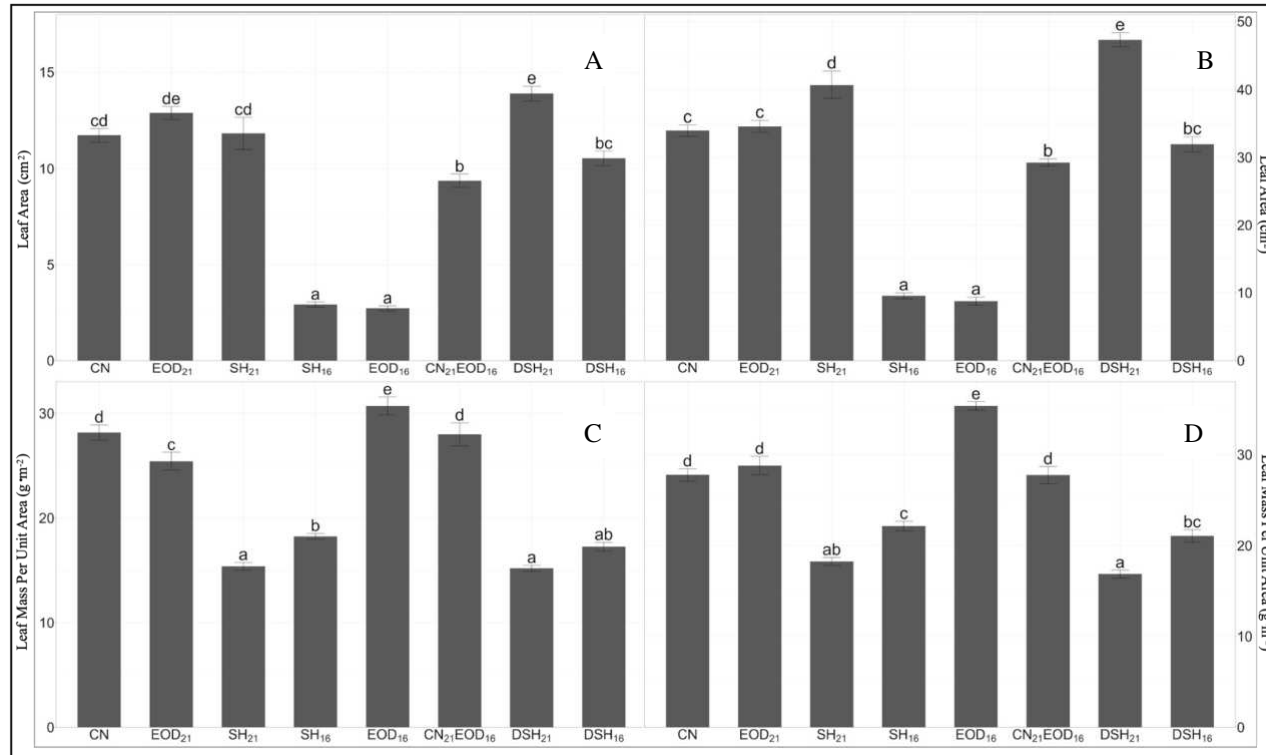


Fig. 5. Leaf area and leaf mass per unit area of *Petunia x hybrida* 'Dreams Midnight' seedlings 21 (A and C) and 28 (B and D) d after treatment initiation under a high red to far-red (R:FR) ratio for 17.25-hours and a one-hour end-of-day white treatment at 21 °C (control; CN), end-of-day far-red treatment at 21 °C (EOD₂₁) or 16 °C; a high R:FR for 17.25-hours at 21 °C and then end-of-day far-red at 16 °C (CN₂₁EOD₁₆), or a low R:FR shade light treatment for 17.25 hours at 21°C (SH₂₁) or 16°C (SH₁₆) with no end-of-day treatment. Dynamic treatments DSH₂₁ and DSH₁₆ were grown under CN conditions for 14 days post treatment initiation, and moved under SH₂₁ and SH₁₆, respectively, for the last 14 days prior to harvest. Means sharing a letter are not statistically different by Tukey's honestly significant difference (HSD) test at $P \leq 0.05$.

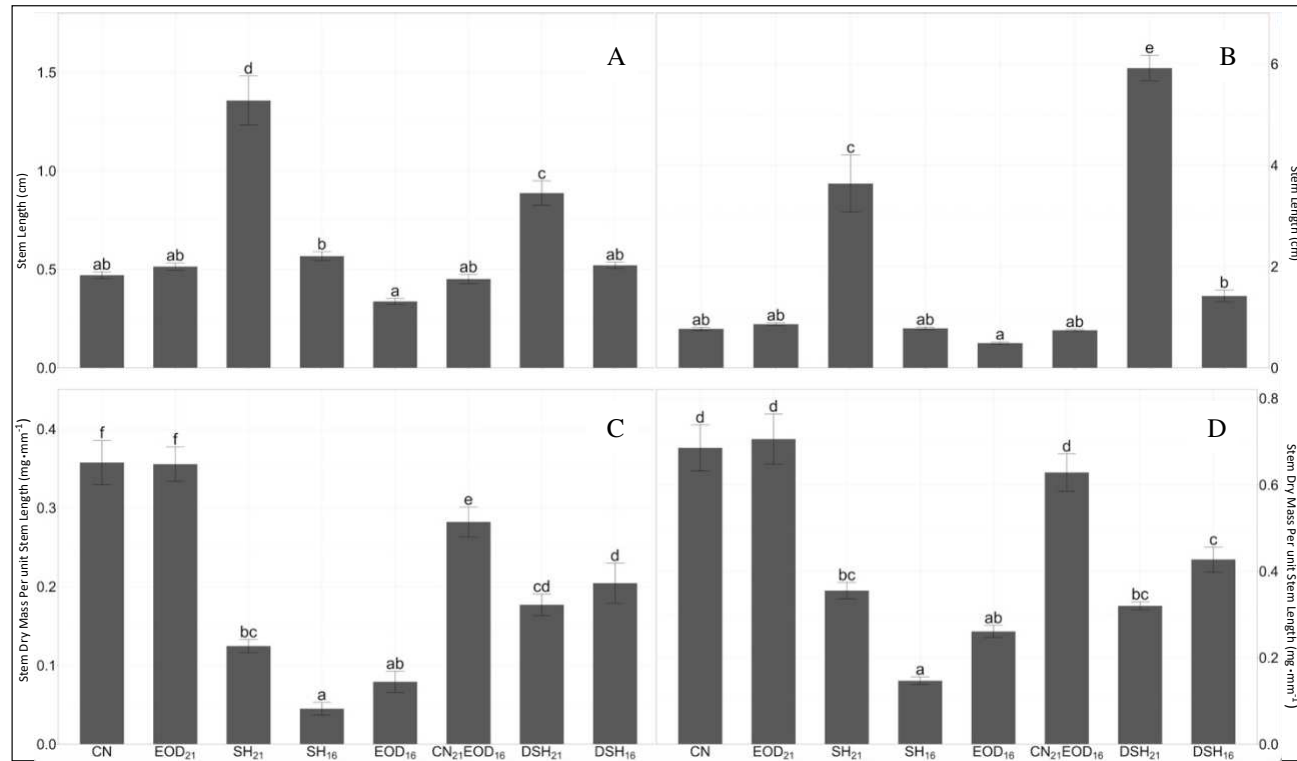


Fig. 6. Stem length and stem dry mass per unit stem length of *Petunia x hybrida* 'Dreams Midnight' seedlings 21 (A and C) and 28 (B and D) d after treatment initiation under a high red to far-red (R:FR) ratio for 17.25-hours and a one-hour end-of-day white treatment at 21 °C (control; CN), end-of-day far-red treatment at 21 °C (EOD₂₁) or 16 °C; a high R:FR for 17.25-hours at 21 °C and then end-of-day far-red at 16 °C (CN₂₁EOD₁₆), or a low R:FR shade light treatment for 17.25 hours at 21 °C (SH₂₁) or 16 °C (SH₁₆) with no end-of-day treatment. Dynamic treatments DSH₂₁ and DSH₁₆ were grown under CN conditions for 14 days post treatment initiation, and moved under SH₂₁ and SH₁₆, respectively, for the last 14 days prior to harvest. Means sharing a letter are not statistically different by Tukey's honestly significant difference (HSD) test at $P \leq 0.05$.

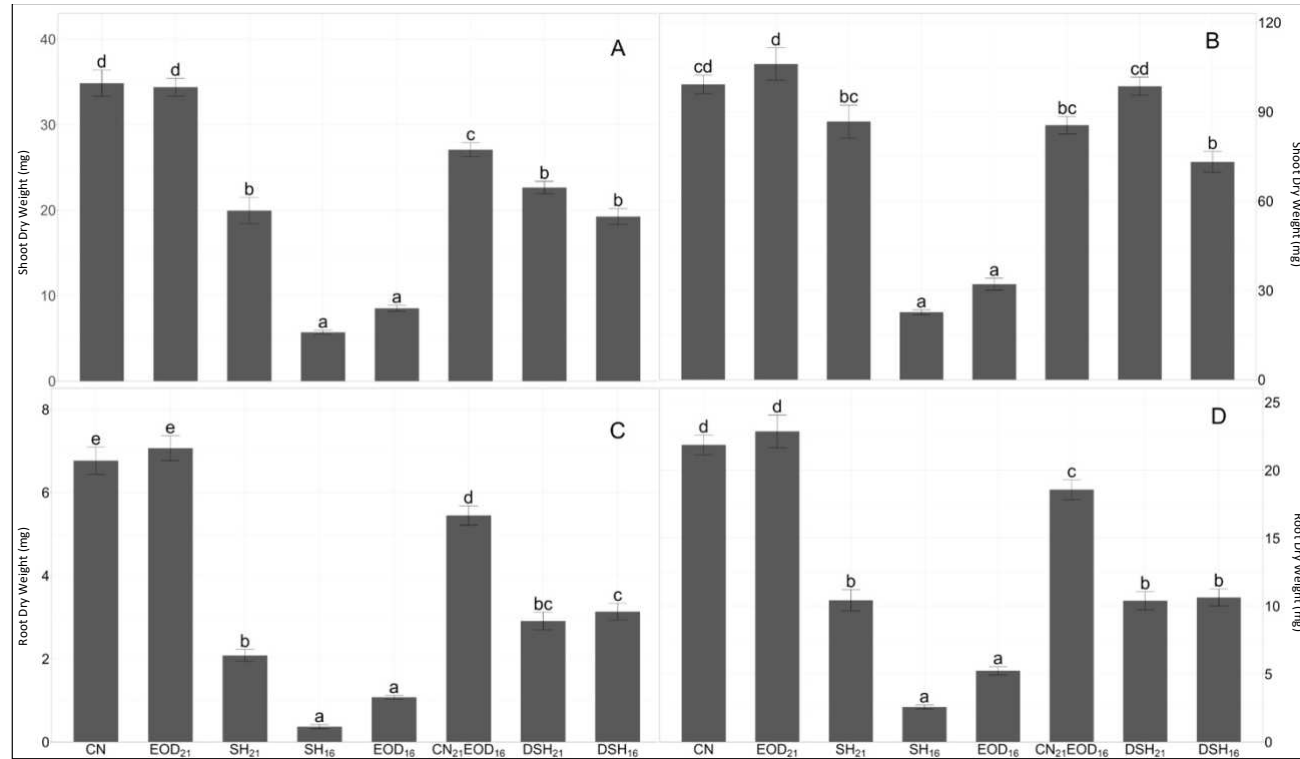


Fig. 7. Shoot dry mass and root dry mass of *Petunia × hybrida* ‘Dreams Midnight’ seedlings 21 (A and C) and 28 (B and D) d after treatment initiation under a high red to far-red (R:FR) ratio for 17.25-hours and a one-hour end-of-day white treatment at 21 °C (control; CN), end-of-day far-red treatment at 21 °C (EOD₂₁) or 16 °C; a high R:FR for 17.25-hours at 21 °C and then end-of-day far-red at 16 °C (CN₂₁EOD₁₆), or a low R:FR shade light treatment for 17.25 hours at 21 °C (SH₂₁) or 16°C (SH₁₆) with no end-of-day treatment. Dynamic treatments DSH₂₁ and DSH₁₆ were grown under CN conditions for 14 days post treatment initiation, and moved under SH₂₁ and SH₁₆, respectively, for the last 14 days prior to harvest. Means sharing a letter are not statistically different by Tukey’s honestly significant difference (HSD) test at $P \leq 0.05$.

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CHAPTER 4. FINDINGS SUMMARY AND INDUSTRY TAKEAWAY

4.1 Findings summary

Electric lighting for controlled environment crop production makes up a significant portion of energy costs to growers, but young plants with small leaves have a reduced capacity for light interception resulting in wasted energy. Two experiments were conducted to investigate the effects of far-red light on leaf expansion for plug production in controlled environments, as early leaf expansion in young plants may improve light capture and reduce wasted supplemental light. In the first study (see chapter 2 for details), seedlings of *Petunia ×hybrida* (petunia) ‘Wave Purple’ and ‘Dreams Midnight’ were grown in a greenhouse under a low daily light integral (DLI; $\sim 5 \text{ mol} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$) designed to simulate winter light levels. In a greenhouse, changing light quality using supplemental lighting is difficult due to the quantity of natural light, so an end-of-day lighting strategy was used to provide seedlings with end-of-day far-red (EOD-FR) light using commercially available photoperiodic flowering lamps and research grade LED fixtures to provide intermediate and very low ratios of red to far-red light (R:FR), respectively. End-of-day lighting treatments also varied in the intensity of far-red light and the duration of the treatment. Importantly, no end-of-day treatment resulted in an increase in leaf area for either ‘Dreams Midnight’ or ‘Wave Purple’, but longer end-of-day treatments and a lower R:FR promoted stem elongation and stretching in both cultivars leading to low-quality plugs not acceptable for commercial production.

The low DLI may have limited leaf expansion in response to EOD-FR in the first study, so a second study (see chapter 3 for details) was conducted in a highly controlled growth chamber where the DLI was maintained at the recommended minimum value to achieve quality

young annual bedding plant production ($10 \text{ mol} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$) using programmable LED fixtures. In this study, seedlings of petunia ‘Dreams Midnight’ received either EOD-FR for one hour or were grown under light with a low R:FR light for the duration of the photoperiod (shade light). Somewhat surprisingly, no increase in leaf area was observed for seedlings that received EOD-FR compared to control seedlings (no EOD-FR). In contrast to the first experiment, no significant stem elongation or stretching was seen in response to EOD-FR which may have been due to the increased DLI or photoperiod length. Shade light treatments dramatically promoted leaf expansion, but excessive stem elongation and leaf expansion resulted in seedlings unacceptable for quality production. The interaction between temperature and far-red light was also examined in the second study where plants were grown at either 16 or 21 °C or shifted from 21 to 16 °C for the EOD-FR treatment and the following dark period. Growth at lower temperatures generally resulted in stunted growth of seedlings.

Our results suggest that end-of-day lighting with a very low R:FR does not promote early leaf expansion in seedlings of petunia independent of whether plants are grown under a recommended or low DLI. However, significant stretching responses seen in response to end-of-day lighting when the DLI was limiting for growth were not observed when light was not limiting, showing that some EOD-FR responses are reduced in petunia by sufficient light quantity or long photoperiods. This work further shows species-specific responses to far-red radiation as crops like *Lactuca sativa* (lettuce) have shown leaf expansion in response to EOD-FR light. Other potentially interacting factors such as the long duration of the photoperiod in the second study may have also influenced treatment responses. Using far-red light to promote increased light interception by young plants may still prove useful in limiting wasted artificial light, but further research is required to identify appropriate species in a production setting as

well as more closely examine potential interactive effects of temperature and photoperiod on responses to far-red light. Additionally, it will be necessary to address to what degree a greater leaf area index may reduce wasted light and whether any proposed strategies are viable in a commercial setting and prove cost effective for growers.

4.2 Industry significance

Currently, far-red light may be included in low intensity lighting for end-of-day or night interruption practices to promote flowering in some long-day plants (photoperiodic lighting), but utilizing far-red light to promote changes in plant morphology, such as the expansion of leaves, may also lead to improved production practices through enhanced light capture. However, the information gathered from this work highlights that additional research is required before any such strategies should be implemented in a commercial setting. For example, in chapter 2 we found that applying EOD-FR light with a very low R:FR (0.15) using research-grade LEDs to petunia ‘Dreams Midnight’ and ‘Wave Purple’ growing under a low daily light integral (DLI; $\sim 5 \text{ mol} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$) led to low-quality stretched plugs with no practical impact on leaf area. These results illustrate a potential pitfall, and caution is recommended when choosing photoperiodic lighting sources that provide a low ratio of red light (600-700 nm) to far-red light ($> 700 \text{ nm}$) if the DLI is severely limiting for normal growth and development of horticultural crop species to avoid undesired stretching or stem elongation responses.

The second experiment in this thesis (chapter 3) was conducted in-part to determine if the low DLI prevented leaf expansion for petunia ‘Dreams Midnight’ in response to EOD-FR. However, when grown at the general minimum recommended DLI for quality plug production ($\sim 10 \text{ mol} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$; ~ 17 -hour photoperiod) with one hour of EOD-FR, no practical increase in leaf area was found compared to plants that did not receive EOD-FR. Importantly, this may be an

example of a species-specific or cultivar-specific response as while ‘Dreams Midnight’ may not respond (increase in leaf area) to end-of-day far-red light, lettuce shows leaf expansion in response to EOD-FR. The existence of species-specific responses as well as an increase in evidence to support expanding the range of photosynthetically active radiation (PAR) to include far-red wavelengths (extended PAR; ePAR) provide justification to further examine what crops may benefit from an EOD-FR strategy. The economic benefit of using far-red light to increase leaf expansion early and improve light capture in production has not been examined to our knowledge, and this is another area of research necessary before clear recommendations can be made.