Environmental and Experimental Botany 168 (2019) 103889

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Far-red radiation increases dry mass partitioning to fruits but reduces *Botrytis cinerea* resistance in tomato

Yongran Ji[a,1], Theoharis Ouzounis[a,1], Sarah Courbier[b], Elias Kaiser[a], Phuong T. Nguyen[a,2], Henk J. Schouten[c], Richard G.F. Visser[c], Ronald Pierik[b], Leo F.M. Marcelis[a,*], Ep Heuvelink[a]

[a] Horticulture and Product Physiology, Department of Plant Sciences, Wageningen University & Research, Wageningen, the Netherlands
[b] Plant Ecophysiology, Department of Biology, Utrecht University, Utrecht, the Netherlands
[c] Plant Breeding, Department of Plant Sciences, Wageningen University & Research, Wageningen, the Netherlands

ARTICLE INFO

Keywords:
Botrytis cinerea
Dry mass partitioning
Far red
Growth component analysis
LED lighting
Solanum lycopersicum

ABSTRACT

The addition of far-red (FR, 700–800 nm) radiation to standard growth light triggers a set of photomorphogenic responses collectively termed shade avoidance syndrome. Recent research showed that additional FR increased fruit yield in greenhouse tomato production. However, the mechanism behind this increase is not clear; nor is it known whether there is a trade-off between growth and defense against plant diseases in tomato under additional FR. The aims of this study were 1) to quantify the effect of additional FR on tomato fruit growth, 2) to explain this effect based on underlying growth components and 3) to examine the FR effect on resistance against the necrotrophic fungus *Botrytis cinerea*. Tomato (*Solanum lycopersicum* 'Moneymaker') plants were grown for four months with 30 or 50 μmol m^{-2} s^{-1} of FR added to 150 μmol m^{-2} s^{-1} red + blue or white background LED lighting. Growth and development parameters were recorded, and a growth component analysis was conducted. Bioassays for resistance against *B. cinerea* were conducted on leaf samples collected from each light treatment. Additional FR increased total fruit dry mass per plant by 26–45%. FR affected multiple growth components, among which the fraction of dry mass partitioned to fruits was the most prominent with a 15–35% increase. Truss appearance rate was increased 11–14% by FR while instantaneous net photosynthesis rate was not affected. FR also resulted in more severe disease symptoms upon infection with *B. cinerea*. In conclusion, additional FR increases tomato fruit production mainly by increasing dry mass partitioning to fruits, rather than improving photosynthesis or increasing total plant biomass. However, FR also reduces resistance of tomato leaves against *B. cinerea*.

1. Introduction

The shade avoidance syndrome (SAS), a set of adaptive changes that plants deploy when exposed to light with a low red (R, 600–700 nm) to far-red (FR, 700–800 nm) ratio, is among the most intensively studied set of plant responses to changes in their light environment. Plants perceive changes in the R: FR ratio via a family of photoreceptors named phytochromes, which exist as two photo-interconvertible isoforms: the biologically inactive red-light absorbing form (P_r) and the biologically active far-red-absorbing form (P_{fr}) (Chen et al., 2005). P_{fr} translocates to the nucleus and mediates different photomorphogenetic responses (Ruberti et al., 2012). Typical SAS responses such as stem elongation (Huber and Wiggerman, 1997), leaf hyponasty (Michaud et al., 2017; Pantazopoulou et al., 2017), reduced branching (Finlayson, 2007), and accelerated flowering (Devlin, 1998) have been studied intensively in *Arabidopsis thaliana* (for review, see Casal, 2012). Unlike in nature, where a low R: FR ratio often coincides with a decrease in photosynthetic photon flux density (PPFD), recent research typically features the addition of FR in a defined background light. The development of efficient light-emitting diodes (LEDs) in the past decade also stimulated the study of FR responses in a wide range of crop species including ornamental crops (Park and Runkle, 2017), leafy vegetables (Li and Kubota, 2009; Zhen and van Iersel, 2017) and fruit crops (Hao et al., 2017). Frequently, a positive FR effect on plant dry mass production is reported. To explain this, some authors demonstrated that additional FR may alter plant architecture to increase light interception

* Corresponding author.
E-mail address: leo.marcelis@wur.nl (L.F.M. Marcelis).
[1] Authors contributed equally to this research.
[2] Present address: Faculty of Agronomy, Vietnam National University of Agriculture, Hanoi, Viet Nam.

https://doi.org/10.1016/j.envexpbot.2019.103889
Received 23 July 2019; Received in revised form 17 September 2019; Accepted 17 September 2019
Available online 20 September 2019

(Kalaitzoglou et al., 2019), while in other studies additional FR was shown to increase leaf net photosynthesis rates (*A*) (Zhen and van Iersel, 2017; Cao et al., 2018) or whole-plant photosynthesis (Park and Runkle, 2017). However, it is also worth noting that the FR effect on photosynthesis varies between studies (Kim et al., 2019; Zhang et al., 2019). Additional FR was also reported to affect dry mass partitioning among plant organs, often increasing partitioning to shoot over root (Keiller and Smith, 1989; Page et al., 2009). In contrast to the abundance of research on relatively young plants, detailed studies of FR effects during the fruiting stage of crops such as tomato (*Solanum lycopersicum*) are less frequent. Recently, Kalaitzoglou et al. (2019) reported that additional FR increased total dry mass of tomato plants in the vegetative growth stage, as well as the fruit number per plant, fruit fresh weight per plant and average fruit fresh weight. Similarly, Zhang et al. (2018) reported higher total plant dry mass and higher fruit yield in tomato under additional FR radiation. However, neither study provided sufficient insights on how additional FR increases fruit growth in the fruiting stage of the crop, which is a key step in understanding the FR induced yield improvement in fruit crops like tomato.

Besides the increase of plant growth under additional FR, the promotion of SAS may negatively impact plant immunity (McGuire and Agrawal, 2005; Izaguirre et al., 2006). FR has been reported to downregulate both salicylic-acid and jasmonic-acid-induced plant defense responses in *A. thaliana* (De Wit et al., 2013). *Botrytis cinerea*, a necrotrophic fungal pathogen causing grey mold disease in many plant species, has been studied intensively due to its destructive effects on crop production (reviewed by Van Kan, 2006). In *A. thaliana*, exposure to a low R:FR light reduced plant resistance against *B. cinerea* (Cerrudo et al., 2012; Cargnel et al., 2014). Although no direct FR effect on tomato resistance against *B. cinerea* is known, reduced constitutive defenses and reduced jasmonic-acid-induced direct defenses have been reported under low R:FR light conditions (Cortés et al., 2016). Taken together, it is reasonable to expect that additional FR may reduce the resistance of tomato against *B. cinerea*.

Growth component analysis, which is an analysis that subdivides growth into underlying morphological and physiological components (Jolliffe and Courtney, 1984), can be a useful tool to evaluate the contribution of these processes to fruit growth (Higashide and Heuvelink, 2009). Here, we aimed to identify and quantify the key components of tomato fruit growth as affected by additional FR and study whether additional FR affects resistance against *B. cinerea* in fruiting tomato plants grown with supplemental LED lighting. We hypothesized that additional FR would accelerate plant development (flowering, truss appearance rate), increase total fruit dry mass and fraction of dry mass partitioned to fruits and decrease plant defense against *B. cinerea*. To test these hypotheses, we conducted an experiment with tomato plants for four months in a greenhouse with different levels of FR added to different LED light combinations. Growth components were monitored and bioassays were conducted to evaluate plant resistance against *B. cinerea*.

2. Material and methods

2.1. Plant materials and growth conditions

The experiment was conducted at Wageningen University (52 °N, 6 °E, Wageningen, the Netherlands.). Tomato (*Solanum lycopersicum* 'Moneymaker') seeds were sown in the greenhouse (20 °C, relative humidity 80%) on 28 Nov. 2016 and germinated under natural light. On 15 Dec. 2016, uniform seedlings were transplanted into black 7.5-liter plastic pots filled with river sand and moved into another greenhouse. The greenhouse was divided into 15 compartments separated by double-sided light-impermeable white plastic sheet. In each compartment, 14 plants were placed on two gutters, including two border plants at both ends. Plant density was 4 plants m^{-2}. Climate in the greenhouse was controlled by greenhouse climate control computer (Hoogendoorn, Vlaardingen, The Netherlands). Measured day/night temperature was 22 ± 0.5/18 ± 0.3 °C (mean ± standard deviation) until plants started flowering (35 days after transplanting). Thereafter, temperatures were adjusted to 20 ± 0.7/16 ± 0.3 °C to facilitate fruit set. Temperature was recorded every 10 min with PT500 temperature sensors (Hoogendoorn) placed in the center of each plot. Measured daily average relative humidity was 78 ± 5% and CO_2 partial pressure was 408 ± 11 μbar. The plants were irrigated with nutrient solution (electrical conductivity 2.1 dS m^{-1}, pH 5.5) containing 1.2 mM NH_4^+, 7.2 mM K^+, 4.0 mM Ca^{2+}, 1.8 mM Mg^{2+}, 12.4 mM NO_3-, 3.3 mM SO_4^{2-}, 1.0 mM PO_4^{2-}, 35 μM Fe^{3+}, 8.0 μM Mn^{2+}, 5.0 μM Zn^{2+}, 20 μM B, 0.5 μM Cu^{2+}, 0.5 μM MoO_4^{2-}. The EC and pH level of the nutrient solution were measured twice a week and the nutrient solution was refreshed frequently. Manual pollination with an electronic bee (Vibri Vario, Royal Brinkman, Gameren, the Netherlands) was applied three times per week. Side shoots and same number of old leaves were removed weekly from all plants.

2.2. Light treatments and experimental set-up

Five overhead light treatments were applied: white (W), white + 30 μmol m^{-2} s^{-1} FR (WFR30), red + blue (RB), red + blue + 30 μmol m^{-2} s^{-1} FR (RBFR30) and RB + 50 μmol m^{-2} s^{-1} FR (RBFR50) (Fig. 1, Table 1). The spectral distribution and photon flux density of the supplementary light was measured with a spectroradiometer (USB 2000 + UV-VIS, Ocean Optics, Duiven, the Netherlands), on 6 evenly distributed locations in each plot at the top of the canopy. Phytochrome photostationary state (PSS) in each treatment was calculated based on the measured spectra as the ratio of P_{fr} to the total of P_{fr} and P_r according to Sager et al. (1988). The RBFR50 treatment was included because it had the same PSS value as WFR30 (Table 1). The blue, red and far-red spectra in this experiment peaked at 453 nm, 666 nm and 735 nm, respectively. Photoperiod was set to 16 h (0400 h–2000 h). On average, solar daily photosynthetic photon flux density (PPFD, 400–700 nm) contributed 12% to the total daily PPFD integral during the whole experiment at canopy level (Fig. S2).

All supplementary lighting was provided by LED modules (W: GreenPower LED-TL-DR/W-MB-VISN, RB: GreenPower LED -TL-DR/B-150, FR: GreenPower LED -PM-FR-150, Philips, Eindhoven, the Netherlands). The height of the LEDs was adjusted weekly to maintain the desired PPFD at the top of the canopy (150 μmol m^{-2} s^{-1}, Table 1). When the LEDs reached the maximal height of the greenhouse, the top of the canopy was lowered weekly, as is usual in a modern high wire cultivation system. A spectroradiometer was used to ensure that both PPFD and PSS values were kept constant every time the height of lamps or that of the plants was adjusted. The experiment was set up as a randomized complete block design, where the five light treatments were repeated in three blocks.

2.3. Non-destructive measurements

2.3.1. Growth and development parameters

Numbers of leaves (length ≥ 1 cm) per plant, flowering buds per truss, fully opened flowers per truss and set fruits (diameter ≥ 5 mm) per truss were recorded weekly for three plants per block.

2.3.2. Leaf net photosynthesis rate

Measurements of leaf net photosynthesis were performed using the LI-6400XT photosynthesis system (Li-Cor Biosciences, Lincoln, NE, USA). Acclimation of leaf net photosynthesis rate (*A*) to FR was assessed using CO_2 (A/C_i) and light response curves (A/PPFD), by means of the leaf chamber fluorometer (leaf area: 2 cm^2) with the built-in RB LED light source. Instantaneous *A* under the treatment spectra was additionally assessed using a transparent leaf chamber (leaf area: 6 cm^2). Conditions in the chamber were: 400 μbar CO_2 partial pressure, 0.9–1.2 kPa leaf-to-air vapor pressure deficit, 25 °C cuvette

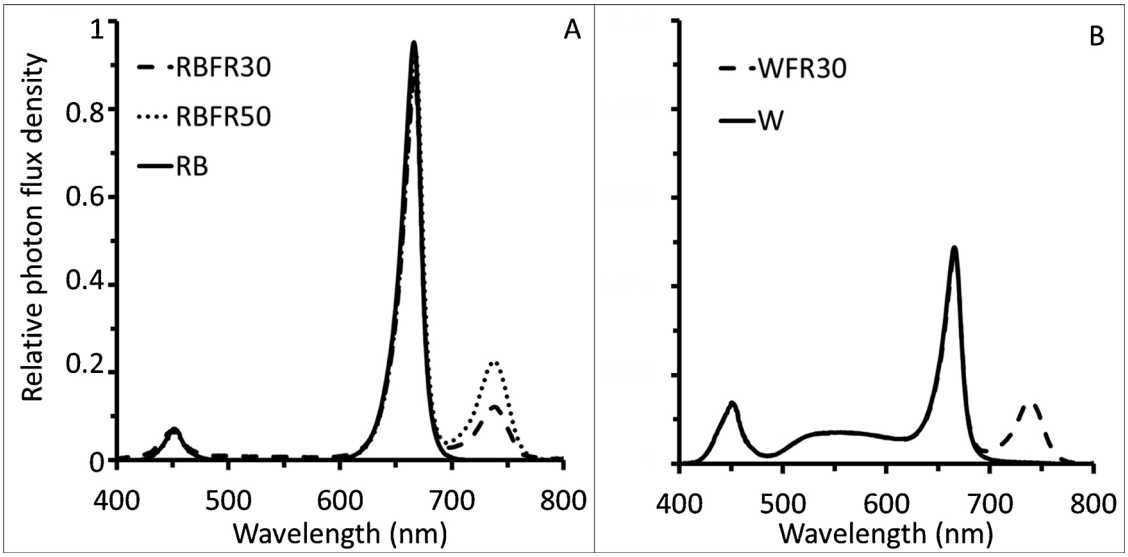

Fig. 1. Spectral composition of light treatments provided by light-emitting diodes (LEDs) measured at the top of the canopy. (A) 150 μmol m^{-2} s^{-1} background light of red/blue (RB) without far red (FR), with 30 or 50 μmol m^{-2} s^{-1} FR. (B) 150 μmol m^{-2} s^{-1} background light of white (W) LEDs, without FR, or with 30 μmol m^{-2} s^{-1} FR.

temperature, and 400 μmol s^{-1} air flow rate, unless described otherwise. The third or fourth leaflet of the fifth leaf (counting from the top of plant, the first leaf being longer than 5 cm) of one plant per plot was used for measurements. Measurements were conducted for two time periods (1) from 16 to 23 Jan. 2017 (32–39 days after transplanting) and (2) from 19 to 24 Feb. 2017 (66–71 days after transplanting).

2.3.2.1. A/C_i curves. Leaves were first adapted to 500 μbar CO_2 and 1500 μmol m^{-2} s^{-1} PPFD for 10–15 min, after which CO_2 partial pressure was increased to 2000 μbar. Then, CO_2 was reduced to 1500, 1000, 800, 600, 400, 200, 100, 50 and 40 μbar, with each step taking ~5 min. Data was logged every five seconds, and averages of six stable values at each CO_2 step were calculated. PPFD was maintained at 1500 μmol m^{-2} s^{-1}. Parameters of a biochemical photosynthesis model (Farquhar et al., 1980), namely the maximum rate of carboxylation of Rubisco (V_{cmax}), maximum electron transport rate at 1500 μmol m^{-2} s^{-1} PPFD (J_{1500}), and maximum rate of triose phosphate use (TPU) were estimated by using the fitting procedure by Sharkey et al. (2007).

2.3.2.2. A/PPFD curves. Directly after A/C_i curve measurements, CO_2 partial pressure was adjusted to 400 μbar, and PPFD remained at 1500 μmol m^{-2} s^{-1} until *A* and stomatal conductance were stable. Then, PPFD was reduced stepwise to 1000, 800, 600, 400, 200, 150, 100, 50 and 30 μmol m^{-2} s^{-1}. Data were logged as described above. Quantum yield (QY), a curvature parameter (Θ) and maximum *A* (A_{max}) were determined by fitting a non-rectangular hyperbola (Ögren and Evans, 1993).

2.3.2.3. Instantaneous A. Opposite leaflets of those used in A/C_i and *A*/PPFD curves were clamped into the transparent leaf chamber. After waiting for approximately two minutes for CO_2 partial pressures and water vapor to equilibrate, data were logged for 30 s, as described above. This was repeated at 1000 h, 1200 h and 1400 h on the same leaflets and the same day. Instantaneous *A* was later expressed as a function of PPFD inside the transparent leaf chamber, which was 88.4% of PPFD measured above the transparent leaf chamber.

2.4. Destructive measurements

On 30 Jan. 2017 (46 days after transplanting), 6 Mar. 2017 (81 days after transplanting) and 24 Apr. 2017 (130 days after transplanting), three replicate plants per compartment (experimental unit) were destructively measured. Total leaf area per plant (LI -3100 area meter, Li-Cor) and fruit number were measured. Leaves, stem, fruits and roots were separated and dried in the oven for 72 h at 105 °C to obtain the dry mass. The number and dry mass of harvested ripe fruits and removed old leaves was recorded and included in the calculation of total fruit dry mass and total plant dry mass.

Table 1
Photosynthetic photon flux density (PPFD), photon flux density (PFD) of blue, green, red, and far red, ratios of red: blue, red: far red and phytochrome photostationary state (PSS) value of the LED supplementary light in the five light treatments measured at the canopy level in the absence of solar radiation.

Parameter	Unit	Light Treatment				
		W	WFR30	RB	RBFR30	RBFR50
PPFD	μmol m^{-2} s^{-1}	153 ± 8	144 ± 9	155 ± 9	146 ± 9	146 ± 8
Blue	μmol m^{-2} s^{-1}	25 ± 3	23 ± 3	9 ± 2	8 ± 3	9 ± 1
Green	μmol m^{-2} s^{-1}	38 ± 4	34 ± 3	3 ± 0.2	4 ± 0.1	3 ± 0.2
Red	μmol m^{-2} s^{-1}	86 ± 4	79 ± 7	141 ± 8	133 ± 6	136 ± 8
Far red	μmol m^{-2} s^{-1}	1 ± 0.1	30 ± 3	3 ± 0.3	28 ± 1	49 ± 2
Red: blue		3 ± 0.3	3 ± 0.1	16 ± 0.4	17 ± 0.3	16 ± 0.4
Red: far red		79 ± 4	3 ± 0.1	49 ± 5	5 ± 0.1	3 ± 0.1
PSS		0.87	0.80	0.88	0.84	0.80

[1]All values are means ± s.e.m. s.e.m of PSS was very small (0.001–0.002) and is therefore not shown.
[2]For the calculations of ratios, PFD was integrated over 100 nm intervals for blue (400–500 nm), green (500–600 nm), red (600–700 nm) and far red (700–800 nm).

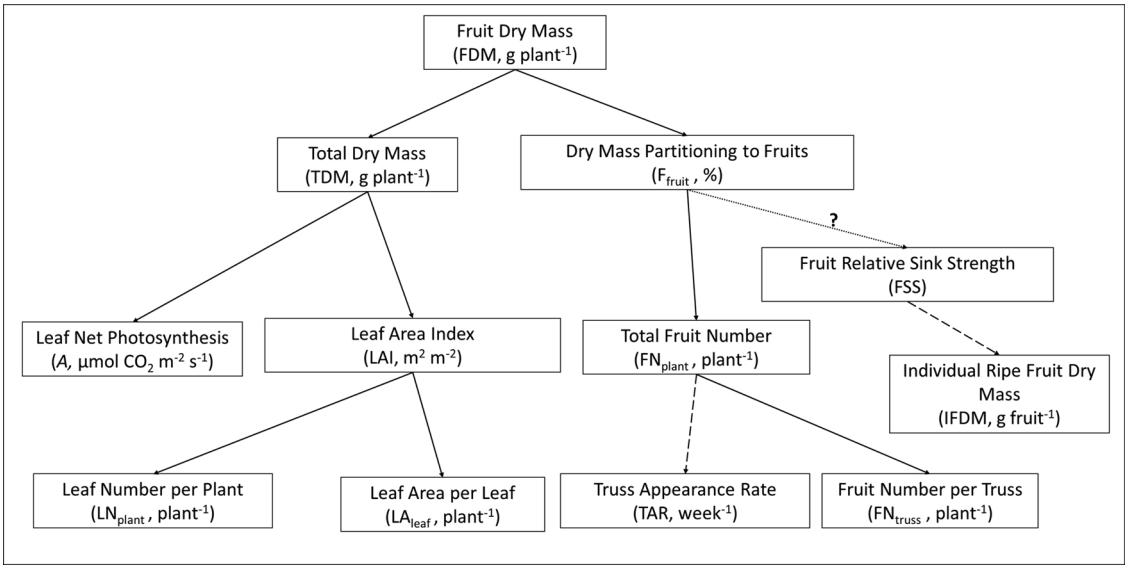

Fig. 2. General scheme of a top-down growth component analysis of total fruit dry mass. Abbreviations and units are included in brackets. Dashed lines indicate that the component was estimated instead of being directly measured.

2.5. Growth component analysis

Effects of light treatment on plant growth were analyzed by separating growth in its underlying components (Fig. 2). Fruit dry mass (FDM) was analyzed as the product of total plant dry mass (TDM) and fraction of dry mass partitioned to fruits (F_{fruit}). F_{fruit} was further divided into fruit number (FN_{plant}) and fruit relative sink strength (FSS, fruit sink strength relative to total sink strength of fruit and vegetative organs). FSS was not directly measured but can be evaluated by examining individual ripe fruit dry mass (IFDM). FN_{plant} can be explained by truss appearance rate (TAR) and fruit number per truss (FN_{truss}). All comparisons were based on the destructive harvests 46 and 130 days after transplanting, unless described otherwise. Instantaneous *A* was the average of the two measurement periods in January and February. Leaf area index (LAI), total leaf number per plant (LN_{plant}), and average leaf area per leaf (LA_{leaf}) were calculated from daily values estimated from linear interpolation of the data measured 46, 81 and 130 days after transplanting. TAR was calculated based on leaf appearance rate assuming that after formation of every three leaves a truss was formed.

2.6. Bioassays with B. cinerea

The *B. cinerea* strain *B.c* 05.10 was maintained for two weeks under natural daylight conditions on a half strength Potato Dextrose Agar medium (BD Difco, Sparks, MD, USA). The spore suspension was prepared according to Van Wees et al. (2013) and diluted to a final concentration of 1.5×10^5 spores mL^{-1} in half strength Potato Dextrose Broth (BD Difco) prior to inoculation. Leaflets of the sixth fully expanded leaf from the top of the plants were detached and placed in a 12 × 12 cm petri dish on water-soaked filter paper (Sigma-Aldrich, Zwijndrecht, the Netherlands). Leaflets from the same leaf rank were randomly collected across the whole greenhouse on 25, 47 and 81 days after transplanting with 10–30 leaflets per light treatment per harvest time. Each harvest time was considered a block in the statistical analysis. The adaxial side of the leaflets was drop-inoculated several times with 5 µl of *B.c* 05.10 spore suspension. Infected leaflets were placed under white LED (GreenPower LED-RM-W, Philips, Eindhoven, the Netherlands) with a photoperiod of 16 h at a PPFD of 150 µmol m^{-2} s^{-1}. Pictures were taken three days after inoculation and lesion areas were measured using ImageJ (Version 1.52e, Schneider et al., 2012).

2.7. Statistical analysis

Light effects on total fruit dry mass, dry mass partitioning to organs and lesion size after *B. cinerea* infection were tested using analysis of variance (ANOVA). Photosynthetic parameters were derived from two measurement periods, where each period was considered a block in the ANOVA. Homogeneity and normality of residuals in the ANOVA were tested using Bartlett's test and the Shapiro-Wilk test, respectively. When data did not fulfil the assumptions, they were log transformed and the tests on residuals were repeated. If the log-transformed data failed to satisfy the assumptions, the Kruskal-Wallis test was used to analyses the original non-transformed data. Fisher's protected least significant difference (LSD) test was used as post-hoc test for the parameters with significant treatment effect in ANOVA while Dunn's test was used for the data analyzed with the Kruskal-Wallis test. For the data used in the growth component analysis, each component was analyzed with simple linear regression with FR PFD as the regressor. A slope significantly different from zero indicated a significant FR effect. Each regression was conducted with W and RB as two groups and differences in the model were tested between the two groups. A common slope was assumed if slopes of the two groups were not significantly different. All statistical analyses were performed in Genstat (18th Edition, VSN International Ltd., Hemel Hempstead, UK) except for Dunn's test, which was performed using the "dunn.test" package in R (Version 3.5.0, R Core Team, 2013). All tests were conducted at $\alpha = 0.05$.

3. Results

3.1. Growth component analysis

During the generative growth period (46–130 days after transplanting), adding 30 µmol m^{-2} s^{-1} of far red (FR) to white (W) or red + blue (RB) background light resulted in 33% and 26% higher total fruit dry mass, respectively (Fig. 3A). Adding 50 µmol m^{-2} s^{-1} of FR in an RB background increased total fruit dry mass by 45%. Similar effect was observed for the individual ripe fruit dry mass (Fig. 3B).

In both RB and W backgrounds, adding 30 µmol m^{-2} s^{-1} of FR significantly increased the fraction of dry mass partitioned to fruits (F_{fruit}), and truss appearance rate (TAR, Fig. 4A, B). These two components further increased when FR was increased from 30 to 50 µmol m^{-2} s^{-1} (Fig. 4C). FR increased significantly (9–10%) the leaf number per plant (LN_{plant}) during this period, however only in the RB background

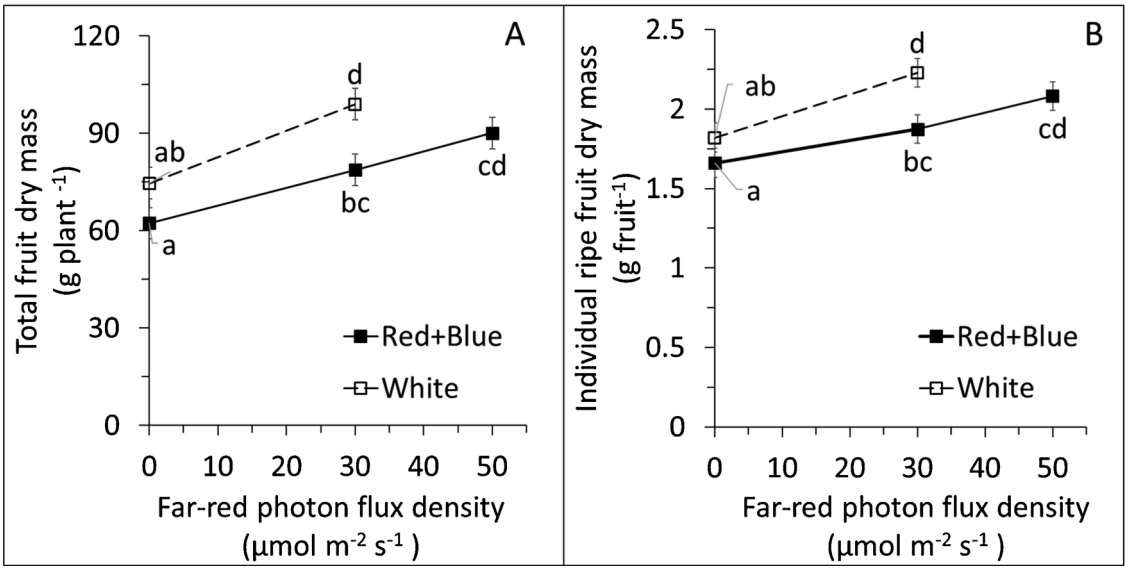

Fig. 3. Effects of additional far red on cumulative fruit dry mass (A) and individual ripe fruit dry mass(B) in white or red + blue background light during generative growth (46–130 days after transplanting). Error bars represent s.e.m. Different letters denote significant differences according to Fisher's protected LSD test ($\alpha = 0.05$).

(Fig. 4). No significant effect of additional FR was found for other components. Absolute values of all components are shown in Table S1.

3.2. Additional FR shifts dry mass partitioning towards fruits and stem at the expense of leaves

After 130 days of growth with additional FR in a W or RB background, the fraction of dry mass partitioned to fruits and stem increased, whereas that partitioned to the leaves was reduced (Fig. 5). There were no significant differences in the fraction of dry mass partitioned to roots between light treatments (Fig. 5D). Similar patterns were observed 46 and 81 days after transplanting (Fig. S1).

3.3. Acclimation to additional FR tends to reduce leaf photosynthetic capacity

No difference was observed in net photosynthesis rate (A) measured with a RB light source in the A/C_i or the A/PPFD curve when comparing plant grown in W and RB without FR, while plants grown under additional FR showed lower A (Fig. 6). This difference was most clear at higher light intensities (Fig. 6A) and CO_2 partial pressures (Fig. 6B). Quantum yield (QY) was not significantly affected by growth under additional FR, except that compared to RB, RBFR30 resulted in a significantly lower QY (Fig. 6A, inset). Additional FR significantly reduced the maximum electron transport rate in W background (J_{1500}, Fig. 6B, inset). Other parameters derived from the fitted model were not

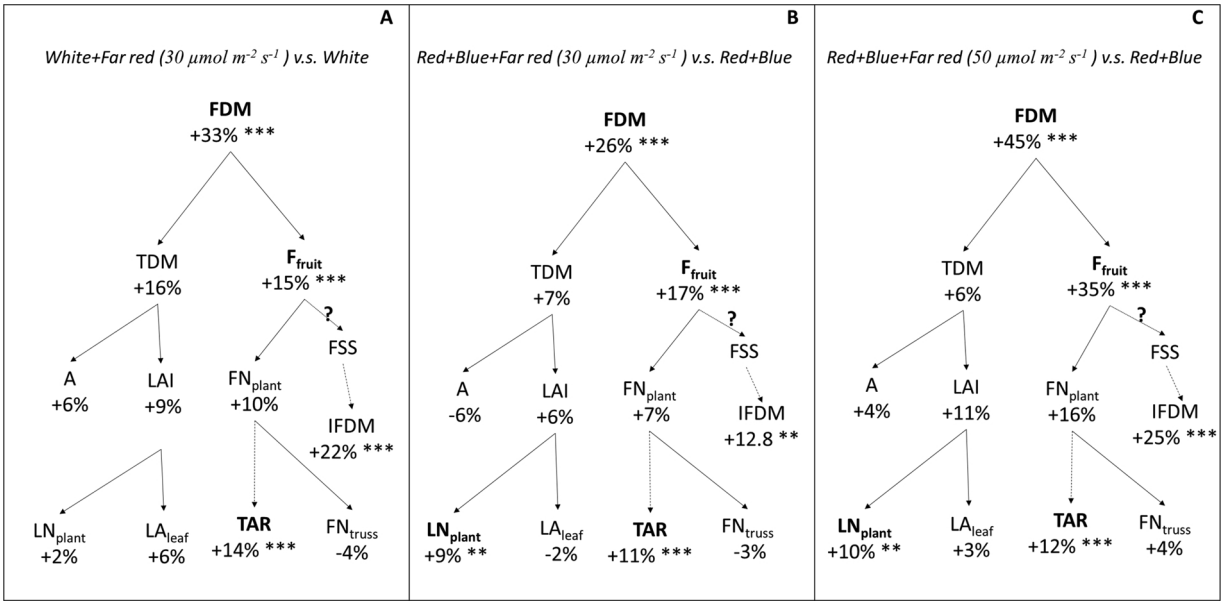

Fig. 4. Effects of additional far red in white (A) or red + blue (B, C) background light on the growth components 46–130 days after transplanting. Abbreviations in this figure: FDM (fruit dry mass), TDM (total dry mass), F_{fruit} (dry mass partitioning to fruits), A (leaf net photosynthesis), LAI (leaf area index), FN_{plant} (total fruit number per plant), IFDM (individual ripen fruit dry mass), LN_{plant} (leaf number per plant), LA_{leaf} (leaf area per leaf), TAR (truss appearance rate), FN_{truss} (fruit number per truss). Dashed lines indicate that the parameter was based on estimation. Asterisks denote significant effects of additional FR as tested by linear regression (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Fruit sink strength was not determined.

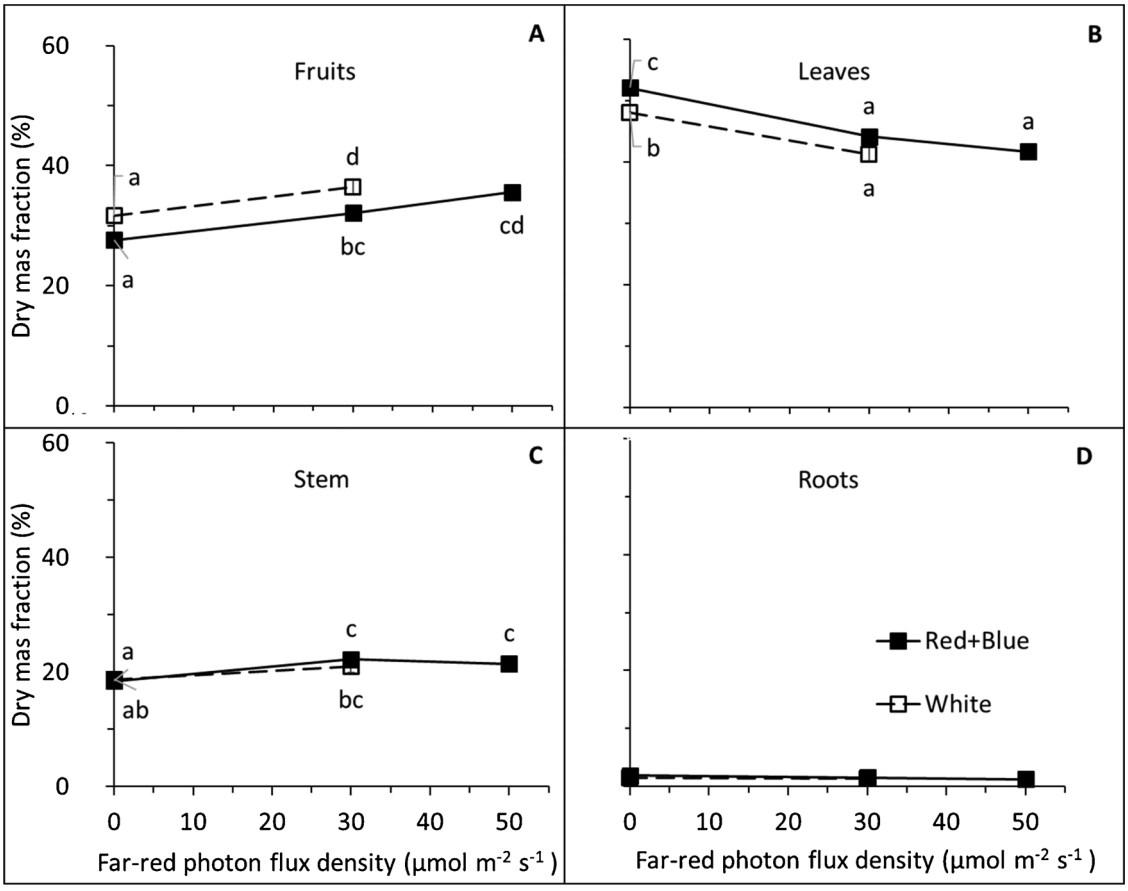

Fig. 5. Effects of additional far red in white or red + blue background light on the fraction of dry mass partitioned to fruits (A), leaves (B) stem (C) and roots (D). Error bars represent s.e.m. Different letters denote significant differences for the dry mass fraction of the same organ according to Fisher's protected LSD test ($\alpha = 0.05$).

significantly different, although reduction in A_{max} ($P = 0.056$) and TPU ($P = 0.052$) when grown under additional FR was close to significant. Instantaneous A, which is the net photosynthesis rate measured under actual growth light, was not significantly affected by the light treatments, neither as absolute rates nor when normalized for PPFD during the measurement (Table S1).

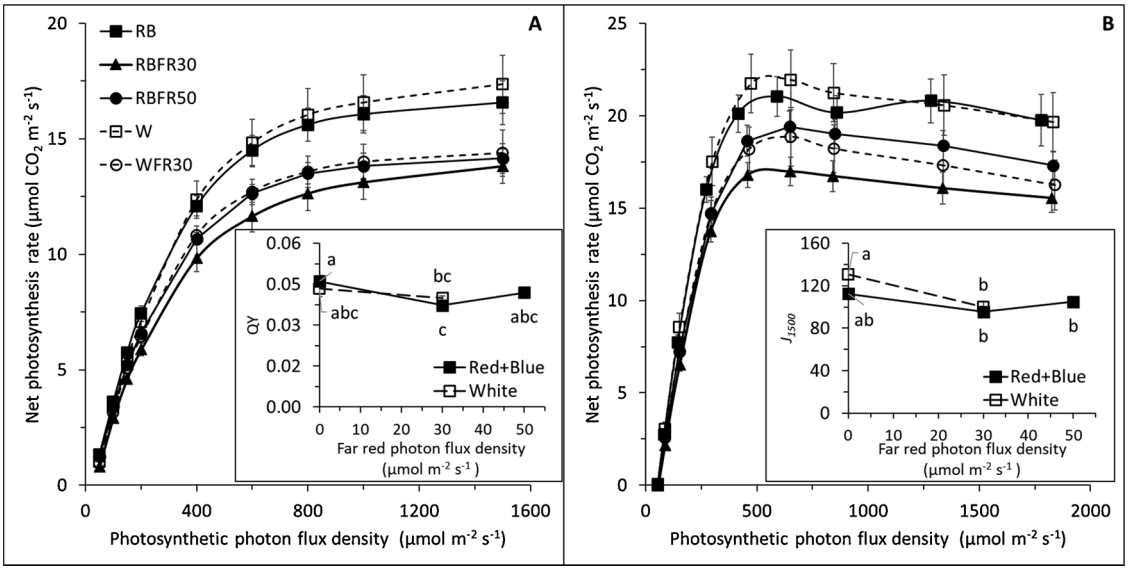

Fig. 6. Light (A) and CO_2 (B) response curves of tomato leaves grown in white (W) or red + blue (RB) background, with 0, 30 or 50 μmol m^{-2} s^{-1} far-red (FR) radiation. The insets show the effects of additional FR on quantum yield (QY) and electron transport rate (J_{1500}) with W or RB background light. During measurements, all leaves were illuminated by RB light. Error bars represent s.e.m. Different letters denote significant differences according to Fisher's protected LSD test ($\alpha = 0.05$).

3.4. Plants grown with additional FR show reduced resistance against B. cinerea

In the RB background, additional FR resulted in significantly larger lesion size, which further increased as FR increased from 30 to 50 μmol m^{-2} s^{-1}. In the W background, lesion size was not significantly affected by the addition of 30 μmol m^{-2} s^{-1} FR, although there was a tendency for lesion size to increase with increased FR.

4. Discussion

4.1. Additional FR increases fruit growth mainly by increasing dry mass partitioning to fruits

Additional FR in both W and RB backgrounds increased total fruit dry mass (Fig. 3A), confirming earlier findings in experiments that used either continuous or end-of-day FR treatments (Hao et al., 2016; Kalaitzoglou et al., 2019; Zhang et al., 2019). FR has been shown to increase leaf area, especially in the early stage of plant growth (Cao et al., 2018; Kalaitzoglou et al., 2019). In the present study we observed a similar but smaller FR effect (Fig. 4), which could be a result of the difference in developmental stage and the fact that we used a less extreme FR treatment in this compared to previous experiments. Despite a negative FR effect on photosynthetic capacity, total plant dry mass was not significantly affected by additional FR (Fig. 4). This may be the result of an improved light distribution within the plant canopy as the elongated internodes under additional FR led to a more open plant architecture, which allowed radiation to penetrate deeper into the canopy (Zhang et al., 2019). This is further supported by a model simulation showing that increasing internode length indeed increases canopy light absorption in the upper part of the canopy and increased canopy photosynthesis rate by up to 10% (Sarlikioti et al., 2011).

Exposure to FR may increase the efficiency of photosystem II electron transport by balancing the excitation of both photosystems, thereby increasing net photosynthesis rate and decreasing non-photochemical quenching in the short term; this has been described as the Emerson enhancement effect (Emerson et al., 1957; Pettai et al., 2005; Zhen and van Iersel, 2017). Here, we observed a tendency towards a decreased photosynthetic capacity and a reduced efficiency for CO_2 fixation per unit leaf area in FR acclimated leaves when these were measured using a light source containing only RB light (Fig. 6). This negative effect may be related to a lower chlorophyll content per unit leaf area, which was often reported in plants grown with additional FR (Tucker, 1981; Heraut-Bron et al., 2000; Kalaitzoglou et al., 2019). When measured with a transparent chamber exposed to the different treatment spectra, we found no significant effect of FR on *A* (Figs. 4 and S1), which was similar to the results reported by Zhang et al. (2019). Previous FR acclimation studies showed positive (Kalaitzoglou et al., 2019), negative (Barreiro et al., 1992) or no effects on *A* (Kasperbauer, 1988; Chow et al., 1990). In the present study, we observed a higher specific leaf area under additional FR (Table S2), which is in line with decreased photosynthetic capacity per unit leaf area. Here, we argue that the FR effect on total plant growth via affecting photosynthesis, on the long run, is rather limited. A low R:FR is a signal for light competition and plants may respond by optimizing their architecture for better light interception. For young tomato plants, the increase in total plant dry mass under additional FR mainly resulted from an increased light interception due to a higher total leaf area index (Kalaitzoglou et al., 2019). In a fruiting tomato canopy, which usually has a leaf area index higher than three and typically intercepts about 90% incident light (Heuvelink et al., 2018), total light interception can only be improved marginally. This is in accordance with our finding that total plant dry mass was not significantly affected by FR during generative growth. Further, we speculate that the positive short-term effect (Emerson enhancement effect) and slightly negative long-term acclimation effect of additional FR on *A* may cancel each other out.

Additional FR significantly increased the fraction of dry mass partitioned to fruits during generative growth (Fig. 4), which was at the expense of partitioning to leaves. This reduced partitioning to leaves is in accordance with a meta-analysis on young plants by Poorter et al. (2012), which associated low R:FR with a decreased leaf mass fraction, in that case accompanied by increased stem mass fraction. Similar changes of partitioning patterns were also reported by Kalaitzoglou et al. (2019). Recently, Kim et al. (2019) showed a tendency that an increased fraction of dry mass was partitioned to tomato fruits when additional FR was provided in an intra-canopy R lighting, however the effect was not statistically significant. In contrast, Zhang et al. (2019) showed small negative effects of additional FR on dry mass partitioning to fruits when overhead additional FR was provided, while RB was provided as intra-canopy lighting. In that research, additional FR increased leaf dry mass remarkably and hence influenced the partitioning pattern. Also, for a fully-grown tomato canopy, whose uppermost leaf layer is responsible for most of the light capture (Acock et al., 1978), intra-canopy lighting may result in different responses to FR compared to that of a plant grown solely under overhead supplemental lighting. To our knowledge, this is the first study to demonstrate a FR induced increase in dry mass partitioning to fruits in an overhead LED crop production system.

4.2. Possible mechanisms of how FR may increase dry mass partitioning to fruits

In the present study, we show that during generative growth, additional FR significantly increases the fraction of dry mass partitioned to fruits in both light backgrounds (Fig. 5). In tomato, fruit load may influence fraction of dry mass partitioned to fruits (Heuvelink, 1997). In this study, fruit number per plant tended to increase by 7–16% (Fig. 4, Table S1). Following the concept of sink strength as a determinant of dry mass partitioning (Marcelis, 1996; Heuvelink, 1997), this could increase the fraction of dry mass partitioned to fruits by up to 11% (Table S3). Hence, increased fruit number can only partly explain the observed 22–42% increase in fraction of dry mass partitioned to the fruits (Fig. 4, Table S1). Collectively, this points to the possibility that additional FR may affect dry mass partitioning by increasing individual fruit sink strength, which is the intrinsic capacity to compete for photosynthetic assimilates (Heuvelink, 1997). To our knowledge, no direct effect of additional FR on fruit sink strength has been reported, but we may derive some clues from existing studies. For example, individual fruit size or mass, which is an indication for fruit sink strength, were shown to be increased by additional FR (Hao et al., 2016; Kim et al., 2019). In the present study, we have also observed a significant increase in individual ripe fruit dry mass under additional FR (Fig. 3B). Further, phytochromes in tomato fruits have been shown to be involved in the transcriptional regulation of genes crucial for sink activity (Bianchetti et al., 2018; Fridman, 2003; Kocal et al., 2008). Taking these lines of evidence together, we argue that higher individual fruit sink strength may be an important reason for improved fruit growth under FR. Indeed, a decreased leaf sink strength may also partly contribute to the increase in fraction of dry mass partitioned to fruits. However, should that be the case, we would expect the dry mass partitioning to stem and roots to be increased to a similar extent as that in fruits, which was not observed in the present study. These results warrant further investigation into the direct effects of additional FR on fruit sink strength.

4.3. Additional FR alters the trade-off between growth and defense

The FR-induced SAS is a strategy that FR-sensitive plants use to ensure reproductive success. The deployment of such a strategy implies a change in the allocation of resources between different physiological processes. The trade-off between growth and defense, often titled “the dilemma of plants” (Herms and Mattson, 1992), is one of the most well-

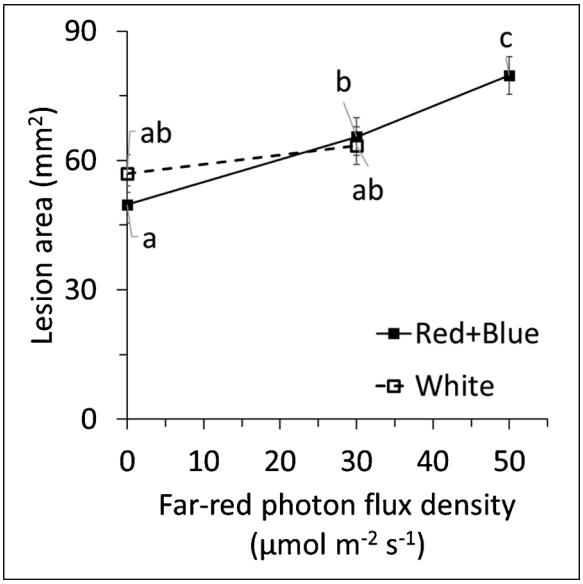

Fig. 7. Effects of additional far red in white or red + blue background light on lesion area after B. cinerea infection. Error bars represent s.e.m. Different letters denote significant differences in lesion area between growth conditions according to Fisher's protected LSD test (α = 0.05).

studied allocation trade-offs. In our study, we observed that tomato plants grown under additional FR showed reduced resistance against *B. cinerea* (Fig. 7). Early works on the effects of canopy density on plant defense against fungal diseases already provided hints for a decreased resistance under high density (low R:FR, for review, see Ballaré, 2014). However, a decreased R:FR is only part of the consequences of a higher planting density. Further work demonstrated the effect of light spectrum, including additional FR, on growth and sporulation of *B. cinerea* (Schumacher, 2017). Research on *A. thaliana* showed that exposure to low R:FR reduced resistance against *B. cinerea* (Cerrudo et al., 2012; De Wit et al., 2013; Cargnel et al., 2014). Also, it has been reported that inactivation of *phyB* in tomato downregulates direct defenses via the jasmonic acid pathway (Cortés et al., 2016), which is closely linked to plant defense against fungal diseases like *B. cinerea*. These results imply that phytochrome mediated FR responses may play important roles in regulating plant's balance between growth and defense.

5. Conclusions

Our results demonstrate that additional FR in supplementary lighting significantly increases tomato fruit dry mass production. This increase is mainly due to the increase in the fraction of dry mass partitioned to fruits, rather than increased photosynthesis or a higher plant biomass. However, additional FR also comes with some negative effects. The larger lesion size observed on leaf samples collected from plants grown under additional FR suggests that additional FR during growth can reduce tomato resistance against *B. cinerea*.

Contributions

Y.J. and T.O. wrote the manuscript. Y.J, T.O, and P.T.N., collected and analyzed the growth component analysis data. S.C collected and analyzed the bioassay data against *Botrytis cinerea*. E.K. collected and analyzed the photosynthesis data. L.F.M.M, E.H., and R.P. provided guidance in the experimental design and provided critical comments on the manuscript. H.J.S., and R.G.F.V., provided critical comments to the overall structure of the manuscript. All authors reviewed and approved the final manuscript.

Uncited-references

Franklin (2008) and Pieterse et al. (2014).

Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgements

This research is part of the "LED it be 50%" program and is supported by LTO Glaskracht, Nunhems (BASF), Rijk Zwaan, Signify, WUR Greenhouse Horticulture and the Netherlands Organization for Scientific Research (NWO), and which is partly funded by the Ministry of Economic Affairs. We thank A. Maassen, M. Peters, S. Geurts, G. Versteeg and E. Schuiling for their technical support.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.envexpbot.2019.103889.

References

Acock, B., Charles-Edwards, D.A., Fitter, D.J., et al., 1978. The contribution of leaves from different levels within a tomato crop to canopy net photosynthesis: an experimental examination of two canopy models. J. Exp. Bot. 29, 815–827. https://doi.org/10.1093/jxb/29.4.815.

Ballaré, C.L., 2014. Light regulation of plant defense. Annu. Rev. Plant Biol. 65, 335–363. https://doi.org/10.1146/annurev-arplant-050213-040145.

Barreiro, R., Guiamét, J.J., Beltrano, J., Montaldi, E.R., 1992. Regulation of the photosynthetic capacity of primary bean leaves by the red:far-red ratio and photosynthetic photon flux density of incident light. Physiol. Plant. 85, 97–101. https://doi.org/10.1111/j.1399-3054.1992.tb05269.x.

Bianchetti, R.E., Lira, B.S., Monteiro, S.S., et al., 2018. Fruit-localized phytochromes regulate plastid biogenesis, starch synthesis, and carotenoid metabolism in tomato. J. Exp. Bot. 69, 3573–3586. https://doi.org/10.1093/jxb/ery145.

Cao, K., Yu, J., Xu, D., Ai, K., Bao, E., Zou, Z., 2018. Exposure to lower red to far-red light ratios improve tomato tolerance to salt stress. BMC Plant Biol. 18, 92. https://doi.org/10.1186/s12870-018-1310-9.

Cargnel, M.D., Demkura, P.V., Ballaré, C.L., 2014. Linking phytochrome to plant immunity: low red: far-red ratios increase Arabidopsis susceptibility to *Botrytis cinerea* by reducing the biosynthesis of indolic glucosinolates and camalexin. New Phytol. 204, 342–354. https://doi.org/10.1111/nph.13032.

Cerrudo, I., Keller, M.M., Cargnel, M.D., et al., 2012. Low red/far-red ratios reduce Arabidopsis resistance to *Botrytis cinerea* and jasmonate responses via a COI1-JAZ10-dependent, salicylic acid-independent mechanism. Plant Physiol. 158, 2042–2052. https://doi.org/10.1104/pp.112.193359.

Chen, M., Tao, Y., Lim, J., Shaw, A., Chory, J., 2005. Regulation of phytochrome B nuclear localization through light-dependent unmasking of nuclear-localization signals. Curr. Biol. 15, 637–642. https://doi.org/10.1016/j.cub.2005.02.028.

Chow, W.S., Goodchild, D.J., Miller, C., Anderson, J.M., 1990. The influence of high levels of brief or prolonged supplementary far-red illumination during growth on the photosynthetic characteristics, composition and morphology of *Pisum sativum* chloroplasts. Plant Cell Environ. 13, 135–145. https://doi.org/10.1111/j.1365-3040.1990.tb01285.x.

De Wit, M., Spoel, S.H., Sanchez-Perez, G.F., et al., 2013. Perception of low red: far-red ratio compromises both salicylic acid- and jasmonic acid-dependent pathogen defences in Arabidopsis. Plant J. 75, 90–103. https://doi.org/10.1111/tpj.12203.

Devlin, P.F., 1998. Phytochrome E influences internode elongation and flowering time in Arabidopsis. Plant Cell Online 10, 1479–1488. https://doi.org/10.1105/tpc.10.9.1479.

Emerson, R., Chalmers, R., Cederstrand, C., 1957. Some factors influencing the long-wave limit of photosynthesis. Proc. Natl. Acad. Sci. U. S. A. 43, 133–143. https://doi.org/10.1073/pnas.43.1.133.

Farquhar, G.D., von Caemmerer, S., Berry, J.A., 1980. A biochemical model of photosynthetic CO2 assimilation in leaves of C3 species. Planta 149, 78–90. https://doi.org/10.1007/BF00386231.

Finlayson, S.A., 2007. Arabidopsis TEOSINTE BRANCHED1-LIKE 1 regulates axillary bud outgrowth and is homologous to monocot TEOSINTE BRANCHED1. Plant Cell Physiol. 48, 667–677. https://doi.org/10.1093/pcp/pcm044.

Franklin, K.A., 2008. Shade avoidance. New Phytol. 179, 930–944. https://doi.org/10.1111/j.1469-8137.2008.02507.x.

Fridman, E., 2003. Functional divergence of a syntenic invertase gene family in tomato, potato, and Arabidopsis. Plant Physiol. 131, 603–609. https://doi.org/10.1104/pp.014431.

Hao, X., Guo, X., Zheng, J., Celeste, L., Kholsa, S., Chen, X., 2017. Response of greenhouse

tomato to different vertical spectra of LED lighting under overhead high pressure sodium and plasma lighting. Acta Hortic. 1170, 1003–1010. https://doi.org/10.17660/ActaHortic.2017.1170.129.
Hao, X., Little, C., Zheng, J.M., Cao, R., 2016. Far-red LEDs improve fruit production in greenhouse tomato grown under high-pressure sodium lighting. Acta Hortic. 1134, 95–102. https://doi.org/10.17660/ActaHortic.2016.1134.13.
Heraut-Bron, V., Robin, C., Varlet-Grancher, C., et al., 2000. Light quality (red: far-red ratio): does it affect photosynthetic activity, net CO_2 assimilation, and morphology of young white clover leaves? Can. J. Bot. 77, 1425–1431. https://doi.org/10.1139/b99-099.
Herms, D.A., Mattson, W.J., 1992. The dilemma of plants: to grow or defend. Q. Rev. Biol. 67, 283–335. https://doi.org/10.1086/417659.
Heuvelink, E., 1997. Effect of fruit load on dry matter partitioning in tomato. Sci. Hortic. 69, 51–59. https://doi.org/10.1016/S0304-4238(96)00993-4.
Heuvelink, E., Li, T., Dorais, M., 2018. Crop growth and yield. In: Heuvelink, E. (Ed.), Tomatoes. CABI, Wallingford, UK, pp. 93.
Higashide, T., Heuvelink, E., 2009. Physiological and morphological changes over the past 50 years in yield components in tomato. J. Am. Soc. Hortic. Sci. 134, 460–465. https://doi.org/10.21273/JASHS.134.4.460.
Huber, H., Wiggerman, L., 1997. Shade avoidance in the clonal herb *Trifolium fragiferum*: a field study with experimentally manipulated vegetation height. Plant Ecol. 130, 53–62. https://doi.org/10.1023/A:1009702611270.
Izaguirre, M.M., Mazza, C.A., Biondini, M., Baldwin, I.T., Ballare, C.L., 2006. Remote sensing of future competitors: impacts on plant defenses. Proc. Natl. Acad. Sci. 103, 7170–7174. https://doi.org/10.1073/pnas.0509805103.
Jolliffe, P.A., Courtney, W.H., 1984. Plant growth analysis: additive and multiplicative components of growth. Ann. Bot. 54, 243–254. https://doi.org/10.1093/oxfordjournals.aob.a086788.
Kalaitzoglou, P., van Ieperen, W., Harbinson, J., et al., 2019. Effects of continuous or end-of-day far-red light on tomato plant growth, morphology, light absorption and fruit production. Front. Plant Sci. 10, 322. https://doi.org/10.3389/fpls.2019.00322.
Kasperbauer, M., 1988. Phytochrome involvement in regulation of the photosynthetic apparatus and plant adaptation. Plant Physiol. Biochem. 26, 519–524. https://doi.org/10.1016/j.bbabio.2018.03.003.
Keiller, D., Smith, H., 1989. Control of carbon partitioning by light quality mediated by phytochrome. Plant Sci. 63, 25–29. https://doi.org/10.1016/0168-9452(89)90097-6.
Kim, H.J., Lin, M.Y., Mitchell, C.A., 2019. Light spectral and thermal properties govern biomass allocation in tomato through morphological and physiological changes. Environ. Exp. Bot. 157, 228–240. https://doi.org/10.1016/j.envexpbot.2018.10.019.
Kocal, N., Sonnewald, U., Sonnewald, S., 2008. Cell wall-bound invertase limits sucrose export and is involved in symptom development and inhibition of photosynthesis during compatible interaction between tomato and *Xanthomonas campestris* pv *vesicatoria*. Plant Physiol. 148, 1523–1536. https://doi.org/10.1104/pp.108.127977.
Li, Q., Kubota, C., 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. Environ. Exp. Bot. 67, 59–64. https://doi.org/10.1016/j.envexpbot.2009.06.011.
Marcelis, L.F.M., 1996. Sink strength as a determinant of dry matter partitioning in the whole plant. J. Exp. Bot. 47, 1281–1291. https://doi.org/10.1093/jxb/47.Special_Issue.1281.
McGuire, R., Agrawal, A.A., 2005. Trade-offs between the shade-avoidance response and plant resistance to herbivores? Tests with mutant *Cucumis sativus*. Funct. Ecol. 19, 1025–1031. https://doi.org/10.1111/j.1365-2435.2005.01047.x.
Michaud, O., Fiorucci, A.-S., Xenarios, I., Fankhauser, C., 2017. Local auxin production underlies a spatially restricted neighbor-detection response in Arabidopsis. Proc. Natl. Acad. Sci. 114, 7444–7449. https://doi.org/10.1073/pnas.1702276114.
Ögren, E., Evans, J.R., 1993. Photosynthetic light-response curves. Planta 189, 182–190. https://doi.org/10.1007/BF00195075.
Page, E.R., Tollenaar, M., Lee, E.A., Lukens, L., Swanton, C.J., 2009. Does the shade avoidance response contribute to the critical period for weed control in maize (*Zea mays*)? Weed Res. 49, 563–571. https://doi.org/10.1111/j.1365-3180.2009.00735.x.
Pantazopoulou, C.K., Bongers, F.J., Küpers, J.J., et al., 2017. Neighbor detection at the leaf tip adaptively regulates upward leaf movement through spatial auxin dynamics. Proc. Natl. Acad. Sci. 114, 7450–7455. https://doi.org/10.1073/pnas.1702275114.
Park, Y., Runkle, E.S., 2017. Far-red radiation promotes growth of seedlings by increasing leaf expansion and whole-plant net assimilation. Environ. Exp. Bot. 136, 41–49. https://doi.org/10.1016/j.envexpbot.2016.12.013.
Pettai, H., Oja, V., Freiberg, A., Laisk, A., 2005. Photosynthetic activity of far-red light in green plants. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1708, 311–321. https://doi.org/10.1016/j.bbabio.2005.05.005.
Poorter, H., Niklas, K.J., Reich, P.B., Oleksyn, J., Poot, P., Mommer, L., 2012. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytol. 193, 30–50. https://doi.org/10.1111/j.1469-8137.2011.03952.x.
Pieterse, C.M.J., Pierik, R., Van Wees, S.C.M., 2014. Different shades of JAZ during plant growth and defense. New Phytol. 204, 261–264. https://doi.org/10.1111/nph.13029.
R Core Team, 2013. R: a Language and Environment for Statistical Computing. http://www.r-project.org/.
Ruberti, I., Sessa, G., Ciolfi, A., Possenti, M., Carabelli, M., Morelli, G., 2012. Plant adaptation to dynamically changing environment: the shade avoidance response. Biotechnol. Adv. 30, 1047–1058. https://doi.org/10.1016/j.biotechadv.2011.08.014.
Sager, J.C., Smith, W.O., Edwards, J.L., Cyr, K.L., 1988. Photosynthetic efficiency and phytochrome photoequilibria determination using spectral data. Trans. ASABE 31, 1882–1889. https://doi.org/10.13031/2013.30952.
Sarlikioti, V., De Visser, P.H.B., Buck-Sorlin, G.H., Marcelis, L.F.M., 2011. How plant architecture affects light absorption and photosynthesis in tomato: towards an ideotype for plant architecture using a functional–structural plant model. Ann. Bot. 108, 1065–1073. https://doi.org/10.1093/aob/mcr221.
Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675. https://doi.org/10.1038/nmeth.2089.
Schumacher, J., 2017. How light affects the life of Botrytis. Fungal Genet. Biol. 106, 26–41. https://doi.org/10.1016/j.fgb.2017.06.002.
Sharkey, T.D., Bernacchi, C.J., Farquhar, G.D., Singsaas, E.L., 2007. Fitting photosynthetic carbon dioxide response curves for C_3 leaves. Plant Cell Environ. 30, 1035–1040. https://doi.org/10.1111/j.1365-3040.2007.01710.x.
Tucker, D.J., 1981. Phytochrome regulation of leaf senescence in cucumber and tomato. Plant Sci. Lett. 23, 103–108. https://doi.org/10.1016/0304-4211(81)90031-6.
Van Kan, J.A.L., 2006. Licensed to kill: the lifestyle of a necrotrophic plant pathogen. Trends Plant Sci. 11, 247–253. https://doi.org/10.1016/j.tplants.2006.03.005.
Van Wees, S.C.M., Van Pelt, J.A., Bakker, P.A.H.M., Pieterse, C.M.J., 2013. Bioassays for assessing jasmonate-dependent defenses triggered by pathogens, herbivorous insects, or beneficial rhizobacteria. In: Goossens, A., Pauwels, L. (Eds.), Jasmonate Signaling. Methods in Molecular Biology (Methods and Protocols). Humana Press, Totowa, NJ, pp. 35–49. https://doi.org/10.1007/978-1-62703-414-2_4.
Zhang, Y., Zhang, Y., Yang, Q., Li, T., 2019. Overhead supplemental far-red light stimulates tomato growth under intra-canopy lighting with LEDs. J. Integr. Agric. 17, 62–69. https://doi.org/10.1016/S2095-3119(18)62130-6.
Zhen, S., van Iersel, M.W., 2017. Far-red light is needed for efficient photochemistry and photosynthesis. J. Plant Physiol. 209, 115–122. https://doi.org/10.1016/j.jplph.2016.12.004.