Unraveling Light Spectrum Effects on Fruit Set in Sweet Pepper

Sijia Chen

Propositions

1. Far-red light-enhanced apical dominance increases competition for assimilates, reducing fruit set.
   (this thesis)

2. Light spectrum effects observed for plants grown in climate chambers are not directly applicable to greenhouse production, or vice versa.
   (this thesis)

3. Resilience is an unrecognized quality for researchers.

4. Continuously monitoring biological processes *in vivo* will become increasingly important in life sciences.

5. Academic writing is like pruning plants: a masterpiece requires meticulous shaping.

6. To optimize work efficiency, organization expansion demands proportional facility expansion.

7. Dependence on caffeine intake to enhance short-term work performance decreases the long-term performance.

Propositions belonging to the thesis, entitled
Unraveling light spectrum effects on fruit set in sweet pepper

Sijia Chen
Wageningen, 21 June 2024

Unraveling light spectrum effects on fruit set in sweet pepper

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Chapter 1

General introduction

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1 Fruit set and abortion

Fruit set indicates the initiation of growth and development of the female flower part (gynoecium) into a fruit, and the cessation of this process is termed as abortion. In nature, many species commonly produce mature fruits from only a small portion of their female flowers, where they regularly abort both flowers and immature fruits (Stephenson, 1981). These species are remarkably diverse, including many economically important crops (Fig. 1).

In an agricultural and horticultural context, a rational level of flower and premature fruit abortion can increase fruit size and quality, while an excessive abortion can lead to significant yield losses (Shi *et al.*, 2023). Compared to the later stages of fruit development, much less is known about the physiological mechanisms regulating fruit set or abortion (Ruan *et al.*, 2012). It is essential to understand the regulation of fruit set for at least two reasons. Firstly, fruit set, as the earliest stage, is likely to have a profound impact on the later stages of fruit development and determines yield potential. Secondly, compared to later stages of fruit development or vegetative growth, fruit set is highly sensitive to external stresses in terms of water, light, temperature, and biotic factors (Ruan *et al.*, 2012; Shi *et al.*, 2023; Fig. 2). This makes fruit set a particularly crucial step in the plant life cycle.

In the upcoming 30 years, the gap between global food supply and demand has been predicted to gradually increase (Tian *et al.*, 2021). Increasing crop yield to reduce this gap will be more and more important in the future. Moreover, climate change has been shown to increase the frequency of regional extreme weather (Fischer *et al.*, 2016), e.g. extreme high or low temperatures, drought, or flooding, which will have a great impact on fruit set and yield. Therefore, investigating the ecophysiology in fruit set or abortion is of great importance to optimize growth conditions or plant properties for yield improvements.

2 Physiological mechanisms behind flower and fruit abortion

2.1 Hormones

Flower and fruit abortion in many crop species, appearing as flower and immature fruit shedding, is an abscission process (Shi *et al.*, 2023). Abscission occurs specifically at the abscission zone, formed by highly differentiated cells. Abscission zone is usually located at the pedicels for flower and premature fruit abscission (Shi *et al.*, 2023). Once abscission is triggered, the cells at abscission zone expand, and

the middle lamella is dissolved by hydrolytic enzymes, which allows organ separation (Patharkar & Walker, 2018). Based on analyses of tomato abscission mutants, *JOINTLESS* and *BLADE-ON-PETIOLE* (*BOP*) were found to be two crucial genes involved in the activity of the abscission zone. The transcription factor *BOP* is essential for the formation of the abscission zone (McKim *et al.*, 2008; Wu *et al.*, 2012). The *JOINTLESS* gene has been identified to be tissue specific in the abscission zone of the pedicel, and it controls the tomato flower abscission zone development (Mao *et al.*, 2000).

Figure 1. Examples of crop species that produce mature fruits from only a small portion of their female flowers (in alphabetical order). Species have been selected from a review by Stephenson (1981). Sources of images are listed in Supplementary Table S1.

The activation of abscission zone is primarily controlled by the balance between auxin and ethylene (Taylor & Whitelaw, 2001). Increased ethylene production is positively correlated to apple fruit drop (Zhu *et al.*, 2008) and tomato flower abscission (Lanahan *et al.*, 1994). Ethylene promotes fruit abscission, while auxin hinders this process and reduces the sensitivity of the abscission zone to ethylene (Taylor & Whitelaw, 2001). High auxin flux through the abscission zone keeps the abscission zone inactive, inhibits cell separation, and subsequently increases fruit set (Botton *et al.*, 2011).

Other than auxin and ethylene, gibberellins and abscisic acid are also involved in regulating fruit set (Fig. 2). In general, fruit set and development depend on successful pollination and fertilization. Following that, the developing seeds produce hormones to control growth and differentiation in the young fruits (Stephenson, 1981). In unpollinated ovaries, high abscisic acid and ethylene signals are proposed to restrain cell division and keep the ovaries in a defending and dormancy status (Vriezen *et al.*, 2008). Pollination and fertilization increases auxin and gibberellins signals, which inhibit abscisic acid and ethylene biosynthesis and signaling (Vriezen *et al.*, 2008), and lead to fruit set. Moreover, auxin or gibberellin application can induce seedless (parthenocarpic) fruit growth in multiple species (reviewed by Sharif *et al.*, 2022), suggesting their crucial functions in inducing fruit set, regardless of fertilization. Auxin is suggested to be the major inducer of fruit set, as gibberellin biosynthesis was suggested to be a downstream response of auxin-induced fruit set (Serrani *et al.*, 2008; Tiwari *et al.*, 2012). Stephenson (1981) suggested that when an immature fruit is going to abscise, the production of growth promotors (auxin, gibberellins) diminishes, and the amount of growth inhibitors (abscisic acid, ethylene) increases in the fruit.

2.2 Carbohydrates

Resource availability is a central factor to determine fruit set (Stephenson, 1981; Fig. 2), where photoassimilate limitation is a primary driver of flower, fruit and seed abortion in grain and fruit crops (Ruan *et al.*, 2012). Therefore, this section focuses on the role of carbohydrates in fruit set, even though the fruit and seed set can also be limited by other essential nutrients (Ruan *et al.*, 2012).

In general, photosynthetic leaves are the primary carbon source supporting early reproductive development. Factors constraining carbon supply from source to sink (new fruit) influence fruit set. Failures in early reproductive development not only relate to inadequate rates of source leaf photosynthesis, but also relate to inadequate phloem import rate into developing reproductive organs (Ruan *et al.*, 2012). Sucrose is the main form of photoassimilate for transportation in most crop species. Feeding sucrose inhibited flower abortion in sweet pepper (Aloni *et al.*,

1997). These authors suggested that the translocated sucrose into flowers can inhibit abscission, by enhancing the activity of sucrose synthase and invertases, which ensures sucrose continuously enters the developing flower and sustains its growth.

Invertases, which hydrolyze sucrose into hexoses, is an important modulator for seed and fruit set. Firstly, invertase activity is crucial for pollen development. Suppression a pollen-specific cell wall invertase gene in tobacco results in unviable pollen, associated with loss of starch and cell wall integrity (Goetz *et al.*, 2001). Consistently, impaired activities of vacuolar and cell wall invertases in anthers was suggested to be the internal cause of pollen sterility under water stress (Koonjul *et al.*, 2005). Secondly, invertases can also regulate programmed cell death genes, which can trigger abortion (Ruan *et al.*, 2012). Ovary abortion in maize induced by water deficit was associated with reduced activities of vacuolar and cell wall invertases, and thus reduced glucose concentration (McLaughlin & Boyer, 2004). Feeding sucrose partly reversed these effects, blocked the expression of programmed cell death genes (*RIP2* and *PLD1*), and eventually restored seed set by 70% (McLaughlin & Boyer, 2004). Moreover, high activities of vacuolar and cell wall invertases is associated with high heat tolerance in tomato (Li *et al.*, 2012). A heat tolerant genotype had a higher sucrose import rate, a lower expression of a programmed cell death gene *LePLDa1*, and a higher fruit set under heat stress compared to a heat sensitive genotype (Li *et al.*, 2012). Therefore, it was proposed that the glucose signaling generated by invertases can repress programmed cell death genes and promote cell division, which together lead to seed and fruit set (Boyer & McLaughlin, 2007; Ruan *et al.*, 2012).

Other than invertases, many other components in sugar metabolism are also important to seed and fruit set. Apple fruit abscission was associated with repressed expression of sorbitol and the sucrose transporter genes (*MdSOT* and *MdSUT*) (Zhu *et al.*, 2011). Inhibiting sucrose synthase activity by constitutive expression of the antisense RNA, reduces sucrose unloading capacity and fruit set in tomato (D'Aoust *et al.*, 1999). Moreover, Botton *et al.* (2011) suggested that trehalose-6-phosphate, *SnRK3*-like gene, and sucrose synthase gene could mediate early abscission signals in apple fruits as a response to carbon starvation. A kinetic model of sucrose metabolism predicted that fruit set at least partly requires the activity of fructokinase (Shinozaki *et al.*, 2020). In fact, compared to ovaries without growing, fruit set rewires the central carbon metabolism pathways, which promotes sucrose uptake and carbon fluxes for producing the constituents of biomass and for producing energy for rapid ovary growth (Shinozaki *et al.*, 2020).

Carbohydrates crosstalk with hormones to determine fruit set or abortion (Botton *et al.*, 2011; Zhu *et al.*, 2011). Hormones produced by developing seeds can play a

leading role in the mobilization of resources into developing fruits (Stephenson, 1981). Reduced sugar content was associated with enhanced ethylene production in pepper flower buds and in cotton bolls under shading (Guinn, 1976; Wien *et al.*, 1989). These supported the idea of Stephenson (1981) that inadequate resources in a fruit can probably promote the production of growth inhibitors.

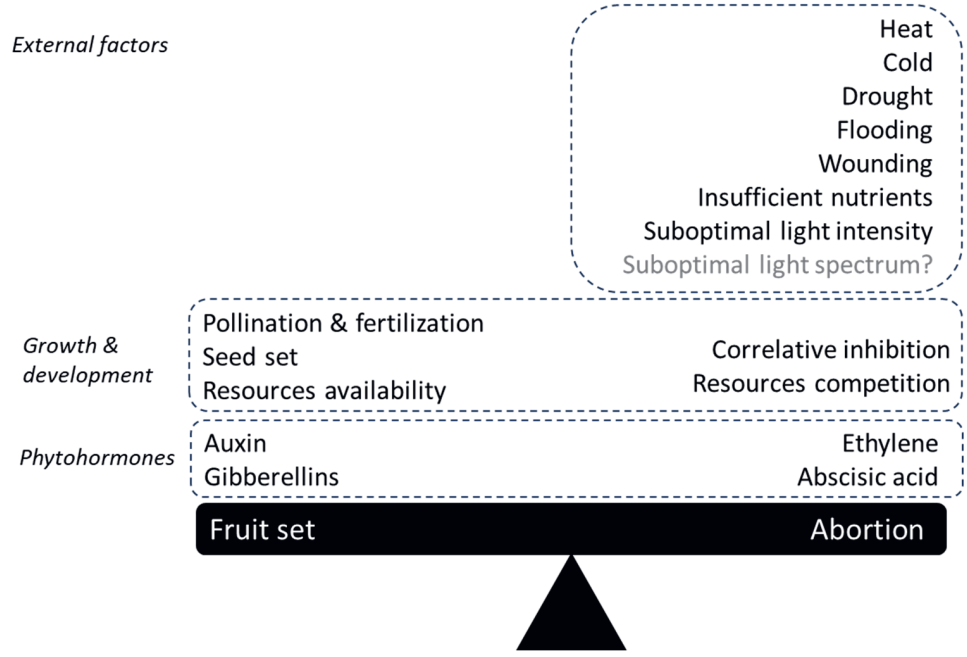

Figure 2. Factors influencing fruit set and abortion. The hormonal balance between growth promotors (auxin and gibberellins) and growth inhibitors (ethylene and abscisic acid) is tightly associated with fruit set or abortion. Pollination and fertilization, and consequently seed set can influence the hormonal balance, while they are not obligatory for fruit set in some species given the existence of parthenocarpic fruits. Correlative inhibition and competition for resources with other organs can reduce the available resources for fruits and triggers hormonal responses to reduce fruit set. External factors can influence fruit set by affecting plant growth and development, and hormonal balances.

2.3 Growth of competing organs

Growth of other organs can influence fruit set through competition for resources and/or correlative inhibition. Studies in apple showed that vigorous shoot growth is associated with more flower and immature fruit drop, whereas shoot tip removal increased fruit set and yield (Quinlan & Preston, 1971; Making *et al.*, 1986; Yuan & Greene, 2000). These studies suggest that competition for photoassimilate between vegetative and reproductive organs can influence fruit set. In sweet pepper, the presence of fruit as a competitor reduced the sucrose translocation from source leaves to new flowers (Aloni *et al.*, 1991). Consistently, flower and fruit abortion positively correlated with the growth rate of earlier formed competing fruits (Marcelis

et al., 2004). This effect was explained by a combination of competition for photoassimilate and dominance among fruits (Marcelis *et al.*, 2004). The dominance among fruits and seeds has been described in a wide range of species, including cucumber, wheat, soybean, oilseed rape and tomato (reviewed by Walker & Bennett, 2018). As apical dominance, the dominance among fruits and seeds is a correlative inhibition phenomenon, where one part of the plant suppresses the growth and development of another part (Walker & Bennett, 2018).

Based on the canalization model, auxin transport auto-inhibition is the key in the dominance phenomenon, where an actively growing organ is able to inhibit the auxin export from other organs directly (Bangerth, 1989). Being essential for the outgrowth of a lateral bud (Morris, 1977), auxin export is also essential for fruit retention (Xie *et al.*, 2013). Auxin export only occurs if the organ creates a canalized link between itself and the polar auxin transport stream in the stem, which could be prevented by auxin derived from other organs via saturating the transport capacity of the main stem (Prusinkiewicz *et al.*, 2009). Bangerth (1989) suggested that, if auxin export from the flower or fruit is inhibited, the formation of an abscission layer can occur. Supporting this idea, the interruption of auxin export from developing fruits by 2,3,5-triiodobenzoic acid (TIBA) promoted fruit abscission in sweet cherry (Else *et al.*, 2004).

Regarding the effect of vegetative growth on fruit set, removing the shoot tip of an apple, grape or bean plant, resulted in higher fruit set and more auxin export from nearby fruits (Bangerth 1989; Bangerth *et al.*, 2000). Parthenocarpic fruits were even formed when apical dominance was released in tomato and pea (Serrani *et al.*, 2010; Rodrigo & García-Martínez, 1998). Regarding the interaction among fruits, a larger seed number increased the inhibitory effect of a sweet pepper fruit on set and growth of later-developing fruits (Marcelis & Hofman-Eijer, 1997). Other than an increased fruit sink strength, this inhibitory effect was also attributed to a stronger dominance from the earlier-developing fruits, due to more hormone production from the developing seeds (Marcelis & Hofman-Eijer, 1997). This can also explain why a plant can bear much more parthenocarpic fruits compared to seeded fruits, which has been observed in multiple species (reviewed by Walker & Bennett, 2018). These findings suggest that the growth of vegetative organs and earlier formed reproductive organs can control fruit set, not only through competition for photoassimilate, but also through correlative inhibition (dominance), which might be mediated by auxin (Fig. 2).

Hormones other than auxin, and sugar could be involved in correlative inhibition as well. Other theories on apical dominance suggest that auxin indirectly controls the outgrowth of buds by regulating the synthesis of secondary messengers, cytokinins and strigolactones, which can move into lateral buds and regulate branching (Brewer

et al., 2015). Furthermore, the role of sugar in apical dominance has also been emphasized. Sugar translocation into the buds was earlier than changes in auxin content during the outgrowth of buds after decapitation (Morris *et al.*, 2005; Mason *et al.*, 2014). Moreover, sucrose supply can directly promote bud release, which suggests that enhancing sugar supply to lateral buds is sufficient for overcoming apical dominance (Mason *et al.*, 2014). Thus, sugar demand could be another regulator in correlative inhibition.

3 Potential effects of light spectrum on fruit set

3.1 Sweet pepper

Fruit set is sensitive to various environmental cues. Light spectrum is one important environment factor, which can greatly influence plant growth and development. However, its effect on fruit set has barely been investigated. In this thesis, I focus on the effect of light spectrum on fruit set (section 3.2, 3.3), where sweet pepper (*Capsicum annuum* L.) was chosen as model species for the following reasons.

Even in a carefully controlled production environment, abortion of flower and fruit in sweet pepper is high and can reach up to 70-80% of all flowers (Wubs *et al.*, 2009). Moreover, in crops like pepper (Heuvelink *et al.*, 2004) and cucumber (Marcelis, 1992), fruit set shows a strong cyclical pattern, where periods with high fruit set are alternated with periods with almost no fruit set (Fig. 3A). This leads to a relatively unstable labor demand, market supply and product price in pepper production. Hampered by low and fluctuating fruit set, sweet pepper became a good candidate for this research, considering its economic relevance.

In greenhouses, usually indeterminate varieties of sweet pepper are grown. Sweet pepper shows a dichotomous branching (Patel & Shah, 1977), where the stem is monopodial until the first flower onsets. Afterwards, each apex divides dichotomously into two apices at every node, where normally one flower, one leaf and one axillary bud appear at each node (Fig. 3B).

Under shading and heat stress, sweet pepper flower and fruit are most susceptible to abortion from a few days before anthesis until about two weeks after anthesis (Marcelis *et al.*, 2004). This finding supports the statement of Stephenson (1981) that most fruit abortion occurs before their exponential growth period. Thus, this period (from one week before anthesis to two weeks after anthesis) is also considered as abortion susceptible period in this thesis.

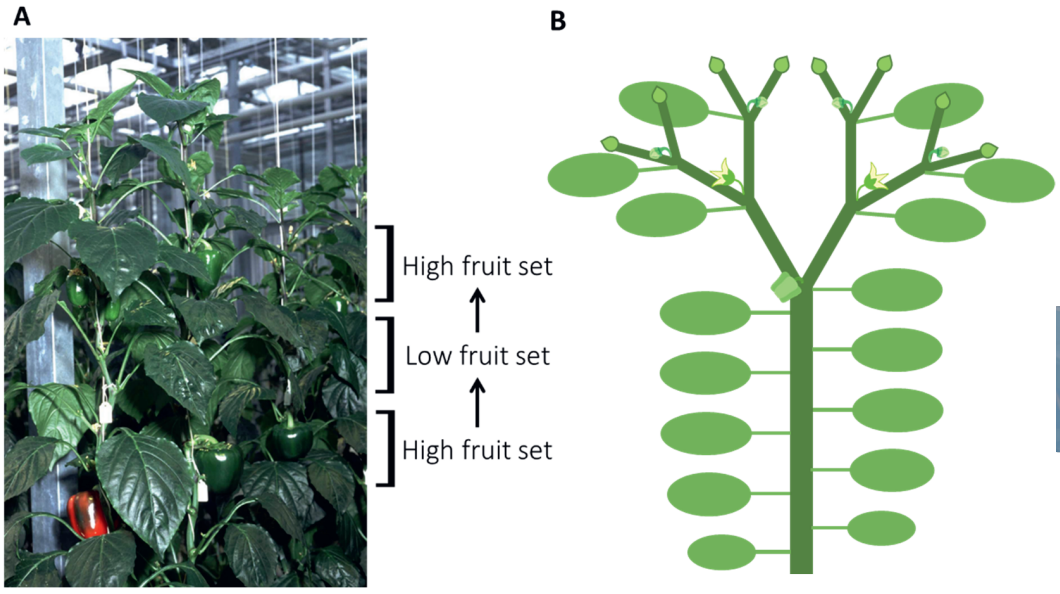

Figure 3. (A) Fruit set fluctuation in sweet pepper. Nodes with fruits (high fruit set) are alternated with nodes with empty axils (low fruit set). (B) Schematic drawing of pepper plant architecture with dichotomous branching.

3.2 Red: far-red ratio (R:FR)

Red: far-red ratio (R:FR) is an important aspect of light spectrum. R:FR ratio allows plants to sense the seasons, the time during the day, and neighboring plants (Smith, 2000; Kotilainen *et al.*, 2020). Within a canopy, R:FR ratio tends to be lower than at the top of the canopy, since the transmittance of red light through leaves is low whereas the transmittance of FR is relatively high. Therefore, a low R:FR ratio delivers a signal of shading to plants. Low R:FR ratio (together with low light intensity) leads to a shade-avoidance syndrome in most plants, which is typically characterized by increased elongation of stems and petioles, and elevated leaf angles (Franklin, 2008; Ballaré & Pierik, 2017). A short-term far-red (FR) exposure at the end of day (EOD) can cause similar but less pronounced morphological responses compared to low R:FR during the day, such as stem elongation (Kalaitzoglou *et al.*, 2019).

Red light inhibited dark-induced leaf abscission (Craker *et al.*, 1987; Curtis, 1978; Decoteau & Craker, 1983; Mao & Craker, 1990; Mao *et al.*, 1989), while FR either reversed this effect or enhanced abscission in these studies. Red light delayed whilst far-red light accelerated flower abscission in *Hibiscus rosa-sinensis* L. (van Meeteren & van Gelder, 2000). Red light also reduced soybean reproductive abscission and increased its pod set *in vitro* compared to FR (Myers *et al.*, 1987). Preliminary data obtained before starting this thesis research (L.F.M. Marcelis,

unpublished) showed that only 30 minutes EOD red light increased the number of fruits compared to 30 minutes EOD FR in sweet pepper (2.8 vs. 2.2 fruits per plant). Therefore, it is expected that R:FR influences fruit set in sweet pepper.

Red and FR light are both perceived by photoreceptor phytochromes. Plant responses depend on the fraction of active phytochrome Pfr (far-red light absorbing form) in the total amount of phytochromes, called photostationary state of phytochrome (PSS). Phytochromes exert their biological activity via interaction with different families of transcription factors, where PHYTOCHROME INTERACTING FACTORS (PIFs) are the best characterized. PIFs regulate various aspects of plant development (reviewed by de Lucas & Prat, 2014).

A low R:FR can promote apical dominance (Leduc *et al.*, 2014), which is related to its effect on promoting auxin synthesis and possibly auxin transport (Küpers *et al.*, 2020; Song *et al.*, 2024). Stronger apical dominance may inhibit the auxin export of new flowers or fruits (as discussed above) and cause more abortion. Moreover, R:FR also influences growth-inhibiting hormones: ethylene and abscisic acid. Low R:FR can promote ethylene production, as in tobacco and pea seedlings (Pierik *et al.*, 2004; White & Mattoo, 2017). Low R:FR also up-regulated the expression of ABA key biosynthetic gene *NCED3* (Reddy *et al.*, 2013), and increased ABA levels in lateral buds of *Arabidopsis* (Holalu & Finlayson, 2017).

Moreover, R:FR also increases fruit sink strength in tomato (Ji *et al.*, 2020), and thus regulates photoassimilate allocation. With a higher sink strength, the earlier-developing fruits in sweet pepper can probably enlarge their advantages to attract photoassimilate when competing with later-developing fruits. A reduced photoassimilate import could enhance flower and fruit abscission, considering the inhibitory effect of translocated sucrose on abscission as suggested by Aloni *et al.* (1997).

3.3 Blue:red ratio (B:R)

Besides R:FR, blue:red ratio (B:R) is also an important aspect of light spectrum. A mixture of blue and red light is commonly used for supplementary lighting in practice, considering their high absorption by plants. Blue light is mainly perceived by blue light receptors, such as cryptochromes and phototropins. Similar to a spectrum rich in FR, a spectrum rich in blue light reduces PSS (Kong *et al.*, 2018), thus a very high B:R may influence fruit set through phytochromes. Phytochromes and blue light receptors control plant morphogenesis together, such as shade avoidance response. The phyB and cry1 pathways appeared to converge in their requirement for PIF4 and PIF5 to elicit the shade avoidance syndrome, even though low R:FR and blue light induced distinct hormonal cascades (Keller *et al.*, 2011).

B:R ratio may affect fruit set through regulating hormones and carbohydrates. On the one hand, high B:R could be beneficial to fruit set, as activated cryptochromes led to a decreased abscisic acid concentration in wild-type *Arabidopsis* leaves compared to the *cry1cry2* mutant leaves (Boccalandro *et al.*, 2012). Moreover, blue light also postponed petal senescence of carnation cut flowers, which was linked to the down-regulated expression of ethylene biosynthetic genes (Aalifar *et al.*, 2020). On the other hand, low B:R could be beneficial to fruit set, because cultivation under higher fractions of blue light led to a lower starch content in leaves (Larsen *et al.*, 2022; Shengxin *et al.*, 2016), and a lower plant dry matter production (He *et al.*, 2017; Wang *et al.*, 2016; Warrington & Mitchell, 1976), reflecting a reduced source strength.

In dwarf sweet pepper, the flower initiation and fruit set tended to decrease when increasing B:R from 1:19 to 1:5 (Naznin *et al.*, 2019). However, the underlying mechanism is still unknown. Furthermore, the range of B:R in the study of Naznin et al. (2019) was relatively small and did not affect PSS. To the best of our knowledge, there was no report on the effect of higher B:R on fruit set.

4 Thesis outline

Optimizing light spectrum is an important topic in greenhouse production and vertical farming. Light spectrum has the potential to regulate fruit set in sweet pepper, however it has hardly been investigated. In this thesis, I focus on two important aspects of light spectrum: R:FR ratio and B:R ratio. My main objective is to investigate how R:FR and B:R can influence fruit set in sweet pepper, and the underlying mechanisms regarding plant growth and morphology, carbohydrates, and hormones (Fig. 4).

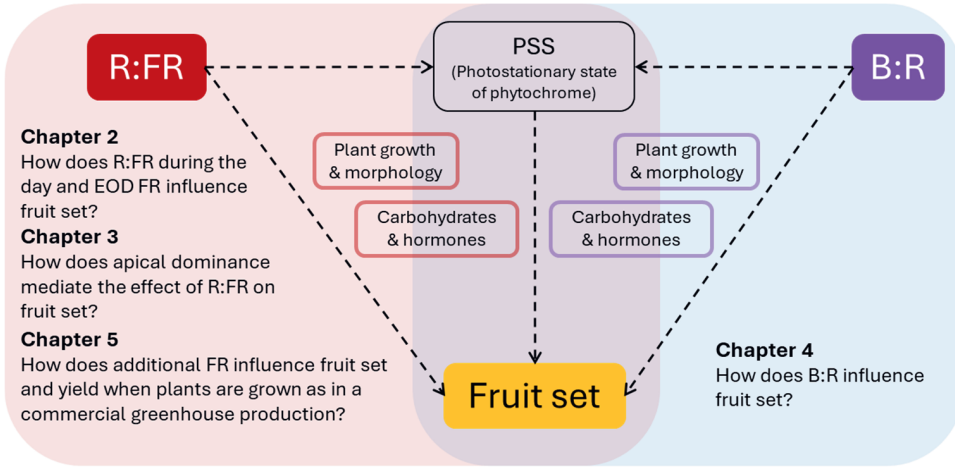

Figure 4. Research scheme and key research questions for this thesis. Red: far-red ratio (R:FR) and blue: red ratio (B:R) are the two aspects of light spectrum investigated here. Plant growth, morphology, carbohydrates, and hormones were the main focus of underlying mechanisms when investigating these key research questions.

The following hypotheses were investigated in this thesis:

1) A low R:FR during the day and EOD FR reduce fruit set in sweet pepper (Chapter 2)
2) At low R:FR, the enhanced apical dominance reduces fruit set by elevating the level of apically derived auxin (Chapter 3)
3) Increasing B:R reduces fruit set by reducing available carbohydrates, especially at high B:R when PSS is lowered (Chapter 4)
4) A lower R:FR in greenhouses reduces fruit set but increases fruit size due to a higher fruit sink strength (Chapter 5).

Experiments in Chapters 2 to 4 were conducted in climate chambers to allow for fully controlled environments. In these experiments, the interaction between earlier and later-developing fruits was minimized by restricting the number and location of flowers. The experiment in Chapter 5 was conducted in a greenhouse to investigate the effect of R:FR in a growth environment employed in practice. This thesis ends with a general discussion (Chapter 6) connecting findings from Chapters 2 to 5 and bringing forward perspectives for future research.

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Supplementary information

Table S1. Images used in Fig. 1.

Species	Family	Image attribution	License link
Anacardium occidentale	Anacardiaceae	"Ripe cashew apples" by Abhishek Jacob, used under the CC BY-SA 3.0 DEED license. The image was cropped from original.	https://creativecommons.org/licenses/by-sa/3.0/deed.en.
Capsicum annuum	Solanaceae	"Capsicum annuum cultivars" by Eric Hunt, used under the CC BY-SA 3.0, 2.5, 2.0 and 1.0 DEED license. The image was cropped from original.	https://creativecommons.org/licenses/by-sa/3.0/deed.en; https://creativecommons.org/licenses/by-sa/2.5/deed.en; https://creativecommons.org/licenses/by-sa/2.0/deed.en; https://creativecommons.org/licenses/by-sa/1.0/deed.en.
Citrus × limon	Rutaceae	"Lemon fruit and flower" by Ellen Levy Finch (Elf), used under the CC BY-SA 3.0 DEED license. The image was cropped from original.	https://creativecommons.org/licenses/by-sa/3.0/deed.en.
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Cucumis melo	Cucurbitaceae	"Isang milong musko" by Seth Vidal, used under the CC BY-SA 2.0 DEED license. The image was cropped from original.	https://creativecommons.org/licenses/by-sa/2.0/deed.en.
Cucurbita maxima	Cucurbitaceae	"Gartenkürbis (Cucurbita) im September 2007" by Maja Dumat, used under the CC BY 2.0 DEED license. The image was cropped from original.	https://creativecommons.org/licenses/by/2.0/deed.en
Gossypium herbaceum	Malvaceae	"Gossypium herbaceum, Malvaceae, Levant Cotton, fruits; Botanical Garden KIT, Karlsruhe, Germany. The fresh, inner rootbark is used in homeopathy as remedy: Gossypium herbaceum (Goss.)" by H. Zell, used under CC BY-SA 3.0 DEED license. The image was cropped from original.	https://creativecommons.org/licenses/by-sa/3.0/deed.en.
Malus domestica	Rosaceae	"Apples are an all-American success story-each of us eats more than 19 pounds of them annually." by Scott Bauer, USDA. The image is in the public domain.	
Mangifera indica	Anacardiaceae	"Mangoes (Magnifera indica) from West Bengal, India. Photo taken by Yogabrata Chakraborty, on June 9, 2022." by Billjones94, used under the CC BY-SA 4.0 DEED license. The image was cropped from original.	https://creativecommons.org/licenses/by-sa/4.0/deed.en.
Persea americana	Lauraceae	"Avocados (Persea americana)" by B.navez, used under the CC BY-SA 3.0, 2.5, 2.0 and 1.0 DEED license. The image was cropped from original.	https://creativecommons.org/licenses/by-sa/3.0/deed.en; https://creativecommons.org/licenses/by-sa/2.5/deed.en; https://creativecommons.org/licenses/by-sa/2.0/deed.en; https://creativecommons.org/licenses/by-sa/1.0/deed.en.
Prunus cerasus	Rosaceae	"Prunus cerasus" by Diako1971, used under the CC BY-SA 4.0 DEED license. The image was cropped from original.	https://creativecommons.org/licenses/by-sa/4.0/deed.en
Prunus domestica	Rosaceae	"Fruits of Prunus domestica" by YAMAMAYA, used under the CC BY-SA 3.0, 2.5, 2.0 and 1.0 DEED license. The image was cropped from original.	https://creativecommons.org/licenses/by-sa/3.0/deed.en; https://creativecommons.org/licenses/by-sa/2.5/deed.en; https://creativecommons.org/licenses/by-sa/2.0/deed.en; https://creativecommons.org/licenses/by-sa/1.0/deed.en.

Prunus persica	Rosaceae	"Prunus persica - Peach Hungary" by Takkk, used under the CC BY-SA 3.0 DEED license. The image was cropped from original.	https://creativecommons.org/licenses/by-sa/3.0/deed.en
Pyrus communis	Rosaceae	"Though the pears pictured do not have a texture suitable for good eating, scientists at the ARS Appalachian Fruit Research Station in Kearneysville, West Virginia, will combine their fire blight-resistant qualities with other lines possessing traits sought in commercial pear varieties." by Keith Weller. The image is in the public domain.	
Theobroma cacao	Malvaceae	"Cacao (Theobroma cacao)" by Luisovalles, used the CC BY 3.0 DEED license. The image was cropped from original.	https://creativecommons.org/licenses/by/3.0/deed.en.

Chapter 2

Far-red radiation increases flower and fruit abortion in sweet pepper (*Capsicum annuum* L.)

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Abstract

Fruit set is a crucial plant developmental process, determining yield in many crops. Pepper (*Capsicum annuum* L.) is a crop with poor fruit set as typically about two-thirds of all flowers abort. A higher light integral improves fruit set. However, the role of light spectrum has hardly been investigated. Opportunities for detailed investigation of light spectrum effects on fruit set have strongly increased with the introduction of narrow-band LED lighting.

To investigate whether additional far-red light (FR) influences the fruit set of sweet pepper, a climate chamber experiment was conducted. Four light treatments were applied to pepper plants grown under 130 µmol m^{-2} s^{-1} of red/white LED light. Treatments consisted of different intensities of FR (0, 50, 100 µmol m^{-2} s^{-1}) applied throughout the day or applied at the end of day (EOD, 30 minutes, 30 µmol m^{-2} s^{-1}). Treatments resulted in phytochrome photostationary state (PSS) values of 0.88, 0.77. 0.70 and EOD 0.16 respectively. Fruit set was determined 3 weeks after the last anthesis of the studied flowers.

Additional FR light reduced fruit set in sweet pepper, regardless of whether FR light was provided during the whole day or only at the end of the day. Meanwhile, FR led to more stem elongation, more upright branches, and more dry mass partitioning to stems. Additional FR during the daytime increased total shoot dry weight but not when FR was applied at the end of day. Possible reasons for stimulated flower and fruit abortion by additional FR are discussed.

Key words:

Abscission; *Capsicum annuum* L.; end of day lighting; fruit set; R:FR ratio.

1 Introduction

Fruit set, the initiation of fruit growth shortly after flowering, is a crucial step in plant reproduction. The cessation of flowering and fruit set is termed abortion. Due to abortion, only a small portion of the flowers can produce mature fruits in many species (Stephenson, 1981), which limits the yield in various crops, e.g., peppers, melons, squashes, and various fruit trees. In sweet pepper (*Capsicum annuum* L.), flower and fruit abortion are high and can reach up to 70-80% of all flowers (Wubs *et al.*, 2009). In crops like pepper (Heuvelink *et al.*, 2002) and cucumber (Marcelis, 1992), fruit set shows a strong cyclical pattern, where periods with high fruit set are alternated with periods with almost no fruit set. Thus, it leads to a relatively unstable market supply and product price in pepper production.

Fruit set can be influenced by various environmental signals (Wubs *et al.*, 2009). Light spectrum is an important environmental signal but its effect on fruit set is barely known. Nowadays, thanks to the development of LEDs, it is readily feasible to manipulate light spectrum. LEDs are increasingly used as supplementary lighting source in greenhouses or as sole lighting source in indoor cultivation (Sipos *et al.*, 2020). Therefore, the interest in using this opportunity to regulate plant growth and plant defense by manipulating light spectrum is currently expanding (Demotes-Mainard *et al.*, 2016; Lazzarin *et al.*, 2021).

Plants perceive light spectrum by photoreceptors. Among them, phytochromes perceive red (R, 600–700 nm) and far-red (FR, 700–800 nm) light (Li *et al.*, 2015), which plays a critical role in mediating plant growth and development (Smith, 2000; Legris *et al.*, 2019). Phytochromes can reversibly convert between the biologically in-active R-absorbing form (Pr) and the biologically active FR-absorbing form (Pfr). Plant responses depend on the equilibrium of Pr and Pfr forms, which is quantified by the ratio of amount of active phytochrome Pfr to the total amount of phytochrome, the so-called photostationary state of phytochrome (PSS) (Sager *et al.*, 1988). R:FR ratio is commonly used (also in this paper) to reflect the level of PSS, and to link light treatments to phytochrome-mediated morphological responses (Franklin, 2008). Recently, FR fraction (FR/(R+FR)) was suggested to be an improved metric for phytochrome-mediated responses (Kusuma & Bugbee, 2021).

R:FR ratio allows plants to sense the seasons, the time during the day, and neighboring plants (Smith, 2000). In nature, a low R:FR ratio usually coincides with decreased photosynthetic active radiation (PAR). Within the canopy, the R:FR ratio usually is lower than at the top of the canopy, since red light is mostly absorbed, while far-red is mostly reflected or transmitted by leaves. Therefore, a low R:FR

ratio delivers a message to plants that they are probably under shading conditions. In response to the low R:FR ratio, most plants show a shade-avoidance syndrome, which is typically characterized by increased elongation of stems and petioles, and elevating leaf angles (Franklin, 2008; Ballaré & Pierik, 2017). Some morphological responses after a short-term, end-of-day (EOD) FR exposure seem similar but less pronounced as those under whole-day FR light, especially stem elongation (Kalaitzoglou *et al.*, 2019). This makes EOD FR lighting a potential interesting practical option since less energy is needed compared to whole day FR lighting.

The FR-induced morphological adaptations, on one hand, may hinder productivity, since FR light promotes stem and petiole elongation at the expense of the growth of other parts, such as less root growth and less chlorophyll content (Demotes-Mainard *et al.*, 2016). On the other hand, these changes may help plants to capture more light energy with a more open plant structure (Sarlikioti *et al.*, 2011), and/or with a promoted leaf expansion, resulting in enhanced plant growth, for example in tomato (Kalaitzoglou *et al.*, 2019) and lettuce (Meng *et al.*, 2019; Jin *et al.*, 2021). Recently, it was also reported that FR may enhance partitioning to the fruits in tomato (Ji *et al.*, 2020). However, only very few greenhouse studies (Hao *et al.*, 2018; Schuddebeurs, 2021; Lanoue *et al.*, 2022) have investigated the effect of FR light on pepper plants in their generative growth phase, while to the best of our knowledge, there is no report investigating the effect of FR light on fruit set in pepper.

Abortion of flowers and/or fruits in peppers is an active process involving the formation of an abscission layer in the pedicel (Wubs *et al.*, 2009). Craker *et al.* (1987) found that red light in a very low amount already could reduce leaf abscission in Coleus, while FR enhanced this process. Similarly, red light delayed the flower abscission in *Hibiscus rosa-sinensis* L., while far-red light accelerated it (van Meeteren & van Gelder, 2000). As an abscission process, it is therefore likely that flower and fruit abortion in sweet pepper is influenced by FR light. However, this has not been investigated yet. Therefore, the objectives of this study were to identify: 1) the effect of FR on flower and fruit abortion in sweet pepper, 2) how FR influences the morphology of sweet pepper plants. For this purpose, we conducted an experiment in a controlled environment growth chamber with different levels of FR, during the daytime or EOD.

2 Materials and Methods

2.1 Plant material

Sweet pepper plants (*Capsicum annuum* L. cv. Frazier) rooted in stone wool cubes with the first visible flower buds were obtained from a plant raising company (Beekenkamp Plants B.V., Netherlands). Pepper plants have a dichotomous branching pattern, where every apex ends in a flower and two new apices, which will turn into two branches, so-called 'splitting'. During this experiment, plants were pruned to two main stems like commercial practice, with side shoots stopped at one leaf. To limit the interaction between fruits of different ages, each plant was pruned to have only 8 flowers retained. The location of target flowers on the plants is shown in Fig. 1A. To prevent accidental damage to nearby tissues, we pruned the shoots when their stems were longer than 3 cm, and the flower buds when their petals appeared, which is usually 3-5 days before their anthesis. In pepper, pollination normally occurs by self-pollination; in line with commercial production of sweet pepper, no measure was taken to stimulate pollination of flowers.

2.2 Growth conditions and light treatments

Plants were transplanted on stone wool slabs (Grodan, Roermond) in a controlled environment growth chamber, where they were cultivated under four light treatments with different amounts of additional far-red (Table 1, Supplementary Fig. S1). The photosynthetically active radiation (130 µmol m^{-2} s^{-1} in all treatments) was provided by light-emitting diodes (LEDs, Philips GreenPower deep red/white LED production module, 16 W). Light intensity was maintained constant at the top of the plants during the experiment by adjusting the height of lamps, with irradiance measurement every 2 weeks using a spectroradiometer (type SS-110, Apogee Instruments, Inc). Additional far-red (provided by Philips GreenPower far-red LED research module, 10 W) was 0, 50 or 100 µmol m^{-2} s^{-1} throughout the day (photoperiod 12 h), or 30 µmol m^{-2} s^{-1} for half an hour after the main light was switched off as end of day lighting (EOD). This resulted in phytochrome photostationary state (PSS) values of 0.88, 0.77. 0.70 and 0.16 EOD respectively, based on Sager *et al.* (1988).

The temperature was 22/18 °C (day/night), the relative humidity was maintained at 80%, and no CO_2 enrichment was applied. The plants were irrigated with a drip system 3 times a day with a nutrient solution (electric conductivity 2.0 dS m^{-1}, pH 6.1) containing 1.2 mM NH_4^+, 9.5 mM K^+, 5.4 mM Ca^{2+}, 2.4 mM Mg^{2+}, 16 mM NO_3^-, 4.4 mM SO_4^{-2}, 1.5 mM PO_4^{2-}, 25.0 µM Fe^{3+}, 10.0 µM Mn^{2+}, 5.0 µM Zn^{2+}, 30.0 µM B^+, 0.75 µM Cu^{2+}, and 0.5 µM MoO_4^{2-}.

Table 1. Light treatments with different amounts of additional far-red, their PSS (phytochrome photostationary state) values and R:FR ratio. PAR (photosynthetically active radiation)=400-700 nm. Blue=400-500 nm. Green=500-600 nm. Red=600-700nm. FR=700-800nm. Within PAR, Blue: Green: Red = 9: 18: 73. Mean ± SEmean, indicates that average light intensity and standard error based on 40 measurements. EOD = end-of-day, indicating the lighting during the first 0.5 hour of dark period.

Treatments	PAR ($\mu mol\ m^{-2}\ s^{-1}$)	Far-red ($\mu mol\ m^{-2}\ s^{-1}$)	PSS	Red: Far-red
FR 0	133.9±2.2	0.4±0.2	0.88	>50
FR 50	131.8±1.4	53.6±1.8	0.77	1.95±0.07
FR 100	133.5±2.8	96.2±2.8	0.70	1.10±0.04
EOD FR	131.7±2.0	31.6±1.0	0.88 → 0.16	>50→<0.1

2.3 Morphological measurements

The anthesis and abortion of flowers were recorded daily from day 17 till day 56 after transplanting. Final morphological measurements were performed on day 56 after transplanting. The plant height was determined by averaging the length of both main stems, from the base of the plant till its topmost node. The fresh and dry weight of leaves and stems (105 °C ventilated oven, 24 h), leaf area (LI-COR 3100 area meter), and the angle between two branches at the first and second splitting node (averaging the value from two main stems) (illustration in Supplementary Fig. S2) were measured on every plant. Fruit location on the plants was recorded as well as fruit length and width excluding the pedicels, and their fresh and dry weight including the pedicels (105 °C ventilated oven, 48 h) were measured. The sum of stem, leaf and fruit dry weight is total shoot dry weight. The calculations used for plant parameters are as below.

$$\text{Fruit set percentage at Layer i per plot (\%)} = \frac{\text{Total number of fruit set at Layer i}}{\text{Total number of flower at Layer i}} \times 100\%\ (\text{i} = 1, 2, 3)$$

$$\text{Volume of an individual fruit (cm}^3\text{)} = \pi \times \frac{\text{width (cm)}^2}{2} \times \text{length (cm)}$$

$$\text{Fruit or leaf or stem dry matter percentage (\%)} = \frac{\text{Fruit or leaf or stem dry weight per plant (g)}}{\text{Total shoot dry weight (g)}} \times 100\%$$

$$\text{Specific stem length (cm g}^{-1}\text{)} = \frac{\text{Plant height (cm)}}{\text{Stem dry weight (g)}}$$

$$\text{Specific leaf area (cm}^2\ \text{g}^{-1}\text{)} = \frac{\text{Leaf area (cm}^2\text{)}}{\text{Leaf dry weight (g)}}$$

2.4 Statistical analysis

The 4 light treatments were allocated to the 8 cells (as 8 independent experimental units) in the climate chamber according to a complete block design. Thus, there were 2 statistical replicates, where each replicate had 8 individual plants. The mean values of different parameters for each of these 8 experimental units were used in a one-way Analysis of Variance (ANOVA) (Genstat 19th edition). Fisher's protected LSD test at P=0.05 was used for mean separation. Homogeneity of variances was not tested but assumed as the experiment had only 2 statistical replicates and normality of residuals was judged graphically by a quantile quantile plot (Q-Q plot). When the normality assumption was not met, we transformed the data for further analysis.

3 Results

Adding far-red light (FR) reduced fruit set in sweet pepper, regardless of whether FR light was provided during the whole day or only at the end of the day (Fig. 1B). This also resulted in reduced fruit fresh weight and dry weight per plant under additional FR (Supplementary Fig. S3; Fig. 1D). Growth of some fruits was arrested, reaching a fresh weight of less than 5 g even when they were already 25-35 days after anthesis (see an example of arrested fruit in Supplementary Fig. S4). Of all fruits in each treatment, 9%, 9%, 15%, and 32% were arrested fruits under FR 0, FR 50, FR 100, and EOD FR light condition, respectively.

Target flowers were located at 3 different layers on the plants (Fig. 1A), and the response of fruit set to FR differed among layers. Although it was not statistically significant, FR strongly reduced the fruit set percentage of the first layer (the eldest layer), while it showed less FR effect in the later layers (Fig. 1C). We observed an increasing tendency in fruit set from layer 1 to 3 for plants under whole-day FR, but a decreasing tendency in the absence of FR. Treatment EOD FR showed a pattern where fruit set first increased then decreased (Fig. 1C).

The higher flower and fruit abortion under additional FR started approximately one week after the first anthesis (Supplementary Fig. S5). We performed our observations until 3 weeks after the last anthesis of the target flowers (day 56 after transplanting), however, the fruit abortion in all treatments became already stable 2 weeks after the last anthesis (Supplementary Fig. S5).

With applying light treatments 17 days before the first anthesis, FR showed little effect on anthesis time at all layers, though there was a tendency that FR slightly delayed the anthesis of flowers in the later layers (Table 2).

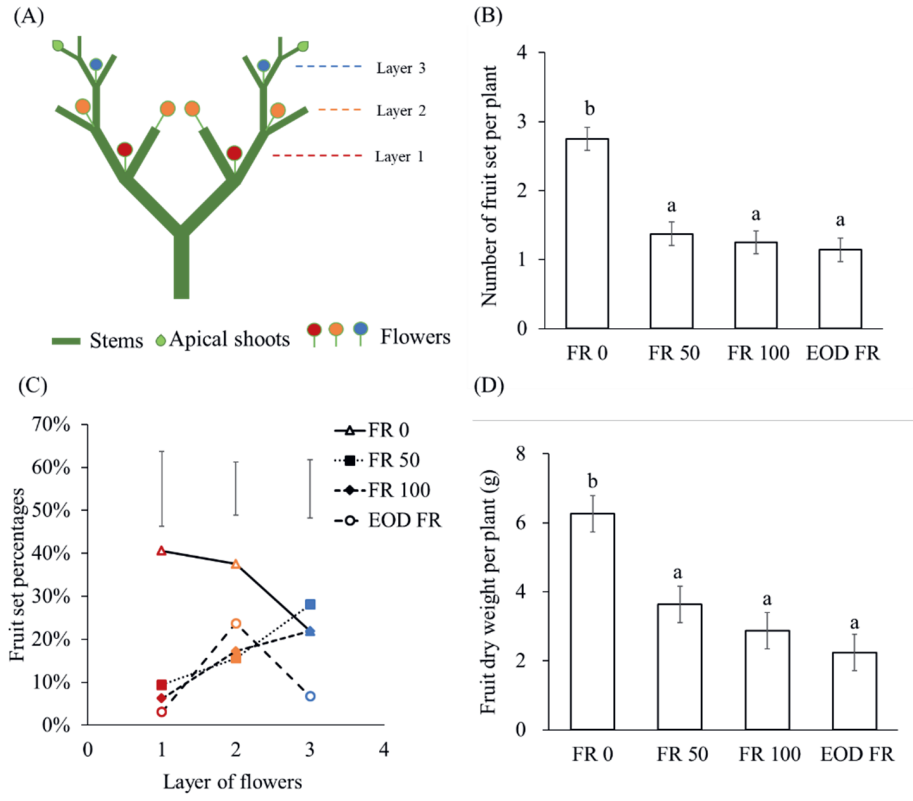

Figure 1. Effect of additional far-red (FR) on fruit set. (A) Plant structure and the location of 8 target flowers on the plants. Flowers at the same node number counted from the bottom of the plant were called the flowers at the same layer. Layer 1, 2 and 3 included 2, 4 and 2 flowers per plant respectively, which are represented by red, orange, and blue circles. Leaves are not shown in this illustration. (B) The average number of fruits per plant out of target flowers on day 56 after transplanting (day 40 after the first anthesis). (C) The percentage of fruit set in each flower layer. The color of symbols in (C) indicates the specific flower layer with the same color as in (A), where layer 1 is the lowest layer and layer 3 is the top layer on the plant. (D) The total fruit dry weight per plant. Each data point was the mean value derived from 2 replicates each based on 8 individual plants. One-way ANOVA was performed, where F-probability for light treatment is (B) 0.007; (C) 0.107, 0.288, and 0.190 for layer 1-3, respectively; (D) 0.020. The error bars indicate ±standard error of means based on common variance. Different lowercase letters indicate significant differences between treatments according to Fisher's protected LSD test at P=0.05.

There was no significant effect of FR on fruit dry matter content and individual fruit dry weight, however, both variables showed a slight tendency to increase when FR was present (Fig. 2A, 2B). Interestingly, adding FR led to a lower fruit length: width ratio, which indicates a change in fruit shape by FR (Fig. 2E). This was probably due to the slight increase of individual fruit width, while individual fruit length was barely influenced (Fig. 2C, 2D). All treatments shared the same linear relationship

between fruit fresh weight and calculated fruit volume (Supplementary Fig. S6), which means FR did not affect this relationship.

Adding FR also strongly impacted the morphology of the plants (Fig. 3). Plants showed significant elongation under FR, and the effect was the largest in FR 100, followed by FR 50, and the effect of EOD FR on plant elongation was the least (Fig. 3; Table 3). At the highest FR intensities (FR 50 and FR 100), specific stem length was lowest, and correspondingly, the stem dry matter content was the highest (Table 3). Plant leaf area was not significantly influenced by light treatments (Table 3). The response of specific leaf area seemed related to the dose of additional FR light: treatment FR 50 and EOD FR both had higher specific leaf area; while FR 100 had lower specific leaf area compared to FR 0 (Table 3). Similar to stem dry matter content, the dry matter content of leaves was also significantly higher under whole day far-red lighting compared to the treatment without FR or EOD FR (Table 3). The angles between branches at the first and second splitting were significantly narrower in the treatments with additional FR (Table 3).

Adding FR throughout the day led to a significantly higher total shoot biomass, however, with a very limited number of fruits (Fig. 4). Probably due to a smaller number of fruits, adding FR throughout the day or EOD led to significant lower dry matter partitioning to the fruits (Fig. 4). Meanwhile, additional FR led to significant higher dry matter partitioning to the stem, and less partitioning to the leaves when FR was applied during the day (Fig. 4). This could explain a higher ratio between stem and leaf dry weight when FR was applied (Table 3).

Table 2. Average anthesis time (days after transplanting) for each layer of flowers. Different intensity of additional far-red (FR, 0, 50, 100 µmol m^{-2} s^{-1}) was applied throughout the day, or applied as end of day lighting (EOD, 30 minutes, 30 µmol m^{-2} s^{-1}). *P* = F-probability for treatment effect in one-way ANOVA. N=2, each replicate was based on 8 individual plants.

Treatments	Layer of flowers		
	Layer 1	Layer 2	Layer 3
FR 0	17.9	23.9	29.1
FR 50	17.9	23.9	29.8
FR 100	17.8	24.5	30.6
EOD FR	18.3	24.4	29.8
SE_{mean}	0.22	0.27	0.27
P	0.576	0.430	0.060

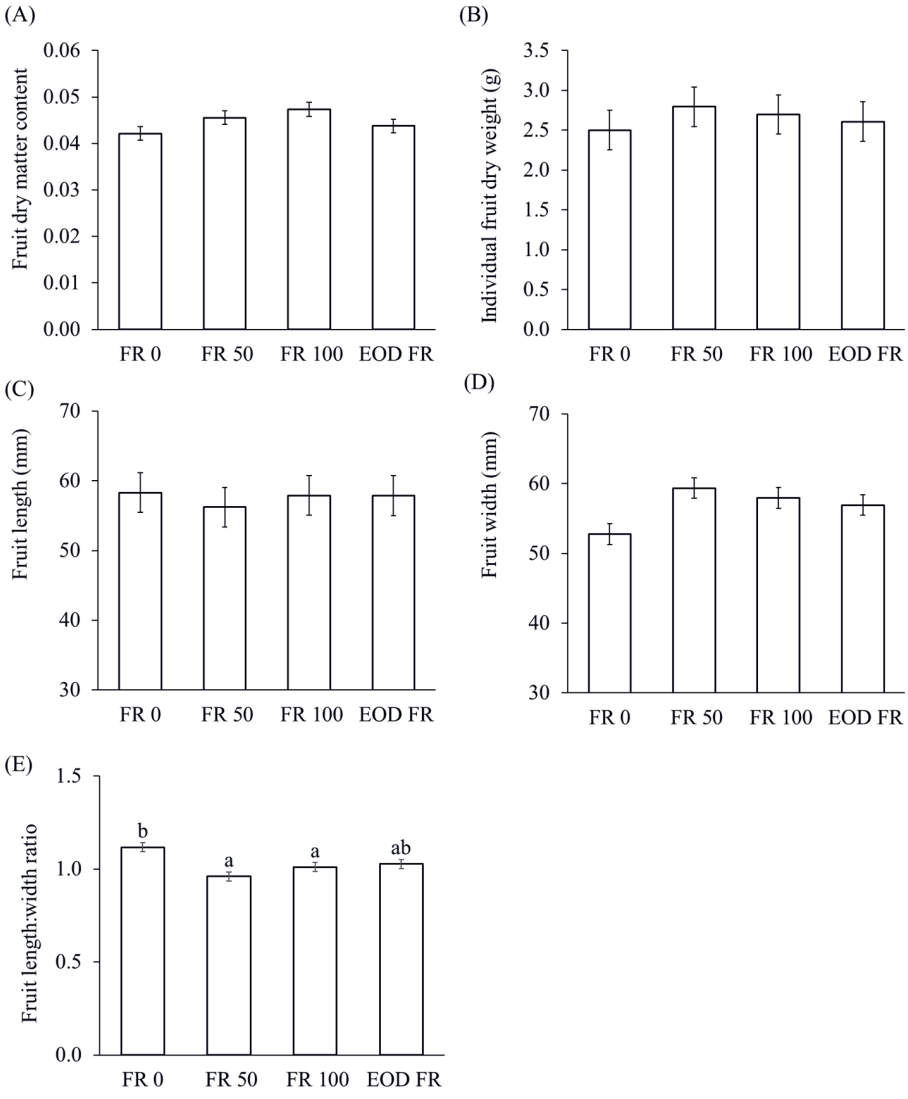

Figure 2. Effect of additional FR light on fruit growth. (A) Fruit dry matter content; (B) Individual fruit dry weight; (C) Fruit length; (D) Fruit width; (E) Ratio of fruit length to width. Data was the mean value derived from 2 replicates each based on all fruits from 8 individual plants. All fruits were harvested on day 56 after transplanting. One-way ANOVA was performed, where the F-probability for light treatment was (A) 0.218, (B) 0.855, (C) 0.955, (D) 0.119 and (E) 0.042 respectively. The error bars indicate ±standard error of means based on common variance. Different lowercase letters indicate significant differences between treatments according to Fisher's protected LSD test at *P*=0.05.

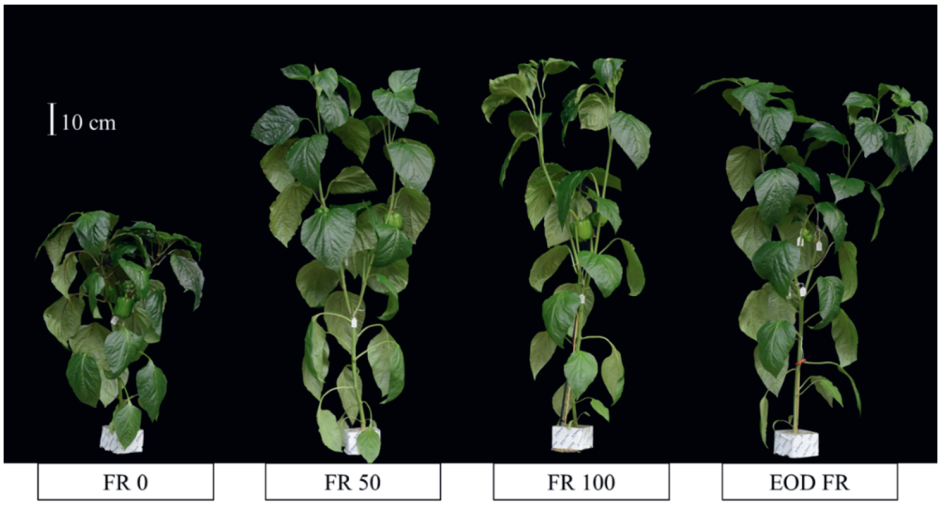

Figure 3. Sweet pepper plants on day 56 after transplanting. From left to right, the plants were cultivated under different intensity of additional far-red (FR, 0, 50, 100 µmol m^{-2} s^{-1}) applied throughout the day, or applied as end of day lighting (EOD, 30 minutes, 30 µmol m^{-2} s^{-1}). The white bar indicates 10 cm.

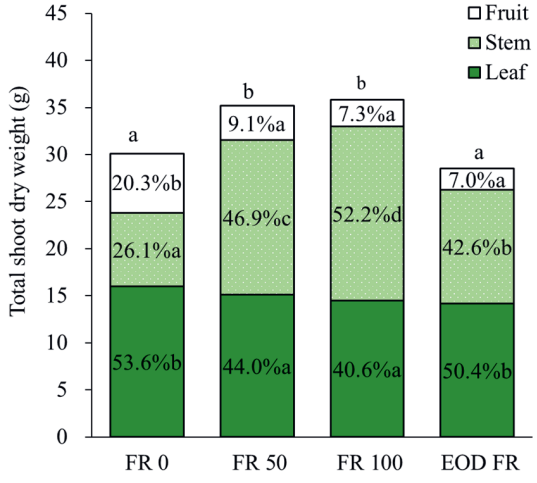

Figure 4. Effects of additional FR on total shoot dry weight (aboveground), which was composed of fruit, leaf, stem, on day 56 after transplanting. Different intensity of additional far-red (FR, 0, 50, 100 µmol m^{-2} s^{-1}) was applied throughout the day, or applied as end of day lighting (EOD, 30 minutes, 30 µmol m^{-2} s^{-1}). Data was the mean value derived from 2 replicates each based on 8 individual plants. One-way ANOVA was performed, where F-probability for light treatment is 0.003 for total shoot dry weight, 0.014, 0.006, and <0.001 for fruit, leaf, and stem dry weight percentage, respectively. Different lowercase letters indicate significant differences between treatments according to Fisher's protected LSD test at P=0.05.

Table 3. Effects of additional FR on the morphology of sweet pepper plants on day 56 after transplanting. Different intensity of additional far-red (FR, 0, 50, 100 µmol m^{-2} s^{-1}) was applied throughout the day, or applied as end of day lighting (EOD, 30 minutes, 30 µmol m^{-2} s^{-1}). DMC = dry matter content. *P* = F-probability for treatment effect in one-way ANOVA. N=2, each replicate was based on 8 individual plants. Means followed by different letters differ significantly according to Fisher's protected LSD test at *P*=0.05.

Parameters	Treatments					
	FR 0	FR 50	FR 100	EOD FR	SE_{mean}	*P*
Plant height (cm)	66.25 a	107.09 c	116.09 d	95.04 b	3.94	<.001
Specific stem length (cm g^{-1})	8.714 b	6.864 a	6.407 a	8.139 b	0.705	0.002
Stem DMC	0.0955 a	0.1213 b	0.1202 b	0.1074 ab	0.0159	0.030
Leaf area (cm^2)	5686	5645	4869	5827	935.6	0.140
Specific leaf area (cm^2g^{-1})	361.3 b	380.1 c	343.2 a	416.6 d	17.3	0.001
Leaf DMC	0.0867 a	0.0952 b	0.0971 c	0.0902 ab	0.0054	0.018
Stem dry weight /leaf dry weight	0.4887 a	1.0873 c	1.2868 d	0.8505 b	0.0829	<.001
First splitting angle (°)	55.00 b	34.87 a	36.94 a	35.92 a	7.08	0.004
Second splitting angle (°)	59.19 b	28.91 a	31.97 a	33.61 a	5.13	<.001

4 Discussion

4.1 Far-red light reduces fruit set

Far-red is an important light signal that can regulate plant growth by mediating crucial physiological processes, which has fascinated plant scientists for generations. Here, we reveal for the first time that FR light reduced fruit set. This effect was shown for both the whole day FR and EOD FR in sweet pepper (Fig. 1). We did not find a dose-effect of FR lighting on fruit set (FR at 50 or 100 µmol m^{-2} s^{-1} had the same effect on fruit set). Both levels of FR lighting were relatively high, so perhaps both close to the saturation end in the Michaelis-Menten-type dose-response curve of FR described by Chia & Kubota (2010).

FR light was reported to enhance leaf abscission or flower abscission, while the red light was reported to delay these processes (Craker *et al.*, 1987; van Meeteren & van Gelder, 2000), suggesting photocontrol of abscission mediated by phytochromes. Flower and fruit abortion in sweet pepper is also an abscission process (Wubs *et al.*, 2009). Thus, the higher flower and fruit abortion under additional FR in sweet pepper was consistent with those findings and may share the same mechanisms.

In contrast to our experiment in climate-controlled rooms with sole artificial lighting, in few greenhouse experiments with supplemental lighting, adding FR showed a beneficial effect on yield in the early phase of production (Hao *et al.*, 2018; Schuddebeurs, 2021). In these greenhouse experiments, light intensities were generally higher while a relatively lower amount of FR was added, e.g., up to 24 μmol m^{-2} s^{-1} of FR was added to 160-180 μmol m^{-2} s^{-1} supplementary HPS light in winter (Hao *et al.*, 2018). One of the reasons for the different findings could be that at higher daily light integral (DLI) fruit set is promoted due to a high source/sink balance such that the relative effects of far-red diminish. Another reason could be that the effect of FR might follow a dose-effect pattern, or a threshold effect pattern. In either case, FR may have little effect on stimulating abortion when FR level is low as was the case in the greenhouse experiments. Moreover, the different background light spectrum could be a reason for contrasting results, as the interaction of FR with other light colors could play a role (Brown & Klein, 1971; Zhang *et al.*, 2022).

4.2 FR stimulated abortion could be due to altered hormonal balance at abscission zone

Flower and fruit abortion in peppers involves the formation of an abscission layer in the pedicel, controlled by the balance between auxin and ethylene (Taylor & Whitelaw, 2001).

Ethylene emissions were increased by additional FR in tomato leaves (Courbier *et al.*, 2021) and *Arabidopsis* (Pierik *et al.*, 2009). However, lower ethylene level was observed under lower R:FR conditions in sunflower (Kurepin *et al.*, 2007) and tomato seedlings (Kurepin *et al.*, 2010). In an early study on cuttings of mung beans, Craker *et al.* (1987) suggested that FR may increase the sensitivity of the plant to ethylene without affecting ethylene production. These contrasting results make the role of ethylene remain obscure in the observed FR effect.

Auxin, as opposed to ethylene, mitigates flower and fruit abscission, and reduces the sensitivity of the abscission zone to ethylene (Taylor & Whitelaw, 2001). High auxin flux through the abscission zone makes the abscission zone stay inactive, inhibits cell separation, and subsequently increases fruit set (Taylor & Whitelaw,

2001; Botton *et al.*, 2011). R:FR is known to affect auxin synthesis and transport in plants (Küpers *et al.*, 2020), thus this might be an explanation for the effect of additional FR on flower and fruit abortion.

4.3 FR stimulated abortion could be due to correlative inhibition and competition for assimilates

Except for the possible direct effect of FR on target flowers or fruits, a low fruit set may also result from the effect of FR on other organs in the plants: a dominance effect and/or an effect via competition for assimilates.

Based on Bangerth's theory (2000), young fruit abscission is probably due to the correlative influences exerted on them by more dominant fruit and/or shoots. These correlative influences are mediated by the autoinhibiting effect from the auxin stream of the more dominant organ on the auxin export of the more dominated organ. And this autoinhibition mechanism can probably downregulate IAA transport through the abscission zone of these fruits and finally triggers their abscission.

Seed number is an important determinant of auxin export of a particular fruit (Callejas & Bangerth, 1998). Low seed number can lower the probability of fruit set, while an increase in seed number increased the inhibitory effect of a fruit on the setting and the growth of later-developing fruits, through larger dominance effect and increased sink strength in the competition for assimilates (Marcelis & Baan Hofman-Eijer, 1997; Wubs *et al.*, 2009).

However, in our study, FR influenced fruit set the most in the first layer of flowers (Fig. 1C). The competition and dominance effect usually are from the elder fruits to younger fruits. Thus, the low fruit set under FR seemed not to result from stronger dominance or competition from elder fruits, as there were no elder fruits for the first layer flowers.

We noticed when the first layer of flowers had a low probability of fruit set, later layers showed a higher fruit set probability. This cancelled out the effect of FR on fruit set in later layers, and FR might even allow more fruit set later. This corresponds to the findings of the interactions between fruits (Marcelis & Baan Hofman-Eijer, 1997). In several crops, including sweet pepper, fruit production shows great cyclic fluctuations during a growing season (Marcelis, 1992; Heuvelink *et al.*, 2002). This means in long-term cultivation, the interaction between older and younger fruits cannot be neglected when applying different light spectra to regulate the fruit set in sweet pepper, since the light effect can be entangled with the flushing pattern.

Besides earlier-formed fruits, the apical shoot can also compete for assimilates and exert a dominance effect on flowers and fruits. Apical dominance can be promoted by a low R:FR ratio (Leduc *et al.*, 2014). If the dominance effect between vegetative and generative organs shares the same mechanism as apical dominance on the outgrowth of axillary buds (Walker & Bennett, 2018), the low fruit set could be the result of a strongly stimulated shoot growth under additional FR.

Dominance effects attract much attention. However, we cannot overlook the effect of competition for assimilates. Libenson *et al.* (2002) suggested that a reduction of sunflower yield under low R:FR is likely the consequence of enhanced stem growth and the competition for resources between the reproductive structures and the stem, which can also be an explanation for our findings.

4.4 FR stimulated abortion cannot be explained by FR effect on plant growth

Additional FR during the day increased total shoot dry weight substantially (17% for FR 50 and 19% for FR 100; Fig. 4). As the root dry weight of pepper is only up to 10% of total dry weight in fruiting plants (Nielsen & Veierskov, 1988; Bennett *et al.*, 1979), it is likely that additional FR during the day increased the total plant dry weight too. In fact, effects on total plant dry weight might be even slightly larger as FR may also increase root biomass (Lee *et al.*, 2016). Although a higher dry matter accumulation of the plant suggests a higher source strength, which normally increased fruit set (Marcelis *et al.*, 2004), these plants under additional FR had fewer fruits, compared to the treatment without FR. Hence the effects of FR on fruit set are not likely explained by effects on source strength of the plants.

The higher shoot biomass was not related to an increase in leaf area, as, under the highest FR intensity (100 µmol m^{-2} s^{-1}), leaf area was even 14% lower compared to no FR (though this difference was not statistically significant; Table 3). The positive effect on total shoot biomass might partly be due to a more open plant structure with elongated internodes (Fig. 3), improving light distribution and absorption (Sarlikioti *et al.*, 2011; Kalaitzoglou *et al.* 2019). Recently, Zhen & Bugbee (2020) proposed that additional FR is as photosynthetic effective as PAR light. Hence, improved leaf photosynthesis rate might also be the reason for the increased biomass. This may explain why EOD lighting promoted stem elongation while it had no significant effect on total shoot biomass (Table 3), similar as found in tomato (Kalaitzoglou *et al.*, 2019).

Dry matter partitioning towards stems was greatly increased with additional FR (Fig. 4). The vigorous growth of stems seemed supportive to our speculation that, apical dominance and/or assimilate competition between different plant organs may be responsible for the additional flower and fruit abortion under additional FR.

5 Conclusions

Adding far-red (FR) either during the daytime or EOD reduced fruit set in sweet pepper. This was not likely explained by the effects of FR on plant growth and morphology. FR applied during daytime promoted shoot biomass, but not when applied only at the end of day. Adding FR led to more stem elongation, more upright branches, and more dry mass partitioning to stems. The FR induced flower and fruit abortion might be regulated through an altered hormonal balance at the abscission zone, correlative inhibition and/or competition for assimilates. The possible mechanisms have been discussed and need further investigation.

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Supplementary information

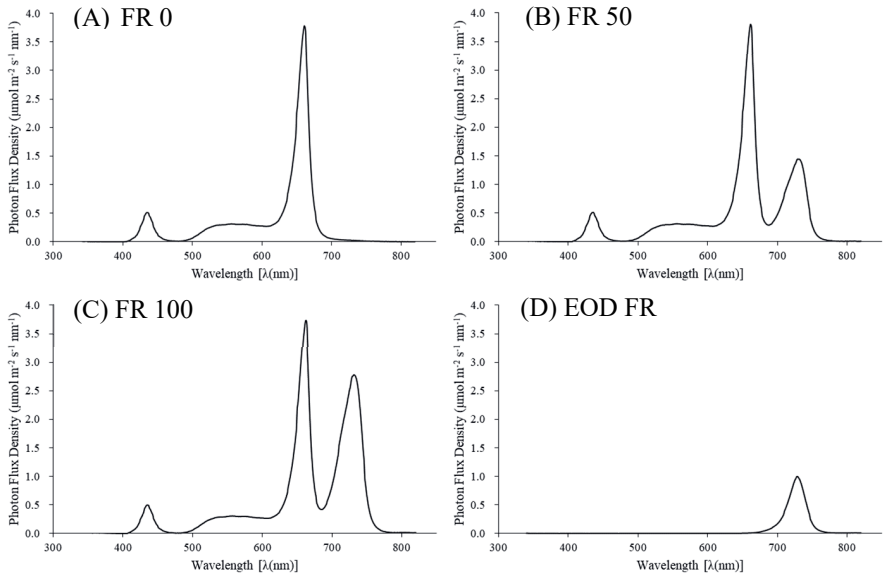

Figure S1. Light spectrum of 4 light treatments provided by light-emitting diodes (Philips GreenPower deep red/white LED production module, 16 W, & Philips GreenPower far-red LED research module, 10 W).

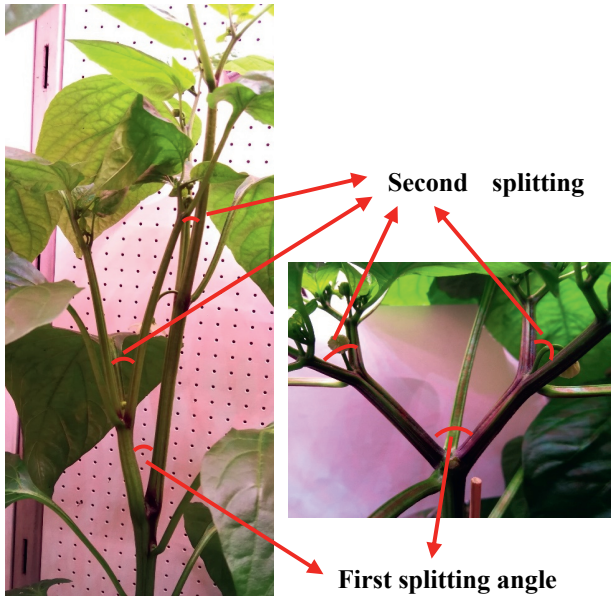

Figure S2. The illustration of where we measured the angles of the first and second splitting nodes. The second splitting angle was determined by averaging the angles from two main stems. The left plant was under FR 50, and the right one was under FR 0.

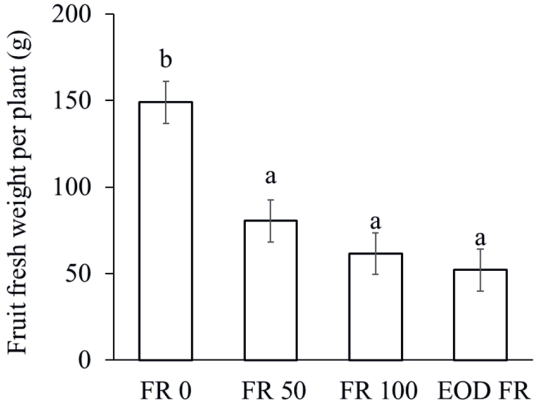

Figure S3. Total fruit fresh weight per plant on day 56 after transplanting. One-way ANOVA was performed and F-probability for light treatment is 0.015. The error bars indicate ±standard error of means based on common variance. Different lowercase letters indicate significant differences between treatments according to Fisher's protected LSD test at *P*=0.05.

Figure S4. Arrested fruit (the left photo) under FR 100 on its day-31 after anthesis (fresh weight 0.74g, length 1 cm), compared to a normal fruit (the right photo) under FR 100 on its day-32 after anthesis (fresh weight 51.33g, length 6 cm).

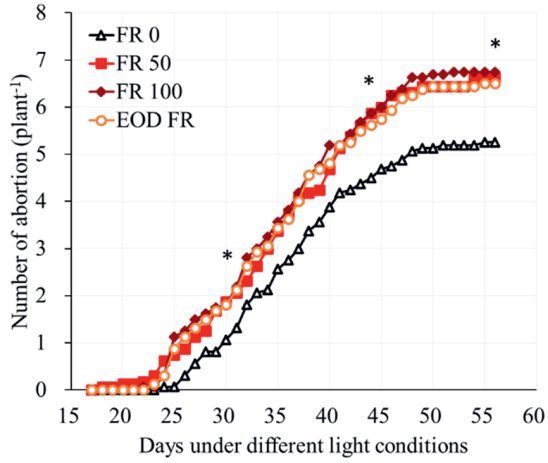

Figure S5. The number of average flower and fruit abortion per plant every day from day 17 till day 56 after plants transplanted to cells with different light conditions. Each data point was the mean value derived from 2 replicates of 8 plants. One-way ANOVA was performed on the data of day 30, day 44 and day 56, where star symbol ('*') indicate significant difference (F-probability for light treatment =0.036, 0.018, 0.011 respectively). $LSD_{0.05}$ (least significant differences of means for 5% level) = 0.560, 0.772, 0.734, respectively.

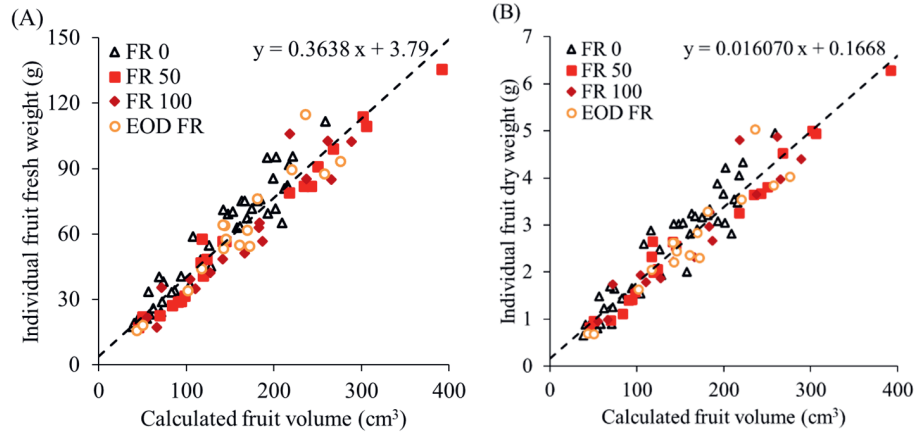

Figure S6. (A) Linear regression between individual fruit fresh weight and calculated fruit volume based on measured fruit length and width on 56 days after transplanting. (B) Linear regression between individual fruit dry weight and calculated fruit volume. Linear regression analysis was performed with Genstat 19th edition, and there is no treatment effect.

Chapter 3

Far-red light-enhanced apical dominance stimulates flower and fruit abortion in sweet pepper

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Abstract

Far-red radiation affects many plant processes, including reproductive organ abortion. Our research aimed to determine the role of apical dominance in far-red light-induced flower and fruit abortion in sweet pepper (*Capsicum annuum* L.). We conducted several climate room experiments where plants were grown under white or red-rich LED light, with or without additional far-red light. Additional far-red light enhanced apical dominance: it increased auxin levels in the apices of dominant shoots, and caused a greater difference in internode length and apical auxin levels between dominant and subordinate shoots. Additional far-red light stimulated fruit abortion in intact plants but not in decapitated plants, suggesting a crucial role of shoot apices in this effect. However, reducing basipetal auxin transport in the stems with N-1-naphthylphthalamic acid (NPA) did not influence far-red light-stimulated fruit abortion, although auxin levels in the stem were largely reduced. Applying the synthetic auxin 1-naphthaleneacetic acid (NAA) on decapitated apices did not influence fruit abortion. However, applying the auxin biosynthesis inhibitor yucasin to shoot apices reduced fruit abortion regardless of the light conditions, accompanied by slight shoot growth retardation. These findings suggest that the basipetal auxin stream does not mediate far-red light-stimulated fruit abortion. Far-red light-stimulated fruit abortion was associated with reduced sucrose accumulation and lower invertase activities in flowers. We suggest that under additional far-red light conditions, increased auxin levels in shoot apices promote fruit abortion probably through enhanced competition for assimilates between apices and flowers, which limits assimilate import into flowers.

Key words:

Auxin; sugar; *Capsicum annuum* L.; far-red radiation; fruit set.

1 Introduction

Flower and fruit abortion, which comprises the cessation of development of these organs and their subsequent abscission, is an important cause of yield loss in fruit crops. In sweet pepper (*Capsicum annuum* L.), flower and fruit abortion can reach up to 70-80% (Wubs *et al.*, 2009). Flower and fruit abortion can be induced by various environmental signals, such as far-red radiation (FR; 700-800 nm). Generally, FR is an important signal for plants (Demotes-Mainard *et al.*, 2016). Plants perceive FR with the phytochrome (PHY) light receptors, which are activated by red light (R; 600-700nm) and inactivated by FR. Low R:FR ratios trigger shade avoidance syndromes in various species, characterized by strong internode elongation, less outgrowth of lateral buds, and more biomass partitioning to stem (Demotes-Mainard *et al.*, 2016). In our previous work, we observed that fruit abortion in sweet pepper was promoted by additional FR during the day or at the end of day (Chapter 2). However, the mechanism of this phenomenon is still unknown.

Flower and fruit abortion in peppers involves the formation of an abscission layer in the pedicel, controlled by the balance between auxin and ethylene (Taylor & Whitelaw, 2001). Ethylene promotes fruit abscission, while auxin hinders this process and reduces the sensitivity of the abscission zone to ethylene (Taylor & Whitelaw, 2001). Low auxin flux through the abscission zone activates the abscission zone, causes cell separation, and subsequently leads to flower or fruit abortion (Botton *et al.*, 2011). In the formation of an abscission layer, correlative inhibition may play an important role (Bangerth, 1989). Correlative inhibition is an important regulatory mechanism in plants, where one part of the plant suppresses the growth and development of another part. Apical dominance is a form of correlative inhibition, where the shoot apical meristem suppresses the outgrowth of lateral meristems, and potentially suppresses the growth of other organs as well (Walker & Bennett, 2018). A low R:FR ratio can promote apical dominance (Leduc *et al.*, 2014), which makes apical dominance a candidate to explain FR-stimulated abortion. Dominance of earlier formed fruits can also lead to abortion of later-formed fruits or flowers in pepper (Marcelis *et al.*, 2004). However, this cannot explain the FR-stimulated abortion, as in our previous work, the FR-stimulated abortion was already shown among the first fruits of a plant (Chapter 2).

Apical dominance has been mainly studied in the outgrowth of lateral buds, where the controlling role of auxin can be direct or indirect (Domagalska & Leyser, 2011; Beveridge *et al.*, 2023). Based on the canalization model, auxin transport auto-inhibition is the key to the dominance phenomenon, where an actively growing organ is able to inhibit the export of auxin from other organs directly (Bangerth,

1989). Auxin export from a lateral bud is essential for its outgrowth (Morris, 1977). This only occurs if the bud creates a canalized link between itself and the polar auxin transport stream in the stem, which could be prevented by apically derived auxin via saturating the transport capacity of the main stem (Prusinkiewicz *et al.*, 2009). Another explanation suggests that auxin indirectly controls the outgrowth of buds by regulating the synthesis of secondary messengers, cytokinins and strigolactones, which can move into lateral buds and regulate branching (Brewer *et al.*, 2015). Furthermore, the role of sugar in apical dominance has also been emphasized. Sugar translocation into the buds was one of the earliest observations during the outgrowth of buds after decapitation, whereas changes in auxin content in the adjacent stem happened much later than the bud outgrowth (Morris *et al.*, 2005; Mason *et al.*, 2014). Moreover, sucrose supply can directly promote bud release, which suggests that enhancing sugar supply to lateral buds is sufficient for overcoming apical dominance (Mason *et al.*, 2014). Thus, the sugar demand of shoot tips was suggested to be one of the initial regulators of apical dominance instead of auxin. However, the sustained growth of buds will require continued sucrose supply and auxin depletion in the adjacent stem (Cao *et al.*, 2023).

Similar to the outgrowth of a lateral bud, auxin export is also essential for fruit retention (Xie *et al.*, 2013). The interruption of auxin export from the developing fruit by 2,3,5-triiodobenzoic acid (TIBA) promoted fruit abscission in sweet cherry (*Prunus avium* L.) (Else *et al.*, 2004). If auxin export from the flower or fruits is inhibited by apically derived auxin, the formation of an abscission layer and thus the abscission of the inhibited organ can happen (Bangerth, 1989). On the other hand, when the shoot tip of an apple (*Malus domestica* Borkh.), grape (*Vitis vinifera* L.) or bean (*Vicia faba* L.) plant was removed, nearby fruits showed higher fruit set and started to export more IAA (Bangerth 1989; Bangerth 2000). Parthenocarpic fruits were even formed in tomato (*Solanum lycopersicum* L.) and pea (*Pisum sativum* L.) when apical dominance was released (Serrani *et al.*, 2010; Rodrigo & García-Martínez, 1998). These findings suggest an important role of apical shoots in controlling fruit set, which may share the same mechanism as controlling the outgrowth of lateral buds.

The promotion of apical dominance by low R:FR ratio (Leduc *et al.*, 2014) is related to its effect on promoting auxin synthesis and possibly auxin transport (Küpers *et al.*, 2020; Song *et al.*, 2024). The auxin export of a sink organ is essential for its vascular tissue differentiation, which determines the import of assimilates, water and minerals into the sink organ. Auxin may have a direct effect on assimilate transport, which then favors dominant organs with their higher auxin diffusion rate (Bangerth 1989; Morris & Arthur, 1987; Patrick & Steains 1987). This brought up the

possibility that the enhanced apical dominance under a low R:FR ratio may not only inhibit the auxin export from flowers and fruits through auxin transport autoinhibition but also limit assimilate import to flowers and fruits. In pepper flowers, decreased sugar accumulation was closely linked to the subsequent flower abortion under a low light intensity or extra leaf removal (Aloni *et al.*, 1996). The presence of fruit as a competitor reduced the sucrose translocation from source leaves to new flowers, followed by a lower sugar level in flowers (Aloni *et al.*, 1991). Likewise, NAA (1-Naphthaleneacetic acid) and shading-induced flower drop in apple were associated with repressed expression of sorbitol and the sucrose transporter genes in the fruit abscission zone (Zhu *et al.*, 2011). Furthermore, feeding sucrose inhibited pepper flower abortion (Aloni *et al.*, 1997). Aloni *et al.* (1997) suggested that the translocated sucrose into flowers can inhibit abscission, by enhancing the activity of sucrose synthase and invertases, which are responsible for sucrose cleavage. Thus, it may ensure sucrose continuously enters the developing flower and sustains its growth.

To elucidate the role of apical dominance in FR-stimulated flower and fruit abortion, a series of experiments was conducted in which 1) plants were decapitated, 2) the basipetal auxin stream in stems was inhibited chemically by the auxin transport inhibitor NPA (N-1-naphthylphthalamic acid), 3) synthetic auxin NAA (1-naphthaleneacetic acid) was applied on decapitated plants, and 4) auxin biosynthesis at apices was inhibited chemically with yucasin, when plants were grown under LED light with or without FR. We hypothesized that limiting basipetal auxin transport or the level of apically derived auxin reduces FR-stimulated flower and fruit abortion. Besides measurements of abortion and auxin content, sugar content and enzyme activities for sucrose cleavage in flowers were analyzed to determine whether FR-stimulated abortion was associated with a lower sugar accumulation and a lower level of sucrose cleavage activity.

2 Materials and methods

2.1 Plant materials and growth conditions

Seeds of sweet pepper (*Capsicum annuum* L. cv. Gialte) were provided by Enza Zaden (Enkhuizen, the Netherlands). Unless mentioned otherwise, seeds were sown in potting mix soil (Lensli, Bleiswijk, the Netherlands). One week after germination, seedlings were transplanted in stonewool cubes (Grodan, Roermond, the Netherlands) and cultivated in a glasshouse for around 6 weeks before each experiment started. Plants were irrigated with ebb and flow system with nutrient solution (Supplementary Table S1).

Unless mentioned otherwise, in the first week of each experiment, plants were transplanted onto stonewool slabs with a drip system in a climate room and were exposed to acclimation light without additional far-red (Table 1). In the climate room, the temperature was controlled at 22/18 °C (day/night), the humidity was 70%, and no CO_2 enrichment was applied. The plants were irrigated 4 times a day with the same nutrient solution.

Table 1. Light conditions in various experiments. See Supplementary Fig. S1 for Light spectra. PAR indicates photosynthetic active radiation (400-700 nm), where 400-500 nm was considered as blue light, 500-600 nm as green light, and 600-700 nm as red light. PPFD stands for photosynthetic photon flux density within the range of PAR. Light in the range of 700-800nm was considered as FR (far-red). PSS = Photostationary State of Phytochrome (calculated based on Sager *et al.* 1988). The light intensity was expressed as the mean ±standard error of the mean, where the standard error was based on the variance between statistical replicates (plots) for each light treatment.
* This light treatment also served as the acclimation light in the first week of the experiment. The light spectrum and intensity of acclimation light for NAA and Yucasin experiment can be found in Supplementary Fig. S1. Photoperiod of acclimation light was the same as the light treatments in the respective experiment.

Experiments	Light treatments	Composition of PAR			PPFD ($\mu mol\ m^{-2}\ s^{-1}$)	FR ($\mu mol\ m^{-2}\ s^{-1}$)	PSS	Day length (h)
		Blue	Green	Red				
Vegetative growth EXP; NPA EXP	-FR*	19%	43%	38%	192.2±2.3	4.1±0.1	0.86	16
	+FR				195.2±8.2	64.4±4.1	0.69	
Decapitation EXP	-FR*	6%	18%	76%	138.1±2.9	1.0±0.2	0.88	12
	+FR				138.5±3.8	52.0±1.4	0.78	
NAA EXP; Yucasin EXP	-FR	10%	0%	90%	122.4±1.4	0.7±0.2	0.88	14
	+FR				124.9±2.1	56.2±1.8	0.78	

Pepper plants follow a dichotomous branching, where every apex ends in a flower and two new apices, which turn into two branches, so-called 'splitting'. Unless mentioned otherwise, plants were pruned to four main stems, with the weaker shoots out of two twin shoots stopped at one leaf. Other than splitting shoots, all the other shoots emerging from the axil of a leaf were considered as lateral shoots.

To limit the interaction between fruits of different ages, each plant was pruned to have only 8-12 flowers retained. The shoots were pruned when longer than 3 cm, and the flower buds when the petals were visible, which was usually 3-5 days

before their anthesis (flower opening). In pepper, pollination normally occurs by self-pollination; in line with the commercial production of sweet pepper, no measure was taken to stimulate pollination.

2.2 Light treatments

In all experiments, 6-week-old plants were cultivated under two light conditions: with or without additional FR. The light was provided by light-emitting diodes (LEDs; Table 1; Supplementary Fig. S1). For each experiment, light intensity at the top of the plants was maintained constant during the experiment by adjusting the height of the lamps weekly. We used spectroradiometers (type SS-110, Apogee Instruments, Inc) for all the irradiance measurements.

The susceptible period of abortion in sweet peppers is between one week before anthesis and two weeks after anthesis (reviewed in Wubs *et al.*, 2009). All the light treatments started at least one week before the first anthesis and ended at least two weeks after the last retained flower reached anthesis. Flower abortion in this study was defined as the abortion before anthesis, while fruit abortion as the abortion after anthesis.

2.3 Vegetative growth experiment

The climate room was separated into four compartments with white plastic sheets. Two replicates of two light conditions '-FR' and '+FR' were randomized over four compartments. Light treatments started when the first flower bud was visible and lasted for 23 days. There were 12 plants in each compartment resulting in a planting density of 11.5 plants m^{-2}. All flowers were removed before the petals appeared. The shoots of plants were not pruned and were allowed to grow and branch freely. The internodes were labeled from bottom to top (Fig. 1A). Six plants per compartment were used for final morphological measurements. Plant tissues on the other six plants per compartment were collected for further analysis.

2.4 Decapitation experiment

The experiment was conducted in a glasshouse with blackout screens, which blocked almost all light from outside during the experiment. The experiment was conducted from 5th Feb to 26th March 2020, at Wageningen, Netherlands (51°N, 5°E). The glasshouse was divided into six compartments by white plastic sheets, where three replicates of two light conditions '-FR' and '+FR' were distributed over these compartments randomly. The light treatments started around one week before the first anthesis and lasted for 44 days.

Adult plants rooted in rockwool cubes were provided by Beekenkamp (Maasdijk, the Netherlands). These plants were transplanted on top of the potting soil in 2.5 L

plastic pots, mixed with around 10g slow-release fertilizer (Osmocote Exact Standard 8-9M, ICL Specialty Fertilizers, Waardenburg, the Netherlands). Daily irrigation with tap water was done manually. There were 16 plants in each compartment resulting in a planting density of 9.5 plants m^{-2}. Plants were maintained to have four main stems carrying eight flowers (Fig. 2A). The lateral shoots and extra flowers were pruned weekly. Half of the plants per compartment were decapitated when the higher layer of flowers reached anthesis. Decapitated plants were elevated to have their canopy surface at the same height as non-decapitated plants. Plants were rotated weekly within one compartment. All 16 plants per compartment were used for final morphological measurements.

2.5 NPA experiment

The experiment had the same setup as the vegetative growth experiment. Plants were maintained to have four main stems carrying 12 flowers (Fig. 3A). Light treatments started around two weeks before the first anthesis and lasted for 52 days. In each compartment, we divided plants into two subplots, six plants were treated with NPA (N-1-naphthylphthalamic acid) mixed with lanolin, and the other six were treated with sole lanolin. NPA was dissolved in 2 ml DMSO (Dimethyl sulfoxide), and then the solution was added to 100 g warm lanolin to reach a final concentration of 0.5% (w/w) NPA. In the control treatment, 2 ml DMSO was added to 100 g warm lanolin. Control and NPA treatment were applied as a complete ring of lanolin paste on the second internode above the higher layer of flowers to prevent chemicals from directly contacting flowers (Fig. 3A). Chemicals were applied on all four main stems of each plant when most of the flowers reached anthesis (day 26 after light treatments). On day 52 since light treatments, final morphological measurements were conducted on all plants, and plant tissues were collected at the same time for further analysis.

2.6 NAA experiment

The experiment was conducted in a climate room, which was separated into eight compartments with white plastic sheets. There were six plants per compartment resulting in a planting density of 5.5 plants m^{-2}. Plants were maintained to have four main stems carrying eight flowers (Fig. 4A). Two replicates of four treatments were distributed randomly over these eight compartments: plants were 1) grown without additional FR (-FR); 2) grown with additional FR (+FR); 3) grown under '+FR' and decapitated at first anthesis; 4) grown under '+FR', decapitated at first anthesis, and NAA (1-Naphthaleneacetic acid) applied immediately after decapitation. NAA was dissolved in 2 ml 96% (v/v) ethanol first, then mixed with 50 g warm lanolin to reach a final concentration of 1% (w/w) NAA. In the 3rd treatment, plants were applied with sole lanolin (with 2 ml ethanol mixed in 50 g lanolin). Lanolin paste

with or without NAA was applied as a thick layer on the cutting surface after decapitation. The application was renewed once a week for three weeks. Light treatments started around two weeks before the first anthesis and lasted for 53 days until final measurements and collection of plant tissues.

2.7 Yucasin experiment

This experiment had the same setup as the NAA experiment. Plants were maintained to have four main stems carrying eight flowers (Fig. 5A). Two light conditions '-FR' and '+FR' with four replicates were randomized over eight compartments. In each compartment (replicate), we divided plants into two subplots, three plants were sprayed with yucasin solution, and the other three plants were sprayed with only the solvent. Yucasin (5-(4-Chlorophenyl)-2,4-dihydro-[1,2,4]-triazole-3-thione) was dissolved in 5 ml DMSO, then the solution was added to 100 ml distilled water to reach a final yucasin concentration of 50 mM for the first application, and a concentration of 25 mM for the second to fourth application. The chemical treatment was sprayed evenly on the apices every week, until the apices were fully covered by a thin layer of solution. While spraying, a paper cone was placed below the application area, to prevent contact between the solution and the flowers (Fig. 5A). On day 46 after light treatments, final morphological measurements and plant tissues collection were performed.

2.8 Morphological observations and measurements

Anthesis, flower and fruit abortion were observed weekly. The reproductive organ abortion in our experiments happened mostly (>90%) one week after anthesis, which was therefore considered as mainly fruit abortion. Lateral shoots were counted when they had a stem longer than 0.2 cm and one leaf longer than 1.5 cm. At the start and end of each experiment, plant height was recorded from the surface of the substrate to the highest visible nodes. Only leaves longer than 3 cm were counted and used to determine leaf area. The dry weight of stems, leaves, and lateral shoots was determined after drying in a ventilated oven for 24 h at 105 °C, while fruits were dried for 72 hours. Fruits with a diameter larger than 2cm were cut open to count the number of seeds.

2.9 Collection of plant tissues

Apex samples of 1 cm were collected with a sharp knife as the apical meristems with surrounding young flower buds and leaves. Flowers were collected including pedicels. Leaves were sampled with a puncher with a diameter of 1.5 cm, where 5 leaf discs at random spots per leaf were collected. Four leaves from the four main stems of each plant were used for sampling. Samples were placed in liquid nitrogen immediately after collection and stored in a -80°C freezer. Frozen samples

were ground into fine powder with a ball mill (Mixer Mill, Retsch) for further analysis.

To determine the carbohydrate level in different plant tissues, a new batch of plants was cultivated in the same light conditions as for the yucasin experiment. Four flowers were kept per plant with one flower per main stem. From these plants, 1 cm apices, flowers, 1 cm nodes where the flowers were attached, leaf discs of adjacent leaves to the flowers, and leaf discs of topmost mature leaves were collected. Samples were collected at the end of the light period ± 0.5 hours, as at this part of the day the highest levels of carbohydrates are expected, which could allow for a better distinction between treatments. Plants were harvested at three stages regarding flowers: 7 days before anthesis; at anthesis (when flower petals just opened), and 7 days after anthesis (Supplementary Fig. S2). Flowers from spare plants were used for pollen viability test and pollen quantification as described in Supplementary Fig. S3.

2.10 IAA extraction and quantification

To measure the concentration of free auxin (indole-3-acetic acid, IAA), 1 ml MeOH containing labelled auxin ($^{13}C_6$-IAA, final concentration 100 pmol) was added to each ground sample (~20 mg fresh weight). Afterwards, the samples were sonicated for 10s and placed on an orbital shaker in darkness at 4°C overnight. The samples were then centrifuged at 10000 rpm for 10 min at 4°C. Auxin was extracted from the supernatant by Solid Phase Extraction cartridges (amino) as previously described (Ruyter-Spira *et al.*, 2011), and the auxin concentration was determined by MRM-UPLC-MS/MS analysis as previously described (Schiessl *et al.*, 2019; Gühl *et al.*, 2021). The quantification method for ABA, ACC, SA, JA and cytokinins is described in Supplementary Fig. S4; and the quantification of ethylene emission is described in Supplementary Fig. S5.

2.11 Carbohydrates extraction and quantification

The soluble sugars and starch were extracted from around 15 mg freeze-dried tissue powder as described by Min *et al.* (2021), using the same equipment with minor modifications. After mixing with 5ml 80% ethanol (v/v), samples were vortexed thoroughly and shaken in an 80 °C water bath for 20 min, followed by a vortex again. After extraction, samples were centrifuged at 8500 rcf at 4 °C for 5 min, and 1ml supernatant was vacuum dried (Savant SpeedVac SPD2010, Thermo Fisher Inc.). Then 1 ml Milli-Q water was added to dissolve the carbohydrates with an ultrasonic bath (Branson 2200, Branson Ultrasonics) for 10 min at room temperature. Then the solutions were centrifuged at 21300 rcf at 4 °C for 10 min. The samples from flowers were diluted 10 times, while the other samples were

diluted 5 times with Milli-Q water for quantification of glucose, fructose and sucrose.

The remaining pellet after extraction was washed 3 times with 80% ethanol, and vacuum dried. Then 2 ml alpha-amylase solution (1 mg/ml) was added, followed by shaking in a 90°C water bath for 30 min. Then 1 ml amyloglucosidase (0.5 mg/ml in 50 mM citrate buffer, pH 4.6) was added, followed by shaking in a 60 °C water bath for 10 min. Afterwards, samples were centrifuged at 8500 rcf at 4 °C for 5 min. The supernatant was centrifuged at 21300 rcf for 15 min (4 °C). The solution from stems and flowers was diluted 10 times, while that from the other samples was diluted 20 times with Milli-Q water for quantification of glucose (for starch quantification).

Soluble sugars were eluted with 45mM NaOH, and starch-derived glucose was eluted with 100 mM NaOH + 25 mM NaOAc at a flow rate of 0.25 ml min^{-1}, which were both quantified with a high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD; Dionex™ ICS-5000, Thermo Fisher Scientific), equipped with a Dionex™ CarboPac™ PA1 column (2 ×250 mm; Thermo Fisher Scientific).

2.12 Enzyme assay

The methods to determine the activity of invertases and sucrose synthases were adapted from Aloni *et al.* (1991, 1996). Finely ground samples (around 100 mg fresh weight) were homogenized in 1.5 ml ice-cold extraction buffer (pH 7.2) containing: 25 mM HEPES (N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid), 5 mM $MgCl_2$, 0.5 mM Na_2EDTA (Ethylenediaminetetraacetic acid disodium salt), 2 mM DDT (DL-Dithiothreitol), 3 mM DIDCA (Diethyldithiocarbamic acid), 1% (w/v) PVP (Polyvinylpyrrolidone), and 0.1% (v/v) Triton X-100. The mixture was centrifuged at 21300 rcf for 20 min at 4 °C. The supernatant was collected; and the pellet was resuspended in 0.3 ml of the extraction buffer, after being washed twice with the same buffer. Bradford protein assay was performed with the supernatant for protein content quantification, with bovine serum albumin as standard solutions.

Aliquots of 50 µl of the supernatant or the suspended pellet were incubated in 500 µl 0.1 N phosphate citrate buffer (pH 5.0) with 20 mM sucrose, to determine the activity of soluble acid invertase or insoluble cell wall invertase respectively. Aliquots of 50 µl of the supernatant were incubated in 500 µl 0.1 N phosphate citrate buffer (pH 7.5) with 20 mM sucrose, to determine the activity of soluble neutral invertase. The activity of sucrose synthase was determined as sucrose breakdown where aliquots of 50 µl were incubated in 0.1 N phosphate citrate buffer (pH 7.0) with 200 mM sucrose and 5 mM UDP. The additional fructose produced at pH 7.0 in the presence of 5 mM UDP compared to reactions without UDP, was

attributed to sucrose synthase activity. Boiled enzymes were used as the blank for each reaction.

The incubation was carried out for 60 min at 37 °C and terminated by adding 500 µl dinitrosalicylic acid reagent. After boiling for 15 min, 200 µl 40% (w/v) Rochelle salt (dissolved in Milli-Q water) was added. After cooling, the resulting reducing sugars were determined colorimetrically at 540 nm. The samples for cell wall acid invertase were centrifuged at 21300 rcf shortly before the colorimetric determination.

2.13 Statistical analysis

Data that had been assessed on several plants per plot were first averaged to give one value per plot. The number of plots for each treatment is the number of replicates (n). One way ANOVA was carried out on the data of the vegetative growth experiment (n=2) and the NAA experiment (n=2). ANOVA with split plot design was carried out for the decapitation experiment (n=3), the NPA experiment (n=2), the yucasin experiment (n=4), and the data for sugar determination and enzyme assay (n=4). In all analyses, the critical level of significance was α=0.1 instead of the common α=0.05 motivated by the limited degrees of freedom for the residual. Homogeneity of variances was assumed and could not be tested because of the low number of replicates. Fisher's unprotected least significant difference (LSD) test at P=0.1 was used for mean separation: unprotected since mean separation for interaction averages was also conducted when the F-test showed no significant interaction effect.

3 Results

3.1 FR enhances dominance of the larger apical shoots in pepper

Pepper plants follow a dichotomous branching pattern. Between two apical shoots derived from the same apex, one usually is slightly larger than the other one, which suggests that one apical shoot has dominance over the other. To study if FR-stimulated fruit abortion is mediated by enhanced dominance of the larger apical shoot, we first determined whether this dominance was enhanced by FR. Six weeks old plants were grown under white light without far-red ('-FR') or supplemented with FR ('+FR'). Plants were only allowed to have vegetative growth by removing all flower buds.

After 23 days of cultivation, additional FR led to an increasing difference in dry weight and length between the two twin shoots starting from the third layer of internodes (internode 3) (Fig. 1B, C; Supplementary Fig. S6). Additional FR had less

influence on the unbalanced growth of the first and second layer of internodes (internode 1 and 2), which was probably related to the developmental stages of internodes when light treatments started (Supplementary Fig. S7). A significantly higher level of auxin was observed for the apices of the larger shoots compared to the smaller shoots when grown without additional FR, and this difference was enhanced by additional FR (Fig. 1D). Additional FR also completely inhibited the outgrowth of lateral shoots during the experimental period, which appeared mostly below the first splitting node (Fig. 1E; Supplementary Fig. S6). This effect of FR was also found in our other experiments (Supplementary Fig. S8). All these findings suggest that additional FR enhanced the dominance of the larger apical shoots over the smaller apical shoots and over lateral shoots.

3.2 Apical shoots mediate FR-stimulated fruit abortion

To test our hypothesis that an increase in dominance of the larger apical shoots was responsible for the FR-stimulated fruit abortion, plants were cultivated with or without additional FR, while these plants were pruned to keep 4 main shoots potentially carrying 8 flowers (Fig. 2A). Additional FR was added 11 days before the first anthesis. Half of the plants in both light conditions were decapitated above the last formed flowers (marked yellow) when these reached anthesis (Fig. 2A).

Additional FR enhanced the growth of apical shoots, shown as an increased plant height (Supplementary Fig. S9), and a higher dry mass accumulation and partitioning to stems (Supplementary Fig. S10). The effect of FR on stem elongation and dry mass partitioning to the stem was reduced when the plants were decapitated. In addition, we noticed that flowers and young fruits under additional FR had substantially longer pedicels in both intact and decapitated plants (Supplementary Fig. S11). Pedicel length might influence the rate of assimilates import as a longer pedicel requires a longer transport path. However, we did not find a relation between pedicel length and fruit abortion.

Additional FR stimulated fruit abortion in non-decapitated plants. Interestingly, decapitation increased the number of fruits per plant under both light conditions which resulted in no influence of FR on fruit abortion in decapitated plants (Fig. 2B). This implies that the presence of the apical shoot has a stimulating effect on fruit abortion, and suggests that the apical shoot mediates the FR-stimulated fruit abortion in pepper.

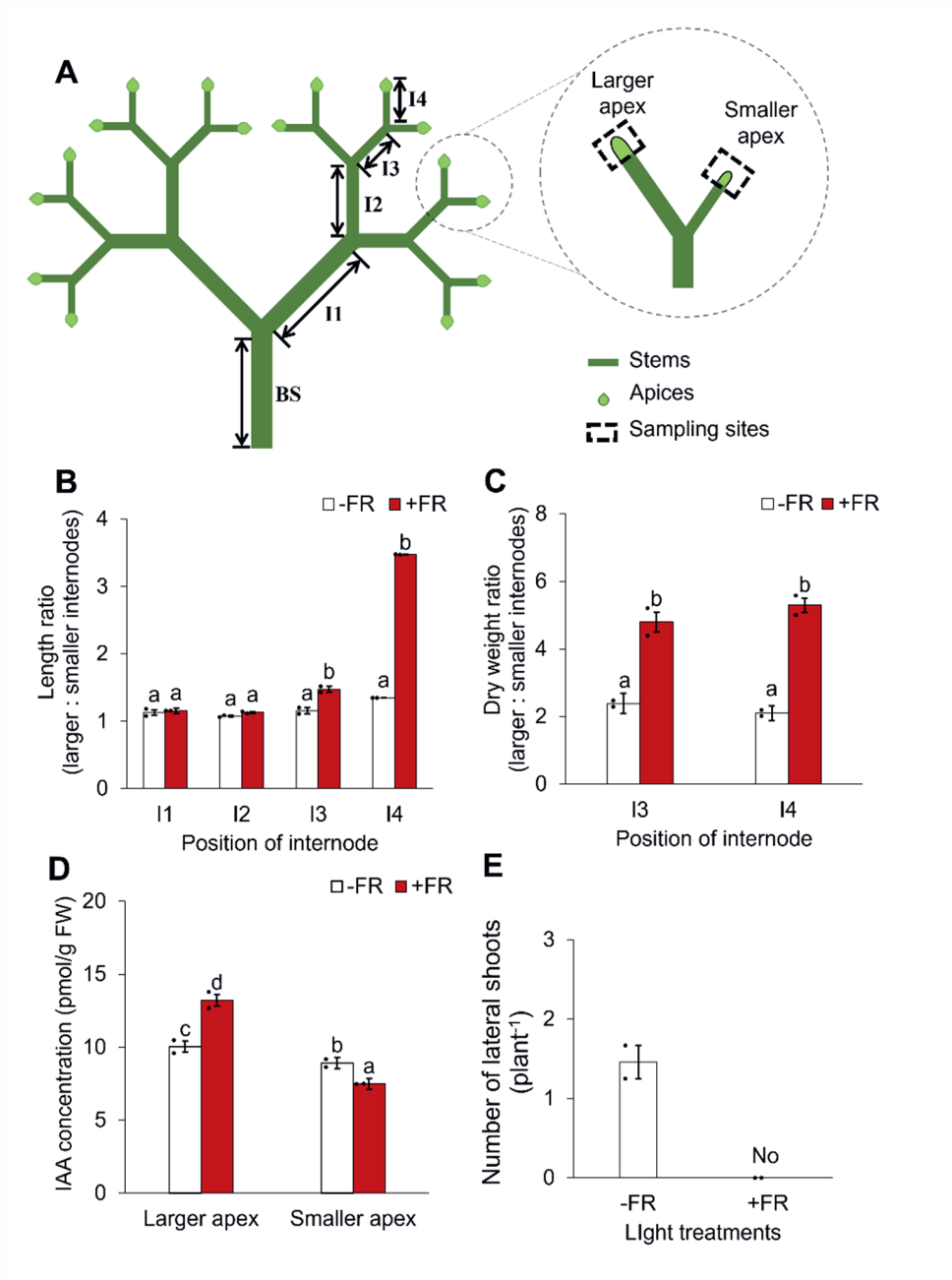

Figure 1. FR stimulates dominance from apical shoots in pepper plants. (A) Plant shoot architecture during the vegetative growth experiment. All flowers were removed. Leaves are not shown. BS=bottom stem, the stem before splitting; I1-I3=internode 1 to 3; I4=internode 4 and the apex above. Plants were cultivated with or without far-red (FR). (B)(C) Length ratio and dry weight ratio of the two internodes connected to the same node below, which was calculated by using the value of the

larger shoot divided by the smaller one. (D) Levels of IAA (indole-3-acetic acid)in 1 cm of apices from larger and smaller shoots respectively. FW stands for fresh weight. (E) Number of lateral shoots with a stem longer than 0.2 cm and a leaf longer than 1.5 cm. 'No' indicates no lateral shoots in +FR treatment. Mean values were derived from 2 statistical replicates, each based on 6 plants. Black dots indicate individual data of each statistical replicate. One-way ANOVA was performed for each internode in (B) and (C). Two-way ANOVA was performed for (D). Error bars in (B) to (E) indicate ±standard error of mean based on the common variance. Different lowercase letters indicate significant differences between treatments according to Fisher's unprotected LSD test at P=0.10.

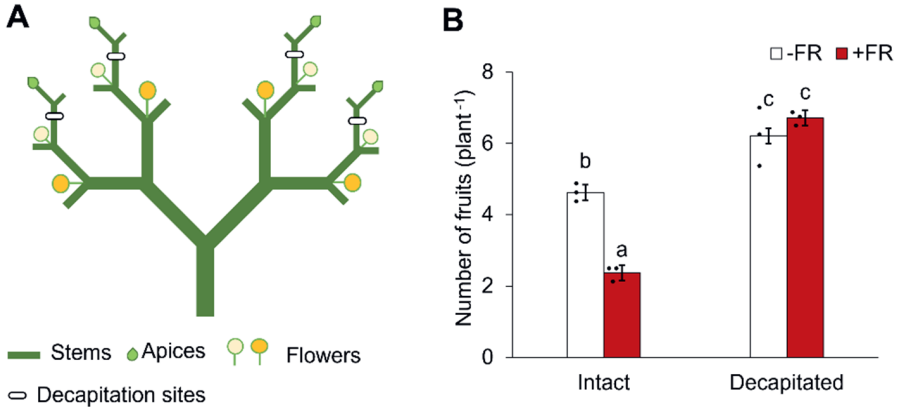

Figure 2. FR has no influence on fruit abortion in decapitated pepper plants. (A) Plant shoot architecture during decapitation experiment. Plants were cultivated with or without far-red (FR). Plants were pruned to have 4 main shoots carrying 8 flowers. Leaves are not shown. Decapitation was performed when the upper layer of flowers reached anthesis. (B) Number of fruits per plant on day 44 since light treatments. Mean values were derived from 3 statistical replicates, each based on 8 plants. Black dots indicate individual data of each statistical replicate. ANOVA based on split-plot design was performed. Error bars indicate ±standard error of means based on the common variance. Different lowercase letters indicate significant differences between treatment means according to Fisher's unprotected LSD test at P=0.10.

3.3 Auxin transport from the apical shoot is not responsible for the FR-stimulated fruit abortion

Basipetal auxin transport is considered as a core component of dominance from apical shoots. We, therefore, tested reducing basipetal auxin transport from the apex with the auxin transport inhibitor NPA (1-naphthylphthalamic acid). Application of NPA on the main stems above the highest flowers did not affect the FR-stimulated fruit abortion compared to the control groups (Fig. 3B).

To determine the effectiveness of NPA application, we sampled the apices and stem segments above (stem A) and below (stem B) the NPA application site (Fig. 3A). Additional FR generally increased auxin levels in these tissues, and NPA application increased the auxin levels in the apex and stem above the application

site (stem A). In the stem below NPA application site (stem B), NPA application resulted in a substantial drop in the auxin level under both light conditions (Fig. 3C). This result indicates that NPA had the desired effect of reducing basipetal auxin transport from the apex to the flowers, and that basipetal auxin transport does not mediate the stimulating effect of the apical shoot on fruit abortion.

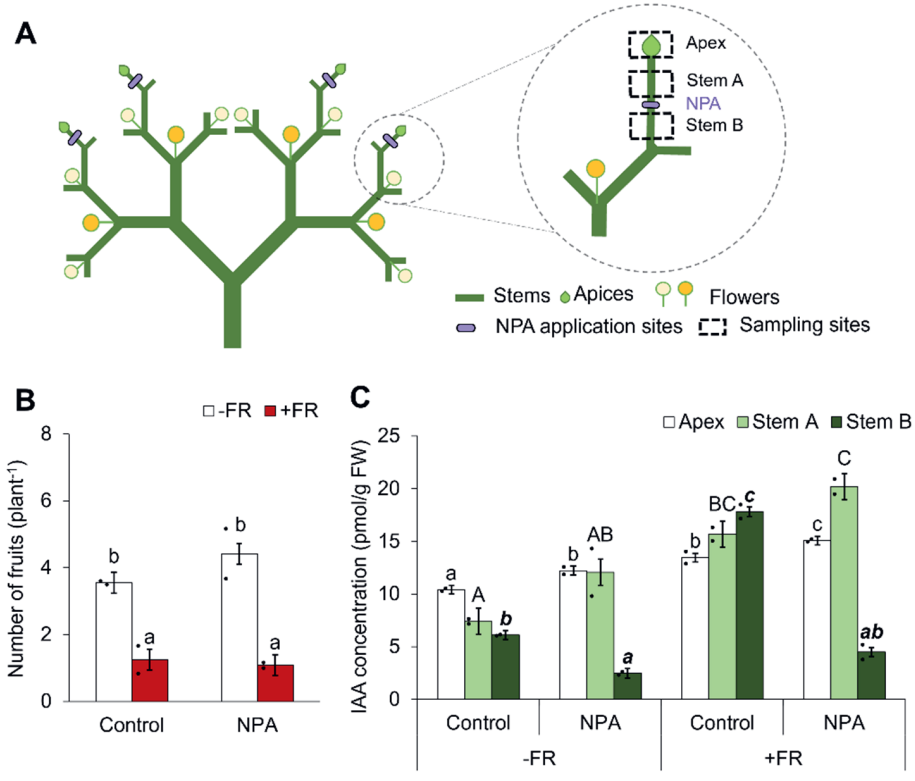

Figure 3. NPA application reduces auxin basipetal transport but does not influence fruit abortion. (A) Plant shoot architecture in the NPA experiment. Plants were pruned to have 4 main shoots carrying 12 flowers. Leaves are not shown. Plants were cultivated with or without far-red (FR). NPA (N-1-naphthylphthalamic acid, 5mg/g) was applied in lanolin paste as a ring around the stems when the upper layer of flowers reached anthesis, while sole lanolin was applied to the control group. 1 cm of apices; 1 cm of stem above the NPA application site (stem A); 1 cm of stem below the NPA application site (stem B) were collected for auxin determination. (B) Number of fruits per plant on day 52 since light treatments. (C) Levels of IAA (indole-3-acetic acid) in apex, stem A, and stem B. FW stands for fresh weight. Mean values were derived from 2 statistical replicates, each based on 6 plants. Black dots indicate individual data of each statistical replicate. ANOVA based on split-plot design was performed for (B)(C). Error bars indicate ±standard error of means based on the common variance. Different letters for the same tissue indicate significant differences between treatment means according to Fisher's unprotected LSD test at P=0.10. In (C), small letters are for apex, capital letters are for stem A, and small letters in bold and Italics are for stem B.

3.4 Synthetic auxin NAA application does not promote fruit abortion

To investigate if NAA can replace the role of apical shoots to promote fruit abortion in pepper, we applied the synthetic auxin 1-naphthylacetic acid (NAA) to the decapitated apices. In this experiment, we had four treatments: intact plants cultivated with or without additional FR; or decapitated plants cultivated under additional FR with or without NAA applied to the decapitation site. The fruit number on non-decapitated plants was very low: despite the consistent trends with previous experiments that additional FR reduced fruit number, the effect was not statistically significant (Fig. 4B), which might be due to the variations among starting materials from their seedling stages in greenhouses. Decapitation significantly reduced fruit abortion, while adding NAA on decapitated stems did not promote fruit abortion (Fig. 4B). Endogenous IAA was quantified in all treatments including the one with exogenous application of NAA, considering that NAA may influence IAA level via a potential effect on local IAA biosynthesis. Additional FR significantly elevated IAA levels in the apex, lower node, and topmost mature leaves compared to no additional FR (Fig. 4C). Decapitation reduced IAA levels in both lower and higher nodes, and decapitation plus NAA application caused an even stronger reduction, suggesting that exogenous NAA application did affect endogenous IAA level (Fig. 4C). Furthermore, we noticed fewer lateral shoots in the NAA treated plants (Supplementary Fig. S8) indicating that the NAA application was effective in partially restoring apical dominance with respect to shoot branching.

3.5 Auxin biosynthesis inhibitor yucasin applied on shoot apices reduces fruit abortion independent of FR

To test whether the increased auxin concentration in the shoot apex under additional FR is causing the observed dominance, the apices were treated with the auxin biosynthesis inhibitor yucasin (5-(4-Chlorophenyl)-2,4-dihydro-[1,2,4]-triazole-3-thione). Yucasin inhibits the activity of the YUCCA proteins, which are enzymes in a rate-limiting step of auxin biosynthesis (Nishimura *et al.*, 2014). Spraying plant apices with yucasin resulted in an increase in fruit number, i.e., less fruit abortion in both plants grown with or without additional FR (Fig. 5B). Especially under additional FR, yucasin application reduced IAA levels in all tissues except for the topmost mature leaf (Fig. 5D). Furthermore, we noticed a substantial reduction in plant height of 4.4 cm or 6.6 cm by yucasin treatment when grown with or without additional FR, respectively (Fig. 5C). This indicates that the reduced fruit abortion by inhibiting auxin biosynthesis in apices corresponded with a suppressed shoot growth.

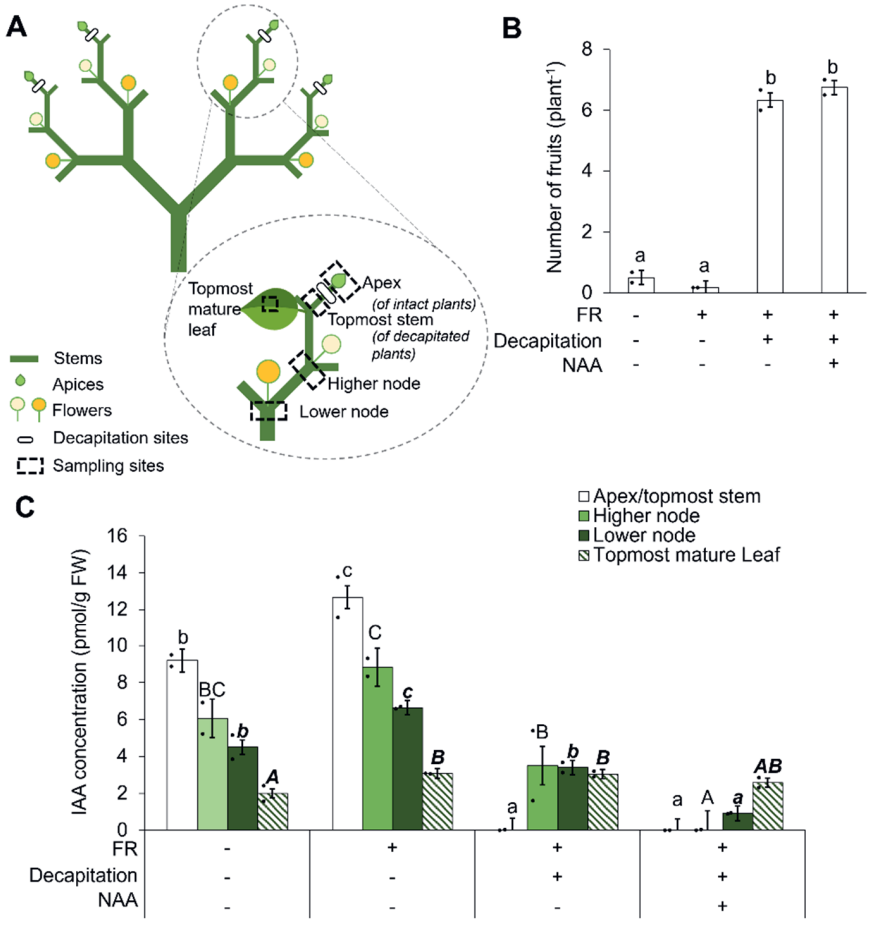

Figure 4. NAA application to decapitated shoots does not promote fruit abortion. (A) Plant shoot architecture in NAA experiment. Plants were pruned to have 4 main shoots carrying 8 flowers. Plants were cultivated with or without far-red (FR). NAA (1-Naphthaleneacetic acid, 1% w/w) was applied in lanolin paste on the cutting surface after decapitation, while sole lanolin was applied to the control group. Decapitation and NAA application was performed on the same day when the upper layer of flowers reached anthesis. Afterwards, NAA was renewed every week for 2 more weeks. Topmost tissues of each plant (either 1 cm of apices in intact plants, or 1 cm of topmost stem segment in decapitated plants); 1 cm of nodes where the higher and the lower layer flowers were attached to (higher node & lower node); leaf discs from the topmost mature leaves were collected for auxin determination. (B) Number of fruits per plant on day 53 since light treatments. (C) Levels of IAA (indole-3-acetic acid) in and plant apex/topmost stem, lower node, higher node and leaf. FW stands for fresh weight. Mean values were derived from 2 statistical replicates, each based on 6 plants. Black dots indicate individual data of each statistical replicate. One-way ANOVA was performed for (B) and (C) for each tissue type. Error bars indicate ±standard error of means based on the common variance. Different letters for the same tissue indicate significant differences between treatment means according to Fisher's unprotected LSD test at P=0.10. In (C), small letters are for apex/topmost stem, capital letters are for higher node, small letters in bold and Italics are for lower node, and capital letters in bold and Italics are for topmost mature leaf.

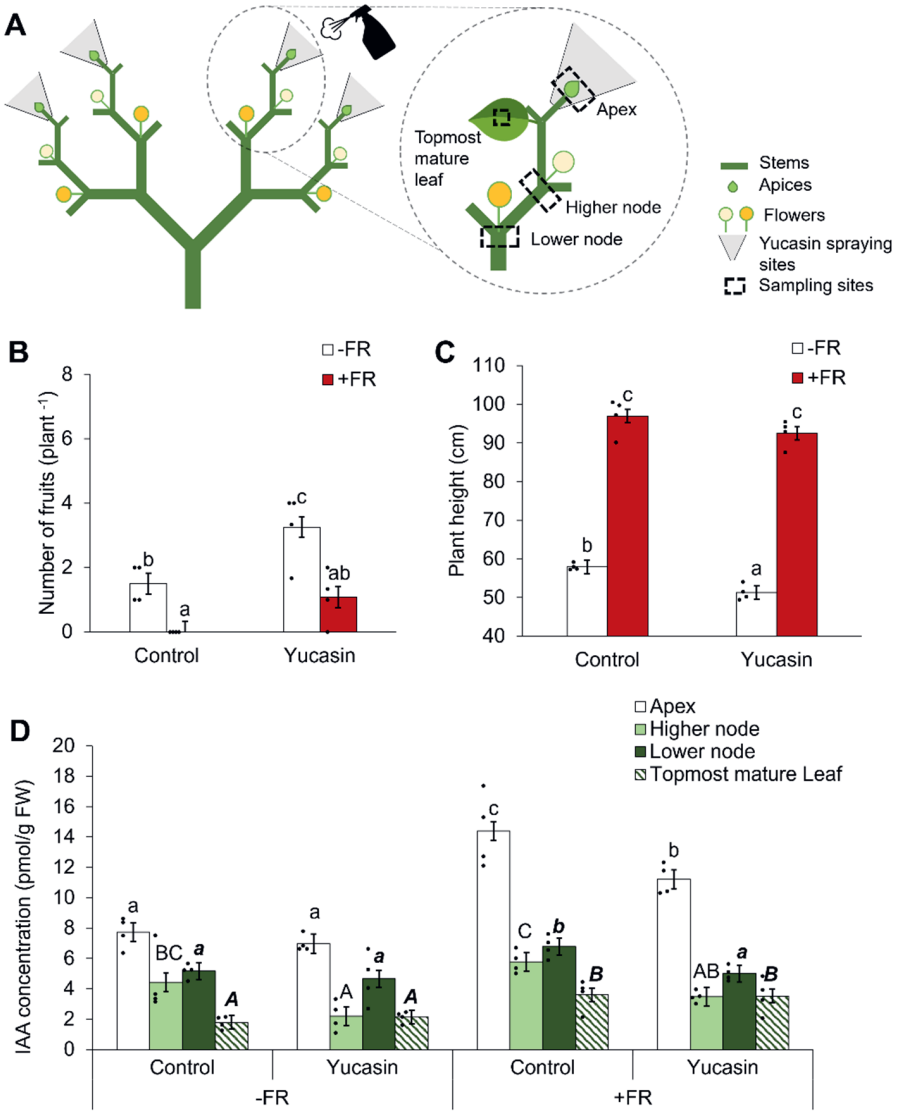

Figure 5. Inhibition of auxin biosynthesis in shoot apices reduces fruit abortion independent of FR. (A) Plant shoot architecture upon treatment with the auxin biosynthesis inhibitor yucasin. Plants were cultivated with or without far-red (FR). Plants were pruned to have 4 main shoots carrying 8 flowers. Yucasin solution (50mM for 1st application, and 25mM for 2nd - 4th application) was sprayed on the apical shoots every week since the anthesis of lower layer of flowers. The control group was sprayed with the solvent. One week after the last application, 1 cm of apices; 1 cm of nodes where the higher and the lower layer flowers were attached to (higher node & lower node); leaf discs from the topmost mature leaves were collected for auxin determination. (B) Number of fruits per plant on day 60 since light treatments. (C) Plant height on day 60 since light treatments. (D) Levels of IAA (indole-3-acetic acid) in apex, higher node, lower node, and leaf. FW stands for fresh weight. Mean values were

derived from 4 statistical replicates, each based on 3 plants. Black dots indicate individual data of each statistical replicate. ANOVA based on split-plot design was performed for (B)-(D). Error bars indicate ±standard error of means based on the common variance. Different letters for the same tissue indicate significant differences between treatment means according to Fisher's unprotected LSD test at P=0.10. In (D), small letters are for apex, capital letters are for higher node, small letters in bold and Italics are for lower node, and capital letters in bold and Italics are for topmost mature leaf.

3.6 FR causes a lower sucrose content and lower sucrose cleavage enzyme activities in flowers

From the above experiments, we realized that, even though additional FR did increase IAA levels in the shoot apex and inhibition of auxin biosynthesis in the shoot apex reduced fruit abortion, the basipetal auxin transport was unlikely to be the direct reason for enhanced flower and fruit abortion under additional FR. This brought us to investigate if the competition for assimilates between apex and flowers could be an explanation for the FR-stimulated fruit abortion. We hypothesized that the shoot apex may demand more assimilates under additional FR – corresponding with a higher auxin level – and may cause carbohydrate shortage in flowers and nearby tissues.

Plants grown in the same light conditions as in the yucasin experiment were used for tissue sampling for carbohydrates (Fig. 6A) and hormonal analysis (Supplementary Fig. S4). Additional FR increased the total soluble sugar and non-structural carbohydrates to different extents in different tissues (Supplementary Fig. S12). In the sampled vegetative tissues, additional FR generally elevated the levels of all sugars: glucose, fructose, sucrose, and starch (Fig. 6B), which indicates that there was unlikely a sugar shortage in the tissues surrounding the flowers. In flowers, the concentration of glucose, fructose, sucrose, and starch showed a peak at anthesis compared to 7 days before and 7 days after anthesis. Additional FR led to a slight elevation of this peak for glucose, fructose, and starch, but to a substantial reduction of sucrose (Fig. 6B). In addition, we noticed that flowers on day 7 after anthesis were generally smaller when grown under additional FR (Supplementary Fig. S13), which suggests a suppression on fruit growth by additional FR.

We examined the enzyme activity of multiple groups of invertases and sucrose synthases, which are responsible for sucrose cleavage and linked closely to sink strength (Wang *et al.*, 1993; Zrenner *et al.*, 1995; Morey *et al.*, 2018). In flowers, additional FR led to a significantly lower level of cell wall invertases (CWI) at anthesis, and a lower acid invertase (AI) activity at, and after anthesis (Fig. 7). In shoot apices, the CWI activity was much lower than in flowers, and additional FR led to a reduction before anthesis. The AI activity in the apex was reduced by FR before anthesis but slightly increased after anthesis. Additional FR led to much

less reduction of CWI and AI activity in the apex than in flowers. The effect of additional FR on sucrose synthase (Susy) was very small in both tissues. Neutral invertases (NI) showed very low activity in both tissues, and FR did not have a significant effect on their activity except causing a slight reduction in apex before anthesis (Fig. 7). Moreover, additional FR reduced the protein content in flowers but increased the protein content in apex after anthesis (Supplementary Fig. S14).

Hormone analysis revealed that additional FR resulted in substantially lower IAA, and higher salicylic acid (SA) levels in flowers on day 7 after anthesis (Supplementary Fig. S4). Additional FR also led to a higher ethylene emission rate in flowers on day 1-3 after anthesis, but not anymore on day 4-7 after anthesis (Supplementary Fig. S5). Abscisic acid (ABA) and the ethylene precursor ACC can promote abscission. However, additional FR did not influence ACC levels in flowers at all three flower stages; and only lowered ABA level in flowers before anthesis, but not anymore from anthesis onwards (Supplementary Fig. S4).

Furthermore, we also examined the pollen quality of pepper flowers, as pollen may be susceptible to stress. The pollen quality can influence fertilization and therefore seed development. The number of seeds could influence the auxin export and the fruit's sink strength in the competition for assimilates (Marcelis & Baan Hofman-Eijer, 1997; Wubs *et al.*, 2009). The number of pollens per flower, the fraction of viable pollen, as well as the average seed number per fruit, were all not or hardly influenced by additional FR (Supplementary Fig. S3 & S15). Thus, compromised pollination or fertilization is unlikely to explain FR-stimulated abortion.

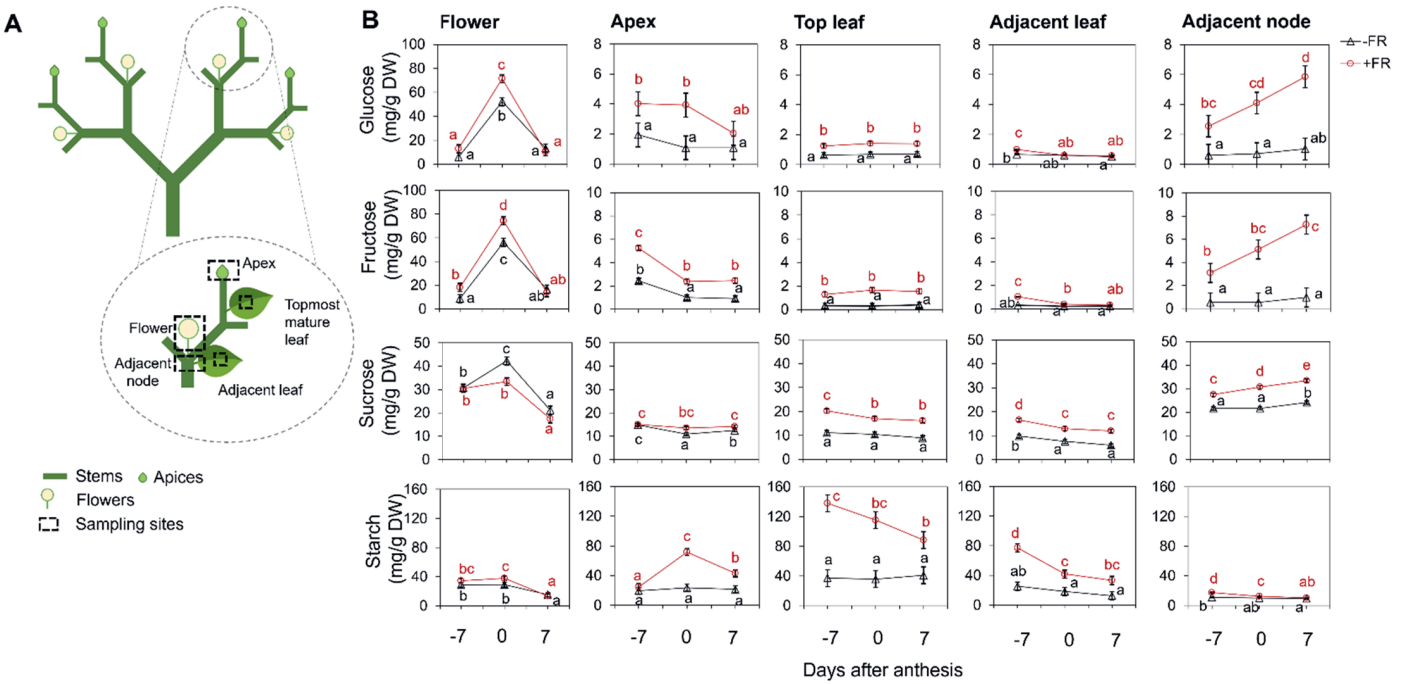

Figure 6. Additional FR leads to higher sugar content except for sucrose content in flowers. (A) Plant shoot structures when sampling tissues for sugar and hormone measurements. Plants were pruned to have 4 main shoots, each carrying 1 flower. Plants were cultivated with or without far-red (FR). Sampled tissues are flowers (including pedicels); 1 cm of apex; 1 cm of node where the flowers are attached to, and leaf discs from the topmost mature leaf and the leaf adjacent to the flowers. Samples were collected within the last hour of light period. (B) The glucose, fructose, sucrose and starch levels in the indicated tissues sampled on day 7 before anthesis, at anthesis, and on day 7 after anthesis. DW stands for dry weight. Means values were derived from 4 statistical replicates, each based on 2 plants. Split-plot ANOVA was performed on all parameters. Error bars indicate ±standard error of means based on the common variance. Different lowercase letters indicate significant differences between treatment means according to Fisher's unprotected LSD test at P=0.10.

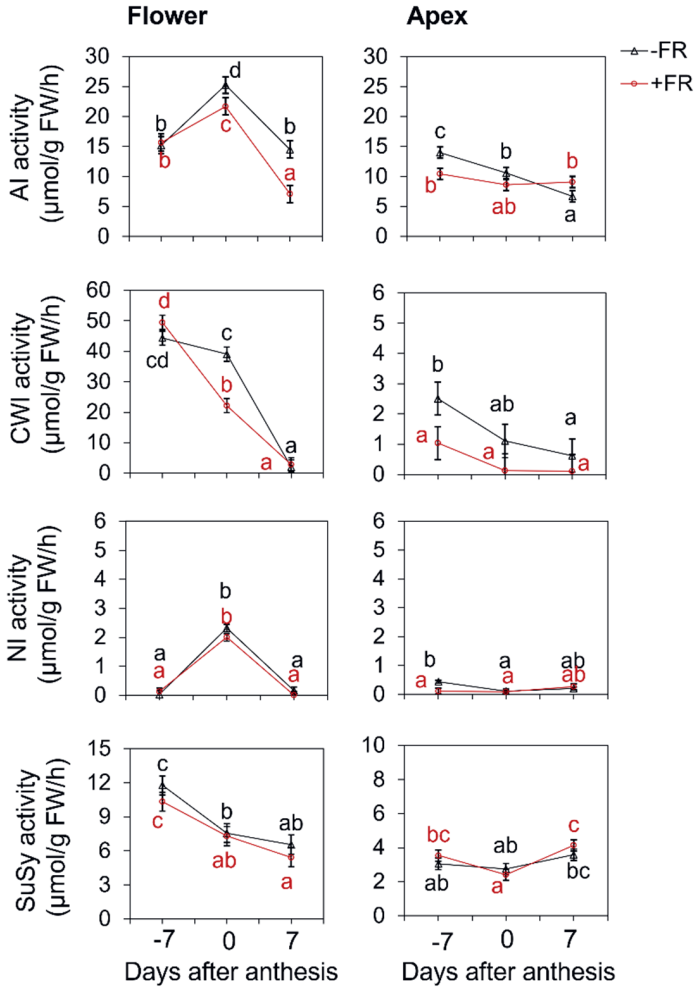

Figure 7. Effect of additional FR on the activity of sucrose cleavage enzymes. Plants were cultivated with or without far-red (FR). The activity of soluble acid invertase (AI), cell wall invertase (CWI), neutral invertases (NI) and sucrose synthase (SuSy) in flowers and apex on day 7 before anthesis, at anthesis, and on day 7 after anthesis. FW stands for fresh weight. Samples were collected within the last hour of light period. Mean values were derived from 4 statistical replicates, each based on 2 plants. Split-plot ANOVA was performed on all parameters. Error bars indicate ±standard error of means based on the common variance. Different lowercase letters indicate significant differences between treatment means according to Fisher's unprotected LSD test at P=0.10.

4 Discussion

4.1 FR causes a stronger apical dominance in pepper

FR, as an important component of solar radiation, triggers a wide range of plant responses and has a critical influence on plant developmental processes. In our previous study, we showed a negative effect of FR on fruit set in sweet pepper (*Capsicum annuum* L.) (Chapter 2). Here we investigated whether this negative effect is related to an increased apical dominance under additional FR. As a common form of correlative inhibition, where one part of the plant suppresses the growth and development of another part, apical dominance has an important role in regulating plant growth in resource-limited environments. Our study shows that apical dominance plays a role in the suppressing effect of additional FR on fruit set, i.e., stimulating fruit abortion by additional FR.

First, we showed that additional FR promotes the dominance of the larger dichotomous apical shoots (dominant) over the smaller apical shoots (subordinate) in pepper, resulting in an increased ratio between the length of dominant and subordinate shoots. This enhanced dominance under additional FR also reduced the number of lateral shoots to almost zero (Fig. 1E; Supplementary Fig. S8). The promoted growth of dominant shoots was accompanied by a higher level of auxin in their apices compared to subordinate shoots (Fig. 1D). This is in line with the apical dominance effect in a dwarf pea (*Pisum sativum* L.) system with two cotyledonary shoots: IAA transport from the subordinate shoot was considerably reduced compared to that from the dominant shoot (Morris, 1977). Thus, we consider the dominance from shoot apices over other organs, including subordinate shoots, lateral shoots and reproductive organs, as apical dominance (a form of correlative inhibition from shoot apices, as discussed in reviews: Beveridge *et al.*, 2023; Kotov *et al.*, 2021; Walker & Bennett, 2018). Based on this, we suggest that the enhanced unbalanced growth between two dichotomous shoots of a plant (Fig. 1B, C) is a suitable morphological indicator for the stronger apical dominance for dichotomous branching species.

Decapitation (removal of all shoot apices) reduced fruit abortion in sweet pepper, which is in line with findings in other species (Bangerth, 1989; Bangerth, 2000). Additional FR did not influence fruit abortion in decapitated plants (Fig. 2), suggesting shoot apices are the key mediators of the FR effect on fruit abortion. The removal of shoot apices not only removes the basipetal auxin transport from these apices, but also removes the strong sinks that are competing for photosynthetic assimilates. Here, we attempt to investigate if the competition for the basipetal auxin stream or assimilates explains the FR-stimulated fruit abortion.

4.2 Basipetal auxin stream is not the reason for FR-stimulated fruit abortion

Polar cell-to-cell auxin transport is considered an important factor in apical dominance. According to the canalization model (Bangerth, 1989; Prusinkiewicz *et al.*, 2009), actively growing shoots under additional FR can inhibit the export of auxin from flowers by saturating the auxin transport capacity of the main stem. We found that additional FR elevated the auxin levels in apices, and in the stems or nodes above flowers. This might cause a stronger basipetal auxin stream, which has the potential to inhibit the export of auxin from young flowers and fruits, stimulating abortion. However, reducing basipetal auxin transport from shoot apices did not reduce fruit abortion (Fig. 3), nor did auxin application on decapitated shoots promote fruit abortion (Fig. 4), suggesting that competition for the basipetal auxin stream is not involved in the FR-stimulated fruit abortion.

Auxin transport inhibitor NPA was reported to relieve the apical dominance, while synthetic auxin NAA can restore the apical dominance over the outgrowth of lateral buds in pea and *Arabidopsis* (Li *et al.*, 1995; Chatfield *et al.*, 2000; Nakajima *et al.*, 2001). However, NPA and NAA did not exert such effects on fruit abortion. NAA application on decapitated shoots did have an inhibitory effect on the outgrowth of lateral shoots and endogenous IAA level, where the combined effect of exogenous NAA and endogenous IAA would require further investigation, e.g. at the transcriptional level. Even though NPA caused an auxin depletion in the stem, it did not stimulate lateral bud growth (Morris *et al.*, 2005, and current study) or fruit set (current study) as decapitation did. We thus suggest that the decapitation may promote fruit set and outgrowth of lateral shoots via an auxin-independent mechanism in pepper plants.

4.3 Assimilate competition could be the main reason for FR-stimulated fruit abortion

The intense demand for sugars of shoot tips has recently attracted attention as an important component of apical dominance (Barbier *et al.*, 2015; Schneider *et al.*, 2019; Kotov *et al.*, 2021). This is probably because the auxin depletion after decapitation happened much later than the initial bud outgrowth, while the changes in sucrose are rather rapid and can correspond better with the timing of early stages of bud outgrowth (Morris *et al.*, 2005; Mason *et al.*, 2014). Axillary bud outgrowth can be promoted by sugars in different plant species (Mason *et al.*, 2014; Barbier *et al.*, 2015; Salam *et al.*, 2021; Xia *et al.*, 2021).

We noticed that promoted fruit set in decapitated plants or yucasin treated plants was accompanied by no or less shoot growth (in terms of plant height), compared to intact plants or plants not treated with yucasin respectively. Moreover, additional FR increased the dry matter partitioning to the stem at the expense of

the leaves, in agreement with previous reports (Chapter 2; Kalaitzoglou *et al.*, 2019; Ji *et al.*, 2019). Additional FR elevated the auxin levels in apices, suggesting a higher demand of apices for assimilates, as high auxin level is positively linked to high assimilate import (Bangerth, 1989; Morris & Arthur, 1987; Patrick & Steains, 1987). Similarly, plant apices probably had a lower demand for assimilates during the attempted inhibition of auxin biosynthesis at the plant apices, which can explain the reduced fruit abortion with or without additional FR. Therefore, the higher demand of plant apices for assimilates seems a more likely reason for the FR-stimulated fruit abortion.

We hypothesize that additional FR would lead to a re-distribution of carbohydrates within the plant. Additional FR led to an elevation of soluble sugars and starch in most tested vegetative organs, including the leaf and stem adjacent to the flowers (Fig. 6), which is in agreement with the findings in tomato (*Solanum lycopersicum* L.), lettuce (*Lactuca sativa* L.), and tobacco (*Nicotiana tabacum* L.) (Courbier *et al.*, 2020; Zou *et al.*, 2023; Kasperbauer *et al.*, 1970). This means an assimilate shortage nearby the flowers is unlikely. However, it remains doubtful how much of the available assimilates were transported into the flowers.

The sucrose accumulation in flowers and fruits reflects the balance between sucrose import and consumption. Sucrose can be stored directly or turned into hexoses through cleavage, serving the synthesis of cell wall constituents, proteins, lipids, fructans and starch (Patrick *et al.*, 2013). FR led to a higher level of glucose, fructose, and starch in flowers at anthesis, but a substantially lower sucrose level (Fig. 6). This suggests that the rate of sucrose import is probably slower than sucrose cleavage. Under additional FR, the higher level of hexoses in flowers could result from a slower metabolism where less hexoses were used for building up the structural biomass, which corresponded to the smaller fruits on day 7 after anthesis (Supplementary Fig. S13).

Sucrose, as the main mobile photosynthetic product, can determine the fate of flowers (Aloni *et al.*, 1997), either as an energy source, or as a signal related to its level, its flux, or its ratio with other sugars (Eveland & Jackson, 2012). Ruan *et al.* (2012) suggested a model that phloem-imported sucrose serves as a primary signal sensed by invertases that generates glucose to repress the programmed cell death and to promote cell division of fruit tissues, thereby allowing seed and fruit set to proceed. Feeding sucrose can reduce the abortion rate, which is probably related to the enhanced activity of sucrose synthase and invertases in pepper flowers (Aloni *et al.*, 1997). Sucrose synthases and invertases (especially cell wall invertases in pedicels and soluble invertases in nucellar tissues) are two important sucrose cleavage enzymes to regulate sucrose import (Wang *et al.*, 1993; Li *et al.*, 2012), which will further influence carbon partitioning, cell differentiation and

development (Sturm & Tang, 1999). Cell wall invertases seem to have a more important role than sucrose synthase in unloading sucrose at the early stages of tomato fruit set and development (Li *et al.*, 2012; Liu *et al.*, 2016). This may explain the relatively low activity of sucrose synthase in our samples, which was not influenced by additional FR. Under additional FR, the lower activity of soluble acid invertases and cell wall invertases was associated well with the lower sucrose accumulation in flowers.

In terms of the activity of sucrose cleavage enzymes, the impact of additional FR on apices was much less compared to flowers. Similarly, heat stress or the presence of a competitor fruit had a much smaller impact on the acid invertase activity in young leaves of pepper than that in pepper flowers (Aloni *et al.*, 1991). The minimum light intensity for a positive daily sugar accumulation in the sink leaves was much lower than in flowers (Aloni *et al.*, 1996), suggesting that flowers could be more susceptible to competition for assimilates than vegetative sink organs.

The flower and fruit abortion in pepper is controlled by the balance between auxin and ethylene (Taylor & Whitelaw, 2001). The alteration of hormonal status (auxin and ethylene) by additional FR happened after anthesis, which seems later than the observed changes in sucrose content. Under additional FR, flowers at 1-3 days after anthesis had a higher ethylene emission than without FR, which could be a downstream response to the low sucrose accumulation. Under additional FR, the lower IAA level in flowers on day 7 after anthesis may further lead to a higher sensitivity to ethylene at the flower abscission zone (Taylor & Whitelaw, 2001). Taken together, we propose that the suggested lower sucrose import to flowers due to a potentially stronger competition for assimilates under additional FR, may serve as the initial trigger for the downstream responses in hormonal status, resulting in a higher flower and fruit abortion.

5 Conclusions

Additional far-red (FR) enhanced apical dominance stimulates fruit abortion in sweet pepper plants. The FR-stimulated flower and fruit abortion is not mediated by the enhanced competition for basipetal auxin stream, but we speculate it to be due to enhanced competition for assimilates between shoot apices and flowers, which still requires further investigation. Under additional FR, the lower sucrose accumulation along with lower invertase activities in flowers could serve as a signal to trigger alterations in hormones and cause flower and fruit abortion (abscission).

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Supplementary information

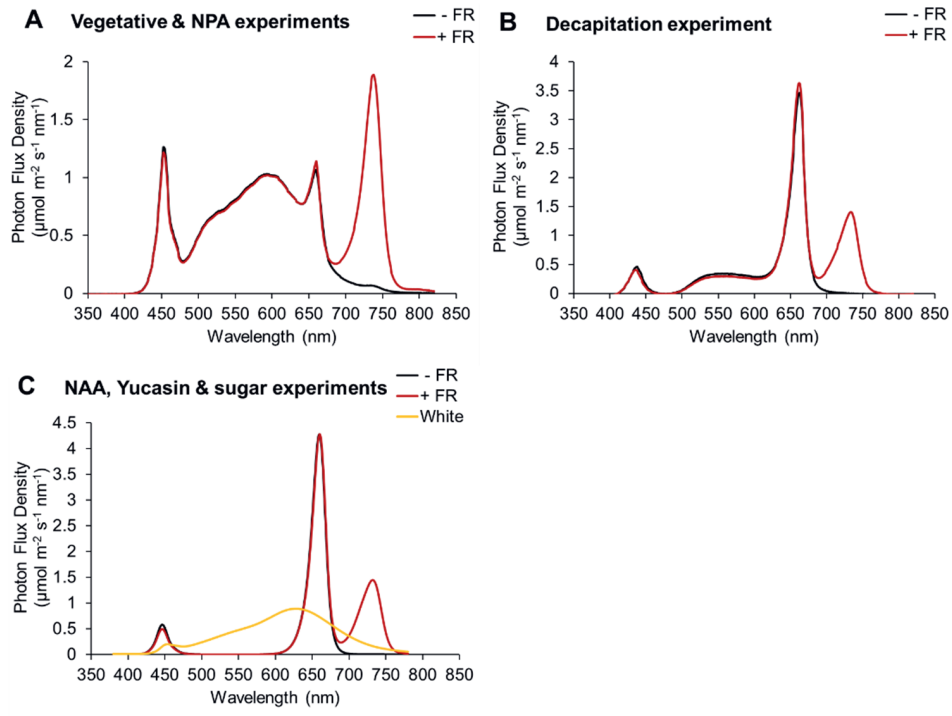

Figure S1. Light spectra used in various experiments. (A) Vegetative growth experiment and NPA experiment (NPA: N-1-naphthylphthalamic acid): PAR light was provided by Fluence VYPR 2P, far-red (FR) was provided by Philips GreenPower production modules; (B) decapitation experiment: Philips GreenPower deep red/white modules and FR modules; (C) NAA (1-Naphthaleneacetic acid), yucasin and sugar experiment: both treatment lights '-FR','+FR', and acclimation light 'White' were provided by OSRAM PHYTOFY® RL, which spectrum can be customized. The white light had an intensity of 130.4 ± 2.3 µmol m^{-2} s^{-1} with a 14-hour photoperiod. The intensity and photoperiod of other light conditions can be found in Table 1.

Figure S2. Three developmental stages of flowers collected for carbohydrate and hormone analysis. (A) At around 7 days before anthesis; (B) at anthesis when petals open at the same day; (C) 7 days after anthesis.

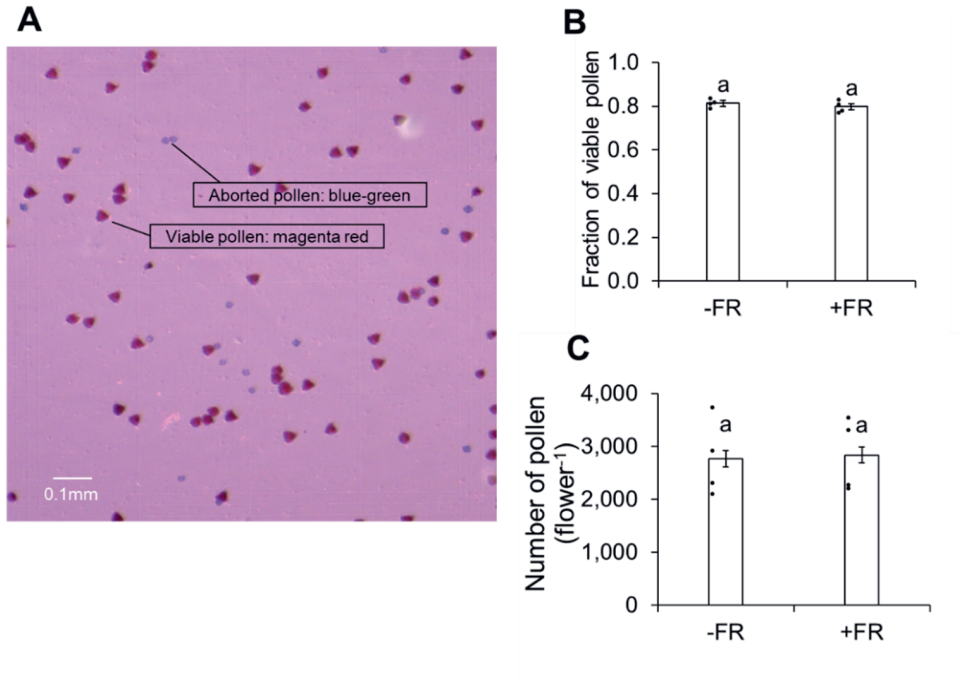

Figure S3. Effect of additional far-red (FR) on pollen number and pollen viability of sweet pepper flowers. Anthers from each flower were first fixed in Carnoy's fixative. After anthers being dried and stained with Alexander's dye, the pollens were released by ultrasonic bath, and further observed with a Fuchs-Rosenthal hemocytometer (4 x 4 x 0.2 mm grid, 3.2 mm^3) under a stereoscope (LEICA MZ APO). Observation of each flower included 2 technical replicates with 6 views each. (A) Example of a view under the stereoscope after pollen staining, where the viable pollens have magenta red color, while the aborted pollens have blue-green color. (B) Fraction of viable pollens. (C) Number of pollens per flower. Mean values were derived from 4 statistical replicates, where 2 replicates contained 8 individual flowers, the other 2 replicates contained 3 individual flowers. Black dots indicate individual data of each statistical replicate. Error bars indicate ±standard error of means based on the common variance. Different lowercase letters indicate significant differences between treatment means according to Fisher's unprotected LSD test at P=0.10.

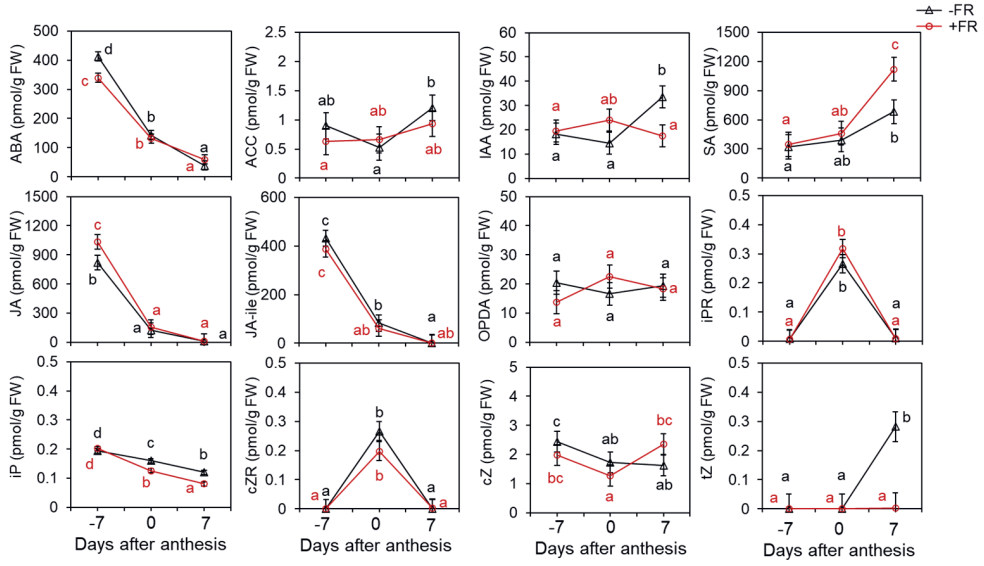

Figure S4. Hormonal profiles of flowers before anthesis, at anthesis and after anthesis. Plants were cultivated with or without far-red (FR). Measured hormones include ABA (abscisic acid), ACC (1-amino-cyclopropane-1-carboxylic acid), IAA (indole-3-Acetic Acid), SA (salicylic acid), JA (jasmonic acid), JA-ile (jasmonic acid isoleucine), OPDA (12-oxo-Phytodienoic acid), iPR (isopentenyl adenosine riboside), iP (isopentyladenine), cZR (cis-zeatin riboside), *c*Z (cis-zeatin), *t*Z (trans-zeatin). FW stands for fresh weight. The level of *t*ZR (trans-zeatin riboside) was not detectable. Samples were collected within the last hour of light period. Ground frozen samples were extracted with 100% methanol containing the appropriate internal standards. The supernatant was fractionated into three fractions, containing ACC, acidic hormones (IAA, SA, OPDA, JA, JA-ile, ABA) and cytokinins (iPR, iP, tZ, tZR, cZR, cZ) respectively, using a 30mg/1cc Oasis MCX cartridge (Waters Corporation, USA) and analyzed by MRM-UPLC-MS/MS analysis as previously described (Bours *et al.*, 2013; Schiessl *et al.*, 2019; Gühl *et al.*, 2021). Mean values were derived from 4 statistical replicates, each based on 2 plants (4 flowers per plant). Split-plot ANOVA was performed on all parameters. Error bars indicate ±standard error of means based on the common variance. Different lowercase letters indicate significant differences between treatment means according to Fisher's unprotected LSD test at P=0.10.

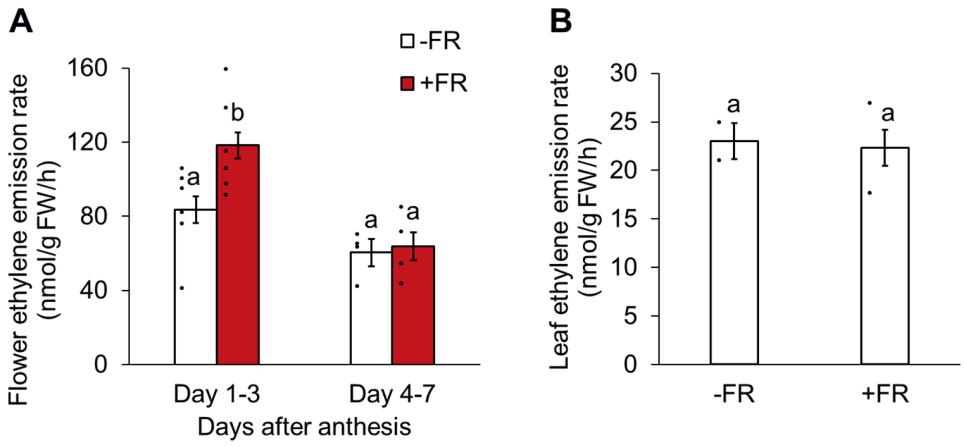

Figure S5. Ethylene emission rate of flowers and leaves grown with or without far-red (FR). (A) Flowers at two different developmental stage: 1-3 days after anthesis (n=6), or 4-7 days after anthesis (n=4). (B) Top mature leaves. Leaf discs were sampled with a puncher (n=2). FW stands for fresh weight. For both (A) and (B), each statistical replicate (n) consists of 3 glass vials, where each had 4-5 individual flowers or 5 leaf discs. Black dots indicate individual data of each statistical replicate. The samples were collected in the morning, incubated in a sealed glass vials for 5 hours at room temperature. Afterwards, the air samples from the vials were analysed with a gas chromatography for the ethylene emission. One way ANOVA was performed for the leaf samples and different groups of flowers separately. Error bars indicate ±standard error of means based on the common variance. Different lowercase letters indicate significant differences between treatment means according to Fisher's unprotected LSD test at P=0.10.

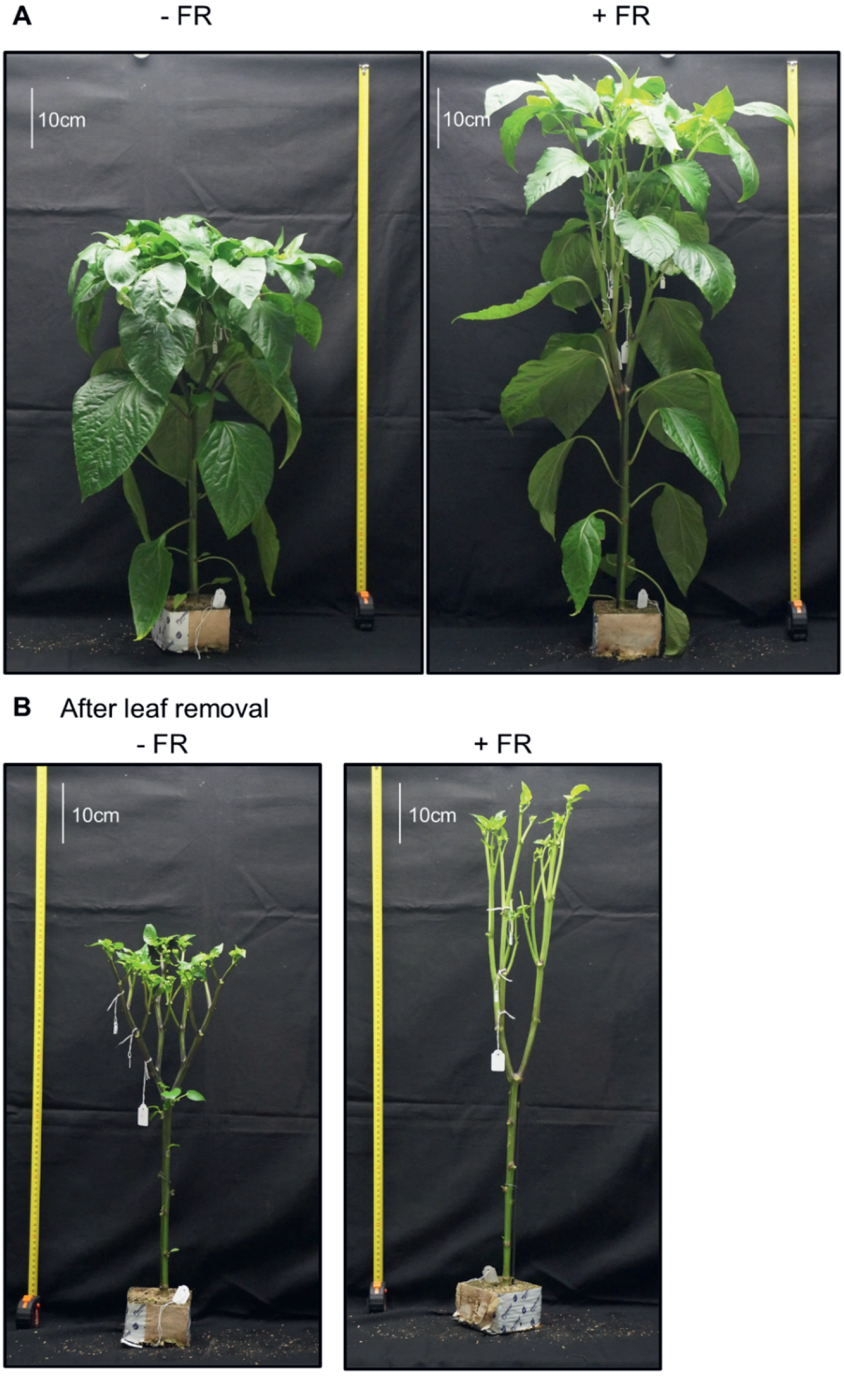

Figure S6. Sweet pepper plants in the vegetative growth experiment. (A) Sweet pepper plants after 23 days of growth with or without additional far-red (FR). (B) Same plants but the mature leaves were removed to show the plant architecture and the outgrowth of lateral buds.

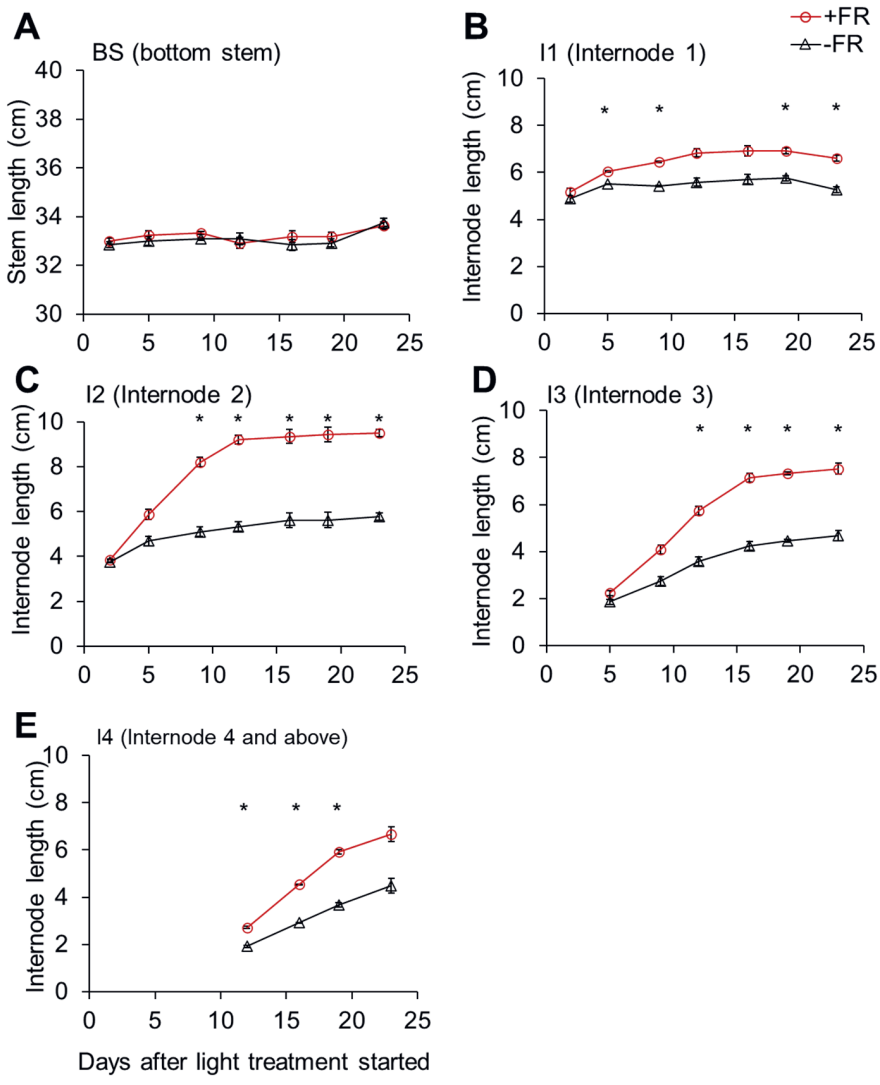

Figure S7. The length of stem and internodes of sweet pepper plants grown with or without additional far-red (FR). (A) Bottom stem; (B) Internode 1; (C) Internode 2; (D) Internode 3; (E) Internode 4 and above (as illustrated in Fig. 1A). Every plant has 1 bottom stem, two times internode 1, four times internode 2, eight times internode 3, and sixteen times internode 4. Mean values were derived from two statistical replicates, each consisting of 3 plants, except data on day 23 where each replicate consisted of 6 plants. Error bars indicate ±standard error of mean based on the common variance. '*' indicates significant differences between treatment means according to Fisher's unprotected LSD test at P=0.10.

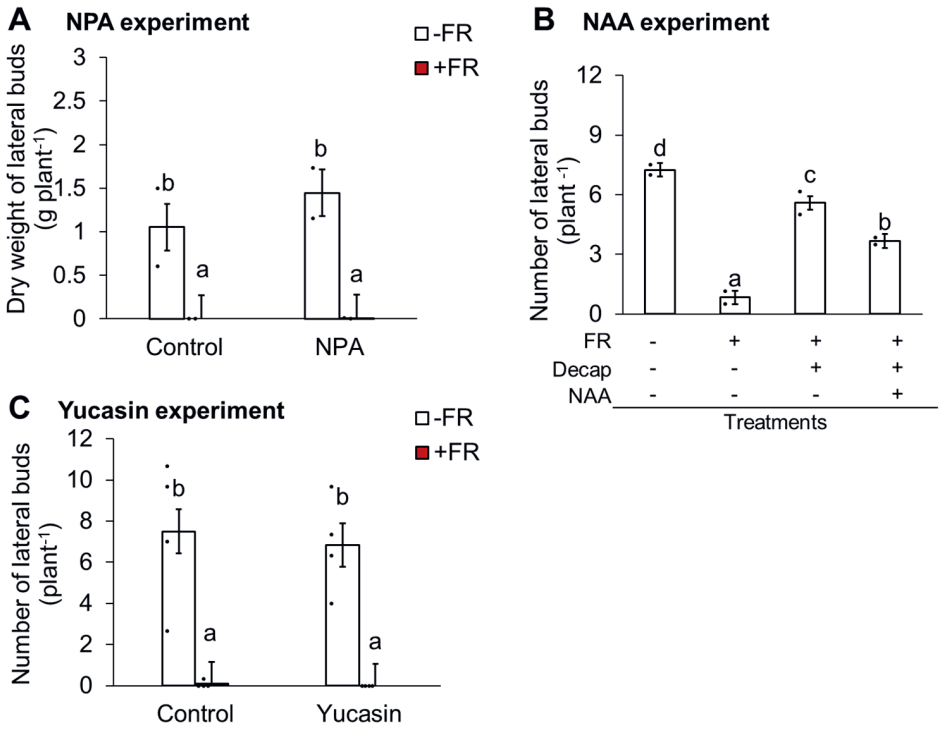

Figure S8. Outgrowth of lateral buds in various experiments. Plants were cultivated with or without far-red (FR). (A) Dry weight of outgrowing lateral buds at the end of the NPA experiment (NPA: N-1-naphthylphthalamic acid). (B)(C) The number of outgrowing lateral shoots in the NAA experiment (NAA: 1-Naphthaleneacetic acid) and yucasin experiment within a duration of 14 days and 28 days respectively (from their first chemical application till the end of the experiment). 'Decap' stands for decapitation. Black dots indicate individual data of each statistical replicate. Error bars indicate ±standard error of mean based on the common variance. Different lowercase letters indicate significant differences between treatment means according to Fisher's unprotected LSD test at P=0.10.

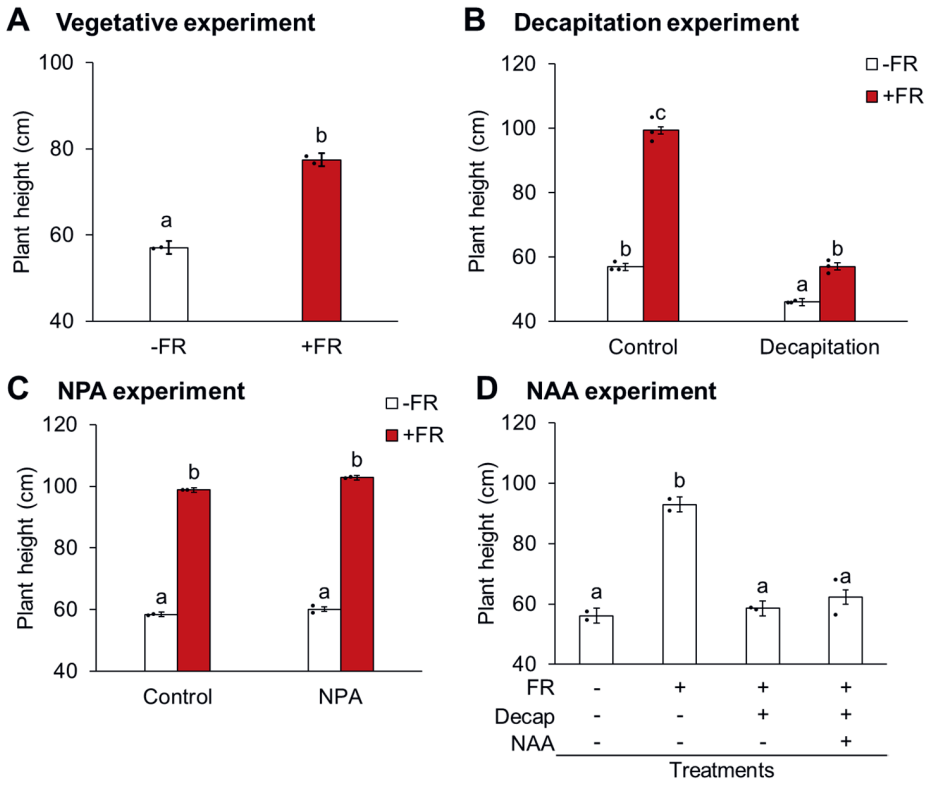

Figure S9. Plant height in various experiments. Plants were cultivated with or without far-red (FR). (A) Vegetative growth experiment; (B) decapitation experiment; (C) NPA experiment (NPA: N-1-naphthylphthalamic acid); (D) NAA experiment (NAA: 1-Naphthaleneacetic acid). 'Decap' stands for decapitation. Black dots indicate individual data of each statistical replicate. Error bars indicate ±standard error of mean based on the common variance. Different lowercase letters indicate significant differences between treatment means according to Fisher's unprotected LSD test at P=0.10.

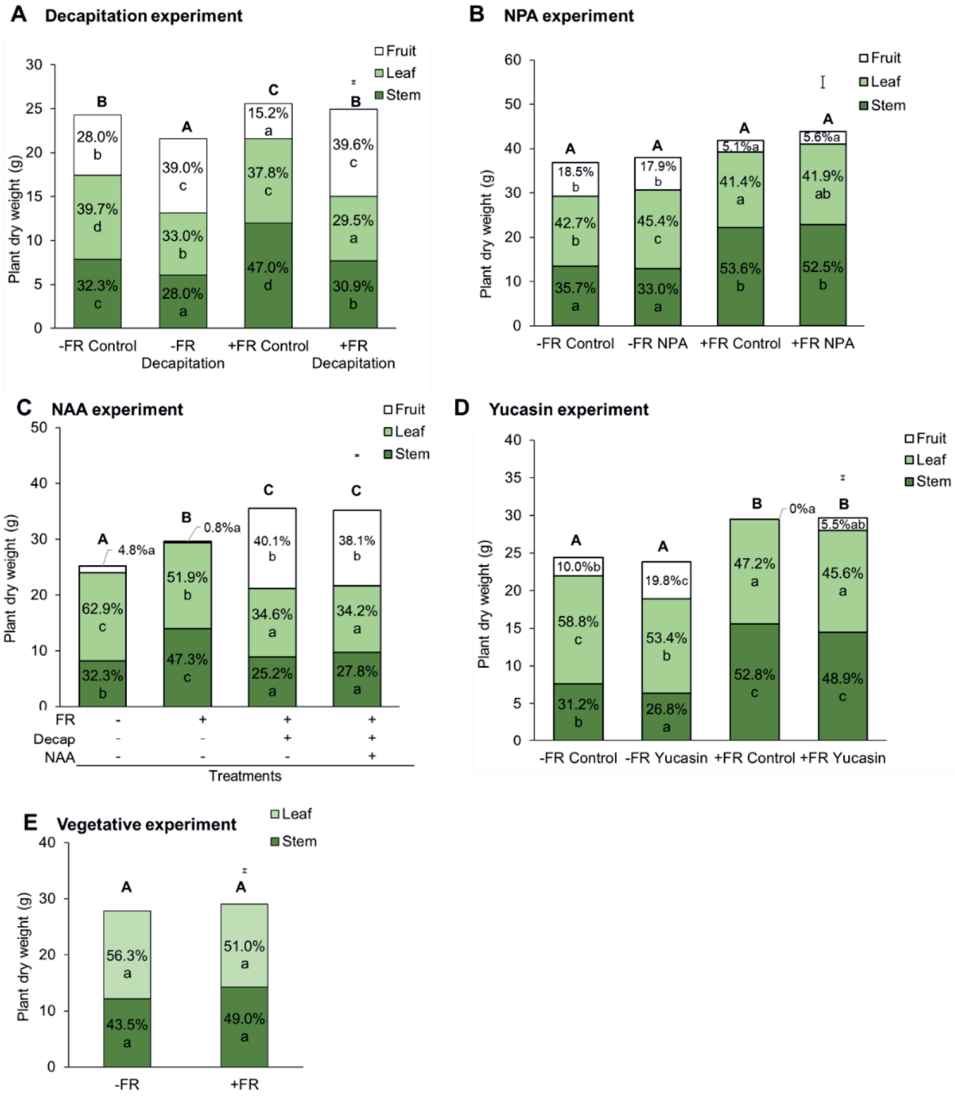

Figure S10. Plant total aboveground dry weight, and the partitioning of dry weight to stem, leaf and fruit in various experiments. Plants were cultivated with or without far-red (FR). (A) Decapitation experiment; (B) NPA experiment (NPA: N-1-naphthylphthalamic acid); (C) NAA experiment (NAA: 1-Naphthaleneacetic acid), where the 'Decap' stands for decapitation; (D) yucasin experiment; (E) vegetative growth experiment. Error bars at the top right corner of each plot indicate standard error of mean based on the common variance in plant total dry weight aboveground. Different letters indicate significant differences between treatment means according to Fisher's unprotected LSD test at *P*=0.10: lowercase letters for dry mass partitioning and uppercase letters for plant total aboveground dry weight.

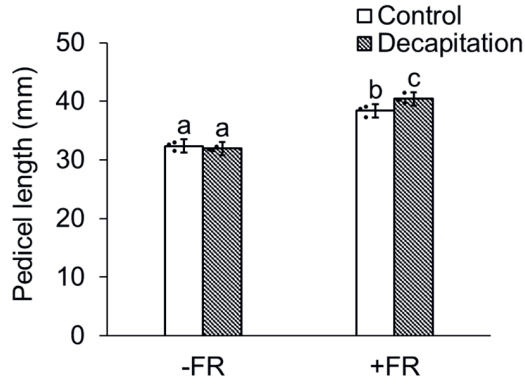

Figure S11. The pedicel length of flowers and young fruits at the end of decapitation experiment. Plants were cultivated with or without far-red (FR). Mean values were derived from 3 statistical replicates, each based on all available fruits from 8 plants. Black dots indicate individual data of each statistical replicate. ANOVA based on split-plot design was performed. Error bars indicate ±standard error of means based on the common variance. Different lowercase letters indicate significant differences between treatment means according to Fisher's unprotected LSD test at *P*=0.10.

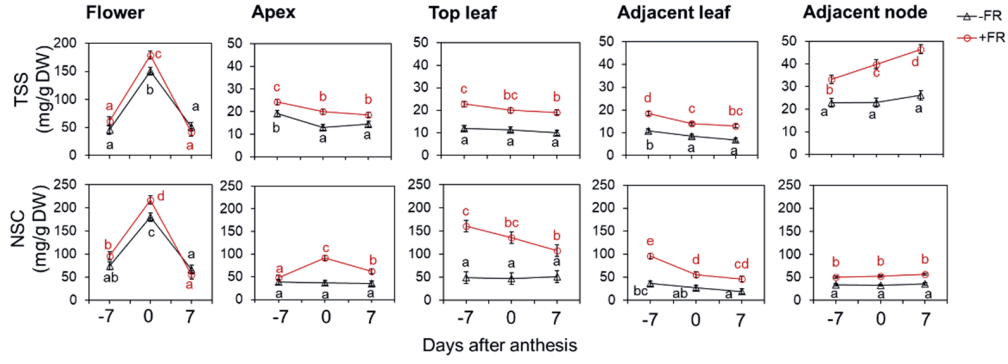

Figure S12. Effect of additional far-red (FR) on total soluble sugar (TSS) and non-structural carbohydrates (NSC) based on results in Fig. 6. Total soluble sugar = glucose + fructose + sucrose. Non-structural carbohydrates = total soluble sugar + starch. DW stands for dry weight. Mean values were derived from 4 statistical replicates, each based on 2 plants. Split-plot ANOVA was performed on all parameters. Error bars indicate ±standard error of means based on the common variance. Different lowercase letters indicate significant differences between treatment means according to Fisher's unprotected LSD test at *P*=0.10.

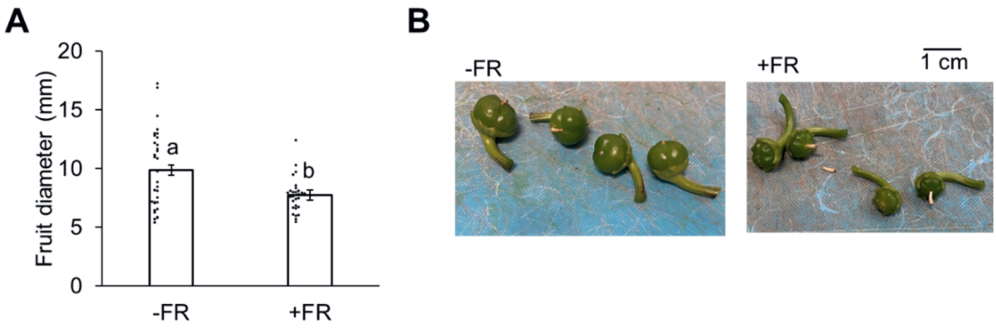

Figure S13. The sample size of young fruits on day 7 after anthesis, for enzyme assay, carbohydrate analysis and hormonal analysis. Plants were cultivated with or without far-red (FR). (A) The average diameters of sample organs when harvested on 7 days after anthesis. The values were achieved by analyzing digital photos with ImageJ. N=32 for '-FR' treatment; N=28 for '+FR' treatment. Black dots indicate individual data of each sample. One way ANOVA was performed. Error bars indicate ±standard error based on the common variances. Different lowercase letters indicate significant differences between treatment means according to Fisher's unprotected LSD test at *P*=0.10. (B) Example photos of samples harvested on day 7 after anthesis under two different light treatments. The photo for treatment '-FR' was also used in Fig. S2C to indicate floral developmental stages.

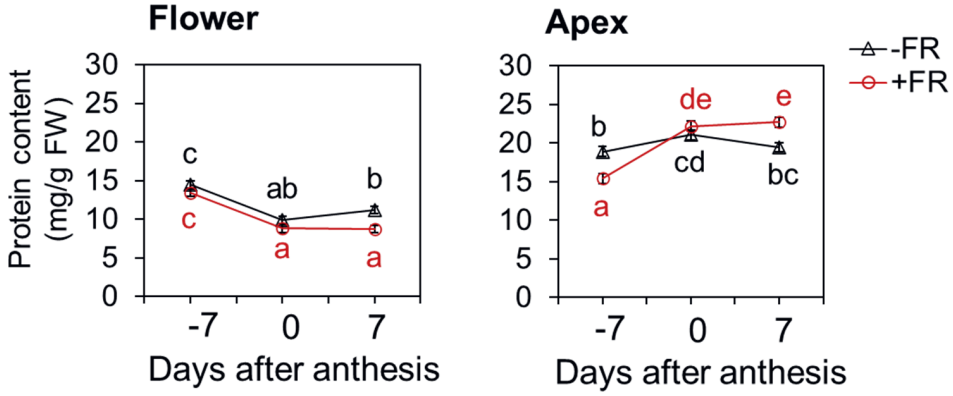

Figure S14. Protein content of flowers and apex samples used in Fig. 7. Plants were cultivated with or without far-red (FR). FW stands for fresh weight. The protein content was quantified by Bradford test, with bovine serum albumin as standard solutions. Mean values were derived from 4 statistical replicates, each based on 2 plants. Split-plot ANOVA was performed on all parameters. Error bars indicate ±standard error of means based on common variance. Different lowercase letters indicate significant differences between treatment means according to Fisher's unprotected LSD test at *P*=0.10.

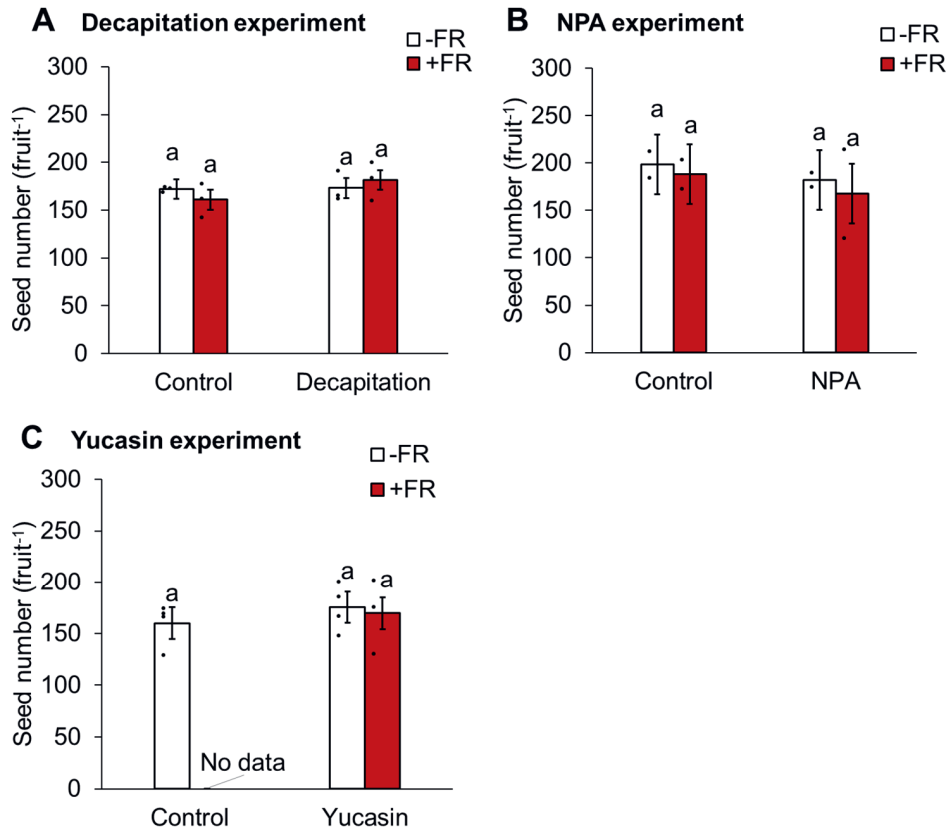

Figure S15. Seed number of all available fruits (when fruit width >2cm) in various experiments. Plants were cultivated with or without far-red (FR). (A) Decapitation experiment, (B) NPA experiment (NPA: N-1-naphthylphthalamic acid), and (C) yucasin experiment. Black dots indicate individual data of each statistical replicate. Error bars indicate ±standard error of means based on the common variance. Different lowercase letters indicate significant differences between treatment means according to Fisher's unprotected LSD test at P=0.10.

Table S1. Nutrient solution recipe. EC=2.0, pH=6. This recipe was used in all experiments except decapitation experiment, where plants were transplanted in soil and tap water was used for irrigation.

Macro elements (mM)		Micro elements (μM)	
NH_4	1.2	Fe	25
K	7.2	Mn	10
Ca	4.1	Zn	5
Mg	1.8	B	30
NO_3	12.4	Cu	0.75
SO_4	3.3	Mo	0.50
P	1.1		

Chapter 4

High ratio of blue:red light reduces fruit set in sweet pepper, which is associated with low starch content and hormonal changes

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Submitted

Abstract

In sweet pepper (*Capsicum annuum* L.), the fruit yield is often negatively affected by fruit abortion. Here we investigated whether fruit abortion is affected by the blue:red light ratio (B:R) and the possible underlying physiological mechanisms related to carbohydrates and hormones. Sweet pepper plants were grown at B:R of 1:10, 1:3, 1:1 or 9:1 with a total photosynthetic photon flux density of 200 µmol m^{-2} s^{-1}, resulting in a phytochrome photostationary state (PSS) of 0.88, 0.88, 0.86 and 0.72, respectively. For fruit set observations, each plant was allowed to retain 12 flowers on 4 main stems. Sweet pepper plants grown at the highest B:R (9:1) showed a low fruit set (around 3 fruits per plant), whereas the other three treatments resulted in higher fruit set (6-7 fruits per plant). This response matched with the changes in PSS, suggesting the B:R effect on fruit set was likely controlled by phytochrome signaling. Plant shoot biomass and leaf area were reduced at B:R of 1:1 and 9:1. The reduced fruit set was associated with a drop in starch content and sucrose synthases activity; and a low auxin, high salicylic acid and high *cis*-Zeatin type cytokinin (*c*Zs) levels in flowers. Flowers in the low fruit set treatment also failed to reduce the abscisic acid and ethylene levels after anthesis. We concluded that both the reduced starch content and the hormonal changes in flowers play a role in triggering fruit abortion at the high B:R of 9:1.

Key words:

Light quality; fruit abortion; carbohydrates; phytohormones; *Capsicum annuum* L.

1 Introduction

Fruit set is a transition process where a flower develops into a fruit. It is a determining factor for the number of fruits on the plant, thus a major determinant of crop yield. Low fruit set, due to high flower and fruit abortion, limits yield in many crops including sweet pepper (*Capsicum annuum* L.). Even in protected cultivation, 70%-80% of the reproductive organs can abort in sweet pepper (Wubs *et al.*, 2009).

Light spectrum can significantly affect fruit set and abortion. Decreasing the ratio of red to far-red light (R:FR) has been shown to induce more flower and fruit abortion in sweet pepper (Chapter 2; Chapter 3). Low R:FR is associated with a low photostationary state of phytochrome (PSS), a parameter that indicates the ratio of active phytochrome to total phytochrome. Along with blue and UV light receptors, phytochromes control plant morphogenesis, such as the shade-avoidance response (Legris *et al.*, 2019). A low PSS value can be achieved by decreasing the R:FR ratio, but it also occurs under a spectrum with a very high blue:red light ratio (B:R). Therefore, it could be speculated that a very high B:R with a low PSS value induces flower and fruit abortion in sweet pepper, similar to a low R:FR ratio. Relating to this, shade avoidance responses at high fractions of blue light have been associated with low PSS (Kong *et al.*, 2018; Kong *et al.*, 2019).

As an abscission process, flower and fruit abortion in sweet pepper is tightly regulated by hormonal balances (Crane, 1969; Sawicki *et al.*, 2015; Taylor & Whitelaw, 2001), where ethylene and abscisic acid are potential abscission accelerators, while auxin and gibberellin are potential abscission inhibitors. B:R can potentially influence fruit set by regulating hormonal balances, such as through blue light receptors cryptochrome and phototropin. Activated cryptochromes led to a decreased abscisic acid concentration in wild-type *Arabidopsis* leaves compared to the *cry1cry2* mutant leaves (Boccalandro *et al.*, 2012). Blue light postponed petal senescence of cutting carnation flowers, which was linked to the down-regulated expression of ethylene biosynthetic genes (Aalifar *et al.*, 2020). This suggests that a high B:R may affect fruit set positively.

Apart from hormones, carbohydrates also play an important role in fruit set. In pepper flowers, decreased sugar accumulation was closely linked to more flower abortion (Aloni *et al.*, 1996), possibly through enhancing abscisic acid and ethylene synthesis (Wien *et al.*, 1989; Sawicki *et al.*, 2015). Additional sugar in the cultivation media reduced abortion of pepper flower explants, probably by enhancing activity of sucrose synthases and invertases (Aloni *et al.*, 1997), which are crucial enzymes in regulating fruit set and development (Nielsen *et al.*, 1991; Ruan *et al.*, 2012).

Light spectrum can influence the carbohydrate status greatly (Heo *et al.*, 2006). Cultivation under higher fractions of blue light leads to a lower starch content in leaves (Larsen *et al.*, 2022; Shengxin *et al.*, 2016); and a lower plant dry matter production (He *et al.*, 2017; Wang *et al.*, 2016; Warrington & Mitchell, 1976). This suggests that a high B:R may affect fruit set negatively by limiting photoassimilate availability.

Considering the potential cross talk between carbohydrates and hormones, it remains inconclusive how B:R could influence fruit set. Here, we aimed to investigate the effect of a wide range of B:R on the fruit set of sweet pepper and the possible mechanisms. We hypothesized that increasing the B:R decreases fruit set, especially at very high B:R, where the PSS is lowered. We hypothesized this will be the result of lower plant dry matter production and lower carbohydrate accumulation in flowers, even with down-regulated ethylene and abscisic acid levels at high B:R. To investigate this hypothesis, sweet pepper plants were grown under four B:R of 1:10, 1:3, 1:1 or 9:1 in climate chambers, and various morphological and physiological parameters were observed.

2 Materials and Methods

2.1 Plant materials and growth conditions

Seeds of sweet pepper (*Capsicum annuum* L. cv. Gialte, Enza Zaden, Enkhuizen, the Netherlands) were sown in potting mix soil (Lensli, Bleiswijk, the Netherlands), and transplanted into stonewool cubes (10x10x6.5 cm; Grodan, Roermond, the Netherlands) a week after germination. Seedlings were cultivated in a glasshouse (51°N, 5°E) for 7-8 weeks before moving into a climate chamber.

The experiment was conducted twice in time (seeds sown in July and September). The climate chamber was divided into eight cells: light treatments were randomly assigned to cells at the start of each replicate experiment. In each cell, 6 sweet pepper plants were grown on stonewool slabs with a density of 5.4 plants m^{-2}. The plants were pruned to maintain four main shoots (Fig. 1A). In the first replicate experiment, four flowers per plant at node 6 and eight flowers at node 7 were used for fruit set observations (section 2.3), where nodes were counted from bottom to top with the first splitting node as node 1. In the second replicate experiment, flowers were two nodes higher, i.e., four flowers per plant at node 8 and eight flowers at node 9 were used for fruit set observations (section 2.3), while four flowers at node 7 were sampled for lab analyses (section 2.4). All other flowers were removed before their anthesis, and extra shoots were removed weekly when they were longer than 3 cm. Based on preliminary experiments, normal self-

pollination was sufficient for good pollination thus no measures were taken to stimulate pollination.

Throughout the experiments, the average temperature was 21.9/18.3 °C (day/night) and the average humidity was 65%. No CO_2 enrichment was applied. Nutrient solution (pH 6.0, EC 2.0 dS m^{-1}) was supplied through drip irrigation four times a day, which consisted of NO_3^- 12.4 mM, SO_4^{2-} 3.3 mM, HPO_4^{2-} 1.1 mM, NH_4^+ 1.2 mM, K^+ 7.2 mM, Ca^{2+} 4.1 mM, Mg^{2+} 1.8 mM, Fe^{3+}/Fe^{2+} 25 µM, Mn^{2+} 10 µM, Zn^{2+} 5 µM, $H_2BO_3^-$ 30 µM, Cu^+/Cu^{2+} 0.75 µM, MoO_4^{2-} 0.5 µM.

2.2 Light treatments

Plants in each cell were illuminated for 14 hours a day using dynamic LED panels (Phytofy® RL, OSRAM GmbH, Berlin, Germany). For the first week in the chamber, plants were grown under white light for acclimatization (Supplementary Table S1). After this, the light spectra were adjusted to the four treatment conditions consisting of only blue and red light with blue: red ratios of 1:10, 1:3, 1:1 or 9:1 (Table 1; Supplementary Fig. S1). The photosynthetic photon flux density (PPFD) of all treatments was about 200 µmol m^{-2} s^{-1} (400-700 nm) (Table 1). Light spectra were measured 20 cm below the LED panels with a spectrometer (LI-180, LI-COR Biosciences, Nebraska, USA). Light intensity was maintained by adjusting the height of the LED panels weekly to keep a constant 20 cm distance between the lamps and the top of the plant canopies.

In the first replicate experiment, the average anthesis date for flowers at nodes 6 and 7 were, respectively, 10 and 16 days after the start of light treatments. In the second replicate experiment, the average anthesis date for flowers at nodes 7 to 9 were, respectively, 8, 14 and 21 days after the start of the light treatments (Supplementary Table S2). Experiments ended about 20 days after the last anthesis: the first replicate experiment lasted 35 days, and the second replicate experiment lasted 42 days from the start of the light treatments.

2.3 Morphological measurements

Anthesis and reproductive organ abortion were recorded every other day, where anthesis was defined as flower opening; flower abortion as the reproductive organ abscission before anthesis and fruit abortion as the reproductive organ abscission after anthesis. Fruit set was determined as the number of fruits obtained from 12 retained flowers per plant.

All plants were used for destructive morphological measurements at the end of a replicate experiment. Plant height was measured as the average height from stonewool surface to the apices of the four main stems per plant. Fruits, leaves and stems were separated. All leaves (including petioles) longer than 3 cm were used

to determine the number of leaves, and the leaf area per plant was measured with a leaf area meter (Li-Cor LI-3100C area meter). The fresh weight of each fruit (including the pedicel) was weighed immediately after removal. All plant materials were dried in a ventilated oven at 105°C for dry weight measurements: stems and leaves for 24 hours, and individual fruits for 72 hours.

Table 1. Four light treatments with different blue:red ratio (B:R). PPFD (photosynthetic photon flux density, 400-700 nm) were similar among treatments, where 400-500 nm was considered as blue light, and 600-700 nm as red light. B% and R% indicate the percentage of blue or red light in PPFD. In these light spectra, UV (380-400nm) was 0.1-0.2 µmol m^{-2} s^{-1}; green (500-600nm) was 0.4-0.6 µmol m^{-2} s^{-1}; and far-red (700-780nm) was 0.03-1.2 µmol m^{-2} s^{-1}. The photoperiod was 14 hours. The mean ± standard error of the mean was derived from 4 replicates with 15 measurements per replicate. PSS (photostationary state of phytochrome) was determined according to Sager *et al.* (1988).

Blue:red ratio	Blue (µmol m^{-2} s^{-1})	Red (µmol m^{-2} s^{-1})	B%	R%	PSS	PPFD (µmol m^{-2} s^{-1})	DLI (mol m^{-2} d^{-1})
1:10	18 ± 2	182 ± 13	9.0	90.7	0.88	200 ± 15	10.1
1:3	50 ± 6	152 ± 11	24.8	75.0	0.88	203 ± 17	10.2
1:1	94 ± 11	101 ± 7	47.9	51.9	0.86	195 ± 18	9.8
9:1	175 ± 21	19 ± 1	90.0	9.8	0.72	195 ± 22	9.8

2.4 Tissue sampling

In the second replicate experiment, four flowers per plant at node 7 were sampled for biochemical analyses (section 2.5, 2.6, 2.7). For each plant, two of the four flowers were collected at anthesis, while the other two were collected 7 days after anthesis. For each cell and developmental stage, flowers from 3 plants were pooled as one sample, resulting in two samples per stage per cell. Samples were collected in the middle of the light period (in the 7th hour of a 14-hour photoperiod). Samples were immediately frozen in liquid nitrogen and stored at -80 °C for further analysis.

After two replicate experiments, a new batch of pepper plants was grown under the same set-up with a blue:red ratio of 1:10 or 9:1 to collect flowers at node 7-9 and top mature leaf samples for ethylene emission assay (section 2.8) and pollen assessment (section 2.9).

2.5 Carbohydrate quantification

The soluble sugars and starch were extracted from around 15 mg of freeze-dried tissue powder as described by Min *et al.* (2021) using the same equipment, with some adaptations as described in Chapter 3. In short, carbohydrates were

extracted with 5 ml 80% ethanol (v/v) in an 80 °C water bath for 20 min. After centrifuging at 4 °C, the supernatant was vacuum dried (Savant SpeedVac SPD2010, Thermo Fisher Inc.), then dissolved in Milli-Q water to quantify soluble sugars. The pellet was vacuum dried, then incubated together with 2 ml alpha-amylase solution (1 mg ml^{-1}) at 90 °C for 30 min. Then, 1 ml amyloglucosidase (0.5 mg ml^{-1} in 50 mM citrate buffer, pH 4.6) was added, followed by an incubation at 60 °C for 10 min. The samples were then centrifuged at 4 °C, where the supernatant was used to quantify starch as glucose. The carbohydrates were quantified with a high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD; Dionex™ ICS-5000, Thermo Fisher Scientific), equipped with a Dionex™ CarboPac™ PA1 column (2 ×250 mm; Thermo Fisher Scientific).

2.6 Enzyme assay

The methods to determine the activity of invertases and sucrose synthases were adapted from Aloni *et al.* (1991, 1996), as described in Chapter 3. In short, around 100 mg (fresh weight) finely ground samples were homogenized in 1.5 ml ice-cold extraction buffer (pH 7.2) containing: 25 mM HEPES (N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid), 5 mM $MgCl_2$, 0.5 mM Na_2EDTA (Ethylenediaminetetra-acetic acid disodium salt), 2 mM DDT (DL-Dithiothreitol), 3 mM DIDCA (Diethyldithiocarbamic acid), 1% (w/v) PVP (Polyvinylpyrrolidone), and 0.1% (v/v) Triton X-100. The mixture was centrifuged at 21300 rcf for 20 min at 4 °C, where the supernatant was used to determine protein content with the Bradford test. The activity of soluble acid invertase or neutral invertase was determined with a 50 µl aliquot of the supernatant incubated in 500 µl 0.1 N phosphate citrate buffer (pH 5.0 or pH 7.5 respectively) with 20 mM sucrose. The activity of insoluble cell wall invertase was determined with a 50 µl aliquot of the suspended pellet incubated in 500 µl 0.1 N phosphate citrate buffer (pH 5.0) with 20 mM sucrose. The activity of sucrose synthase was determined as sucrose breakdown during aliquots of 50 µl of the supernatant incubated in 500 µl 0.1 N phosphate citrate buffer (pH 7.0) with 200 mM sucrose and 5 mM UDP (uridine 5'-diphosphate). Boiled enzymes were used as the blank for each reaction. After incubation at 37 °C for 60 min, the resulting reducing sugars was determined colorimetrically at 540 nm by the dinitrosalicylic acid reaction. The activity of enzymes was expressed as the amount of reaction products per hour on fresh weight basis.

2.7 Hormonal profiling

Ground frozen samples (around 20 mg fresh weight) were extracted with 1 ml 100% methanol containing the appropriate internal standards (Supplementary Table S3) as previously described (Schiessl *et al.*, 2019; Gühl *et al.*, 2021) with minor modifications. Column flow through and wash (formic acid fraction) was collected

and used to quantify ACC (1-aminocyclopropane-1-carboxylate) concentration as previously described by Bours *et al.*, (2013). The concentration of all plant hormones was determined by Multiple Reaction Monitoring Ultra Performance Liquid Chromatography and Tandem Mass Spectrometry (MRM-UPLC-MS/MS) (Waters, Milford, USA) as previously described. Parent–daughter transitions for the different compounds were set using the IntelliStart MS Console. MRM transitions selected for compound identification and quantification are shown in Supplementary Table S3, a 10-point calibration curve was constructed for each compound ranging from 0.1 µM to 19 pM and each dilution also contained a known amount of an appropriate deuterium-labelled internal standard.

2.8 Ethylene emission assay

Flowers were collected at two developmental stages: 1-3 days after anthesis or 4-7 days after anthesis (six replicates per stage). Leaf discs were sampled with a cork borer of 1.5 cm diameter (two replicates). The samples were collected in the morning and incubated in sealed 20 ml glass vials at room temperature (about 20°C). Each statistical replicate consisted of 3 glass vials, where each had 4-5 individual flowers or 5 leaf discs. After 5 hours of incubation, 3 ml air samples from the vials were analysed with a Trace-1300 Gas Chromatogram (InterScience, Breda, NL) coupled with a flame ionization detector, as described by Torres Ascurra *et al.* (2023). Gas Chromatogram was calibrated with a set of certified calibration gases (SOL group, Monza, Italy) before measurements.

2.9 Pollen quantity and viability assessment

Flowers were collected at anthesis for pollen assessment. Anthers from each flower were first fixed in Carnoy's fixative. After the anthers were dried and stained with Alexander's dye, the pollen were released by ultrasonic bath for 5 min, and further observed with a Fuchs-Rosenthal hemocytometer (4 x 4 x 0.2 mm grid, 3.2 mm^3) under a stereoscope (LEICA MZ APO). Pollen with a magenta red color were scored as viable, while pollen with a blue-green color were scored as aborted. Observation of each flower included 2 technical replicates with 6 views each. The total number of pollen per grid in each view was recorded to calculate the number of pollen per flower, based on the ratio of solution volume between the space within each grid and the total dyeing solution used per flower. There were four replicates, where each replicate consisted of 8 individual flowers.

2.10 Statistical analysis

Each light treatment had 4 statistical replicates, composed of 2 replicate cells in each of two replicate experiments. Data that had been assessed on several plants per replicate cell were first averaged to give one value per cell (experimental unit).

The first 2 statistical replicates each consisted of 5 plants, and the second 2 statistical replicates each consisted of 6 plants. Statistics were performed with Genstat software (21st edition). One-way Analysis of Variance (ANOVA) with randomized blocks, where four replicates were considered as four blocks, was used to determine significant differences in morphological parameters among the four light treatments. Fisher's protected least significant difference (LSD) test at P=0.05 was used for mean separation. For enzymatic activity, carbohydrate and hormone quantification, ANOVA with split-plot design was used (light treatments as whole plots, developmental stages as sub-plots), where Fisher's unprotected LSD test at P=0.05 was used, due to the insignificant interactions between both factors for some parameters. Homogeneity of variances was assumed, and normality of residuals was tested with Shapiro-Wilk test at P=0.05 level. If the residuals in ANOVA analysis did not follow normal distribution, the log-transformed data were used for statistical tests. This applied to the concentration of ACC (1-amino-cyclopropane-1-carboxylic acid) and SA (salicylic acid), where their residuals in ANOVA with log-transformed data showed normal distribution.

3 Results

3.1 Fruit set was reduced by the highest blue:red light ratio

Sweet pepper plants, cultivated under four different blue:red light ratios (B:R), were pruned to have only 12 flowers per plant to observe flower and fruit abortion (Fig. 1A; plant photos - Supplementary Fig. S2). The number of days to anthesis was unaffected by B:R (Supplementary Table S2), with almost no flower abortion before anthesis. Hence, the B:R effect on fruit abortion and growth was mainly post-anthesis.

Fruit number and individual fruit dry weight were similar at B:R of 1:10, 1:3 or 1:1. However, at B:R of 9:1, the fruit number was almost reduced by half and the individual fruit dry weight was about 75% lower compared to the lower B:R (Fig. 1B, C). In contrast, the fruit dry matter content gradually increased with increasing B:R and was highest at B:R of 9:1 (Fig. 1D). Fruit abortion at B:R of 1:10 was delayed by 1-1.5 days compared to the higher B:R (from 1:3 to 9:1) (Supplementary Table S4). Fruit abortion was not affected by the number of pollen per flower or the fraction of viable pollen, as these were not influenced by B:R (Supplementary Fig. S3). There was no visual difference in the seed number of harvested fruits.

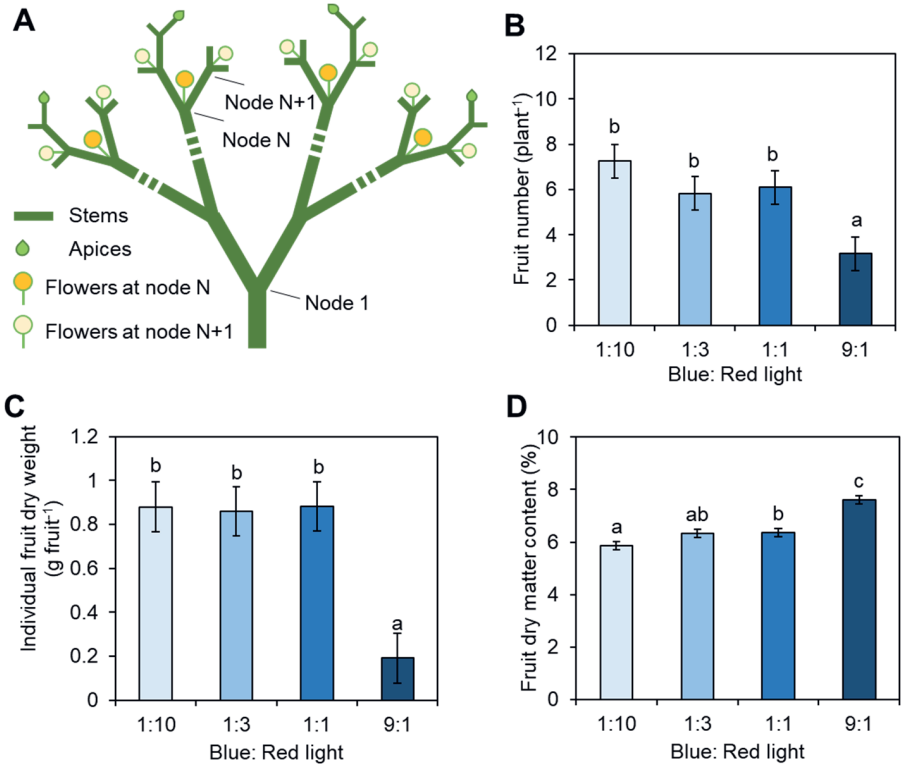

Figure 1. Effect of blue:red light ratios (B:R) on fruit abortion and fruit growth in sweet pepper. (A) Plants were pruned to have 4 main shoots carrying 12 flowers per plant for fruit set observation. Node N=6 in the first replicate experiment, N=8 in the second replicate experiment. (B) Fruit number per plant. (C) Individual fruit dry weight. (D) Fruit dry matter content. Mean values were derived from 4 statistical replicates, each based on 5-6 plants. One-way ANOVA was performed on all variables. Error bars indicate ±standard error of means based on the common variance. Different letters indicate significant differences between treatment means according to Fisher's protected LSD test at P=0.05.

3.2 Higher blue:red light ratios resulted in a lower plant biomass with a lower leaf area

Total shoot biomass was similar for plants at B:R of 1:10 and 1:3, but decreased when B:R increased further (Fig. 2A). The reduction of shoot biomass at B:R of 1:1 was the result of a lower leaf and stem biomass, while at B:R of 9:1, that was the result of lower leaf, stem, and fruit biomass. The dry mass partitioning among plant organs was not influenced by B:R from 1:10 to 1:1, but at 9:1, the partitioning to fruits substantially dropped (Fig. 2B).

The leaf area per plant was reduced at B:R of 1:1 and 9:1, compared to lower B:R (Fig. 2C). Considering that leaf number was not affected by B:R (Fig. 2D), we

suggest that high B:R inhibited leaf expansion in pepper. The specific leaf area and plant height were unaffected by B:R (Fig. 2E, F).

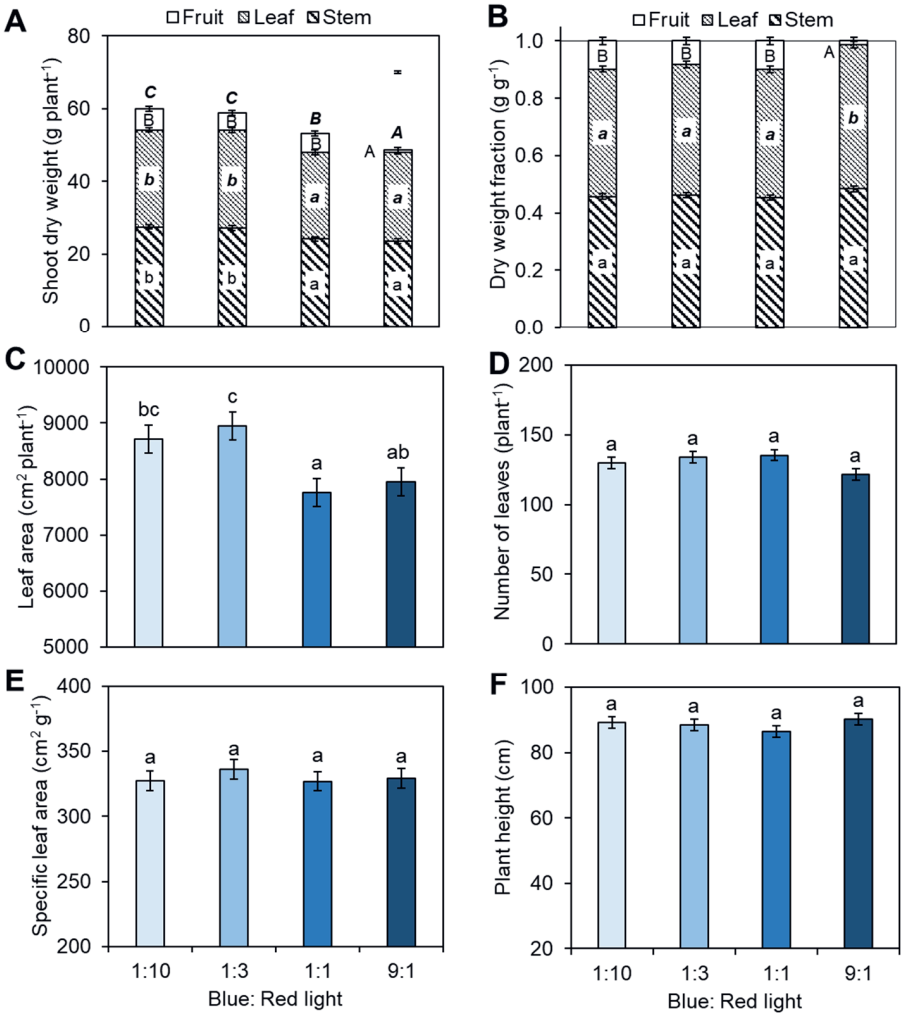

Figure 2. Effect of blue:red light ratios (B:R) on plant morphology in sweet pepper. (A) Plant shoot dry weight and its composition (stem, leaf, and fruit). (B) Fraction of stem, leaf and fruit dry mass. (C) Leaf area per plant. (D) Leaf number per plant. (E) Specific leaf area (leaf area/leaf dry mass). (F) Plant height. Mean values were derived from 4 statistical replicates, each based on 5-6 plants. One way ANOVA was performed for all variables. Error bars indicate ±standard error of means based on the common variance. The error bar at the top right corner of (A) indicates the standard error of means for total shoot dry weight. Different letters indicate significant differences between treatment means according to Fisher's protected LSD test at P=0.05. In (A) and (B), standard small letters are for stem, small letters in bold italics are for leaves, standard capital letters are for fruits and capital letters in bold italics are for total shoot.

3.3 The highest blue:red light ratio reduces starch content in flowers

Most fruit abortion was observed between 9 and 16 days after anthesis (Supplementary Table S4). Thus, the carbohydrate content was analyzed in the flowers on day 0 (at anthesis) and fruits on day 7 after anthesis, which could indicate the carbohydrate status before potential abortion. The carbohydrate content (fructose, glucose, sucrose, and starch) generally dropped on day 7 after anthesis compared to at anthesis (Fig. 3). At both developmental stages, sucrose, fructose, and glucose content were not influenced by B:R (Fig. 3A, B, C). However, starch content at both stages was reduced at B:R of 9:1 compared to the other B:R (Fig. 3D).

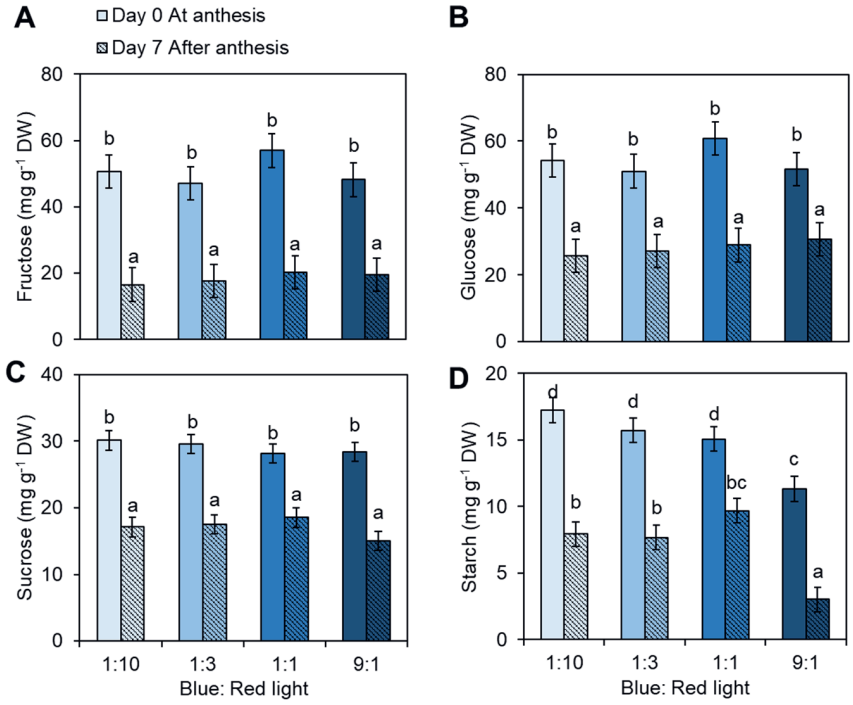

Figure 3. Carbohydrate content of pepper flowers on day 0 (at anthesis) and fruits on day 7 after anthesis. Samples were collected in the middle of the day (in the 7th hour of a 14-hour photoperiod). DW stands for dry weight. Mean values were derived from 2 statistical replicates, each based on 2 samples (3 plants/sample). Split-plot ANOVA was performed with light treatments as the whole plot and developmental stages as the sub-plot. Error bars indicate ±standard error of means based on the common variance. Different letters indicate significant differences between treatment means according to Fisher's unprotected LSD test at P=0.05.

3.4 Effect of blue:red light ratios on sucrose cleavage activities

The activity of soluble acid invertases, cell wall invertases and neutral invertases in flowers/fruits showed a substantial decline on day 7 after anthesis compared to

at anthesis (Fig. 4A, B, C). At anthesis, the activities of these enzymes were not influenced by B:R. On day 7 after anthesis, the soluble acid invertase activity was lower than the other three B:R; the cell wall invertase activity was higher at B:R of 9:1 compared to the B:R of 1:3 and 1:1; whilst the neutral invertase activity was unaffected by B:R (Fig. 4A, B, C).

The activity of sucrose synthases showed different patterns among B:R at the two developmental stages. At anthesis, sucrose synthase activity increased along with the increasing B:R from 1:10 to 1:1, but this tendency did not continue - the sucrose synthase activity showed a substantial drop at B:R of 9:1 (Fig. 4D). On day 7 after anthesis, the sucrose synthase activity was higher at B:R of 9:1 compared to the B:R of 1:3 and 1:1 (Fig. 4D).

In addition, the protein content on day 7 after anthesis was substantially lower at B:R of 9:1 compared to the other B:R, whilst the protein content at anthesis was unaffected by B:R (Supplementary Fig. S4).

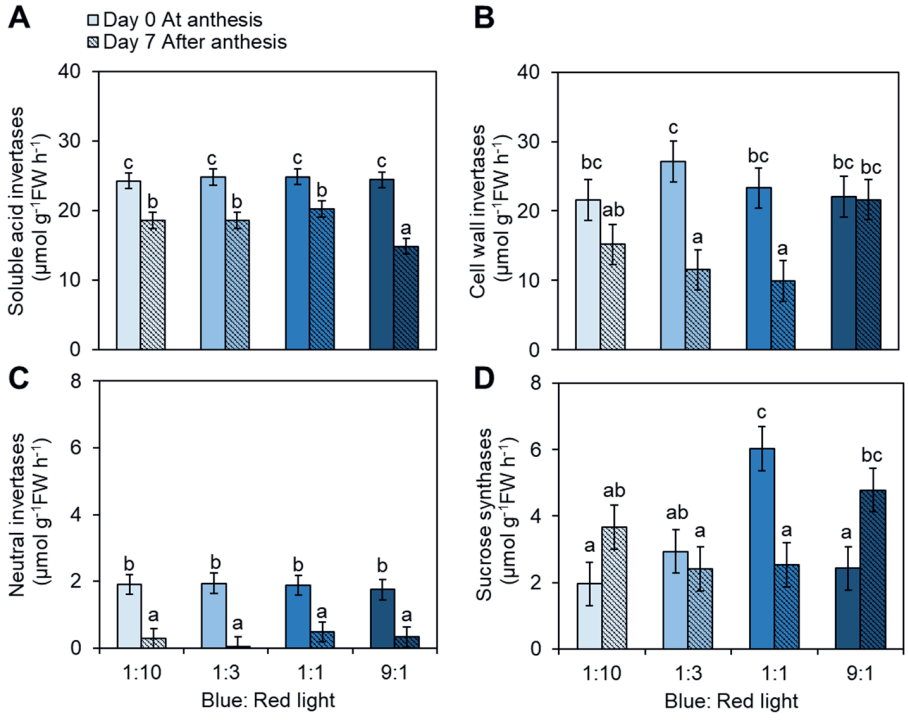

Figure 4. Enzyme activity of invertases and sucrose synthases in pepper flowers on day 0 (at anthesis) and fruits on day 7 after anthesis. Samples were collected in the middle of the day (in the 7th hour of a 14-hour photoperiod). FW stands for fresh weight. Mean values were derived from 2 statistical replicates, each based on 2 samples (3 plants/sample). Split-plot ANOVA was performed with light treatments as the whole plot and developmental stages as the sub-plot. Error bars indicate

±standard error of means based on the common variance. Different letters indicate significant differences between treatment means according to Fisher's unprotected LSD test at *P*=0.05.

3.5 Effect of blue:red light ratios on the hormone concentrations

The effect of B:R on hormone concentrations in flowers/fruits differed between two developmental stages (Fig. 5; Supplementary Fig. S5). At anthesis, the concentration of IAA (indole-3-Acetic Acid) and SA (salicylic acid) were not influenced by B:R (Fig. 5C,D). IAA concentration on day 7 after anthesis was about three times higher compared to at anthesis. SA concentration did not differ between these two stages. This pattern of IAA and SA applies to all B:R except at B:R of 9:1. At B:R of 9:1, IAA was reduced, and SA was doubled on day 7 after anthesis, compared to the other B:R (Fig. 5C,D).

ABA (abscisic acid) concentration was not influenced by B:R at anthesis. Compared to anthesis, the concentration of ABA on day 7 after anthesis decreased and this reduction was less pronounced at the higher B:R. This resulted in a higher ABA concentration on day 7 after anthesis at B:R of 9:1, compared to B:R of 1:10 and 1:3 (Fig. 5A).

At anthesis, B:R influenced neither the concentration of the precursor of ethylene - ACC (1-aminocyclopropane-1-carboxylate), nor the ethylene emission rate (Fig. 5B, E). On day 7 after anthesis, ACC showed a high concentration at B:R of 1:10, which decreased with increasing B:R, showing similarly low levels at B:R of 1:1 and 9:1 (Fig. 5B). After anthesis (from day 1-3 to 4-7 after anthesis), ethylene emission from flowers/fruits was reduced at B:R of 1:10, but not at B:R of 9:1, where ethylene emission maintained at a high level (Fig. 5E). The ethylene emission rates from leaves were not influenced by B:R (Fig. 5F).

The hormone concentrations in the jasmonic acid pathway, including jasmonic acid (JA), jasmonic acid isoleucine (JA-ile, the main active jasmonate), and 12-oxo-Phytodienoic Acid (OPDA, main jasmonate precursor), were not influenced by B:R but differed only between developmental stages (Supplementary Fig. S5), where JA and JA-ile decreased, and OPDA increased on day 7 after anthesis compared to at anthesis.

Different forms of cytokinins (iPR, iP, *c*ZR, *c*Z, *t*Z, *t*ZR) at anthesis were generally not influenced by B:R (Supplementary Fig. S5). On day 7 after anthesis, B:R of 9:1 led to a substantially higher concentration of *c*ZR and *c*Z compared to the other B:R, while B:R did not influence the concentration of the other forms of cytokinins (Supplementary Fig. S5).

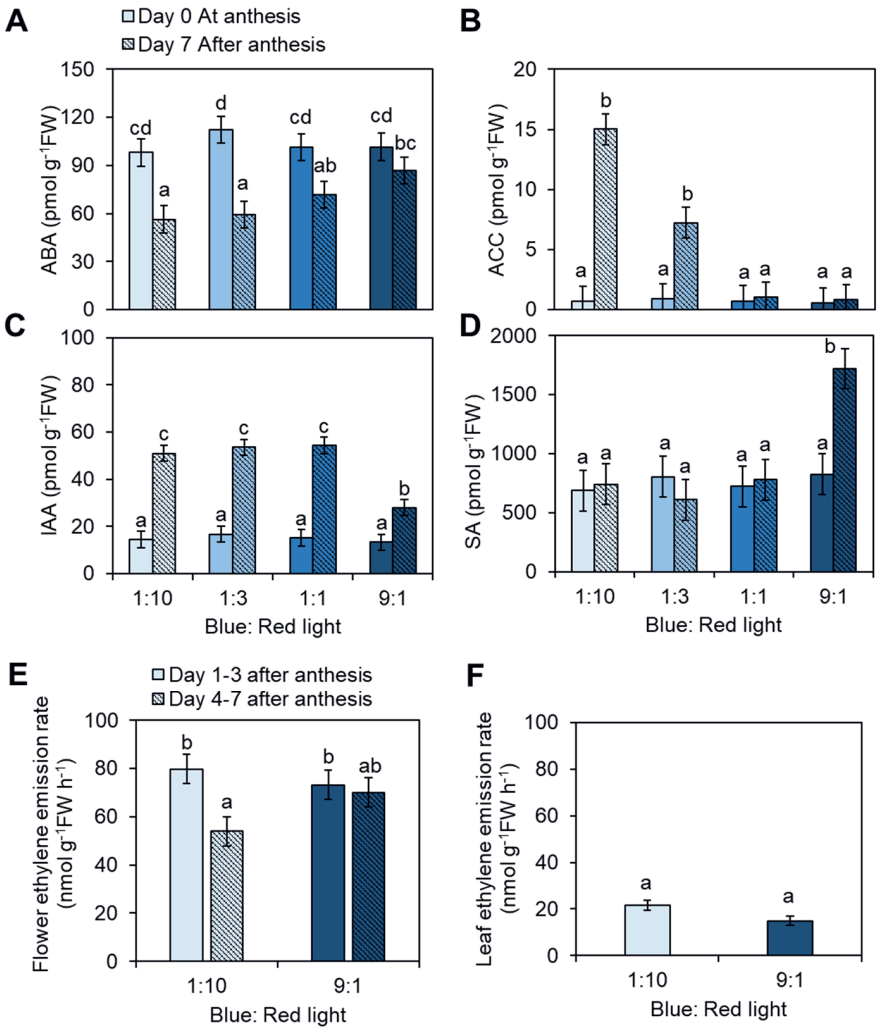

Figure 5. Hormone concentrations in pepper flowers on day 0 (at anthesis) and fruits on day 7 after anthesis. FW stands for fresh weight. For (A) ABA (abscisic acid), (B) ACC (1-amino-cyclopropane-1-carboxylic acid), (C) IAA (indole-3-Acetic Acid) and (D) SA (salicylic acid), mean values were derived from 2 statistical replicates, each based on 2 samples (3 plants/sample). (A)-(D) Samples were collected in the middle of the day (in the 7th hour of a 14-hour photoperiod). For ethylene emission from flowers (E) and leaves (F), the mean values were derived from 6 replicates for flowers, and 2 replicates for leaves. (E)-(F) Each replicate was based on 3 glass vials with 4-5 flowers or 5 leaf discs per glass vial. Samples were collected in the morning. Split-plot ANOVA was performed for (A)–(E) with light treatments as the whole plot and developmental stages as the sub-plot; one way ANOVA was performed for (F). Error bars indicate ±standard error of means based on the common variance. Different letters indicate significant differences between treatment means according to Fisher's unprotected LSD test at P=0.05. Log-transformed data was used for mean separation in (B) and (D) as the residuals in ANOVA analysis based on their original data did not follow normal distribution.

4 Discussion

4.1 Low fruit set at the very high ratio of blue:red light is likely regulated by phytochromes

In this study, we aimed to investigate the effects of different blue:red light ratios (B:R) on the fruit set in sweet pepper and explain effects from physiological and morphological perspectives.

We found the fruit set was unchanged among B:R from 1:10 to 1:1, but it decreased at the highest B:R of 9:1 (Fig. 1B). This pattern, with only the B:R of 9:1 showing a different response, also appeared in the reduced individual fruit weight (Fig. 1C); the reduced starch content in flowers (Fig. 3D); the reduced activity of soluble acid invertases after anthesis (Fig. 4A); the reduced auxin (IAA), the increased salicylic acid (SA) and the increased cytokinin *c*Z and *c*ZR concentrations after anthesis (Fig. 5C,D; Supplementary Fig. S5). These observations are consistent with the changes of PSS (photostationary state of phytochrome) among our treatments: increasing the B:R from 1:10 to 1:1 minimally changes the PSS (0.86-0.88) but increasing the B:R further to 9:1 led to a substantial decrease of PSS to 0.72 (Table 1). Thus, we consider the mentioned observations to be regulated by phytochrome controlled pathways.

In previous study, we observed that additional FR, hence reduced PSS, also caused a reduced fruit set in sweet pepper (Chapter 2), which was also associated with a reduced activity of soluble acid invertases, a reduced IAA concentration, and an increased SA concentration in fruits 7 days after anthesis (Chapter 3). These shared responses further support that the low fruit set at B:R of 9:1 is probably mediated by phytochromes.

4.2 Higher B:R reduces plant source strength

High B:R of 1:1 and 9:1 reduced shoot biomass of sweet pepper plants by around 11% and 18% respectively, compared to lower B:R (Fig. 2A). This biomass reduction could partially be attributed to a 9-13% lower leaf area at B:R of 1:1 and 9:1 (Fig. 2C). Since leaf area and plant biomass decreased from B:R of 1:3 to 1:1, these responses may have been regulated by cryptochrome and phototropin photoreceptors, especially because the PSS minimally changed in this range (Table 1). It is widely reported that increasing the fraction of blue light decreases leaf area (Cope *et al.*, 2014; Kalaitzoglou *et al.*, 2021; Kusuma *et al.*, 2021; Larsen *et al.*, 2020; Snowden *et al.*, 2016), although the presence or magnitude of the response can be species and environment dependent. The reduced leaf area could lead to a reduced light interception at B:R of 1:1 and 9:1.

In addition to the decrease in light interception, blue photons tend to have a slightly lower photosynthetic efficiency than red photons when measured on the same plant (McCree, 1971; Zhen & Bugbee, 2020; Hogewoning *et al.*, 2012). However, it is important to emphasize potential acclimation to B:R, which affects the photosynthetic response. Increasing B:R also tends to increase leaf thickness, chlorophyll content, stomatal density and conductance, which can contrastingly result in increasing photosynthetic efficiencies on a leaf area basis (Wang *et al.*, 2016; Hogewoning *et al.*, 2010). Therefore, the effect of B:R on photosynthesis may be complex, where the photosynthesis may be optimized under some moderate B:R. For example, the highest net photosynthesis rate was shown at B:R of 1:4 in lettuce (Kang *et al.*, 2016), at B:R of 3:2 in cherry tomato (Liu *et al.*, 2018), and at B:R of 1:3 in sweet pepper (Li *et al.*, 2023). This could possibly explain the increased leaf photosynthesis rate with the increasing B:R ratios reported by some researchers, especially below B:R of 1:1 (Wang *et al.*, 2016; Hogewoning *et al.*, 2010). Responses for higher B:R ratios (between 1:1 and 1:0) are rarely studied. Compared to a more balanced B:R, monochromatic blue light can significantly reduce both acclimated and non-acclimated photosynthesis (Hogewoning *et al.*, 2010; Zhen & Bugbee, 2020). The highest B:R (9:1) in the current study is close to monochromatic blue, thus, we speculate that such high B:R could possibly reduce net photosynthesis rate in sweet pepper. Therefore, the reduced plant dry matter production could result from both the lower leaf area and potentially lower photosynthesis rate at the highest B:R.

4.3 Low fruit set is associated with low starch content

High B:R of 9:1 caused a lower starch content in flowers (Fig. 3D). In many perennial species, flowers with sufficiently available sugar at anthesis, especially high starch content, are more likely to develop into fruits successfully (in apricot: Rodrigo *et al.*, 2000; in avocado: Alcaraz *et al.*, 2013; Boldingh *et al.*, 2016; in grapevine: Lebon *et al.*, 2008). The stronger capability to accumulate sugars in flowers, especially starch, is related to a lower sensitivity to flower and fruit abortion among cultivars of grapevine (Lebon *et al.*, 2004) and sweet pepper (Aloni *et al.*, 1996). Thus, the lower floral starch content could be one of the main triggers for the reduced fruit set at very high B:R (Fig. 1B).

The starch content in flowers and fruits could be limited by starch accumulation in source and storage organs. A higher starch content in fruiting branches and source leaves is correlated with a higher fruit set in citrus and avocado (Schaffer *et al.*, 1985; Davie *et al.*, 1995). A higher fraction of blue light (90% blue vs. 9% blue) was found to reduce starch content in basil leaves (Larsen *et al.*, 2022). Blue light signal via cryptochrome 1a was found to induce starch degradation in tomato leaves and

lead to less starch accumulation (Dong *et al.*, 2021). Thus, high B:R may limit starch accumulation in source organs, and limit assimilate translocation from source to sink organs, e.g., flowers and fruits. The reduced plant dry matter production (discussed in section 4.2), which indicates a reduced source strength, also implies a lower assimilate availability for flowers.

Other than assimilate availability, the starch synthesis and accumulation in the flowers is largely dependent on the SuSy (sucrose synthases) activity (Angeles-Núñez & Tiessen, 2010; Baroja-Fernández *et al.*, 2009; Baroja-Fernández *et al.*, 2012; D'Aoust *et al.*, 1999; N'tchobo *et al.*, 1999). In tomato fruit, a reduced SuSy activity was correlated with a reduced starch content, which led to a reduced sucrose unloading capacity in young fruit, a slower fruit growth rate and a reduced fruit set (D'Aoust *et al.*, 1999). In flowers at anthesis, the activity of sucrose synthases gradually increased from a B:R of 1:10 to 1:1, followed by a substantial drop at B:R of 9:1 (Fig. 4D). This drop matches the reduced floral starch content at B:R of 9:1 (Fig. 3D). The increased SuSy activity from B:R of 1:10 to 1:1 could be a response of the flowers to the decreasing source strength, resulting in the need to secure assimilates. Our data suggest that a drop in sucrose synthase activity at anthesis, in combination with a reduction in plant source strength, was probably responsible for the reduced floral starch content at the highest B:R.

4.4 The role of hormones in the reduced fruit set at the highest B:R

High B:R of 9:1 led to changes in hormones in flowers and fruits before potential abortion, where the abortion was mostly observed between 9 and 16 days after anthesis. Auxin (IAA) together with gibberellins act as stimulating signals for fruit set and the subsequent activation of cell division (in tomato Vriezen *et al.*, 2008; in pepper Tiwari *et al.*, 2012), thus the lower IAA level at B:R of 9:1 was in line with the reduced fruit set compared to the other B:R. With antagonistic roles to auxin and gibberellin, ethylene and abscisic acid (ABA) are also involved in regulating fruit set. Their biosynthesis genes were strongly expressed before fruit set but attenuated soon after fruit set (Vriezen *et al.*, 2008). We found that ABA concentration and ethylene emission in fruits on day 7 after anthesis were reduced at low B:R but remained at the high level at B:R of 9:1 (Fig. 5A, E). This supports the roles of ABA and ethylene in promoting abscission of reproductive organs (Lee *et al.*, 2021; Shinozaki *et al.*, 2015; Wilmowicz *et al.*, 2016). Interestingly, the concentration of ethylene precursor ACC showed a contradictory pattern to the ethylene emission rate in flowers: from anthesis to 7 days after anthesis, the ethylene emission was reduced but ACC was increased at low B:R; whilst the ethylene emission stayed high, and ACC stayed low at high B:R (Fig. 5B, E). This implies the ethylene production via ACC oxidase (Wang *et al.*, 2002) could be one

of the processes regulated by B:R. At low B:R, the reduced ethylene production after anthesis could result in more ACC accumulation.

To the best of our knowledge, no research shows the direct roles of salicylic acid (SA) in fruit set. Patharkar *et al.* (2017) and Patharkar & Walker (2018) suggested that SA could be involved in regulating abscission. SA production could be enhanced by reactive oxygen species accumulation (Lukan & Coll, 2022), as a response to cryptochrome activation (El-Esawi *et al.*, 2017). However, this mechanism could not explain our observations, where SA level in flowers was not influenced by B:R from 1:10 to 1:1 (Fig. 5D). Instead, SA is probably under the control of phytochromes (as discussed in section 4.1), considering SA pathway genes are found as key components in shade avoidance responses (Nozue *et al.*, 2018).

The roles of cytokinins (CKs) in fruit set are broad: CKs stimulate cell division in developing fruits (Vriezen *et al.*, 2008), initiate parthenocarpic fruit set (Sharif *et al.*, 2022), but stimulate lemon pistil abscission in vitro (Sipes & Einset, 1983), and trigger seed abortion when seeds perceive sugar depletion (Botton *et al.*, 2011). The different roles of CKs could relate to the CK type and localization, tissue developmental stage and plant species. High B:R of 9:1 increased only *c*Z and *c*ZR, both belonging to the *cis*-Zeatin type of cytokinins (Supplementary Fig. S5). Higher levels of *c*Z and *c*ZR could suggest a higher biosynthesis through turnover or degradation of tRNAs (Schäfer *et al.*, 2015), which is a different biosynthesis pathway than *trans*-Zeatin type (*t*Z, *t*ZR) and the isopentenyladenine type (iP, iPR) cytokinins. *c*Z type is thought to be much less biologically active compared to *t*Z or iP types. Even though the functions of *c*Z and *c*ZR are not fully clear, their accumulation has been associated with limited growth (Gajdošová *et al.*, 2011; Schäfer *et al.*, 2015). This is in line with the lower individual fruit biomass found at the highest B:R (Fig. 1C). Despite a lack of experimental evidence, Schäfer *et al.* (2015) hypothesized in their review that, *c*Z and *c*ZR would become abundant (instead of *t*Z and *t*ZR) under growth-inhibiting conditions (e.g., in response to stress or at certain developmental stages), to maintain a minimal CK activity necessary for plant survival and subsequent recovery. The highest B:R in our study might be perceived as a growth-inhibiting condition for pepper flowers.

It is noteworthy that hormones and carbohydrates were quantified from a pooled collection of flowers before potential abortion took place, and as such some of these flowers may have set fruit while others aborted (see Fig. 1B for approximate fruit set out of 12 flowers). Therefore, hormone and carbohydrate concentrations in aborted and non-aborted flowers may be more extreme than values presented here (Fig. 3, Fig. 5, Supplementary Fig. S5), however we believe that the general trends still stand.

Taken together, the low IAA, high SA, high *c*Z and *c*ZR, the unreduced ABA and ethylene at the highest B:R, seem to indicate the trade-off balance of flowers is leaning towards defense and dormancy instead of growth (Huot *et al.*, 2014). We suggest this change could potentially trigger more fruit abortion at very high B:R. However, to improve the understanding of the dynamics of hormonal regulation in this response, further investigations would be required to increase the resolution at both spatial and temporal levels.

5 Conclusions

Sweet pepper plants grown under blue:red light ratio (B:R) of 9:1 showed a substantially lower fruit set compared to those under B:R of 1:10, 1:3 or 1:1. The reduction in fruit set at B:R of 9:1 was associated with a low starch accumulation in the flowers at anthesis and 7 days after anthesis, where the abortion was mostly observed between 9 and 16 days after anthesis. The reduced fruit set and starch content matched with the reduced phytochrome photostationary state (PSS) at B:R of 9:1, where the PSS among the other three B:R was barely changed. Thus, we suggest that these responses are likely controlled by phytochromes. The reduced starch content at B:R of 9:1 in flowers seemed to relate to a lower source strength and a drop in sucrose synthases activity. Moreover, on day 7 after anthesis, flowers at B:R of 9:1 contained a lower auxin, higher salicylic acid, higher cytokinin *c*Z and *c*ZR concentration compared to the other B:R. Compared to at anthesis, abscisic acid and ethylene emission on day 7 after anthesis was not reduced at B:R of 9:1 as in the lower B:R. We suggest the reduced floral starch content and the changes in hormonal balance both play a role in reducing fruit set at high B:R of 9:1.

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Supplementary information

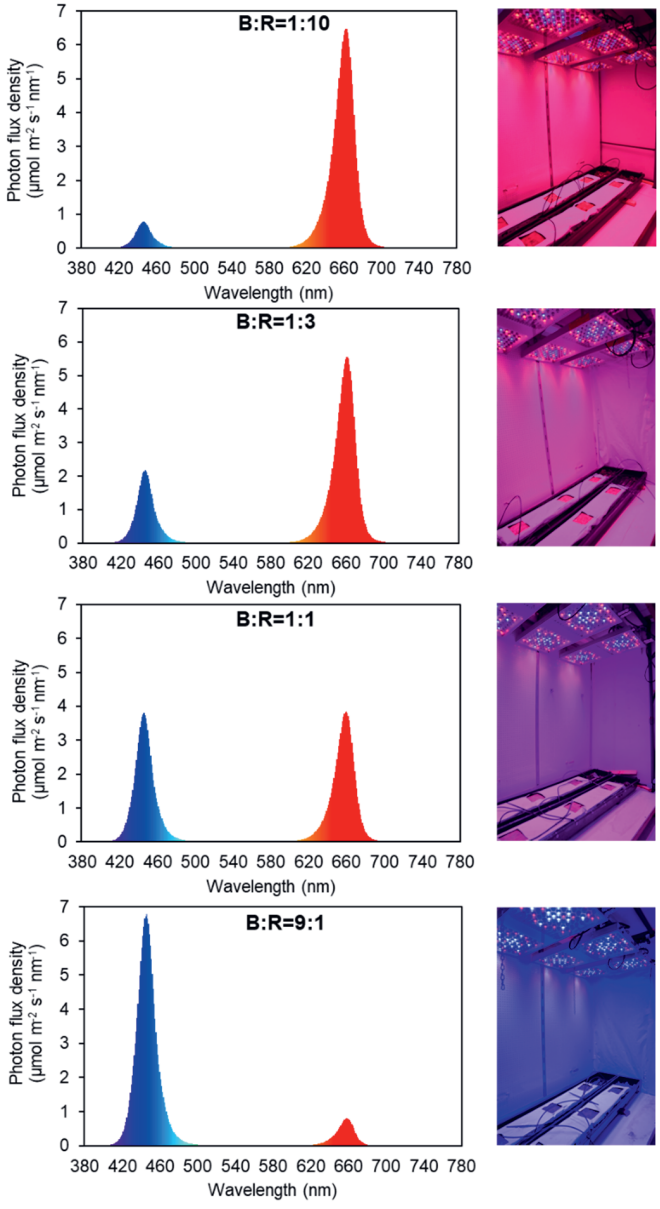

Figure S1. Light spectra of four light treatments with blue:red ratios (B:R) of 1:10, 1:3, 1:1 and 9:1. The light was provided by LED panels (Phytofy® RL, OSRAM GmbH, Berlin, Germany). Light spectra were measured at 15 locations per cell, and at 20 cm below the LED panels with a spectrometer (LI-180, LI-COR Biosciences, Nebraska, USA).

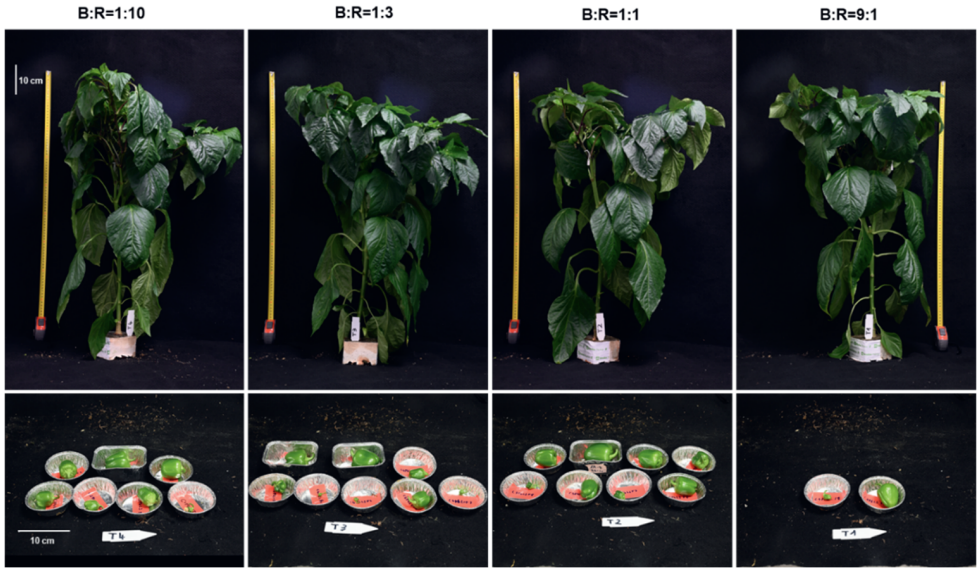

Figure S2. Photos of representative sweet pepper plants and harvested fruits after 35 days cultivation under four light spectra with blue:red ratios (B:R) of 1:10, 1:3, 1:1 or 9:1.

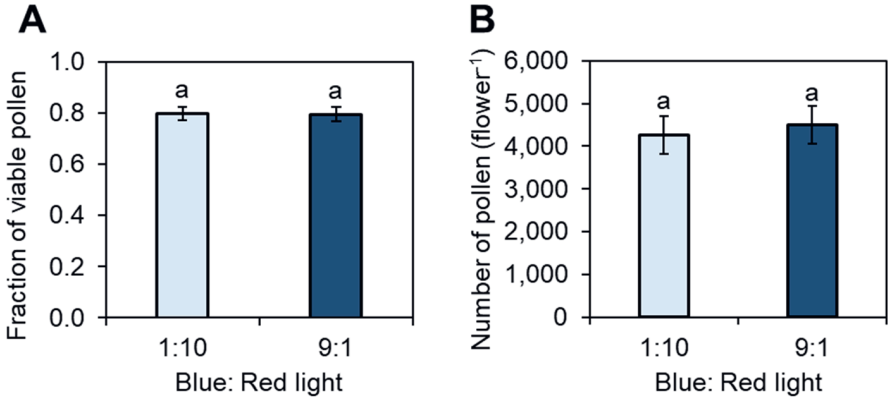

Figure S3. Effect of blue:red light ratios (B:R) on (A) pollen viability and (B) pollen number per pepper flower. Mean values were derived from 4 replicates, where each replicate contained 8 individual flowers. One way ANOVA was performed. Error bars indicate ±standard error of means based on the common variance. Different letters indicate significant differences between treatment means according to Fisher's protected LSD test at P=0.05.

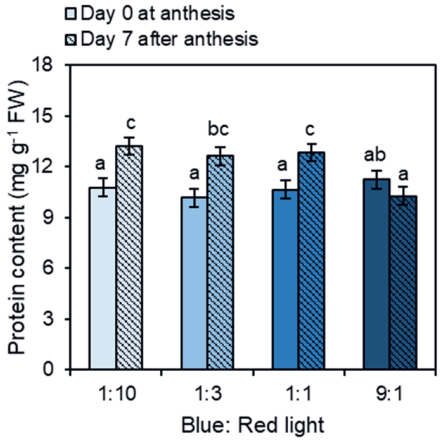

Figure S4. Protein content of pepper flowers on day 0 (at anthesis) and fruits on day 7 after anthesis. Samples were collected in the middle of the day (in the 7th hour of a 14-hour photoperiod). Mean values were derived from 2 statistical replicates, each based on 2 samples (3 plants/sample). Split-plot ANOVA was performed with light treatments as whole plots and developmental stage as sub-plots. Error bars indicate ±standard error of means based on common variance. Different letters indicate significant differences between treatment means according to Fisher's unprotected LSD test at P=0.05.

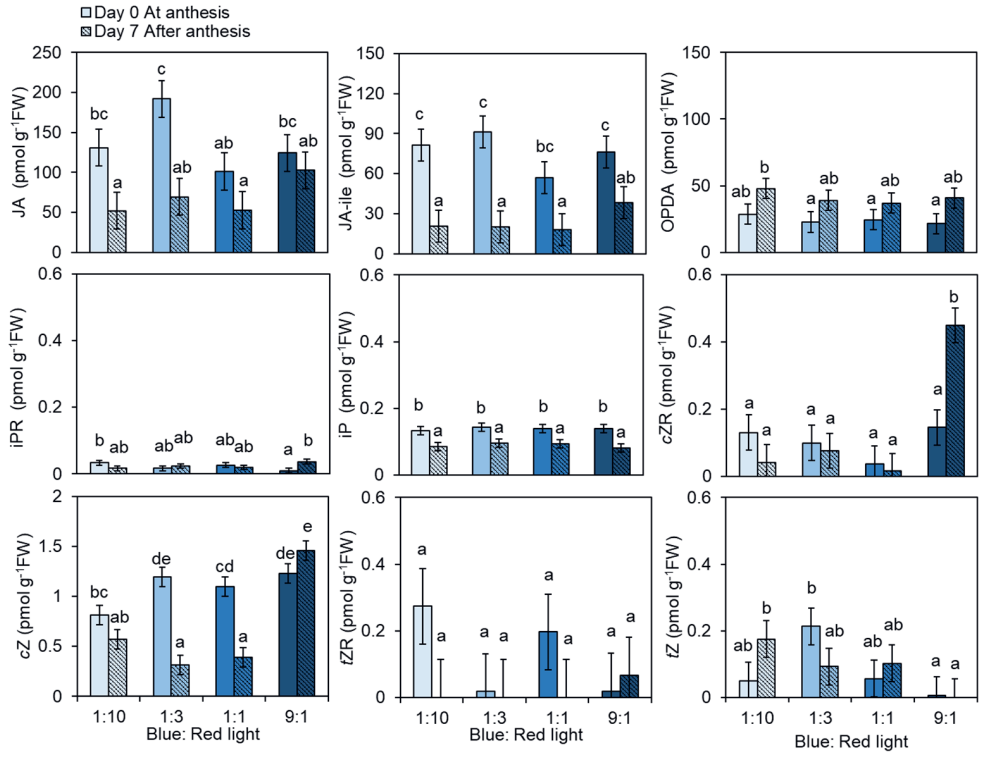

Figure S5. Jasmonic acids and cytokinins concentration of pepper flowers on day 0 (at anthesis) and fruits on day 7 after anthesis. The figures showed the concentration of jasmonic acid (JA), jasmonic acid isoleucine (JA-ile, main active jasmonate), 12-oxo-Phytodienoic acid (OPDA, main jasmonate precursor); and the concentration of each cytokinin: isopentenyl adenosine riboside (iPR), isopentyladenine (iP), *cis*-zeatin riboside (*c*ZR), *cis*-zeatin (*c*Z), *trans*-zeatin riboside (*t*ZR), *trans*-zeatin (*t*Z). Samples were collected in the middle of the day (in the 7th hour of a 14-hour photoperiod). Mean values were derived from 2 statistical replicates, each based on 2 samples (3 plants/sample). Split-plot ANOVA was performed with light treatments as whole plots and floral stage as sub-plots. Residuals of *t*ZR did not follow normal distribution due to a large amount of observations with value 0, thus no measures were taken for data transformation. Error bars indicate ±standard error of means based on the common variance. Different letters indicate significant differences between treatment means according to Fisher's unprotected LSD test at P=0.05.

Table S1. White light spectrum for the plant acclimatization in the first week after moving into the climate chamber. PPFD (photosynthetic photon flux density) = 400-700 nm: blue light = 400-500 nm; green light = 500-600nm; red light = 600-700 nm. UV = 380-400 nm. Far-red = 700-780 nm. The photoperiod was 14 hours. The mean ±standard error of the mean was derived from 8 replicates with 15 measurements per replicate. PSS (Phytochrome Photostationary State) was determined according to Sager *et al.*, 1988.

Light intensity (µmol m^{-2} s^{-1})						PSS	DLI (mol m^{-2} d^{-1})
PPFD	UV	Blue	Green	Red	Far-red		
191 ± 10	0 ± 0	17 ± 1	70 ± 4	105 ± 6	18 ± 1	0.85	9.6

Table S2. Anthesis time (days after light treatment). Pepper flowers at node 7 in replicate experiment 2 were used for biochemical analyses, whilst all other flowers were used for fruit set observation. One way ANOVA was performed, and different letters indicate significant differences between treatment means according to Fisher's protected LSD test at *P*=0.05.

	Replicate experiment 1		Replicate experiment 2		
Blue:red light	Node 6 (d)	Node 7 (d)	Node 7 (d)	Node 8 (d)	Node 9 (d)
1:10	9.5 a	15.2 a	7.8 a	14.6 a	21.6 a
1:3	10.6 a	16.7 a	7.7 a	14.3 a	21.3 a
1:1	10.4 a	16.1 a	6.6 a	12.5 a	20.0 a
9:1	10.2 a	16.3 a	8.2 a	14.2 a	21.3 a
P	0.744	0.546	0.662	0.696	0.780
SE_{mean}	0.713	0.690	0.899	1.305	1.189

Table S3. Multiple reactions monitoring (MRM) transitions table for all plant growth regulators and corresponding internal standards used in this study. *Mass in positive (+) or negative (-) ion mode. ‡Transition used for quantification.

Compound	Retention Time	Mass*	MRM transition	Cone V.	Coll. Energy
IAA	3.34	+176.25	103.2	30	25
			130.2‡	30	15
[13C6]IAA	3.32	+182.1	109.2	30	25
			136.2‡	30	15
SA	3.64	-137.1	93.1‡	40	15
		+139.1	121.0	18	12
[2H4]SA	3.60	-141.1	97.1‡	40	15
		+143.1	124.9	20	14
ABA	4.15	-263.25	153.15‡	30	10
			219.15	30	15
[2H6]ABA	4.12	-269.25	159.15‡	30	10
			225.15	30	15
JA	5.78	-209.06	59.1‡	40	12
		+211.1	133.1	16	16
[2H5]JA	5.76	-214.25	61.9‡	40	12
		+216.2	135.29	14	12
OPDA	12.43	+293.25	95.25	30	22
			133.25	30	20
			275.25‡	30	10
[2H5]OPDA	12.41	+298.25	95.25	30	22
			133.25	30	20
			275.25‡	30	10
JA-Ile	8.38	+324.45	86.25‡	35	20
			151.3	35	15
			278.45	35	10
[2H2]JA-Ile	8.35	+326.4	151.3‡	35	15
			280.45	35	10
iP	8.17	204.1	69.1	40	20
			136.2‡	40	10
[2H2]iP	8.14	210.1	75.08	40	20
			137.1‡	40	10
*t*Z	2.84	220.3	136.25‡	40	15
			148.25	40	15
			202.3	40	10
[2H3]*t*Z	2.80	225.3	137.25‡	40	15
			148.25	40	15
			207.25	40	10

cZ	3.34	220.3	136.25‡	40	15
			148.25	40	15
			202.3	40	10
*t*ZR	5.48	352.1	136.2‡	40	15
			148.2	40	28
			220.2	40	5
$^{[2H5]}$*t*ZR	5.42	357.1	137.2‡	40	15
			148.2	40	28
			225.2	40	10
cZR	6.17	352.1	136.2‡	40	15
			148.2	40	28
			220.2	40	5
iPR	11.84	336.2	136.2‡	50	15
			148.2	50	15
			204.2	50	10
$^{[2H6]}$iPR	11.82	342.3	137.2‡	50	15
			148.2	50	15
			210.2	50	10
ACC	6.38	+272.20	171.05	28	24
			116.00‡	30	46
$^{[2H4]}$ACC	6.40	+276.20	171.05	30	22
			116.00‡	30	46

Table S4. Abortion time (days between abortion and anthesis) of all aborted pepper fruits; fruit age (days between harvest and anthesis) of all harvested pepper fruits at the end of experiments. One way ANOVA was performed, and different letters indicate significant differences between treatment means according to Fisher's protected LSD test at *P*=0.05.

Blue:red light	Time to abortion (d)	Average fruit age (d)
1:10	15.7 b	21.1 a
1:3	14.2 a	20.5 a
1:1	14.7 ab	21.4 a
9:1	14.1 a	18.7 a
P	0.021	0.104
SE $_{mean}$	0.308	0.723

Chapter 5

Additional far-red increases fruit yield of greenhouse sweet pepper mainly through enhancing plant source strength

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Submitted

Abstract

Supplementary lighting is necessary for year-round greenhouse production of fruit vegetables in high-latitude regions. Far-red (FR) radiation can influence plant photomorphogenesis as well as photosynthesis. We aimed to identify the effects of supplementary FR on fruit set and yield of sweet pepper, and its underlying mechanisms via a yield component analysis. A 24-week greenhouse experiment was conducted with cultivars 'Gialte' and 'Margrethe', where FR was added to 190 μmol m^{-2} s^{-1} of white supplementary light in 4 treatments: 0, 50 or 100 μmol m^{-2} s^{-1} FR throughout the whole generative growth phase (since week 8), or 100 μmol m^{-2} s^{-1} FR for only four weeks (week 12 to 16). Fruit yield increased linearly with the cumulative mol of FR in supplementary light. The increased fruit dry weight with additional FR was mainly associated with an increased plant dry weight, accompanied by a marginal increase in the fraction of dry matter partitioned to fruits. The increase in plant dry weight resulted from an increased light use efficiency (plant dry weight per unit of supplementary photosynthetic active radiation (PAR) incident on top of the canopy) and an increased incident PAR (due to taller plants). However, additional FR reduced radiation use efficiency (plant dry weight per unit of total supplementary radiation incident on top of the canopy), indicating that FR was used less efficiently than PAR for biomass production. Additional FR enhanced fruit set percentage and fruit set fluctuations over time, where both long-term and short-term FR application elevated the subsequent fruit set peak after the start of FR application. Additional FR substantially reduced the percentage of fruits with cracking. We concluded that additional FR improved sweet pepper fruit set and yield in greenhouses, mainly by enhancing plant source strength.

Key words:

Far-red; LED lighting; yield component analysis; fruit set fluctuation; *Capsicum annuum* L.

1 Introduction

Light is one of the main limitations for year-round greenhouse production in high-latitude regions, due to the low light intensities and short days especially in winter. Supplemental lighting, therefore, is often used to enable year-round production and improve product quality (Davis & Burns, 2016). Light-emitting diodes (LEDs), as a promising source of supplemental lighting (Davis & Burns, 2016), provides opportunities to manipulate plant growth and development via light spectrum. Sweet pepper, as one of the main greenhouse crops, is popular for its fruits' color, taste and high nutrient value (Pérez-López *et al.*, 2007). However, the greenhouse production of sweet pepper is hampered by low and fluctuating fruit set and yield, where periods with high fruit set and yield alternate with periods with low fruit set and yield (Heuvelink *et al.*, 2004; Wubs *et al.*, 2009).

Far-red (FR, 700-800 nm) radiation is an important component of the natural light spectrum, while it is often lacking in supplementary lighting. If supplementary light lacks FR during winter cultivation, peppers tend to have short internodes resulting in fruit stacking, which can negatively affect fruit shape and increase labor costs for plant maintenance and harvest (Lanoue *et al.*, 2022). A low red: far-red ratio leads to a low photostationary state of phytochrome (PSS), defined as the fraction of active phytochromes in the total phytochromes (Sager *et al.*, 1988). Low PSS triggers a series of shade avoidance syndrome responses, such as increased stem length, leaf hyponasty and reduced branching (Franklin, 2008). These adaptations serve to enhance light interception and overtop competing vegetation, and eventually to enhance the probability of reproductive success (Franklin, 2008). Other than its effect on photomorphogenesis, FR can also enhance photosynthesis when combined with photosynthetic active radiation (PAR, 400-700 nm; Emerson *et al.*, 1957; Zhen & Bugbee, 2020).

Additional FR has been shown to increase fruit yield in sweet pepper greenhouse cultivation (Hao *et al.*, 2018; Kim & Son, 2022; Kim *et al.*, 2023a; Schuddebeurs, 2021). However, the mechanisms explaining such an increase are still largely unknown. In tomato, the increased yield by additional FR in greenhouses (Dorokhov *et al.*, 2021; Hao *et al.*, 2016; Kalaitzoglou *et al.*, 2019) was partly associated with increased total plant biomass, which could result from a higher light interception (Hao *et al.*, 2016; Kalaitzoglou *et al.*, 2019). Light interception can be greatly influenced by leaf area, leaf shape and internode length (Kalaitzoglou *et al.*, 2019; Sarlikioti *et al.*, 2011), which are under the control of PSS. The increased tomato yield by additional FR was also related to promoted biomass partitioning to fruits, mainly due to an enhanced fruit sink strength (Ji *et al.* 2020). Moreover, additional FR accelerated flowering and increased fruit number in tomato (Ji *et al.*,

2019; Kalaitzoglou *et al.*, 2019). In sweet pepper, the increase in plant biomass could also partly explain the increased yield as shown by Kim *et al.* (2023a). However, no report shows if the FR-enhanced yield in sweet pepper is related to a higher biomass partitioning to fruits, which is determined by fruit sink strength and the number of fruits. Some studies suggest that additional FR increased the individual weight of sweet pepper fruits (Kim & Son, 2022; Kim *et al.*, 2023a); whilst the FR effect on sweet pepper fruit set in greenhouse remains ambiguous.

The increased yield in greenhouse studies (Hao *et al.*, 2018; Kim & Son, 2022; Kim *et al.*, 2023a; Schuddebeurs, 2021) could be contrasting to previous observations in climate chambers, where additional FR reduced fruit set (Chapter 2, 3). It is possible that the positive effect of FR on fruit size may outweigh its negative effect on fruit set, resulting in a higher yield with lower fruit number. It is also possible that additional FR did not reduce fruit number in greenhouses, for a few potential reasons: the dose of additional FR in the existing greenhouse studies might be too low to stimulate flower and fruit abortion; a higher daily light integral in greenhouses may reduce the negative effect of FR on fruit set; and the FR effect on fruit set may depend on background light spectrum.

Sweet pepper plants, especially cultivars with large fruits, show strong fluctuations in fruit set. Earlier formed fruit stimulates abortion of later flowers and fruits, due to a dominance effect and competition for assimilates (Marcelis & Hofman-Eijer, 1997; Wubs *et al.*, 2009). Additional FR can potentially regulate fruit set fluctuation: additional FR may lead to a stronger fruit set fluctuation due to its potential effect of increasing individual fruit sink strength, or a less pronounced fluctuation due to its potential effect of reducing fruit set. Moreover, additional FR possibly enables flexible fruit set regulation. For example, if a short-term application of additional FR reduces a fruit set peak, the following bottom in the fruit set cycle would be elevated automatically due to the interaction between fruits, leading to a less pronounced fruit set fluctuation.

Yield and fruit quality are both of high economic importance. Appearance, color, texture, and flavor are important quality attributes for sweet pepper (Sanatombi & Rajkumari, 2020). Additional FR influenced fruit biochemical attributes and improved taste of sweet pepper (Kim & Son, 2022; Kim *et al.*, 2023b). Fruit cracking, a disorder that is observed on the fruit skin and flesh also severely influences fruit quality in sweet pepper (Aloni *et al.*, 1998; Khadivi-Khub, 2015), where the effect of FR on fruit cracking is still unknown.

We aimed to identify the effects of additional FR on fruit yield of sweet pepper in greenhouses, and its underlying mechanisms. For this purpose, a yield component analysis was used, where the contribution of different morphological and

physiological processes to fruit yield can be evaluated systematically and quantitatively (Higashide & Heuvelink, 2009). In addition, we intended to test whether additional FR would affect fruit set of sweet pepper in greenhouses, when plants were grown as in commercial production. Lastly, we intended to test if additional FR influences fruit quality. To address these objectives, different levels of additional FR were applied together with supplemental lighting in greenhouses, and a short-term application of FR was tested in an attempt to regulate fruit set pattern.

2 Materials and Methods

2.1 Plant material and growth conditions

The experiment was conducted in Venlo glasshouses at Wageningen, the Netherlands (52.0°N, 5.7 °E) from October 17th, 2022 until March 31st, 2023 (24 weeks). Two commercial cultivars 'Gialte' (yellow fruit, Enza Zaden, the Netherlands) and 'Margrethe' (red fruit, Enza Zaden, the Netherlands) of sweet pepper (*Capsicum annuum* L.) were grown. On 17th October, 2022, six week old sweet pepper plants grown on stone wool cubes (10x10x6.5cm, Grodan, the Netherlands; plants obtained from Beekenkamp Plants, the Netherlands), were transplanted on stone wool slabs (100x15x7.5cm, Grodan, the Netherlands) in two adjacent glasshouse compartments, each 144 m^2 with eight single gutters, where gutters were placed at 1.5 m distance.

The average temperature inside the glasshouse compartments was 21.5/18.4 °C (day/night), with an average relative humidity of 70/65% (day/night) and CO_2 concentration of 493/513 µmol mol^{-1} (day/night) (Supplementary Fig. S1A). Plants were irrigated with a drip system (Supplementary Fig. S1B) with nutrient solution (electrical conductivity 3.0 dS m^{-1}, pH 6.0) containing 0.5 mM NH_4^+, 8.4 mM K^+, 6.2 mM Ca_2^+, 1.9 mM Mg_2^+, 19.2 mM NO_3^-, 2.2 mM SO_4^{2-}, 1.6 mM PO_4^{2-}, 25 µM Fe^{3+}, 10 µM Mn^{2+}, 5 µM Zn^{2+}, 30 µM B^+, 0.75 µM Cu^{2+}, and 0.50 µM MoO_4^{2-}.

Planting density was 2.67 plants m^{-2}. Sweet pepper plants show dichotomous branching, where we coded the first splitting node as node 0. The plants were pruned to two main stems by pruning side shoots above their first node. Sweet pepper plants usually have one flower per node, where flowers at node 1 and node 2 (counted from bottom to top) were removed at anthesis.

Supplementary light with an intensity of about 190 µmol m^{-2} s^{-1} of PPFD (photosynthetic photon flux density, 400-700 nm) was provided by LEDs (VYPR R8, Fluence, Austin, USA), which light spectrum consisted of 7.1% blue (400-500 nm), 10.6% green (500-600 nm), 80.9% red (700-800 nm), 1.4% far-red (FR, 700-800nm)

(Table 1; Supplementary Fig. S1C). The light intensity and spectrum was quantified with a spectroradiometer (LI-180 by LI-COR Biosciences) after sunset at 2 m above ground. From day 15 after transplanting, the supplementary lighting was applied 4 hours day^{-1}. The hours of supplementary lighting increased gradually based on the growth of the plants, until 16 hours day^{-1} was applied from day 88 after transplanting until the end of experiment (Supplementary Fig. S1D). The supplementary lighting was always turned off half an hour before sunset. Over the whole cultivation, 40% of incident PAR in the greenhouse was from solar radiation (Supplementary Fig. S1D), and 60% from supplementary lighting.

Table 1. Light treatments with different levels of additional far-red (FR) (spectrum in Supplementary Fig. S1C). PPFD (photosynthetic photon flux density) indicates light within 400-700 nm, and FR indicates light within 700-800 nm. FR 0, FR 50, or FR 100 indicates 0, 50 or 100 µmol m^{-2} s^{-1} of additional FR from day 50 after transplanting until the end of the experiment (day 166); FR short indicates 100 µmol m^{-2} s^{-1} of additional FR for only four weeks (from day 78 to 106 after transplanting). Mean ± SEmean were based on two replicates, where each replicate was based on 37 measurements at 2 meters above ground. Phytochrome photostationary state (PSS) of the supplementary light was calculated according to Sager *et al.* (1988). R:FR indicates the ratio between red (600-700 nm) and FR light of the supplementary light.

FR treatments	PPFD (µmol m^{-2} s^{-1})	FR (µmol m^{-2} s^{-1})	PSS	R:FR ratio
FR 0	191.9 ± 2.5	1.5 ± 0.1	0.88	102.5
FR 50	192.8 ± 0.3	55.9 ± 0.5	0.81	2.9
FR 100	192.8 ± 1.1	101.4 ± 0.7	0.77	1.6
FR short	193.1 ± 1.6	100.8 ± 1.0	0.77	1.6

2.2 Light treatments and experimental set-up

Two glasshouse compartments were used as two replicates. Each glasshouse compartment was divided into four plots with white reflective plastic film and four FR treatments were distributed over these four plots. FR was provided by LEDs (Greenpower FR production module 3.1, Philips, the Netherlands) with its peak at 737 nm (light spectrum in Supplementary Fig. S1C). Plants received either 0, 50 or 100 µmol m^{-2} s^{-1} additional FR since most flowers at node 5 reached anthesis until end of experiment (day 50-166 after transplanting), or 100 µmol m^{-2} s^{-1} additional FR for only four weeks (FR short; day 78-106 after transplanting) (Table 1). Additional FR was always applied at the same time as supplementary PPFD (Supplementary Fig. S1D).

Within every plot, one cultivar was grown on the two left gutters, and the other one on the two right gutters (Supplementary Fig. S2). The locations of cultivars were opposite between two glasshouse compartments. In the middle of each plot, eight

plants per cultivar were randomly selected as measurement plants for all measurements; except that spare plants under the same treatments were used to determine leaf proportionality factor α (section 2.3.2), potential fruit growth (section 2.4.2), fruit quality (section 2.4.3), leaf color and photosynthetic pigment contents (section 2.5.2).

2.3 Non-destructive measurements

2.3.1 Plant development

The height and node number of plants were recorded every week. The plant height was determined as the average height of two main stems from the stone wool surface until apex. The node number as the average of two main stems, was counted from bottom to top until the youngest internode longer than 2 cm. Anthesis of all flowers was recorded three times a week together with their location (node number).

2.3.2 Leaf area index

Leaf area index at week 5, 10 and 15 after transplanting was determined non-destructively by measuring the width and length of leaves from four measurement plants per cultivar per plot. A proportionality factor (α) between measured leaf area and the product of leaf length and width was determined based on nine randomly selected full-grown leaves from three spare plants per cultivar per plot. The proportionality factor α was 0.647 for 'Gialte' (no significant difference between light treatments), and 0.630, 0.631, 0.654, and 0.659 for 'Margrethe' for treatment FR 0, FR 50, FR 100 and FR short, respectively. Leaf area index was calculated using the sum of all individual leaf areas for a plant, multiplied by planting density.

2.3.3 Leaf photosynthesis

Leaf photosynthesis rates were measured between week 13-16 after transplanting, with a Licor-6800 (LI-COR biosciences, Nebraska, USA). Six leaves (from both stems of three measurement plants) per cultivar per plot were used for photosynthesis measurements on cloudy days, with one measurement per leaf. Using the topmost mature and fully green leaf, the net photosynthesis rate of a 6 cm^2 area under prevailing light was measured. Relative humidity and temperature values in the measurement chamber were set the same as the greenhouse conditions, and CO_2 concentration was set at 400 ppm. Due to the variations in light intensity for each leaf, the leaf photosynthesis rate was expressed as the ratio between instantaneous photosynthesis and the incident PPFD recorded by the inner sensor of the Licor-6800.

2.4 Fruit measurements

Harvested fruits were classified as ripe fruits (fully colored), which were harvested twice a week; and mature green fruits (not fully colored but have reached their final size), which were only harvested at the end of the experiment (section 2.4.1). Harvested fruits were used to determine fruit yield (fruit fresh weight/m^2). Immature fruits, before mature green stage, were collected at the end of the experiment (section 2.5.1), which were included in fruit dry weight and dry matter partitioning, but not considered as fruit yield.

2.4.1 Weekly fruit harvest

At harvest of each ripe fruit, the node number as fruit location was recorded; the fresh weight was measured with an analytical balance; the maximum diameter and height was measured with a digital caliper; and the peduncle length was measured with a soft measure tape. By cutting fruits into 3-4 pieces along their longitude, the thickness at the middle of the pericarp was measured three times per fruit with a caliper. The individual fruit dry weight was determined after drying the cut open fruits at 105 °C for 72 hours. Number of seeds were counted from 16 random fruits per cultivar per plot. One week before the end of the experiment, all mature green fruits were harvested along with ripe fruits. For mature green fruits, only their locations on the plant, and the fresh and dry weight per plant were determined. Fruit yield includes both ripe fruits and mature green fruits.

2.4.2 Potential fruit growth

Nine spare plants were randomly selected per cultivar per plot in treatments FR 0, FR50, and FR100: Six plants were pruned to have only one fruit per stem (three plants with weekly fruit measurement and the other three without), and three plants were pruned to have two fruits per stem. The retained fruits had their anthesis after the start of FR treatments. These fruits were harvested when they were fully colored in the same way as described in section 2.4.1. Considering that individual fruit dry weight from plants with one fruit per stem was generally higher than those with two fruits per stem (Supplementary Table S1), the former one was taken as potential fruit growth, i.e., fruit growth under non-limiting assimilate supply.

2.4.3 Fruit quality at harvest

Three randomly selected ripe fruits per cultivar per plot were harvested from spare plants in treatments FR 0, FR50, and FR100. Their color was measured with a Chroma meter CR-400 (Konica Minolta Sensing Inc., Japan) using Cielab color

space (L, a*, b*, Chroma, and hue) with five measurements per fruit. In the Cielab color space, L indicates luminescence, a* indicates green-red color (negative to positive respectively), b* indicates blue-yellow (negative to positive respectively), chroma indicates a quantitative attribute of colorfulness, and Hue angle indicates a qualitative attribute of color. The firmness of individual fruits was measured using a fruit texture analyzer GS15 (Güss, Germany), using a 4 mm cylindric flat-ended probe with a 5 mm/s test speed, recording the maximum force (N) needed to penetrate the pericarp of a whole fruit. °Brix and acidity were measured with a Pocket Brix-Acidity Meter PAL-BX (Atago Inc., Japan) and acidity was expressed as a percentage based on sample fresh weight (citric acid as the predominant acid).

2.5 Destructive measurements

2.5.1 Plant growth and development

One day before transplanting, eight randomly selected plants were used to determine initial plant biomass, plant height and number of nodes (as described in section 2.3.1). All leaves longer than 3 cm were separated from the stem, and leaf area was measured per plant with a leaf area meter (LI-3100 area meter, Li-Cor).

In week 24, the eight measurement plants per cultivar per plot were used for measuring final plant height and number of nodes. Leaves and stems of these plants were separated. Fresh weight of leaves, stems, and leaf area were measured. Afterwards, the stem and leaves were dried in a ventilated oven at 105 °C for 24 hours to determine their dry weight. Leaf area index was calculated as leaf area per plant multiplied by planting density. Immature fruits were collected from each measurement plant, where the number of fruits, their locations on the plant, and their fresh weight per plant were recorded. After cutting these immature fruits open, they were dried at 105 °C for 72 hours to determine dry weight.

2.5.2 Leaf color and photosynthetic pigments quantification

One week before the end of the experiment, topmost mature leaves (fully green leaves at node 20 ±1) were collected from three spare plants per cultivar per plot, to measure leaf color with a color meter as described in section 2.4.3. One leaf disc of 1.5 cm diameter per leaf was collected and frozen in liquid nitrogen for chlorophyll and carotenoid quantification. The three leaf discs from three plants were pooled as one sample, which was ground together with liquid nitrogen until obtaining a fine powder. Chlorophyll a, b, and carotenoids were assessed according to López-Hidalgo *et al.* (2021). In brief, the samples were mixed well with 1 ml of cold 80% ethanol, followed by an incubation in ice for 5 minutes, then the tubes were centrifuged for 10 minutes at 10000 g (4°C). Afterwards, the samples were diluted 1:5 and measured three times as technical replicates (150 µl per

technical replicate) in a clear 96-well microplate, where the absorbance was measured at 470, 649, and 660 nm. Chlorophyll a (Chl a) was calculated as $13.36*A_{664} - 5.19*A_{649}$; chlorophyll b (Chl b) was calculated as $27.43*A_{649} - 8.12*A_{664}$; and carotenoids were calculated as $(1000*A_{470} - 2.13*\text{Chl a} - 97.64*\text{Chl b})/209$.

2.6 Yield component analysis

Yield component analysis adapted from Higashide & Heuvelink (2009) was used. For this analysis, total fruit dry weight and the number of fruits includes all fruits harvested and collected during the experiment: ripe fruits, mature green fruits, and immature fruits. Total plant dry weight (aboveground part) includes dry weight of all harvested and collected fruits, leaves and stems. The fraction of dry matter partitioned to fruits was calculated as total fruit dry weight as a percentage of the total plant dry weight. Flower appearance rate was determined as the slope of the linear regression between anthesis location (node number) and anthesis date. Fruit set percentage was determined as the number of fruits as a percentage of the number of flowers (until two weeks before the end of the experiment). Potential fruit growth as a measure for fruit sink strength was estimated as described in section 2.4.2. Photosynthesis rate was measured according to section 2.3.3. Final leaf area index was obtained as described in section 2.5.1.

Based on the relationship between light intensity and the distance between top of the plant and the LED lamps (Supplementary Fig. S3), integral of PPFD or FR was calculated with the consideration that the weekly light intensity at the top of canopy depends on plant height, which varied in different treatments. The integral of PPFD or FR indicates the cumulative mol of supplementary light within the range of 400-700 nm or 700-800 nm from transplanting until the end of the experiment. Light use efficiency (LUE) was calculated as the ratio between total plant dry weight and the integral of supplementary PPFD incident on top of the canopy. Radiation use efficiency (RUE) was calculated as the ratio between the total plant dry weight and the integral of supplementary PPFD plus FR incident on top of the canopy.

2.7 Statistical analysis

The four light treatments were repeated in two glasshouse compartments, as two statistical replicates (blocks). Each cultivar was analyzed separately, thus one-way Analysis of Variance (ANOVA) in two blocks was applied (Genstat 22nd edition). Fisher's protected LSD test at P=0.10 was used for mean separation. Coefficient of variation (cv%) was calculated with weekly fruit set as an indicator of the fruit set fluctuation. Simple linear regression was performed for fruit fresh yield, number of harvested fruits, and all the components in the yield component analysis with the FR integral as regressor. Linear regression was considered significant when $P < 0.1$.

When a variable shows a linear trend in response to FR integral, predicted values were used to calculate the relative change in FR 100 compared to FR 0 treatment in the yield component analysis, otherwise the measured values were used instead.

3 Results

3.1 Additional FR increased fruit yield and harvested fruit number

The fruit yield and the number of harvested fruits both linearly increased with the amount of additional FR (FR integral over the whole cultivation period; Supplementary Table S2) for both cultivars (Fig. 1). The harvested fruits include weekly harvested ripe fruits and the mature green fruits harvested at the end of the experiment. Ripe and mature green fruits collectively contributed 80-88% of the total fruit dry weight, with immature fruits constituting the remainder (Supplementary Fig. S4).

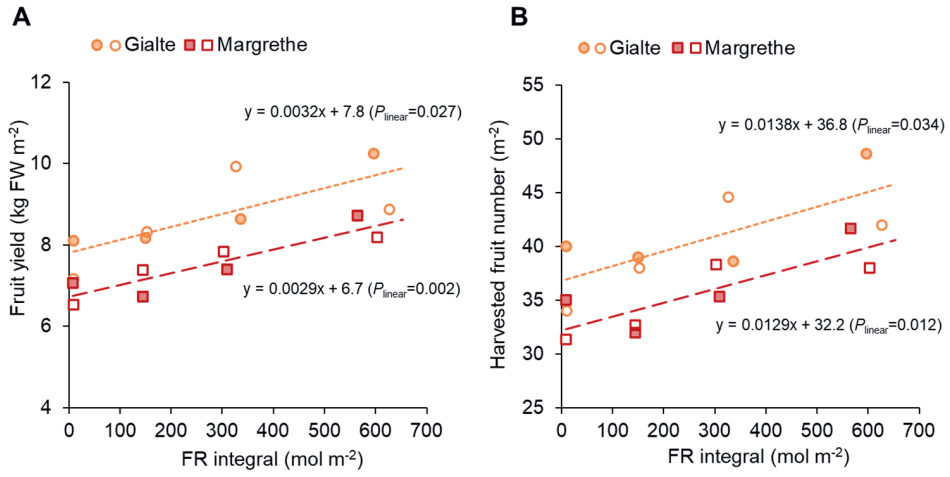


Figure 1. Effect of far-red (FR) on fruit yield and the number of harvested sweet pepper fruit. FR integral indicates the total mol of FR from supplementary lighting during the whole experiment (Supplementary Table S2). FW indicates fresh weight. Circles = 'Gialte'; squares = 'Margrethe'. The linear regression was performed with all four treatments, with two replicates per treatment (closed symbols - Compartment A; open symbols - Compartment B), where each replicate was based on eight individual plants.

3.2 Additional FR resulted in stronger fruit set fluctuation

Fruit set showed 3-4 cycles over the 24 weeks of cultivation (Fig. 2). 'Gialte' plants grown with no additional FR (FR 0) had relatively stable fruit set of 1-1.5 fruits/week from week 7 to 15 (Fig. 2A). Compared to no additional FR, plants grown with long-

term additional FR (FR 50, FR 100), which started at week 8, showed a stronger fluctuation in fruit set. This was shown as a high first peak of about 3 new fruits in week 10, a low bottom of almost no new fruit in week 13, a second peak of about 2 fruits in week 15, and a second bottom of about 0.5 fruits in week 18 (Fig. 2A). Short-term FR application (FR short) during weeks 12 to 16 did not influence the fruit set bottom in week 13, but elevated the second peak in week 15, which was higher than all other treatments (Fig. 2A). Similarly, for 'Margrethe', fruit set at long-term FR application reached a higher first peak compared to no additional FR, and short-term FR application enhanced the second peak of fruit set in week 15 (Fig. 2B). The stronger weekly fruit set fluctuation was also reflected in a higher coefficient of variation in treatments with additional FR, especially short-term FR application (Supplementary Table S3).

Despite a similar peak height, adding 100 µmol m^{-2} s^{-1} FR led to slightly wider peaks compared to 50 µmol m^{-2} s^{-1} FR (Fig. 2), which matched with the higher cumulative fruit number at 100 µmol m^{-2} s^{-1} of additional FR (Fig. 1; Supplementary Fig. S4). Fluctuations in weekly fruit set resulted in fluctuations in weekly fruit yield (Supplementary Fig. S5).

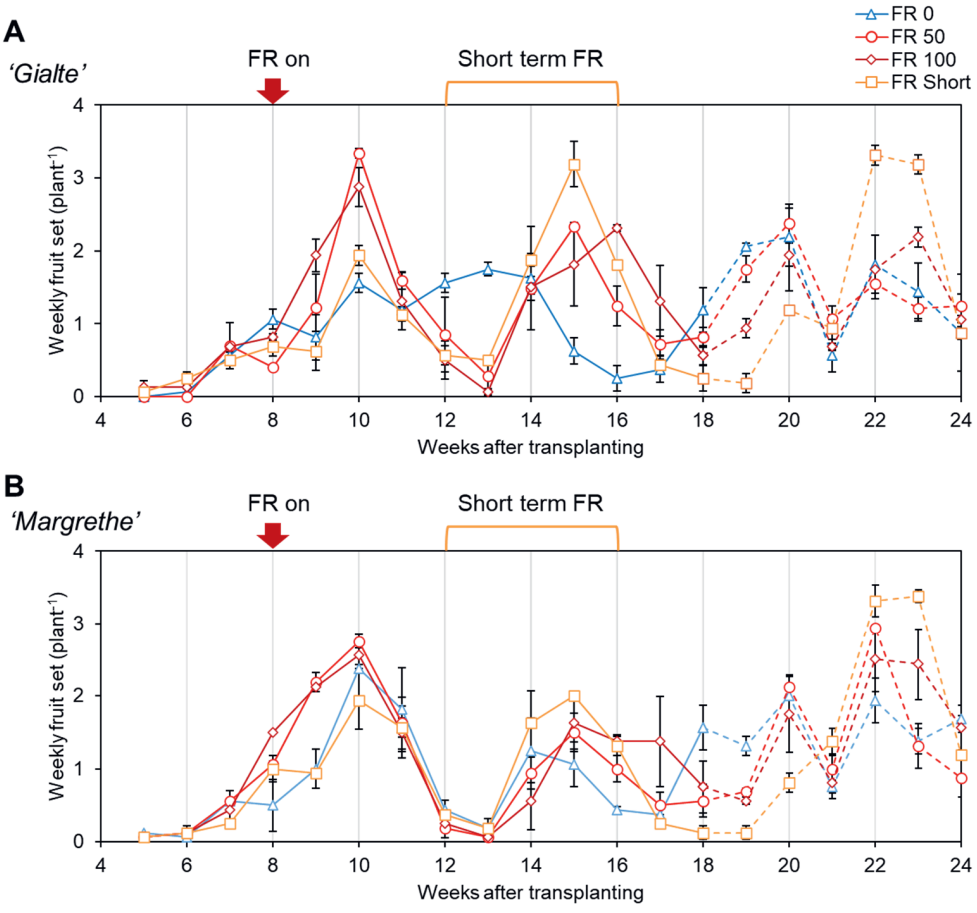


Figure 2. Additional far-red (FR) led to stronger fruit set fluctuations in sweet pepper (A) 'Gialte' and (B) 'Margrethe'. Weekly fruit set indicates the number of newly appeared flowers per week, which had developed into fruits (older than 2 weeks) at the end of the experiment. Solid lines indicate the set fruits that had reached ripe or mature green stage, and dashed line indicates the set fruits that were immature. 'FR 50' and 'FR 100' indicates 50 or 100 µmol m^{-2} s^{-1} of additional FR in the supplementary light since week 8 (day 50-166 after transplanting), and 'FR Short' indicates 100 µmol m^{-2} s^{-1} of additional FR in the supplementary light from week 12 to 16 (day 78-106 after transplanting). Mean values were derived from two replicate plots, where each replicate value was based on eight individual plants. Error bars indicate ±standard error of mean.

3.3 Yield component analysis

Corresponding to the increase in fresh yield, total fruit dry weight also increased linearly with FR integral in both cultivars (Fig. 3; linear regression output Supplementary Table S4). Adding 100 µmol m^{-2} s^{-1} FR led to 24% increase in total fruit dry weight in 'Gialte' and 31% in 'Margrethe' compared to no additional FR. This increase was mainly attributed to an increase in total plant dry weight (19% for 'Gialte' and 26% for 'Margrethe'), with a small contribution from the increased fraction of dry matter partitioned to the fruits (only 4.5% and 4.3% respectively) (Fig. 3).

The increase in plant dry weight was related to increased incident light (Fig. 3) as a result of taller plants under additional FR (Table 2; Supplementary Fig. S6). Additional FR increased light use efficiency (LUE) but decreased radiation use efficiency (RUE) of supplementary light incident on top of the canopy (Fig. 3). Additional FR did not affect leaf area index (LAI) for 'Margrethe' (Fig. 3; Table 2). For 'Gialte', adding 50 µmol m^{-2} s^{-1} FR increased LAI, but adding 100 µmol m^{-2} s^{-1} FR decreased LAI, compared to no additional FR (Table 2). The LAI development over time was hardly affected by additional FR in both cultivars (Supplementary Fig. S7).

Additional FR increased leaf photosynthesis rate in 'Margrethe' but decreased that in 'Gialte', although these observations were not statistically significant (Fig. 3; Supplementary Fig. S8A). Additional FR tended to decrease the stomata conductance of 'Gialte' leaves but did not affect that for 'Margrethe' (Supplementary Fig. S8B). Moreover, from week 12 onwards, some leaves at the top 3-4 nodes of 'Gialte' showed chlorosis, especially plants grown with additional FR (Supplementary Fig. S9). Leaves of 'Margrethe' hardly showed chlorosis even when grown with additional FR (Supplementary Fig. S9). According to a sweet pepper cultivation advisor (Daan Boonman, Vortus B.V., personal communication), the slight leaf chlorosis was also seen in commercial practice with heavy fruit load and not a problem, however, it may have affected leaf photosynthesis measurements.

Supporting the observations in leaf photosynthesis, additional FR tended to increase leaf chlorophyll and carotenoid content in 'Margrethe' but tended to decrease these slightly in 'Gialte' (Supplementary Fig. S8C, E). For both cultivars, the ratio between chlorophyll a and b was hardly affected by additional FR (Supplementary Fig. S8D). The effect of additional FR on leaf color parameters was opposite in two cultivars (Supplementary Fig. S8F-J). Additional FR tended to cause leaf color to be lighter in 'Gialte', whereas additional FR tended to cause leaf color to be slightly darker in 'Margrethe' (Supplementary Fig. S8F).

The slight increase in dry matter partitioning to fruits with additional FR resulted from a higher fruit number for both cultivars, which mainly resulted from a higher fruit set percentage (Fig. 3). Additional FR did not affect flower appearance rate in 'Margrethe' but reduced that in 'Gialte' (Fig. 3). The potential fruit growth - estimated from the individual fruit dry weight when there was only one fruit per stem - was enhanced by additional FR to a different extent for both cultivars (Fig. 3). An increased fraction of dry matter partitioned to fruits under additional FR was associated with a decreased fraction of dry matter partitioned to leaves, whilst the fraction of dry matter partitioned to stems remained unaffected in both cultivars (Table 2).

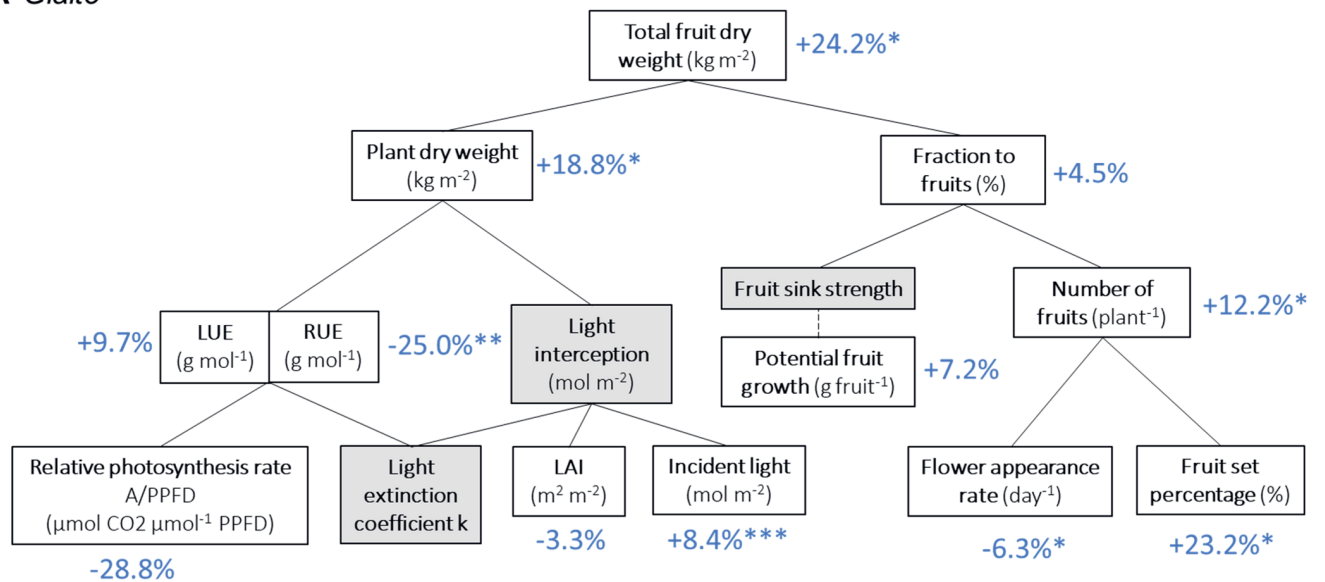
Additional FR increased plant height and did not affect the number of nodes or specific leaf area (Table 2; Supplementary Fig. S6; Supplementary Table S5). Additional FR also significantly increased dry matter content of leaves and stems (Supplementary Table S5).

3.4 Effect of additional far-red on fruit morphology and fruit quality

Additional FR did not influence the fresh or dry weight of individual fruits for 'Margrethe', but FR 50 led to significantly heavier fruits for 'Gialte' compared to the other three treatments (Table 3). The average fruit fresh weight at each node varied only slightly along the plant development, with a small reduction during periods with high fruit load (Supplementary Fig. S10). Additional FR did not influence the fruit shape for 'Margrethe'; but slightly increased fruit diameter for 'Gialte', where FR 50 showed the largest diameter (Table 3). Additional FR also led to a slight but statistically significant increase in pericarp thickness for 'Margrethe' (Table 3). For both cultivars, additional FR increased the length of fruit peduncles by a few millimeters (Table 3). Fruit dry matter content was generally higher for 'Margrethe' than for 'Gialte', while it was unaffected by additional FR. Additional FR also did not affect seed number per fruit (Table 3).

Additional FR did not influence fruit quality in terms of fruit color, firmness, °brix, and acidity (Supplementary Table S6). Additional FR reduced fruit cracking (Fig. 4). For both cultivars grown without additional FR, more than half of the ripe fruits showed fruit cracks varying from slight to severe, and 'Margrethe' seemed to be more susceptible than 'Gialte'. Additional FR, even a short-term FR application for four weeks, substantially reduced the percentage of fruits with cracks and the higher dose of additional FR led to stronger reduction in fruit cracking. Additional FR of 100 µmol m^{-2} s^{-1} led to about only 20% and 25% fruits with cracks (mostly at the slight level) in 'Gialte' and 'Margrethe' respectively (Fig. 4).

A *'Gialte'*


Total fruit dry weight (kg m-2)
+24.2%*
Plant dry weight (kg m-2)
+18.8%*
Fraction to fruits (%)
+4.5%
+9.7%
LUE (g mol-1)
RUE (g mol-1)
-25.0%**
Light interception (mol m-2)
Fruit sink strength
Potential fruit growth (g fruit-1)
+7.2%
Number of fruits (plant-1)
+12.2%*
Relative photosynthesis rate A/PPFD (µmol CO2 µmol-1 PPFD)
-28.8%
Light extinction coefficient k
LAI (m2 m-2)
-3.3%
Incident light (mol m-2)
+8.4%***
Flower appearance rate (day-1)
-6.3%*
Fruit set percentage (%)
+23.2%*

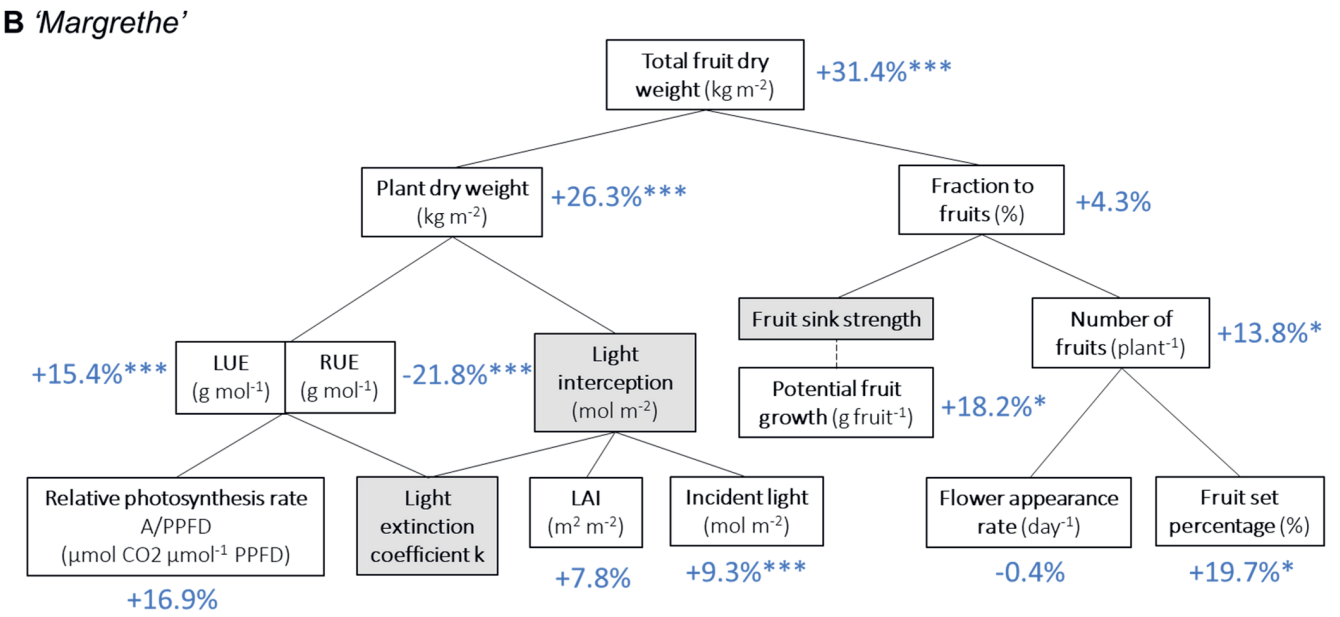


Figure 3. Effect of additional far-red (FR) on yield components in sweet pepper (A) 'Gialte' and (B) 'Margrethe' after 24 weeks of cultivation. For each component, the relative change (%) in treatment FR100 compared to FR0 was based on linear regression with FR integral as regressor (FR integral in Supplementary Table S2). Data used for the yield component analysis and linear regression output are shown in Supplementary Table S4. The predicted values from linear regression outputs were used, except for LAI in 'Gialte', where measured values were used as here LAI did not show a linear relationship with FR integral. PPFD (photosynthetic photon flux density) indicates light within 400-700 nm. LUE indicates light use efficiency (plant dry weight/supplementary PPFD integral of 400-700 nm). RUE indicates radiation use efficiency (plant dry weight/supplementary PFD integral of 400-800 nm). LAI indicates leaf area index (leaf area/ground area). Incident light indicates the integral of PPFD, which is the cumulative mol of PPFD incident on top of the canopy from supplementary light during the whole experiment. Incident light was affected by FR because of the FR effect on plant height. Grey components were not quantified. Potential fruit growth was estimated as the individual fruit dry weight when there was only one fruit per stem. Asterisks denote significant effects of additional FR in linear regression (*P<0.1, **P<0.01, ***P<0.001).

Table 2. Effect of additional far-red (FR) on plant growth parameters after 24 weeks of cultivation. DM indicates dry matter (dry weight). FR lighting in treatment FR 50 and FR 100 starts in week 8 after transplanting, and the short-term FR treatment (FR Short) starts in week 12 and lasted for 4 weeks. The presented mean values were derived from two statistical replicates, each based on eight individual plants. Every plant was pruned to have two main stems. Standard error of mean was based on common variance for each cultivar. One way ANOVA with blocks was conducted to test the effect of additional FR, where the effect was considered significant when $P<0.1$ (marked in bold). Fisher's protected LSD test at $P=0.10$ was used for mean separation.

		Plant height (cm)	Node number (plant^{-1})	Leaf area (m^2 plant^{-1})	Total plant DM (g plant^{-1})	DM partitioning to stem (%)	DM partitioning to leaf (%)	DM partitioning to fruit (%)
'Gialte'	FR 0	191 a	28.2 a	1.357 b	370 a	25.7 a	21.5 c	52.8 a
	FR Short	210 b	27.8 a	1.377 bc	388 a	25.9 a	20.7 bc	53.4 a
	FR 50	223 c	26.4 a	1.400 c	448 b	27.0 a	19.9 b	53.2 a
	FR 100	240 d	25.7 a	1.313 a	436 b	25.9 a	18.7 a	55.4 a
	P	**0.002**	0.134	**0.049**	**0.048**	0.817	**0.028**	0.561
	SE_{mean}	2.3	0.6	120	12.2	1.0	0.3	1.3
'Margrethe'	FR 0	166 a	25.4 a	1.423 a	354 a	23.4 a	22.7 b	53.9 a
	FR Short	182 ab	25.4 a	1.403 a	364 b	23.3 a	21.5 a	55.2 a
	FR 50	189 b	24.4 a	1.548 a	393 c	23.1 a	21.0 a	55.9 a
	FR 100	219 c	24.5 a	1.507 a	442 d	23.2 a	20.3 a	56.5 a
	P	**0.020**	0.632	0.379	**<0.001**	0.994	**0.064**	0.325
	SE_{mean}	5.2	0.7	564	1.2	0.9	0.4	0.8

Table 3. Effect of additional far-red (FR) on fruit growth parameters of all ripe fruits during 24 weeks of cultivation. DMC indicates dry matter content, DW indicates dry weight, FW indicates fresh weight. Ripe fruits, when fruits were fully colored, were harvested twice a week. FR lighting in treatment FR 50 and FR 100 started in week 8 after transplanting, and the short-term FR treatment (FR Short) started in week 12 and lasted for 4 weeks. The presented mean values were derived from two statistical replicates, each based on eight individual plants. Standard error of mean was based on common variance for each cultivar. One way ANOVA with blocks was conducted to test the effect of additional FR, where the effect was considered significant when $P<0.1$ (marked in bold). Fisher's protected LSD test at $P=0.10$ was used for mean separation.

		Fruit height (mm)	Fruit diameter (mm)	Peduncle length (mm)	Seed number (fruit^{-1})	Pericarp thick-ness (mm)	FW (g fruit^{-1})	DW (g fruit^{-1})	DMC (%)
'Gialte'	FR 0	80.5 a	82.0 a	46.2 a	227 a	8.3 a	212 a	12.4 a	5.86 a
	FR Short	81.4 a	83.4 b	47.7 b	226 a	8.4 a	215 a	12.8 a	5.95 a
	FR 50	80.9 a	85.2 d	48.4 b	221 a	8.7 a	228 b	13.4 b	5.90 a
	FR 100	81.3 a	84.1 c	49.8 c	190 a	8.5 a	214 a	12.4 a	5.84 a
	P	0.177	**0.003**	**0.010**	0.526	0.175	**0.006**	**0.062**	0.438
	SE_{mean}	0.23	0.16	0.27	18.3	0.09	1.1	0.17	0.05
'Margrethe'	FR 0	82.2 a	82.1 a	58.9 a	206 a	7.6 a	209 a	14.0 a	6.71 a
	FR Short	84.2 a	84.6 a	61.7 a	198 a	7.9 b	220 a	14.6 a	6.67 a
	FR 50	82.4 a	82.2 a	60.7 a	186 a	7.8 ab	203 a	13.5 a	6.66 a
	FR 100	83.5 a	84.5 a	65.3 b	202 a	8.0 b	214 a	14.6 a	6.85 a
	P	0.408	0.186	**0.064**	0.641	**0.071**	0.138	0.188	0.584
	SE_{mean}	0.83	0.78	0.97	10.9	0.07	3.5	0.31	0.10

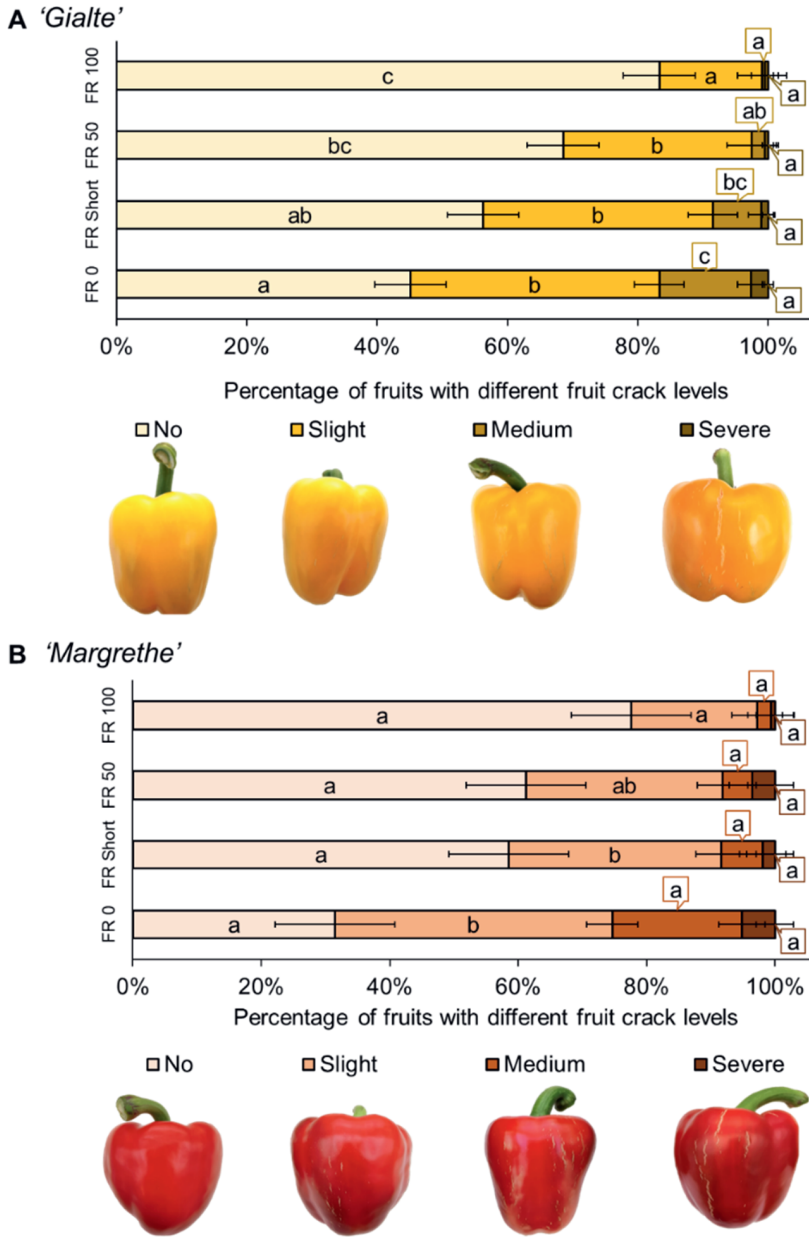


Figure 4. Effect of additional far-red (FR) on the fruit cracking in sweet pepper 'Gialte' (yellow fruit) and 'Margrethe' (red fruit). Fruit cracking of sweet pepper was categorized into four levels: no cracking, slight, medium or severe cracking, where the photo below the legends illustrates each cracking level. Based on EU regulation on sweet pepper fruit classification for the market, fruits with no cracking or slight cracking can be classified as Class I fruits. FR lighting in treatment FR 50 and FR 100 starts at the start of week 8 after transplanting, and the short-term FR treatment (FR Short) starts

at the start of week 12 and lasted for 4 weeks. The presented mean values were derived from two statistical replicates, each based on eight individual plants. One way ANOVA with blocks was conducted to test the effect of additional FR. Different letters for the same category of fruit cracking indicate significant differences between treatment means according to Fisher's protected LSD test at *P*=0.10. Error bars indicate ±standard error of means based on common variance.

4 Discussion

4.1 Higher fruit yield with additional FR mainly results from increased source strength

To identify the effects of additional far-red (FR) on sweet pepper fruit set and yield, we conducted a greenhouse experiment with different levels of additional FR. Fruit yield and the number of harvested fruits showed a positive linear increase with FR integral (Fig. 1). A yield component analysis showed that the increased fruit dry weight at 100 µmol m^{-2} s^{-1} of additional FR (24% for 'Gialte', 31% for 'Margrethe') was primarily associated with an increase in plant dry weight (19% for 'Gialte', 26% for 'Margrethe') (Table 2; Fig. 3), which is an indicator of source strength. The increase in plant dry weight with additional FR is in line with other greenhouse studies (sweet pepper: Kim *et al.*, 2023a; tomato: Hao *et al.*, 2016; Kalaitzoglou *et al.*, 2019). Additional FR only caused a marginal increase in the fraction of dry matter partitioned to fruits (4.5% in for 'Gialte', 4.3% for 'Margrethe') (Fig. 3). This finding is different from what Ji *et al.* (2019, 2020) observed for tomato, where the increased fraction of dry matter partitioned to fruits was the major contributor to the fruit dry weight increase with additional FR. The difference could be related to species and partly related to the spectrum of supplementary light. In the study of Ji *et al.*, (2019), when FR was added to white light instead of red and blue light, plant dry weight and the fraction of dry matter partitioned to fruits equally contributed to the fruit dry weight increase.

One contributor to the higher plant dry weight was the increase in light use efficiency (LUE) by 10-15%, defined as dry weight production per mol of PAR (photosynthetic active radiation) photons (Fig. 3). The increased LUE could be related to light penetration, which could have been improved by stronger internode elongation (Sarlikioti *et al.*, 2011), as observed under additional FR (Supplementary Fig. S6). A more uniform vertical light distribution can promote the whole plant photosynthesis: the photosynthesis of the upper leaves may be close to saturation; but the photosynthesis rate of lower leaves remains unsaturated, and photosynthesis of theses leaves can largely benefit from a deeper penetration of light. Another contributor to the higher plant dry weight was the increase in the incident supplementary light by 8-9% (Fig. 3; Supplementary Table S2). This effect was due to taller plants (Supplementary Fig. S6), i.e., a reduced distance to

overhead LEDs, which resulted in a higher intensity of incident light on top of the canopy for plants grown with additional FR (Supplementary Fig. S3). Although additional FR increased LUE, it reduced radiation use efficiency (RUE), when both PAR and FR photons from supplementary light were taken into account (Fig. 3). This suggests that PAR photons are more efficient than FR photons for biomass accumulation.

Despite the increased LUE in both cultivars, the two cultivars showed opposite responses in leaf photosynthesis rate. Additional FR increased leaf photosynthesis rate for 'Margrethe' but decreased that for 'Gialte', although both effects were not statistically significant (Fig. 3). The increased leaf photosynthesis rate in 'Margrethe' probably results from the Emerson enhancement effect, which indicates the synergistic activity of longer-wavelength photons with PAR photons (Emerson *et al.*, 1957; Zhen & Bugbee, 2020). The decreased leaf photosynthesis rate in 'Gialte' could be related to the effect of additional FR on leaf characteristics. Firstly, additional FR tended to reduce leaf stomata conductance in 'Gialte' (Supplementary Fig. S8B), which is in line with observations in tomato (Kalaitzoglou *et al.*, 2019). Secondly, the more frequent appearance of chlorosis in 'Gialte' under additional FR could affect leaf photosynthesis (Supplementary Fig. S9). This effect may be related to the heavy fruit load of plants grown under additional FR. The appearance of chlorosis, which was 2-3 weeks after the first fruit set peak, may result from stronger nutrient demands from the fruits and thus a reduced nutrient availability for leaf growth, considering sweet pepper fruits have highest fruit sink strength at about 3 weeks after anthesis (Wubs *et al.*, 2009). Even for the leaves without visible chlorosis, the effect of additional FR on leaf color parameters and photosynthetic pigment contents were opposite between two cultivars (Supplementary Fig. S8), supporting their opposite responses in photosynthesis rate. Effects of additional FR on net photosynthesis rate can be contradictory even in one species, e.g., as a result of cultivar differences, as discussed by Kalaitzoglou *et al.* (2019) and Ji *et al.* (2019). The effect of additional FR on the net assimilation rate can vary from negative to positive among different tomato genotypes (Ji *et al.*, 2021).

4.2 Additional FR enhanced fruit set and its fluctuation

Additional FR increased number of fruits (Fig. 3) - in line with studies in tomato by Ji *et al.* (2019) and Kalaitzoglou *et al.* (2019). The higher fruit number did not result from a higher flower number, as the flower appearance rate was unaffected or even reduced by additional FR (Fig. 3). This is different from the findings in tomato where additional FR accelerated truss formation and flowering (Ji *et al.*, 2019; Kalaitzoglou *et al.*, 2019). Instead, the higher fruit number mainly resulted from a

higher percentage of flowers developing into fruits. This finding conflicts with our previous climate chamber study (Chapter 2, 3), where additional FR reduced fruit set.

One possible explanation for the conflicting effects of additional FR on fruit set lies in the different background light spectrum. In the climate chamber study with different levels of additional FR, reducing PSS from 0.88 to 0.77 reduced fruit set, while reducing PSS from 0.77 to 0.7 did not affect fruit set anymore (Chapter 2). This suggests that the effect of additional FR on fruit set may vary at different ranges of PSS, which could be strong at high PSS, e.g., 0.88, but very little at a lower PSS. Due to the presence of solar light, the PSS in the current study were generally much lower than 0.88, as solar light holds a PSS of about 0.72. Moreover, plants in the current study entered the dark period from sole solar light, which usually contains a high fraction of FR at sunset. End-of-day FR also reduced fruit set (Chapter 2). Therefore, the effect of additional FR in the supplementary light to reduce fruit set may have been reduced by the end-of-day FR-enriched solar light. On the other hand, due to the positive effect of additional FR on source strength, fruit set was promoted. Other than this, the lower planting density and higher daily light integral in the current study compared to the climate chamber study (Chapter 2, 3), as well as the different plant pruning and different developmental stage when the FR treatments started, all may have influenced the FR effect on fruit set.

Additional FR led to a stronger fluctuation in fruit set (Fig. 2). This is probably due to additional FR initially enhancing fruit set, which in turn suppressed the fruit set in the subsequent period due to the competition and dominance effect between older and younger fruits (Marcelis & Hofman-Eijer, 1997). To reduce fruit set fluctuation, a short-term application of FR was used during weeks 12 to 16 after transplanting, but it did not elevate the first dip in fruit set at week 13 (Fig. 2). This is probably because of the strong inhibition effect from existing fruits having overruled the effect of FR on enhancing fruit set, especially that the high number of fruits set at week 10 probably have the highest fruit growth rate at about week 13 (at about 3 weeks after anthesis; Wubs *et al.*, 2009). Instead, the short-term FR application enhanced the subsequent fruit set peak (Fig. 2), which again proved the promoting effect of additional FR on fruit set.

4.3 Additional FR improves fruit quality by reducing fruit cracking

With the increased fruit yield, fruit quality of sweet pepper, in terms of size, color, firmness, °Brix and acidity, was not compromised when grown with additional FR (Table 3, Supplementary Table S6). Fruit cracking, which negatively affects fruit quality, was strongly reduced by additional FR (Fig. 4). Fruit cracking in sweet pepper started with mini cracks on the cuticle, which later developed into cracks

traversing the epidermal cell layers (Aloni *et al.*, 1998). Large diel fluctuations in fruit turgor and diameter are probably the cause of the initial cracking of the cuticle (Aloni *et al.*, 1999). Fruit cracking can be associated with many environmental, genetic, morphological, and physiological factors (Khadivi-Khub, 2015). To the best of our knowledge, there was no report about the light spectrum effect on fruit cracking. Here, we reveal for the first time that additional FR reduced fruit cracking in sweet pepper, however, the underlying mechanisms will still require further investigation.

4.4 Practical perspectives

Additional FR showed promising effects to benefit sweet pepper greenhouse production. Additional FR not only increased fruit yield, but also reduced fruit cracking, which improved fruit quality. Moreover, additional FR increased internode elongation, which is usually favored by growers when plants are grown under LED lighting because plants become easier to maintain and fruit stacking can be reduced. However, additional FR reduces RUE, which suggests that additional PAR may have a stronger promoting effect on biomass production and fruit yield compared to the same amount of additional FR.

Compared to a long-term FR application, a short-term FR application could be more appealing in practice. Long-term FR at high intensity may lead to a more frequent appearance of leaf chlorosis and a slower flower appearance rate, as observed in current research. These might cancel out the positive effect of additional FR on fruit yield in a longer cultivation. Moreover, excessive stem elongation caused by a long-term FR application may shorten the production time of sweet pepper due to the height limit of greenhouses. In addition, the promoting effect of additional FR on sweet pepper yield may depend on the season (Kim *et al.*, 2023a) and the developmental stage of the plants (Hao *et al.*, 2018). In terms of regulating fruit set, short-term FR application could be applied flexibly to have a higher yield in a target period (e.g. with a foreseen higher product price). These aspects suggest that a short-term FR application at the right time could be more cost-effective, and at the same time, it could limit the negative effects of long-term FR application. Yet, the short-term and flexible FR application can still increase yield, regulate fruit set, and promote internode elongation to a favorable extent.

5 Conclusions

Additional far-red (FR) enhanced sweet pepper fruit yield in a winter greenhouse cultivation of two commercial cultivars 'Gialte' and 'Margrethe'. This increase was mainly due to an enhanced plant dry matter production (an indicator of source

strength) rather than an enhanced fraction of dry matter partitioned to fruits. The increased source strength resulted from an increased light use efficiency and an increased incident light integral (due to taller plants). However, additional FR reduced radiation use efficiency, indicating FR is less efficient than photosynthetic active radiation for dry matter production. Additional FR enhanced fruit set percentage and fruit set fluctuation, where both long-term FR application (week 8 to 24 after transplanting) and short-term FR application (week 12 to 16 after transplanting) elevated the subsequent fruit set peak after the start of FR application. Moreover, additional FR improved sweet pepper fruit quality as it reduced fruit cracking.

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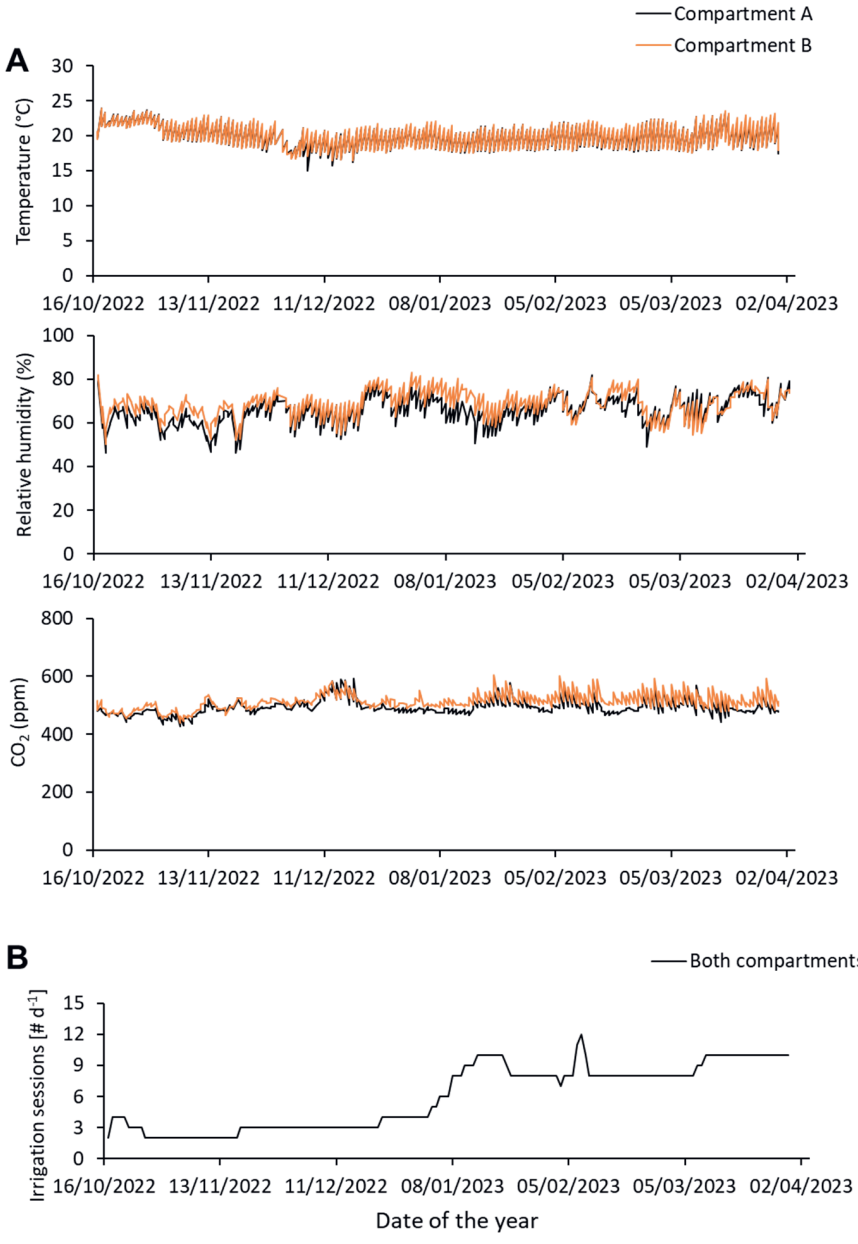
Supplementary information



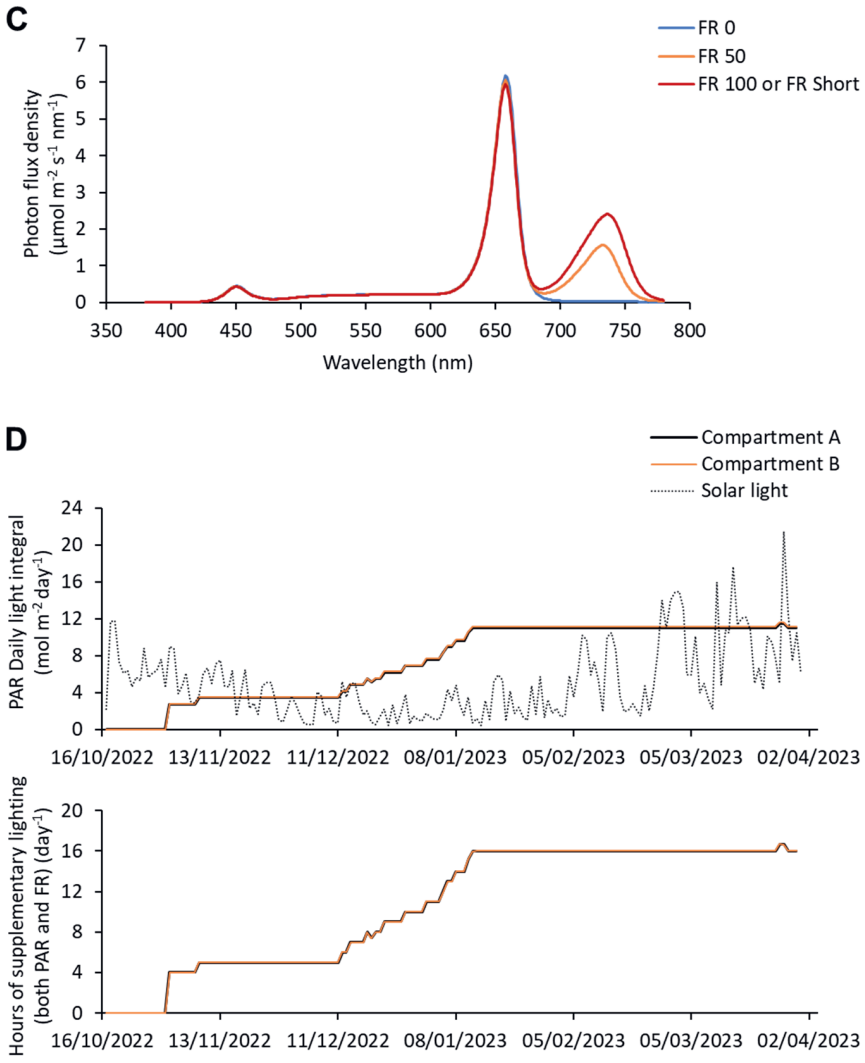


Figure S1. Climate conditions in glasshouses during the experiment from Oct 17th, 2022 to Mar 31st, 2023 (166 days, 24 weeks). (A) Average temperature, relative humidity, CO_2 concentration for each day and night in both compartments. (B) The irrigation frequency with a drip system, where each irrigation session lasted 2-3 min (33ml/min), except in March where each session lasted 4 min. (C) Spectra of supplementary light with 0, 50, or 100 µmol m^{-2} s^{-1} of additional FR. (D) Daily light integral of photosynthetic active radiation (PAR) from supplementary lighting (at 2 meters above ground) in two compartments, and estimated daily light integral of PAR from incident solar light in the glasshouses. The incident solar PAR was estimated based on an assumption that 50% of solar light was PAR, and an assumed transmissivity of 50% between solar light outside and inside greenhouse (achieved from preliminary research with similar setups), considering the effect of plastic curtains between plots, glasses, and other structural components at the top of greenhouses.

Figure S2. Sweet pepper plants on week 22 after transplanting, where the left side is cultivar 'Gialte' and the right side is 'Margrethe'. Photos were taken at the pathway in the middle of each plot. FR 0, FR 50, FR 100 indicates additional FR of 0, 50 or 100 µmol m^{-2} s^{-1} in the supplementary light since week 8 after transplanting until the end of experiment (week 24), while FR Short indicates additional FR of 100 µmol m^{-2} s^{-1} for only four weeks (week 12 to 16).

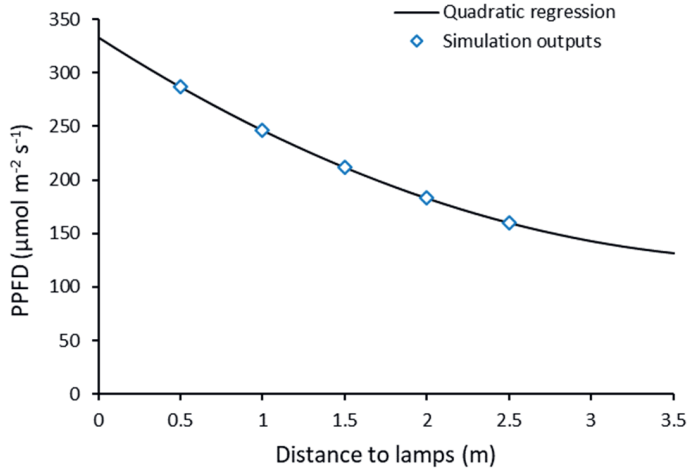


Figure S3. Quadratic relationship between the distance to lamps (m) and PPFD (photosynthetic photon flux density; µmol m^{-2} s^{-1}). Five light intensity simulation outputs (diamonds in the figure) from the LED lamp provider (Fluence, the Netherlands) was used for the quadratic regression, resulting in a relationship of PPFD=11.714*x^2-98.54*x+333.2 (P_{quad} <0.001), where x=distance to lamps. Lamps were about 3.8 m above ground. The measured PPFD at 2 m above ground (191-193 µmol m^{-2} s^{-1}) fits well with the simulated value at 1.8 m below lamps (193.8 µmol m^{-2} s^{-1}). Therefore, this quadratic relationship was used to estimate light intensity at the top of canopy for different plant height, where the base of plants were about 0.4 m above ground, and FR intensity was assumed to change proportionally with PPFD in each treatment.

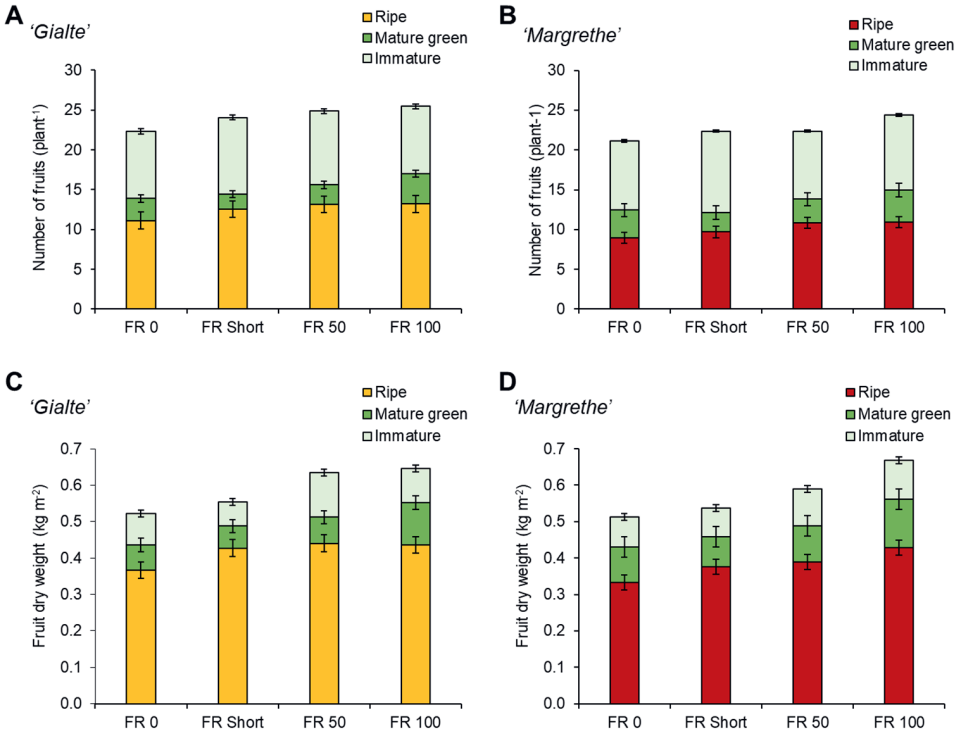


Figure S4. Composition of total fruit number and total fruit dry weight among immature, mature green, and ripe fruits in sweet pepper (A)(C) 'Gialte' and (B)(D) 'Margrethe' grown under four light treatments. FR 0, FR 50, FR 100 indicates additional FR of 0, 50 or 100 µmol m^{-2} s^{-1} in the supplementary light since week 8 after transplanting until the end of experiment (week 24), while FR Short indicates additional FR of 100 µmol m^{-2} s^{-1} for only four weeks (week 12 to 16). Mean values were derived from two statistical replicates, each based on eight individual plants. Error bars indicate ±standard error of mean based on the common variance.

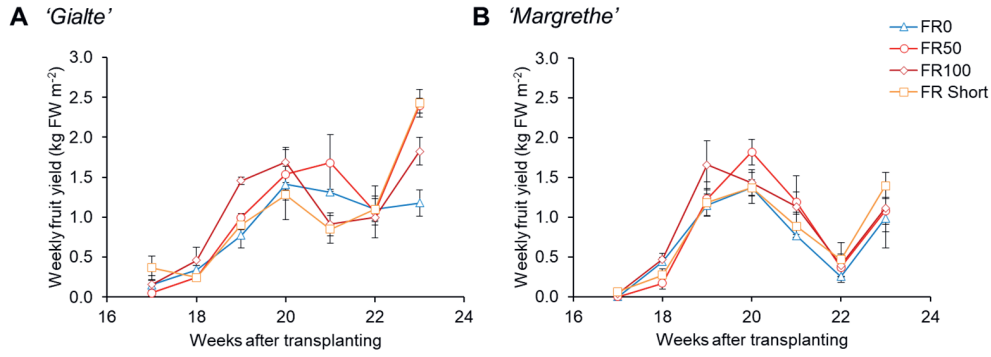


Figure S5. Weekly fruit yield of ripe fruits in sweet pepper (A) 'Gialte' and (B) 'Margrethe' grown under four light treatments. FW indicates fresh weight. FR 0, FR 50, FR 100 indicates additional FR of 0, 50 or 100 µmol m^{-2} s^{-1} in the supplementary light since week 8 after transplanting until the end of experiment (week 24), while FR Short indicates additional FR of 100 µmol m^{-2} s^{-1} for only four weeks (week 12 to 16). Mean values were derived from two statistical replicates, each based on eight individual plants. Error bars indicate ±standard error of mean.

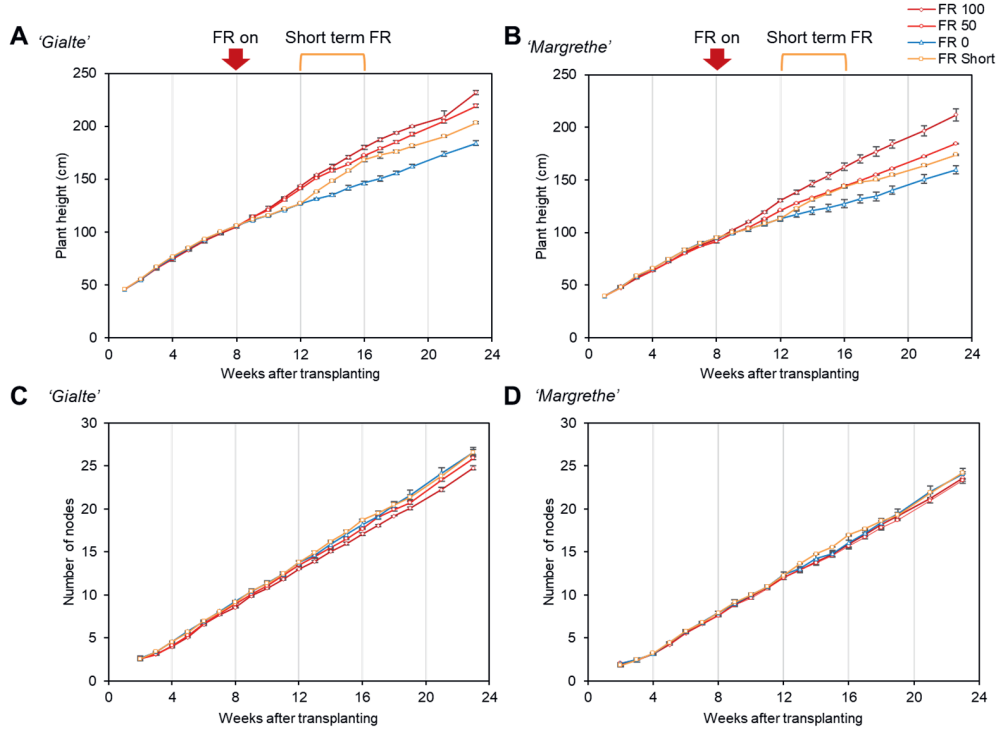


Figure S6. Development of plant height and node number every week in sweet pepper (A)(C) 'Gialte' and (B)(D) 'Margrethe' grown under four light treatments. FR 0, FR 50, FR 100 indicates additional FR of 0, 50 or 100 µmol m^{-2} s^{-1} in the supplementary light since week 8 after transplanting until the end of

experiment (week 24), while FR Short indicates additional FR of 100 µmol m^{-2} s^{-1} for only four weeks (week 12 to 16). Mean values were derived from two statistical replicates, each based on eight individual plants. Error bars indicate ±standard error of mean.

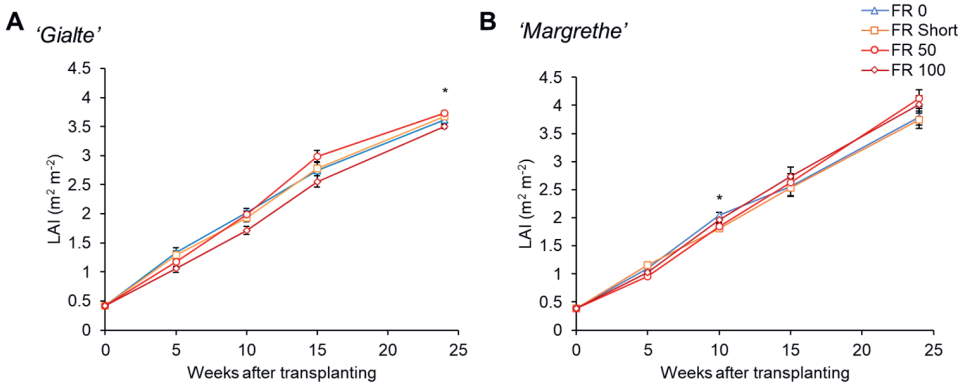


Figure S7. Development of leaf area index (LAI) of sweet pepper plants (A) 'Gialte' and (B) 'Margrethe' grown under four light treatments. LAI was measured destructively at week 0 and 24 after transplanting, where week 0 was before transplanting. LAI was measured non-destructively at week 5, 10 and 15. FR 0, FR 50, FR 100 indicates additional FR of 0, 50 or 100 µmol m^{-2} s^{-1} in the supplementary light since week 8 after transplanting until the end of experiment (week 24), while FR Short indicates additional FR of 100 µmol m^{-2} s^{-1} for only four weeks (week 12 to 16). Mean values were derived from two statistical replicates, each based on eight individual plants. Asterisks denote significant effects of additional FR in one way ANOVA (*P<0.1). Error bars indicate ±standard error of mean.

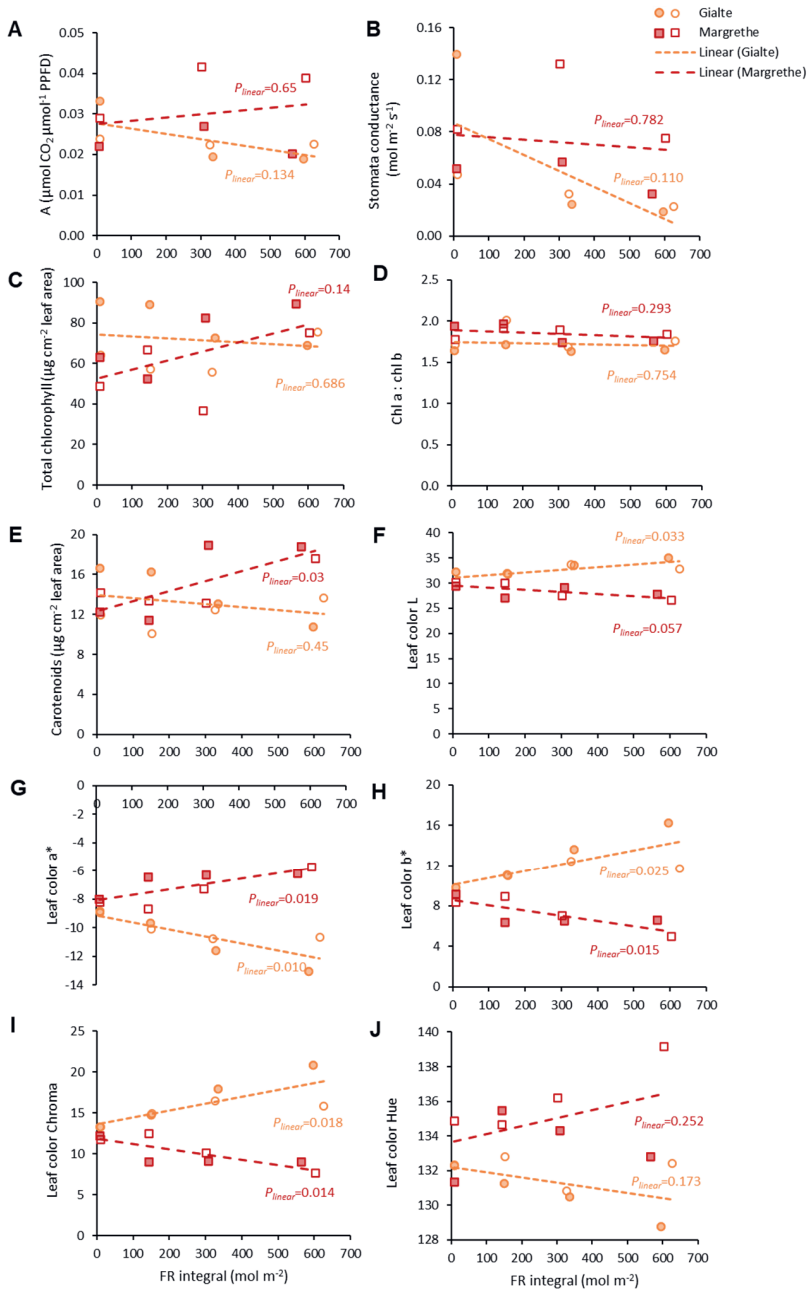
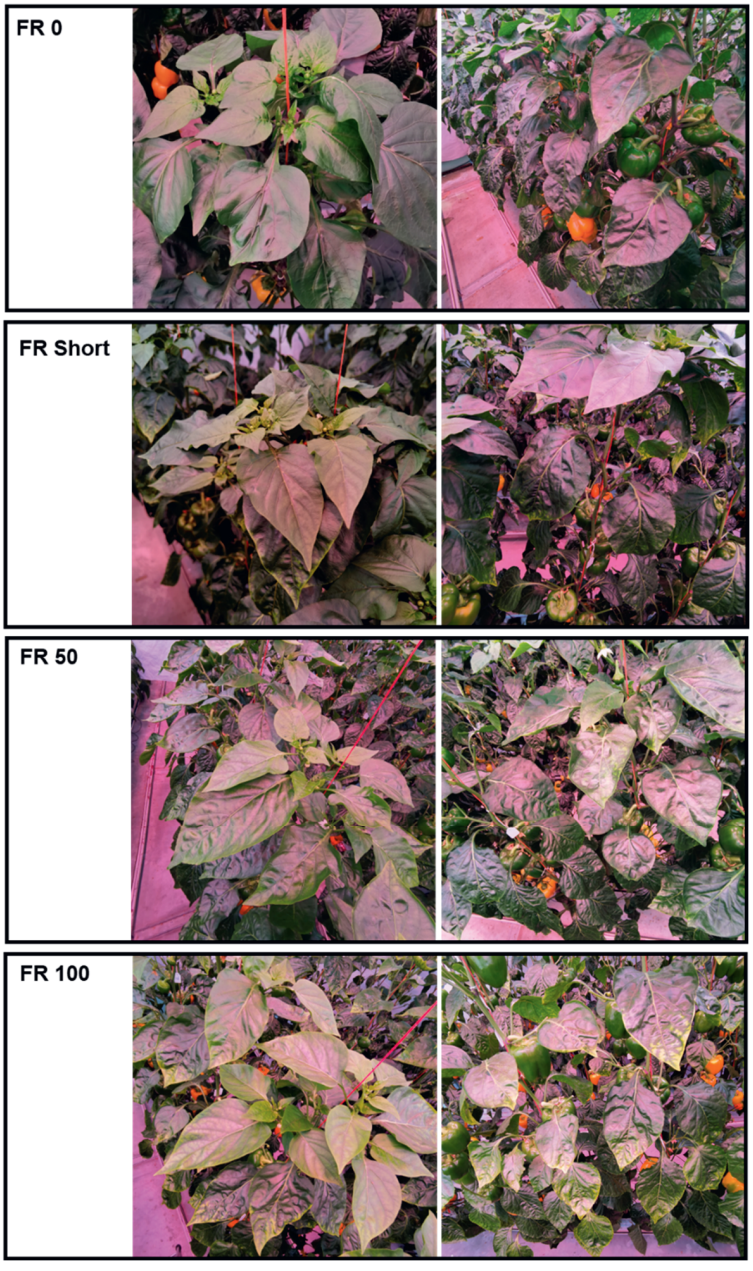


Figure S8. Leaf photosynthesis, leaf color and photosynthetic pigment content in sweet pepper 'Gialte' (circles) and 'Margrethe' (squares). (A) Leaf photosynthesis and (B) stomata conductance were measured at the topmost fully expanded leaves between week 13-16. (C) Total chlorophyll content, (D) the ratio between chlorophyll a and chlorophyll b, and (E) carotenoids content were measured from nine topmost fully expanded leaves (at node 20 ±1) of three plants per plot, were sampled at week 23

after transplanting. Leaf color of these leaves (F) L indicate luminescence, (G) a* indicates green-red color (negative to positive respectively), (H) b* indicates blue-yellow (negative to positive respectively), (I) chroma indicates quantitative attribute of colorfulness, and (J) Hue angle indicates qualitative attribute of color. For each variable, linear regression with FR integral as the regressor was performed with two replicates (closed symbols - Compartment A; open symbols - Compartment B), where *P* values from linear regression were labelled.

A *'Gialte'*



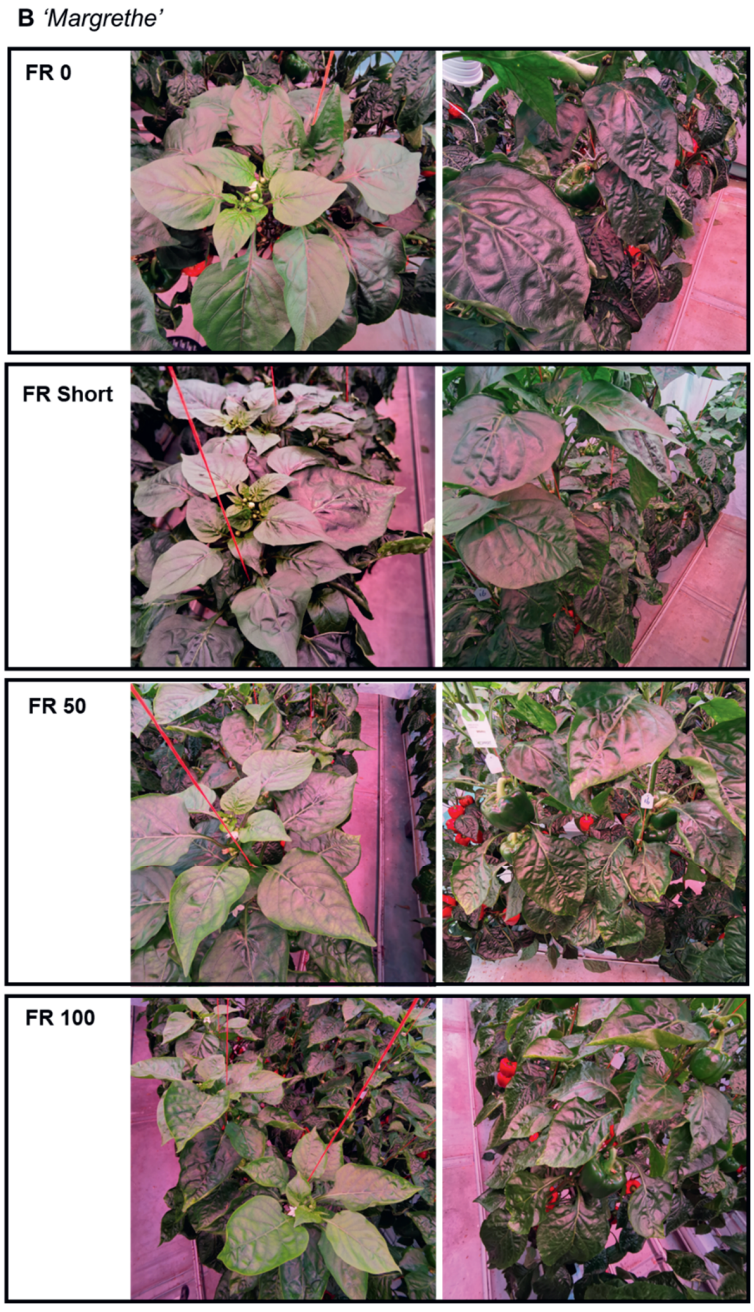


Figure S9. Top canopy of sweet pepper (A) 'Gialte' and (B) 'Margrethe' at week 20 after transplanting. FR 0, FR 50, FR 100 indicates additional FR of 0, 50 or 100 µmol m^{-2} s^{-1} in the supplementary light since week 8 after transplanting until the end of experiment (week 24), while FR Short indicates additional FR of 100 µmol m^{-2} s^{-1} for only four weeks (week 12 to 16). Additional FR led to more chlorosis in 'Gialte', but this effect was not obvious in 'Margrethe'.

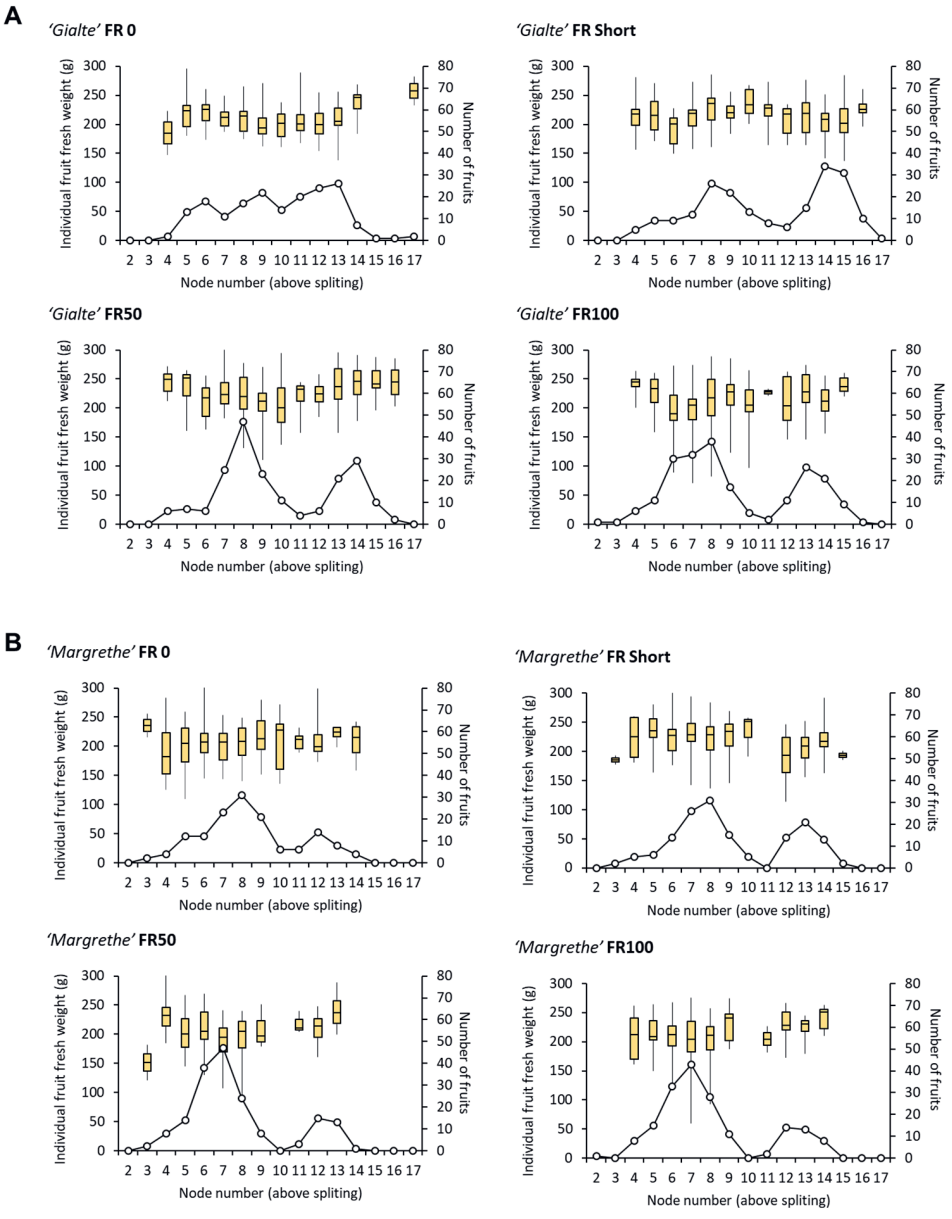


Figure S10. Boxplots of individual fruit fresh weight based on their locations on the plant (node number). The node number was counted from bottom to top, where the first splitting node as node 0. Boxplots used the primary y axis on the left. The number of fruits used for the boxplots were shown as the symbols connected by a line (secondary y axis on the right), which are the sum of fruits from 2 plots per treatment with eight individual plants per plot. FR 0, FR 50, FR 100 indicates additional FR of 0, 50 or 100 µmol m^{-2} s^{-1} in the supplementary light since week 8 after transplanting until the end of experiment (week 24), while FR Short indicates additional FR of 100 µmol m^{-2} s^{-1} for only four weeks (week 12 to 16).

Table S1. Fruit parameters when only one or two fruits were retained per stem. The left side of the table shows the comparison of individual fruit dry weight of ripe fruits from three conditions: one fruit per stem with weekly measurement, two fruits per stem with weekly measurements, and one fruit per stem without weekly measurements. Split-plot ANOVA was conducted for the left side of the table (light – whole plot, group – subplot), where the effect was considered significant when P<0.1 (marked in bold). Due to the significant difference among groups, especially between one or two fruits per stem, the data from one fruit per stem are expected to be closer to potential fruit growth (without source limitation). Therefore, the right side of the table shows the fruit morphology under condition of only one fruit per stem (a combination of fruits with or without weekly measurements), where FW indicates individual fruit fresh weight, DW indicates individual fruit dry weight, DMC indicates dry matter content. One way ANOVA with blocks was conducted for the right side of the table to test the effect of additional FR, where the effect was considered significant when P<0.1 (marked in bold). Standard error of mean was based on common variance. Fisher's protected LSD test at P=0.10 was used for mean separation.

Treatments		Dry weight (g fruit^{-1})			Under one fruit per stem condition (combined touched and untouched fruits)				
		One fruit per stem	Two fruits per stem	One fruit per stem (no weekly measure-ment)	Fruit height (mm)	Fruit dia-meter (mm)	FW (g fruit^{-1})	DW (g fruit^{-1})	DMC (%)
'Gialte'	FR 0	16.1 b	12.7 a	17.6 b	91.6	86.9 a	275.2 a	16.82 a	6.10 b
	FR 50	16.7 b	14.0 a	17.6 b	93.6	89.1 a	286.0 a	17.16 a	5.98 b
	FR 100	17.9 b	15.3 a	18.3 b	92.7	93.2 a	310.0 a	18.12 a	5.82 a
	P	Interaction 0.743; Light 0.491; Group **0.001**			0.310	0.252	0.283	0.555	**0.072**
	SE_{mean}	Interaction 1.02; Light 0.86; Group 0.39			0.68	1.86	11.19	0.75	0.038
'Margrethe'	FR 0	17.54 a	16.06 a	19.67 bc	95.9 a	89.4 a	276.7 a	18.61 a	6.71 a
	FR 50	21.08 cd	17.12 a	22.57 de	101.3 b	94.4 b	326.7 b	21.82 b	6.67 a
	FR 100	23.05 e	19.45 b	20.95 bc	100.6 b	95.8 b	328.2 b	22.00 b	6.69 a
	P	Interaction **0.032**; Light **0.032**; Group **<0.001**			**0.052**	**0.034**	**0.056**	**0.010**	0.975
	SE_{mean}	Interaction 0.57; Light 0.32; Group 0.33			0.68	0.64	7.17	0.19	0.112

Table S2. Light integral from the supplementary lighting. The integral of PPFD (photosynthetic photon flux density) or FR (far-red) indicate the cumulative mol of supplementary light within the range of 400-700 nm or 700-800 nm from transplanting till the end of experiment. A quadratic relationship (Fig. S3) was used to estimate the light intensity at the top of canopy for each week based on weekly plant height (Fig. S6).

Light integral /treatments		PPFD (mol m^{-2})	FR (mol m^{-2})
'Gialte'	FR 0	1151.8 ± 4.6	9.3 ± 0.3
	FR Short	1197.6 ± 3.4	151.6 ± 0.8
	FR 50	1231.3 ± 2.2	331.1 ± 3.1
	FR 100	1253.6 ± 5.6	611.3 ± 10.7
'Margrethe'	FR 0	1094.6 ± 9.9	8.8 ± 0.4
	FR Short	1126.4 ± 2.8	144.4 ± 0.01
	FR 50	1141.0 ± 0.1	305.5 ± 2.3
	FR 100	1199.6 ± 11.9	584.1 ± 13.5

Table S3. Coefficient of variation (cv%) of weekly fruit set (Fig. 2). Mean values derived from two replicates, which were used for calculating cv% with software Genstat (22nd edition).

Fruit set (week $^{-1}$)		Mean	cv%
'Gialte'	FR 0	1.08	61.4
	FR Short	1.18	88.6
	FR 50	1.21	68.5
	FR 100	1.23	65.3
'Margrethe'	FR 0	1.04	67.3
	FR Short	1.10	91.3
	FR 50	1.10	77.7
	FR 100	1.20	69.6

Table S4. Data for yield component analysis. Linear regression was tested between the listed variables and FR integral (Table S2) with 2 replicates per treatment. DW indicates dry weight, No. indicates number, LUE indicates light use efficiency for wavelength range of 400-700 nm, RUE indicates radiation use efficiency for wavelength range of 400-800 nm. Linear regression was considered significant when P<0.1 (marked in bold). Fitted values were used to calculate relative change in treatment FR 100 compared to FR 0. All variables showed a linear trend along the FR integral, except leaf area index in 'Gialte', where the measured values were used to calculate relative change in FR 100 vs. FR 0.

		Incident PAR (mol m^{-2})	Total fruit DW (kg m^{-2})	Total plant DW (kg m^{-2})	Fraction to fruit (%)	No. fruits (plant^{-1})	Flower appearance rate (day^{-1})	Fruit set percentage (%)	Potential fruit growth (g fruit^{-1})	LUE (g mol^{-1} PPFD)	RUE (g mol^{-1} PFD)	Relative Photosynthesis rate (µmol CO_2 µmol^{-1} PPFD)	Leaf area index (m^2 m^{-2})
'Gialte'	P linear regression	**<0.001**	**0.015**	**0.025**	0.231	**0.091**	**0.054**	**0.044**	0.549	0.141	**0.001**	0.134	0.218
	Fitted value FR 0	1165.1	0.533	1.01	52.6	22.9	0.173	25.8	16.7	0.867	0.845	0.028	3.68
	Fitted value FR 100	1263.2	0.662	1.20	55.0	25.7	0.162	31.8	17.9	0.952	0.634	0.020	3.57
	Measured value FR 0	1151.8	0.523	0.987	52.8	22.3	0.172	25.4	16.8	0.857	0.850	0.029	3.62
	Measured value FR 100	1253.6	0.646	1.16	55.4	25.4	0.160	31.7	18.1	0.927	0.624	0.021	3.50
'Margrethe'	P linear regression	**<0.001**	**<0.001**	**<0.001**	0.102	**0.018**	0.884	**0.037**	**0.086**	**<0.001**	**<0.001**	0.650	0.277
	Fitted value FR 0	1095.6	0.507	0.929	54.4	21.3	0.155	26.1	19.1	0.850	0.831	0.028	3.79
	Fitted value FR 100	1197.8	0.666	1.173	56.7	24.2	0.154	31.2	22.5	0.980	0.649	0.032	4.09
	Measured value FR 0	1094.6	0.512	0.944	53.9	21.1	0.155	25.3	18.6	0.862	0.855	0.026	3.80
	Measured value FR 100	1199.6	0.668	1.180	56.5	24.4	0.155	30.7	22.0	0.983	0.662	0.030	4.02

Table S5. Effect of additional FR on leaf and stem dry matter content (DMC), dry weight (DW) of stem, leaf and fruit, and specific leaf area (SLA) after 24 weeks of cultivation. SLA is the ratio between leaf area and leaf dry weight. FR 0, FR 50, FR 100 indicates additional FR of 0, 50 or 100 µmol m^{-2} s^{-1} in the supplementary light since week 8 after transplanting until the end of experiment (week 24), while FR Short indicates additional FR of 100 µmol m^{-2} s^{-1} for only four weeks (week 12 to 16). The presented mean values were derived from two statistical replicates, each based on eight individual plants. One way ANOVA with blocks was conducted to test the effect of additional FR, where the effect was considered significant when P<0.1 (marked in bold). Standard error of mean was based on common variance. Fisher's protected LSD test at P=0.10 was used for mean separation.

		Leaf DMC (%)	Stem DMC (%)	Stem DW (g $plant^{-1}$)	Leaf DW (g $plant^{-1}$)	Fruit DW (g $plant^{-1}$)	SLA (cm^2 g^{-1})
'Gialte'	FR 0	13.5 a	16.1 a	95 a	79 a	196 a	172 a
	FR Short	14.0 a	16.8 a	100 ab	80 a	208 a	173 a
	FR 50	15.5 b	18.5 b	120 c	89 a	239 b	159 a
	FR 100	15.8 b	19.0 b	112 bc	81 a	242 b	163 a
	P	**0.017**	**0.030**	**0.085**	0.243	**0.074**	0.385
	SE_{mean}	0.3	0.4	4.7	2.9	8.8	5.6
'Margrethe'	FR 0	12.1 a	15.4 a	82 a	80 a	192 a	179 a
	FR Short	12.1 a	15.7 a	85 a	78 a	201 b	180 a
	FR 50	13.1 b	17.2 b	90 a	82 a	221 c	189 a
	FR 100	14.1 c	18.5 c	102 b	90 b	250 d	169 a
	P	**0.008**	**0.008**	**0.041**	**0.034**	**0.002**	0.327
	SE_{mean}	0.2	0.3	2.7	1.5	2.7	6.2

Table S6. Effect of additional FR on sweet pepper fruit quality. FR 0, FR 50, FR 100 indicates additional FR of 0, 50 or 100 µmol m^{-2} s^{-1} in the supplementary light since week 8 after transplanting until the end of experiment (week 24). The presented mean values were derived from two statistical replicates, each based on three random ripe fruits. One way ANOVA with blocks was conducted to test the effect of additional FR, where the effect was considered significant when $P<0.1$. Standard error of mean was based on common variance. Fisher's protected LSD test at $P=0.10$ was used for mean separation.

		Fruit color					Firm-ness (N)	°Brix	Acidity	Brix/ Acidity
		L	a*	b*	Chroma	Hue				
'Gialte'	FR 0	55.2 a	-1.5 a	45.9 a	46.0 a	92.0 a	21.4 a	4.95 a	0.30 a	16.8 a
	FR 50	56.5 a	-3.2 a	46.6 a	46.7 a	94.0 a	21.2 a	4.90 a	0.34 a	15.9 a
	FR 100	54.7 a	-3.1 a	44.9 a	45.0 a	94.1 a	19.4 a	4.65 a	0.25 a	18.5 a
	P	0.323	0.431	0.264	0.277	0.438	0.568	0.495	0.552	0.566
	SE_{mean}	0.66	0.85	0.50	0.51	1.1	1.2	0.16	0.047	1.5
'Margrethe'	FR 0	35.0 a	27.1 a	16.1 a	31.6 a	30.5 a	25.5 a	5.67 a	0.38 a	15.0 a
	FR 50	35.0 a	25.0 a	16.1 a	29.9 a	32.8 a	21.9 a	5.67 a	0.36 a	15.8 a
	FR 100	31.1 a	26.8 a	16.3 a	31.4 a	31.4 a	21.5 a	6.23 a	0.34 a	18.5 a
	P	0.975	0.395	0.938	0.291	0.613	0.381	0.111	0.540	0.111
	SE_{mean}	0.30	0.89	0.40	0.61	1.4	1.8	0.12	0.021	0.65

Chapter 6

General discussion

Sijia Chen

Optimizing light spectrum is an important topic in greenhouse production and vertical farming, considering its potential in regulating plant growth and development. In many crops, including pepper, fruit set is an important determinant of yield. This thesis aimed to investigate how light spectrum can influence fruit set in sweet pepper, and to explore the underlying morphological and physiological mechanisms. Two important aspects of light spectrum, i.e., red: far-red ratio (R:FR) and blue: red ratio (B:R) were investigated. In the current chapter, I re-examine the findings, discuss and position them in a broader background, and bring up perspectives for future study.

1 Physiological mechanisms underlying reduced fruit set at additional far-red

In Chapter 2, we observed that additional far-red (FR) reduced fruit set in sweet pepper. Inspired by the unbalanced growth among dichotomous shoots, and vigorous shoot growth at additional FR, we focused on the role of apical dominance in this effect. In Chapter 3, we confirmed that additional FR enhanced apical dominance, which was likely responsible for the FR-reduced fruit set. Auxin has been associated with apical dominance for over a century; thus, it became the first candidate to investigate.

Based on the canalization model, the competition for basipetal auxin transport underlies apical dominance. Therefore, in Chapter 3, we used different approaches to manipulate the basipetal auxin stream in the stems: apex decapitation, reducing auxin basipetal stream with NPA, applying exogenous synthetic auxin NAA to replace the apex, and inhibiting apical auxin biosynthesis with yucasin. Surprisingly, manipulating the basipetal auxin stream did not influence the FR-reduced fruit set, unless the growth of apices was inhibited (or apices were removed). At the end of Chapter 3, we brought up an alternative hypothesis proposing that enhanced apical dominance mediates FR-reduced fruit set through enhancing competition for photoassimilate between apices and flowers/fruits. An enhanced competition for assimilates at additional FR could shift assimilate allocation and might limit assimilate import to the flowers/fruits. Here I will discuss several physiological processes that could play a role in mediating FR-reduced fruit set. The positive effect of FR on fruit set observed in the greenhouse experiment (Chapter 5) will not be discussed here (see section 3).

1.1 Photoassimilate loading and unloading

Additional FR led to higher soluble sugar and starch contents in the leaves and stems adjacent to the flowers as well as in top leaves, compared to plants grown without additional FR (Chapter 3 Fig. 6). This suggests that the source: sink ratio

was probably higher in sweet pepper plants grown with additional FR, considering carbohydrate reserves in source organs are positively related to source: sink ratio (Li *et al.*, 2015; Plaut *et al.*, 1987; Silva *et al.*, 2017). However, these assimilates seem not to be transported from source leaves to flowers/fruits grown with additional FR. Despite the higher non-structural carbohydrate level in these flowers at anthesis, the fruits on day 7 after anthesis were smaller, compared to those grown without additional FR (Chapter 3 Fig. S13). This suggests additional FR led to a lower accumulation of total carbohydrates (structural and non-structural) per flower/fruit within the same amount of time, meaning a lower carbon net influx into flowers/fruits.

A lower carbon influx into flowers/fruits could be an active adjustment to light environments in plants. Species in the Solanaceae family usually use apoplastic phloem loading, where sucrose transporters are essential (Schmitt *et al.*, 2008). These species, compared to species using other phloem loading strategies, seem to have an advantage in flexibly responding to light quality and quantity in terms of carbon partitioning (Slewinski & Braun, 2010). For example, the structure of phloem cells can be influenced by light (Lemoine *et al.*, 2013), as well as the transcriptional regulation, post-translational regulation of subcellular localization and activity of sucrose transporters (Slewinski & Braun, 2010). These allow plants to actively adjust carbon partitioning via transporters.

As a key component in sucrose apoplastic import, cell wall invertases are responsible for the hydrolysis of sucrose at the extracellular space, which also cooperates with downstream sucrose transporters or hexose transporters of recipient sink cells (Braun *et al.*, 2014; Li *et al.*, 2017). Additional FR reduced cell wall invertase activity in sweet pepper flowers at anthesis (Chapter 3 Fig. 7), corresponding to a reduced fruit set with additional FR (Chapter 3). Increasing cell wall invertase activity by genetically silencing its inhibitor improved tomato fruit set under heat stress (Liu *et al.*, 2016), where cell wall invertases were hypothesized to facilitate the generation of glucose signal to activate or stimulate cell division and to block programmed cell death (Li *et al.*, 2017). Silencing genes encoding cell wall invertases downregulated auxin-responsive genes and induced ovule abortion in *Arabidopsis* without causing carbon starvation (Liao *et al.*, 2020). This suggests that the cell wall invertases could be one of the regulators of fruit set at different light spectra.

The effect of additional FR on cell wall invertase activity could be direct or indirect. Silencing fruit-localized phytochrome A (phyA) upregulated the expression of genes encoding cell-wall invertases in immature tomato fruits, while silencing phytochrome B2 (phyB2) did not upregulate these genes (Bianchetti *et al.*, 2018). phyA is physiologically more active under FR, while phyB is activated by red light.

Thus, the local light signal (e.g., R:FR) may alter the cell wall invertase activity in flowers/fruits directly. Moreover, the transcript levels of cell wall invertases can be regulated by hormones, sugars, and other stress-related stimuli (Roitsch *et al.*, 2003). Thus, the reduced cell wall invertase activity might also be a result of metabolic signals caused by additional FR. It is noticeable that the enzyme assay used in Chapters 3 & 4 examines the combined responses of cell wall invertases and their inhibitors to light spectrum and does not allow to distinguish the individual components. Gene expression analysis could provide more detailed information.

1.2 Involvement of other plant hormones

Apart from the nutrient diversion and auxin transport theories about apical dominance, other plant hormones such as cytokinins (CKs) and strigolactones (SLs) were found to play an important role in this process as well. CKs stimulate bud outgrowth whereas SLs inhibit this process (Beveridge *et al.*, 2023; Brewer *et al.*, 2015). In addition, abscisic acid (ABA) and gibberellins (GA) are considered as candidates too, given their levels have often been correlated with bud outgrowth (Beveridge *et al.*, 2023).

Studies on the lateral bud growth regulated by R:FR may give some clues about what the roles of these hormones in FR-reduced fruit set could be. A recent study in tomato showed that SL synthesis is dispensable for the lateral bud growth regulated by R:FR. Instead, this effect was mediated by auxin via suppressing CK and brassinosteroid (BR) synthesis (Song *et al.*, 2024). BR signaling integrates traditional signals including auxin, SLs, CKs, sugars and GA to regulate bud outgrowth in tomato (Xia *et al.*, 2021). The production of BRs activates the BZR1 transcription factor, which suppresses the expression of *BRANCHED1* (*BRC1*) (Xia *et al.*, 2021). BRC1 can inhibit bud outgrowth directly, but also by upregulating ABA levels (Beveridge *et al.*, 2023; González-Grandío *et al.*, 2013). Both ABA levels and the expression of *BRC1* are upregulated by a low R:FR (González-Grandío *et al.*, 2013; Kebrom *et al.*, 2006; Reddy *et al.*, 2013), suggesting the potentially central roles of ABA and BRC1 in the lateral bud growth regulated by R:FR.

In pepper flowers/fruits, additional FR did not affect ABA concentrations at anthesis or 7 days after anthesis (Chapter 3 Fig. S4). Instead, additional FR reduced auxin (indole-3-acetic acid) and CK (isopentyladenine and *trans*-zeatin) concentrations in fruits on day 7 after anthesis (Chapter 3 Fig. S4). Based on an apical dominance model described by Beveridge *et al.* (2023), sucrose promotes CK and auxin levels during bud outgrowth. Therefore, the low auxin and CK in flowers might result from the reduced sucrose content, associated with the stronger apical dominance under additional FR (Chapter 3). These few pieces of

the 'Jigsaw Puzzle' suggest a pivotal role for sugar, but the information is far from complete to fully explain FR-reduced fruit set. Some important pieces are still missing, such as the roles of BRC1 and BR. Moreover, the sensitivity to auxin might dominate over the effect of auxin abundance, which requires further investigation. Low R:FR up-regulated the expression of auxin-responsive genes in *Arabidopsis* compared to high R:FR (Holalu *et al.*, 2021). In one of our experiments, largely reducing auxin concentration in the main stem by chemically inhibiting auxin polar transport did not influence fruit set (Chapter 3 Fig. 3). However, I cannot exclude the possibility that the remaining auxin in the main stem was still sufficient to reduce fruit set due to an enhanced tissue sensitivity to auxin. Last but not least, it was surprising to find a higher salicylic acid (SA) concentration in the flowers/fruits grown at high abortion treatment (Chapter 3 Fig. S4). It is still unknown whether SA plays a role in the apical dominance regulated by R:FR ratio.

2 Differences and similarities between high B:R and low R:FR reduced fruit set

2.1 Photoreceptors

Phytochrome mediated plant responses depend on the photostationary state of phytochrome (PSS), which is expressed as the ratio of the amount of biologically active form Pfr over the total amount of phytochrome (Sager *et al.*, 1988). Low R:FR leads to a low PSS. Along with phytochromes, blue light receptors, including cryptochromes and phototropins, mediate plant responses to light spectrum (Huché-Thélier *et al.*, 2016). Other than influencing the status of blue light receptors, blue light can also influence the status of phytochromes, where a very high fraction of blue light results in a low PSS. Therefore, in Chapter 4, we investigated the effect of four B:R on fruit set and plant growth of sweet pepper. B:R of 1:10, 1:3 and 1:1 resulted in a similar PSS (0.86-0.88), but B:R of 9:1 resulted in a substantially lower PSS of 0.72. Matching with this PSS pattern, fruit set, individual fruit weight, floral starch content, floral auxin and SA concentrations were only affected by B:R of 9:1 compared to the other B:R (Table 1; Chapter 4 Fig. 1, Fig. 3, Fig. 5). Therefore, we consider these responses are more closely associated with the status of phytochromes. This suggests that a lower PSS independent of FR could also reduce fruit set.

Here the differences and similarities between high B:R and FR reduced fruit set are discussed (Table 1), as phytochromes are probably involved in both responses.

2.2 The role of carbohydrates

The study on B:R (Chapter 4) and the study on additional FR (Chapter 3) both point to an important role of carbohydrates on fruit set. A very high B:R and additional FR both led to low carbohydrates import to the flowers/fruits but reflected in slightly different forms (Table 1). B:R of 9:1 reduced starch content in flowers (Chapter 4 Fig. 3); whilst additional FR reduced sucrose content in flowers (Chapter 3 Fig. 6). High B:R and additional FR also had different effects on the activity of sucrose cleavage enzymes in flowers (Table 1). These differences in carbohydrates metabolism suggest that the causes of low carbon import to flowers might be different in the low R:FR and high B:R reduced fruit set.

Despite a low fruit set being observed at low R:FR and very high B:R, additional FR increased plant biomass whereas plant biomass was reduced at high B:R (Chapter 2 Fig.4; Chapter 3 Fig. S10; Chapter 4 Fig. 2). This uncouples fruit set from plant biomass when light spectra differ. A stronger competition for assimilates between flowers and apices, as reflected in a higher fraction of biomass partitioned to the stems with additional FR, cannot explain the observations at high B:R (Chapter 2 Fig.4; Chapter 3 Fig. S10; Chapter 4 Fig. 2).

Moreover, the carbohydrates level in all vegetative tissues grown with additional FR were higher compared to those grown without FR (Chapter 3 Fig. 6). Thus, as discussed in the previous section, the reduced assimilate import to flowers could be an active adjustment, where the cell wall invertases may be involved. Differently, high B:R was reported to reduce starch reserves in source leaves (Dong *et al.*, 2021; Larsen *et al.*, 2022; Shengxin *et al.*, 2016). Moreover, the floral cell wall invertase activity at anthesis was not influenced by B:R, whereas it was influenced by additional FR (Table 1). Therefore, I speculate that, compared to low R:FR reduced fruit set, the high B:R reduced fruit set could be a more passive response resulting from the low available photoassimilate. However, this speculation may not support the idea that low PSS is the main determinate factor for the high B:R to reduce fruit set, which implies an active signaling mediated by phytochromes.

Another different response occurs in fruit growth. Additional FR reduced sizes of fruits on day 7 after anthesis, which are uncertain to set or abort (Chapter 3 Fig. S13); and additional FR did not influence the average fruit size of the surviving immature fruits at the end of experiments, which are unlikely to abort (Chapter 2 Fig. 2). However, high B:R reduced the sizes of the surviving immature fruits at the end of the experiments compared to low B:R (Chapter 4 Fig. 1). This further implies that the high B:R reduced fruit set and size is probably more linked to the nutrient limitation (probably carbohydrates in this case); while the low R:FR reduced fruit set is probably more a signaling effect, where once the fruit is set, its growth will

not be inhibited. Taken together, I suggest that the stimuli causing less carbohydrate import to flowers/fruits are probably different between low R:FR and high B:R.

2.3 The role of hormones

High B:R of 9:1 and additional FR both caused a reduced auxin and a higher SA content in flowers on day 7 after anthesis. Auxin acts as a positive regulator during fruit set and fruit growth (Pattison, *et al.*, 2014). Reduced auxin in flowers on day 7 after anthesis may indicate a repression on auxin signaling, which could increase the tissue sensitivity to ethylene (Taylor & Whitelaw, 2001). This change could trigger abscission through ethylene without elevating its levels.

Increased SA content in high abortion treatments was unexpected, as its direct role in fruit set has not been reported. Increased SA could be a common response prior to flower and fruit abortion, which might suggest the growth of flowers/fruits has ceased. Considering SA is involved in regulating senescence (Peng *et al.*, 2021; Rivas-San Vicente & Plasencia, 2011), and floral organs appear to senesce before they abscise, SA may be involved in regulating abscission as well (Patharkar & Walker, 2018). SA reduced leaf abscission in peach and pepper (Ferrarese *et al.*, 1996), and it can inhibit ethylene biosynthesis (Xu *et al.*, 2023). In contrast, more research shows that SA probably promotes abscission. Reduced SA in transgenic plants delayed stress triggered abscission (Patharkar & Walker, 2019). Applying SA at harvest can induce abscission and reduce fruit attachment force in olives (Hartmann *et al.*, 1968). In *Arabidopsis*, the proteins responsible for abscission zone differentiation belong to the family of NONEXPRESSOR OF PR GENES1 (NPR1), which ensures the induction of defense genes upon accumulation of SA (Olsson & Butenko, 2018). This implies SA could play a role in the formation of an abscission zone, which also suggests a potential role in flower and fruit abortion.

The effects of high B:R and additional FR on CKs were different. Additional FR reduced iP and *t*Z in flowers/fruits, which are CK with high activities; while high B:R increased *c*Z and *c*ZR in flowers/fruits, which are CK with low activities. As discussed before, the reduced level of active CK might result from the reduced floral sucrose with additional FR, which might be a signaling process. High *c*Z and *c*ZR are usually associated with limited growth (Gajdošová *et al.*, 2011; Schäfer *et al.*, 2015). Consistent with previous speculations, the increased level of inactive CK may suggest that the high B:R of 9:1 caused a growth-inhibiting condition for flowers, e.g., a carbon limiting condition.

Table 1. Effects of additional far-red (FR; Chapter 2, 3) and effects of high blue: red light ratio (B:R; Chapter 4) on fruit set and plant growth in sweet pepper. In Chapter 4, four levels of B:R (1:10, 1:3, 1:1, 9:1) were investigated, some effects were only shown in B:R of 9:1, while other effects were shown in a wider range of B:R. Therefore, in the column on effects of B:R, black text indicates the responses which were only influenced by B:R of 9:1 but not by other B:R; while grey texts describe the responses among all B:R. PSS = photostationary state of phytochrome; Susy = sucrose synthases; NI = neutral invertases; AI = soluble acid invertases; CWI = cell wall invertases; ABA = abscisic acid; ACC = 1-amino-cyclopropane-1-carboxylic acid; IAA (auxin) = indole-3-acetic acid; SA = salicylic acid; JA = jasmonic acid; JA-ile = jasmonic acid isoleucine; OPDA = 12-oxo-Phytodienoic acid; iPR = isopentenyl adenosine riboside; iP = isopentyladenine; cZR = *cis*-zeatin riboside; cZ = *cis*-zeatin; *t*ZR = *trans*-zeatin riboside; *t*Z = *trans*-zeatin.

Variables	Effect of additional FR (compared to no additional FR)	Effect of B: R of 9:1 (compared to B:R of 1:10, 1:3 and 1:1)
PSS	0.69-0.78 (vs. 0.86-0.88)	0.72 (vs. 0.86-0.88)
Fruit set (fruit number per plant)	Lower	Lower
Individual fruit diameter or dry weight	Unaffected dry weight of immature fruits at the end of experiment; Smaller diameter of fruits on day 7 after anthesis.	Lower dry weight of immature fruits at the end of experiment
Fruit dry matter content	Unaffected	Higher (gradually increased from B:R of 1:10 to 9:1)
Plant biomass (stem + leaf + fruit)	Higher	Lower (gradually decreased from B:R of 1:3 to 9:1)
Dry mass partitioning	Higher partitioning to stem; Lower partitioning to leaf; Lower partitioning to fruit.	Unaffected partitioning to stem; Higher partitioning to leaf; Lower partitioning to fruit.
Plant height	Higher	Unaffected
Leaf area	Unaffected	Lower than B:R of 1:3
Sugar content in flowers and fruits	(At anthesis) Lower sucrose; higher glucose, fructose, and starch. (7 days after anthesis) Unaffected.	Unaffected sucrose, glucose, and fructose. (At anthesis) Lower starch. (7 days after anthesis) Lower starch.
Protein content in flowers and fruits	(At anthesis) Unaffected. (7 days after anthesis) Lower.	(At anthesis) Unaffected. (7 days after anthesis) Lower.

Activity of sucrose cleavage enzymes in flowers and fruits	Unaffected Susy, NI. (At anthesis) Lower AI; lower CWI. (7 days after anthesis) Lower AI.	Unaffected NI. (At anthesis) Increasing activity of Susy tendency from B:R of 1:10 to 1:1; but had a drop at B:R of 9:1. (7 days after anthesis) Lower AI; higher CWI than B:R of 1:3 and 1:1; higher Susy than B:R of 1:3 and 1:1.
Hormone contents in flowers and fruits	Unaffected ABA, ACC, JA, JA-ile, OPDA, iPR, *c*ZR, *c*Z at both stages. (At anthesis) Lower iP; higher ethylene emission rate. (After anthesis) Lower IAA; higher SA; lower iP; lower *t*Z.	Unaffected JA, JA-ile, OPDA, iPR, iP, ethylene emission rate at both stages. (At anthesis) *t*Z lower than B:R of 1:3; *c*Z higher than B:R of 1:10. (After anthesis) Lower IAA; higher SA; higher *c*ZR; higher *c*Z; higher ABA than B:R of 1:10 and 1:3; lower ACC than B:R of 1:10 and 1:3; *t*Z lower than B:R of 1:10.
Pollen number per flower	Unaffected	Unaffected
Fraction of viable pollen	Unaffected	Unaffected

3 The impact of the experimental set-up on FR-reduced fruit set

In multiple climate chamber experiments, additional FR reduced fruit set in sweet pepper (Chapters 2 & 3). On the other hand, additional FR has been reported to increase fruit fresh mass in greenhouse cultivation of sweet pepper (Hao *et al.*, 2018; Kim & Son, 2022; Kim *et al.*, 2023; Schuddebeurs, 2021), while these studies did not report whether FR affected fruit set. Fruit set, and the consequent fruit number, is one of the most important determinant factors for yield: improved fruit set usually means higher yield. This suggests that the effect of FR observed in the climate chamber studies may differ from what happens in greenhouse cultivation. To investigate this, we conducted a greenhouse experiment (Chapter 5). In this study, one of the cultivars was the same as in previous climate chamber studies (Chapter 3), and the PSS values of the supplementary light spectra (when solar radiation is not considered) were close to those in the climate chamber studies (Fig. 1).

Surprisingly, additional FR did increase fruit set in the greenhouse study (Chapter 5 Fig. 2, Fig. 3). It also resulted in increased yield, which is in line with literature. The reasons for the different FR effect in the greenhouse study compared to the climate

chamber studies remains unclear. Here I will discuss a few potential aspects that may play a role.

3.1 Background light spectrum

One obvious difference between the climate chamber experiments (Chapter 2 & 3) and the greenhouse experiment (Chapter 5) is the background light spectrum. In a chrysanthemum study, flower induction in this short-day plant could be obtained when a short day of red-blue LED light was extended to a long day with monochromic blue light. However, flowering was inhibited when the short day was provided by solar light instead of sole red-blue LEDs (SharathKumar *et al.*, 2021). This difference was caused by the presence of FR in the solar spectrum (SharathKumar, 2023). This shows that light spectrum effect can depend on the background light spectrum.

In the climate room experiments, the control treatments had very little FR, resulting in comparisons between spectra with FR (PSS <0.78) or without FR (PSS of 0.86-0.88) (Fig. 1). In the greenhouse experiment, due to the presence of solar radiation, the comparison was actually made between spectra with different amount of FR, where PSS would vary with the level of solar radiation. At higher solar radiation, the PSS of all treatments are lower, and the PSS difference among treatments can be largely reduced (Fig. 1). Only when solar radiation was below 100 µmol m^{-2} s^{-1}, the PSS in the lowest and highest FR treatments in the greenhouse were comparable to the treatments in the climate chamber studies. However, the incident solar radiation was higher than 100 µmol m^{-2} s^{-1} during 34% of the light periods across the whole greenhouse cultivation (when there was solar light and/or supplementary light, from mid-October 2022 to end of March 2023 in the Netherlands). This could reduce the effect of additional FR treatments.

The effect of FR on fruit set may depend on the PSS range. For example, additional FR may have a strong effect when decreasing PSS from 0.88 to 0.80 but have little effect when decreasing PSS from 0.80 to 0.70. A previous finding (Chapter 2 Fig. 1) supports this hypothesis, where PSS of 0.77 and 0.7 reduced fruit set to the same extent compared to the control with a PSS of 0.88. Another study in lettuce and cucumber shows that, increasing FR fraction from 17% to 33% had a much less pronounced effect on shoot dry mass and leaf area, compared to increasing FR fraction from 2% to 17% (Kusuma & Bugbee, 2023). This also implies that, for certain plant responses, their sensitivity to FR could depend on the background light spectrum.

Supplementary lighting in all treatments was turned off half an hour before sunset, allowing plants to enter the dark period under natural light conditions. Solar radiation usually has a higher fraction of FR at sunset compared to the middle of

the day. Light spectrum during twilight (before dark period) is crucial for photomorphogenesis. The highest sensitivity of hypocotyl elongation to low R:FR occurs at the end of the light period, which is probably related to the circadian clock (Salter *et al.*, 2003). Additional FR at the end of day reduced fruit set, just as whole day FR (Chapter 2 Fig. 1). Thus, one possibility is that the natural solar light at the end of the day reduces the negative effects of whole day FR on fruit set. Instead, only the positive effect of additional FR on fruit set by enhancing source strength would remain.

In the climate chamber, a consistent FR effect on fruit set was found when several different light spectra were used to provide photosynthetic active radiation (PAR): 1) 9% blue + 18% green + 73% red; 2) 19% blue + 43% green + 38% red; 3) 10% blue + 0% green + 90% red (Chapter 2 & 3). This suggests that as long as PSS was unaffected, the composition of blue, green and red light as background spectra was probably less important for the FR-reduced fruit set.

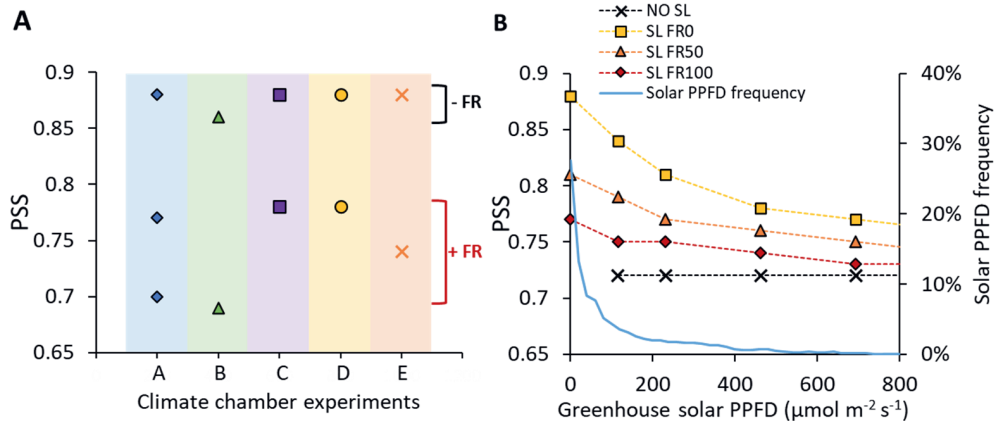


Figure 1. PSS (photostationary state of phytochrome) values in (A) climate chamber experiments and (B) greenhouse experiment. (A) Different symbol shapes represent different experiments in climate chambers. Experiment A: Chapter 2; B: Chapter 3 NPA experiment; C: Chapter 3 decapitation experiment; D: Chapter 3 yucasin and NAA experiments; E: High light intensity experiment (Chapter 6 Supplementary Fig. S1). '-FR' stands for treatments without additional far-red (FR); '+FR' stands for treatments with additional FR. (B) PSS values of additional FR treatments in the greenhouse experiment (Chapter 5), at different levels of incident solar light in the greenhouse. The calculation was based on a standard solar light spectrum (ASTM, 2003). SL stands for supplementary light. FR0, FR50, FR100 indicates the additional 0, 50 or 100 µmol m^{-2} s^{-1} of FR to the 190 µmol m^{-2} s^{-1} supplementary PPFD. PPFD stands for photosynthetic photon flux density between 400-700 nm. The frequency of solar PPFD was expressed as a percentage of all light measurements during light periods (every 5 min), where light period refers to periods with solar light and/or supplementary light.

3.2 Background light intensity

The effect of additional FR could depend on light intensity. In lettuce, increasing fraction of FR decreased leaf expansion at low light intensity, but increased leaf

expansion at high light intensity (Kusuma & Bugbee, 2023). Moreover, phytochrome responses can be affected by light intensity. Compared to high light intensity, photochemical reactions of phytochromes become slower at low light intensity, where thermal reversion has a stronger impact and lowers the proportion of active phytochromes (Sellaro *et al.*, 2019).

FR-reduced fruit set seems to depend on light intensity too. In a climate chamber experiment ('Gialte') with high light intensity (PPFD 250 µmol m^{-2} s^{-1}, photosynthetic photon flux density), additional FR reduced fruit set by 22% (Supplementary Fig. S1). This reduction was much less than observations in Chapter 2 ('Frazier', PPFD 130 µmol m^{-2} s^{-1}) and Chapter 3 ('Gialte', PPFD 120-190 µmol m^{-2} s^{-1}), where fruit set was reduced at least by half with additional FR. In the greenhouse, supplementary light (PPFD 190 µmol m^{-2} s^{-1}) plus solar radiation can easily exceed PPFD of 250 µmol m^{-2} s^{-1}. It is unknown yet whether further increasing light intensity above 250 µmol m^{-2} s^{-1} in a climate chamber condition can reverse the FR effect on fruit set from a negative to a positive effect.

3.3 Source and sink relationships

Plant source strength (assimilate supply) and sink strength (assimilate demand) are highly relevant to fruit set. An increase in source strength increased fruit set, while an increase in sink strength decreased fruit set in sweet pepper (Marcelis *et al.*, 2004; Wubs, 2010; Homma *et al.*, 2022). Fruit set is positively correlated to source: sink ratio until reaching a plateau (in sweet pepper Homma *et al.*, 2022; in tomato Bertin & Gary, 1993; Kang *et al.*, 2011). Wubs *et al.* (2009) suggested a model in which fruit set is regulated by the source: sink ratio. If the source: sink ratio at anthesis is above a certain threshold, the flower will set into a fruit. Source: sink threshold has been used to simulate fruit set in cotton (Lieth *et al.*, 1986), tomato (Bertin & Gary, 1993), cucumber (Marcelis, 1994), and sweet pepper (Wubs *et al.*, 2009). The source: sink threshold for fruit set can also well explain fruit set fluctuations over time (Wubs *et al.*, 2009; Mathieu *et al.*, 2008).

The source: sink threshold for fruit set is related to the potential fruit size, where large-fruited cultivars hold a higher threshold while small-fruited cultivars hold a lower threshold (Wubs *et al.*, 2009). Additional FR was found to enhance potential fruit growth in sweet pepper (Supplementary Fig. S2; Chapter 5 Fig. 3) and in tomato (Ji *et al.*, 2020). This effect was more pronounced in a climate chamber (about 60% increase; Supplementary Fig. S2) than in a greenhouse (about 7% increase; Chapter 5 Fig. 3A). A more demanding fruit probably means a higher source: sink threshold to fruit set, thus additional FR may increase the source: sink threshold to fruit set (Fig. 2A). Moreover, a higher sink strength of earlier-formed fruits usually leads to a higher abortion rate of flowers and fruits formed later

(Marcelis *et al.*, 2004; Ganeshaiah& Uma Shaanker, 1994), due to competition for assimilates and dominance. This suggests that the maximum fruit set could be reduced by additional FR (Fig. 2A).

I argued previously that additional FR may have a stronger effect on fruit set when PSS is decreased from 0.88 to 0.8, compared to a PSS change from 0.8 to lower values. This could also apply to the FR effect on source: sink threshold to fruit set (Fig. 2B), especially considering the FR effect of enhancing fruit potential growth was stronger in climate chamber than in the greenhouse experiment. This means that adding FR in the climate chamber experiments could have a stronger effect on elevating source: sink threshold to fruit set compared to in the greenhouse experiment (Fig. 2B).

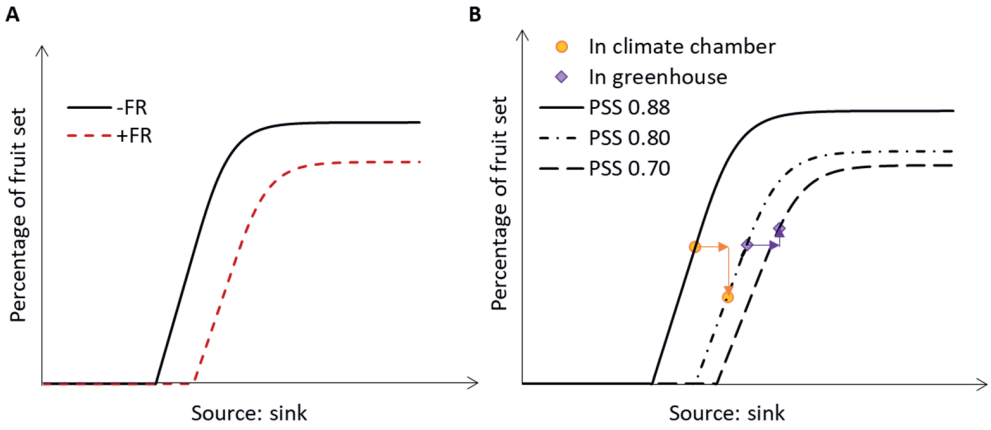


Figure 2. Illustrations of a hypothesized effect of additional FR on source: sink threshold for fruit set. In this hypothetical model, fruit set responds to source: sink ratio positively within a certain range, where the intercept with the x-axis is the source: sink threshold for fruit set. Below this range of source: sink ratio, no fruit set occurs; whilst above this range, fruit set gradually reaches a maximum. (A) Compared to no additional FR (solid line), additional FR (dashed line) increases the source: sink threshold to fruit set and reduces the maximum fruit set. (B) In a hypothetical scenario, adding FR has a larger effect on elevating source: sink threshold to fruit set when PSS was reduced from 0.88 (solid line) to 0.80 (dash-dotted line), but a smaller effect when PSS was reduced from 0.80 to 0.70 (dashed line). Adding FR in climate chamber results in PSS changing from 0.88 to 0.80, while adding FR in greenhouse results in PSS changing from 0.80 to 0.70. Assuming that a same level of fruit set can be achieved in both conditions without additional FR, a same extent of increase in source: sink ratio by additional FR results in a reduced fruit set in climate chamber (orange arrows) but an increased fruit set in greenhouse (purple arrows).

In a hypothetical scenario, the fruit set without additional FR are set at the same level in both climate chamber (PSS 0.88) and greenhouse conditions (PSS 0.80 with supplementary PPFD) (Fig. 2B). Additional FR led to 19% -26% increase in source strength in both climate chamber and greenhouse experiments, using plant dry weight as an indicator for source strength (Chapter 2 Fig. 4; Chapter 5 Fig. 3).

Considering this plus the increased carbohydrates contents in vegetative organs (discussed in section 1.1), additional FR is assumed to increase source: sink ratio. It is noticeable that, if additional FR leads to a same extent of increase in source: sink ratio, it would result in a higher fruit set in greenhouse (PSS from 0.80 to 0.70), but a lower fruit set in climate chamber (PSS from 0.88 to 0.80), due to different magnitude of increase in source: sink threshold (Fig. 2B). This means that the source: sink ratio can (partly) explain the different FR effect on fruit set, when taking into account the potential FR effect on source: sink threshold for fruit set and the dependence of FR effect on the background light.

In this hypothetical scenario (Fig. 2B), plants grown in greenhouse (PSS 0.70-0.80) may require a higher source: sink ratio to reach the same level of fruit set as in the climate chamber without additional FR (PSS 0.88). This hypothesis was supported by the observation that, even with a higher daily light integral (DLI) per plant, the fruit set of the first 12 flowers in the greenhouse, either as fruit number or as percentage, was lower than those in most of the climate chamber experiments (Table 2).

3.4 Local light conditions

In this thesis, light treatments were designed as amount of light per m^2 at the top of the canopy, while plant and shoot density can influence the light perceived by an individual plant, and the local light conditions within a canopy.

Changes in the light spectrum help plants to perceive their neighbors, especially in dense vegetation. Higher planting density not only reduces the intercepted light by each plant and thus the source strength, but also reduces R:FR and fraction of blue light within the canopy. The magnitude of a FR effect on plant growth can vary depending on planting density, as shown in lettuce by Jin *et al.* (2021) and cucumber seedlings by Shibuya *et al.* (2020). Light intensity and R:FR decrease dramatically with canopy depth at a high planting density, while they decrease much more slowly at a low planting density (Postma *et al.*, 2021). Compared to the greenhouse experiment, high planting densities in the climate chamber experiments may have amplified the effect of additional FR (Table 2), perhaps due to a further reduced R:FR within the canopy.

Not only the top of the canopy, but also lower canopy perceives FR, and this can lead to photomorphogenic responses higher in the plant (van der Meer *et al.*, 2023). Usually, the reproductive organs of indeterminant growing sweet pepper are not at the top of the canopy. It is still not clear if the reproductive organs can perceive local light spectrum signals. On the one hand, the light spectrum signal can be perceived by leaves and transmitted to flowers/fruits, as proposed in the study of FR promoting flower bud abscission in *Hibiscus rosa-sinensis* L. (van Meeteren & van

Gelder, 2000). On the other hand, in tomato, silencing fruit-localized phytochromes upregulated genes encoding cell wall invertases (Bianchetti *et al.*, 2018), suggesting a regulatory role of local phytochromes in fruit sink activity. Local light availability was also suggested as an environmental cue to influence the outgrowth of axillary buds (Walker & Bennett, 2018). However, the possibility that local light spectrum and intensity affects fruit set directly through perception by flowers/fruits themselves still requires further investigation. This possibility can perhaps contribute to explaining different FR effects on fruit set in different experiment set-ups, due to different plant density and light distribution within the canopy.

3.5 Plant architecture

Other than the factors mentioned above, plant architecture was also different between climate chamber experiments and the greenhouse experiment (Fig. 3). With different pruning, plants in climate chamber experiments generally had a higher number of shoots (Table 2). The difference in plant architecture may influence the local light conditions as mentioned above (section 3.4) and may influence the interactions between different organs. In the greenhouse, usually no more than 4 flowers per plant would reach anthesis at the same time, whereas in climate chambers, sometimes 8 flowers per plant at the same node number would reach anthesis at the similar time (Fig. 3). Moreover, more shoots per plant could mean a stronger FR-enhanced vegetative sink strength and apical dominance, as suggested in Chapter 3. A higher vegetative sink strength could have a negative effect on fruit set, as shown in a simulation for cucumber (Marcelis, 1994). Thus, a higher number of shoots may enhance the negative effect of additional FR on fruit set.

In addition, the shoot orientation has a profound effect on fruit set in various fruit crops (Budiarto *et al.*, 2018; Khandaker *et al.*, 2020; Lauri *et al.*, 1998; Robbie *et al.*, 1993). In climate chamber, sweet pepper plants grown with additional FR had relatively upright branches, whereas plants grown without additional FR had more horizontal branches, with wider angles between splitting branches (Chapter 2 Fig. 3, Table 3). In greenhouse, additional FR had very limited effect on shoot orientation, as plants are trained on a high wire 'V' grow system, where all plants were growing upright (Chapter 5 Fig. S2). Therefore, the difference in shoot orientation between the climate chamber and the greenhouse experiment may influence the effect of FR on fruit set.

Table 2. Differences in plant management and daily light integral between greenhouse experiment and various climate chamber experiments. Node number was counted from bottom to top, where the first splitting node was recorded as node 0. DLI indicates daily light integral. For climate chamber experiments, DLI was only from supplementary light. For the greenhouse experiment, DLI includes both supplementary light and solar light. DLI shown for the greenhouse experiment is the DLI during the period from one week before the anthesis of first target node (node 3, or 6), until one week after the anthesis of last target node (node 5, or 8). Average DLI across the whole greenhouse experiment was 12.4 mol/m^2. Fruit set is expressed in number of fruits per plant, and in percentage of set fruit in flower numbers; '- vs. +FR' indicates the fruit set of plants grown without vs. with additional FR; 'G','M' and 'F' indicates the sweet pepper cultivar 'Gialte', 'Margrethe' or 'Frazier' respectively.

Experiments	Chapter 5 Greenhouse experiment		Chapter 2 Whole day or EOD FR	Chapter 3 NPA experiment	Chapter 3 Decapi-tation experiment	Chapter 3 NAA & Yucasin experiments	Chapter 6 Fig. S1 High light intensity experiment
Plant density (#/m^2)	2.7		7.1	11.5	9.5	5.4	5.4
Number of shoots (#/plant)	2		2	4	4	4	8
Nodes for fruit set observation	3-5	6-8	1-3	2-3	2-3	2-3	2-3
Flower numbers (#/plant)	12	12	8	12	8	8	12
Start of FR treatments	Later than anthesis of these flowers	At the first anthesis of these flowers	1 week before first anthesis	1 week before first anthesis	1 week before first anthesis	1 week before first anthesis	1 week before first anthesis
DLI (mol/m^2)	6.3	6.8	5.6	11.1	6.0	6.1	12.6
DLI (mol/plant)	2.4	2.6	0.8	1.0	0.6	1.1	2.3
Fruit set (#/plant) (- vs. + FR)	(G) 1.5 vs.1.5 (M) 1.1 vs. 2.3	(G) 2.9 vs. 5.3 (M) 4.3 vs. 6.1	(F) 2.8 vs. 1.3	(G) 3.6 vs.1.3	(G) 4.6 vs. 2.4	(G) 0.5-1.5 vs. 0-0.2	(G) 7.3 vs. 5.6
Fruit set (%) (- vs. + FR)	(G) 12.5 vs.12.5 (M) 9.2 vs.19.2	(G) 24.2 vs.44.2 (M) 35.8 vs.50.8	(F) 35.0 vs.16.3	(G) 30.0 vs.10.8	(G) 57.5 vs.30.0	(G) 6.3-18.8 vs. 0-2.5	(G) 60.8 vs.46.7

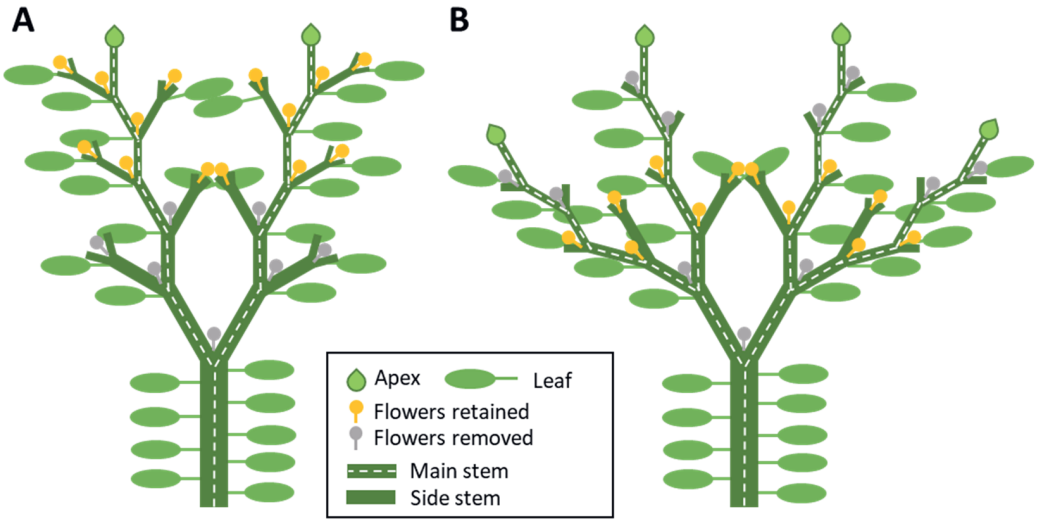


Figure 3. Illustrations of (A) sweet pepper plant architecture in greenhouse and (B) a representative plant architecture in climate chamber experiments. Leaves shown in the figures only indicate the leaf location, but do not represent the reality of leaf shape, size, and angles.

4 The logic of low fruit set in nature

The three sweet pepper cultivars used in this thesis produce relatively large fruits and therefore probably hold a high source: sink threshold for fruit set (Wubs *et al.*, 2009). The high threshold may help to reduce the emergence of new sinks (fruits) under a suboptimal condition, especially a demanding sink. This can help to sustain the growth and development of the existing fruits as well as to sustain the shoot growth with both new vegetative and generative organs, which serves to increase the chance of successful reproduction.

In nature, some plants produce excess flowers, which could be a reason for a low fruit set naturally. The production of excess flowers may indirectly increase reproductive success by allowing selective maturation of fruits of superior quality especially under a higher variance in quality (Burd, 1998; Stephenson, 1981). The excess flowers may simultaneously play other roles such as pollinator attraction, pollen doner, bet hedging, and reproductive assurance mechanisms (Burd, 1998). A self-organizing model (Ganeshaiah & Uma Shaanker, 1994) suggested that any resource molecule moving to a sink, autocatalytically increases its probability of getting further resources. Between two identical ovules, perhaps the initial molecule import is random, while this action autocatalytically leads to further molecule import and dominance of this ovule and the eventual abortion of the other. This mechanism can explain there will be abortions even under resource-

abundance conditions, while resource limitation aggravates the extent of abortion (Ganeshaiah & Uma Shaanker, 1994).

5 Practical potentials in light spectrum-regulated fruit set

Supplementary PAR light in winter greenhouse cultivation in high latitude regions may cause more fruit stacking and higher labor time for plant maintenance and harvest, due to the reduced internode length (Lanoue *et al.*, 2022). Additional FR in the supplementary light can promote internode elongation and alleviate these problems for plants grown under LEDs. Moreover, additional FR, regardless whether applied long-term or short-term, reduced fruit cracking in sweet pepper (Chapter 5 Fig. 4). This suggested a possible application of FR during susceptible fruit development periods or under unfavorable climate conditions to reduce fruit cracking and hence improve fruit quality.

The fluctuation in fruit set matters in a long-term sweet pepper greenhouse cultivation. In crops like pepper (Heuvelink *et al.*, 2004) and cucumber (Marcelis, 1992), fruit set shows a strong cyclical pattern, where periods with high fruit set are alternated with periods with almost no fruit set. This fruit set fluctuation leads to fluctuations in yield, labor demand, and market product price. In an attempt to regulate fruit set pattern with additional FR, temporary FR for four weeks gave a temporary boost in the subsequent peak of weekly fruit set (Chapter 5 Fig. 2). This shows the potential in using a dynamic lighting strategy adjusting light spectrum to regulate plant growth and development. Other than additional FR, alternating between high B:R and low B:R might be another possibility to regulate fruit set fluctuations, as high B:R reduces fruit set and low B:R increases fruit set (Chapter 4 Fig. 1).

Dynamic lighting could make a better use of energy, compared to a long-term use of additional FR. With dynamic lighting, growers could match the high productivity period with the high market demand or high product price period to maximize profit. In addition, excessive elongation caused by long-term FR application may negatively affect the production time of sweet pepper, due to height limits in greenhouses. A dynamic instead of long-term use of FR could avoid this drawback, by promoting internode elongation to a desirable extent, or only prior to fruit set peaks.

However, to apply a dynamic lighting strategy with FR in practice, the optimal timing of adding FR should first be determined. When plants had low fruit load, additional FR since week 8 already increased fruit set in week 9 (Chapter 5 Fig. 2). When plants already had a heavy fruit load, adding FR since week 12 only increased fruit set in

week 14-15 (Chapter 5 Fig. 2). This difference may be related to the number and the developmental stages of fruits present on the plant. Moreover, the dose of FR should be investigated too. The additional FR levels in the greenhouse experiment (Chapter 5) were relatively high for practical use. It is possible that a lower dose of FR would already have a similar effect on fruit set fluctuation, which would be more cost effective considering the number of LED modules and the energy consumption.

Investigations about the effect of FR were done by adding FR on top of PAR in this thesis. Replacing part of PAR with FR may be a more cost-effective approach in practice, to obtain the regulatory effect from FR. FR seems less effective than PAR in terms of biomass accumulation in greenhouse (Chapter 5). This suggests that replacing PAR partly with FR may not result in the same positive effect in source strength as with additional FR. Therefore, in greenhouses where FR-enhanced yield was mainly associated with source strength, replacing PAR partly with FR may give a less positive effect on fruit set and yield compared to additional FR.

Agriculture can benefit from both enhancing and blocking abscission depending on the situation (Patharkar & Walker, 2019). Light spectrum can regulate fruit set as shown in this thesis. On the one hand, yield can be improved by increasing fruit set. On the other hand, intended fruit shedding can lead to bigger fruits (Patharkar & Walker, 2019; Stephenson, 1981), and can assist mechanical harvest (e.g. in olive Hartmann *et al.*, 1968). Perhaps manual fruit pruning at young stages can be replaced or assisted by a suitable lighting treatment in the future.

6 Conclusions

Based on observations in Chapters 2 to 5, I come to the following conclusions:

- Additional FR during the day (reducing PSS from 0.88 to 0.80 or lower), or FR at the end of the day, reduces fruit set in sweet pepper plants grown in climate chambers.
- In sweet pepper plants grown in greenhouses with solar light as in commercial practice, additional FR enhances fruit set. Since additional FR reduces fruit set in climate chambers, whereas additional FR enhances fruit set in greenhouses, the effect of additional FR on fruit set depends on other conditions, e.g., background light spectrum and intensity, source: sink relationships, planting density, and plant architecture.
- In sweet pepper plants grown in climate chambers, FR-reduced fruit set is associated with FR-enhanced apical dominance. Shoot apices have a crucial role in mediating FR-reduced fruit set, as decapitation increases fruit set and diminishes the FR effect of reducing fruit set. Chemically reducing basipetal

auxin transport in the stems, or applying synthetic auxin on decapitated apices, does not influence fruit set. Applying an auxin biosynthesis inhibitor to shoot apices increases fruit set independent of FR, accompanied by slight shoot growth retardation. These findings suggest that manipulating the basipetal auxin stream does not influence fruit set unless the growth of apices is arrested or removed. Therefore, the basipetal auxin stream does not mediate the FR-reduced fruit set. Considering additional FR reduces sucrose accumulation and invertase activities in flowers, the FR-reduced fruit set is proposed to relate to a stronger competition for photoassimilate between apices and flowers.

- In greenhouses, additional FR increases fruit yield of sweet pepper, which is mainly associated with enhanced source strength. Additional FR increases the number of fruits but barely influences individual fruit weight, even though additional FR increases potential fruit growth. In greenhouses, additional FR also enhances fruit set fluctuation and reduces fruit cracking.
- Sweet pepper plants grown under B:R of 1:10, 1:3 and 1:1, with PSS of 0.86-0.88, do not differ in fruit set; however, a very high B:R (9:1), which reduces PSS to 0.72, reduces fruit set. This suggests that a low PSS independent of FR reduces fruit set.
- A very high B:R (9:1) reduces starch content in sweet pepper flowers, which seems to relate to a lower source strength and a drop in sucrose synthase activity in flowers. Moreover, flowers grown at a very high B:R contain a lower auxin, higher salicylic acid, and higher cytokinin *c*Z and *c*ZR concentration, compared to the other B:R. Flowers grown at a very high B:R also maintain a high abscisic acid and ethylene level after anthesis. These findings suggest that the reduced fruit set at very high B:R is associated with reduced starch content and altered hormonal balance in flowers.

7 Future perspectives

- To determine why the FR effect on fruit set differed between the greenhouse and climate chamber experiments, several potential reasons could be investigated:
    - Identifying the potential effects of background light spectrum and different cultivation methods. For this purpose, the FR effect on fruit set could be observed in the following experimental set-ups:

      1) Growing sweet pepper plants in a greenhouse (solar light + LEDs), but with the same cultivation method as in the climate chamber

experiments (i.e., pruning as in Fig. 3B, high planting density). The effect of blocking solar light could also be investigated in this set-up.

2) Growing sweet pepper plants in a climate chamber (with only LEDs), but with the same cultivation method as in the greenhouse experiment (i.e., pruning as in Fig. 3A, low planting density, high wire V grow system). The effect of an artificial solar spectrum, e.g., as investigated in Hogewoning *et al.* (2010), could also be examined in this set-up.

- Identifying the dose response between FR and fruit set with more levels of FR intensities, and thus more levels of PSS values.
- Identifying the effect of additional FR on fruit set at more levels of PPFD.

- To support the hypothesis that enhanced competition for photoassimilate (as part of enhanced apical dominance) mediates the effect of FR-reduced fruit set, further experimental proof in FR-regulated carbon distribution, and its physiological and molecular mechanism are needed. Other than the transcriptional and translational regulation of sugar transporters, perhaps applying radioactive carbon dioxide could be a promising approach to investigate the carbon allocation at different light spectra. This, however, would require developments in facilities to monitor a large plant for a long period and at the desired light spectrum.
- Hormonal regulation of fruit set in response to light spectrum requires further investigation at the tissue level with more timepoints, to increase the resolution at both spatial and temporal levels. Other than hormone content alone, the responsivity/sensitivity to hormones, considering their receptors and responsive genes, should be determined.
- The role of salicylic acid in fruit set requires further investigation, such as through the application of salicylic acid on flowers before fruit set, or genetically manipulating the local biosynthesis of SA in flowers and fruits.
- The local perception of light spectrum and intensity by reproductive organs, and how it will influence fruit set, would require further investigation.
- Rather than adding FR on top of PAR, the effect of replacing PAR partly with FR on fruit set requires further investigation, which is relevant from a practical perspective. Sweet pepper production would also benefit from further investigations on dynamic lighting with additional FR in terms of optimal timing and intensities.
- The effect of light spectrum on fruit set should be verified in other fruit crops.

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Supplementary information

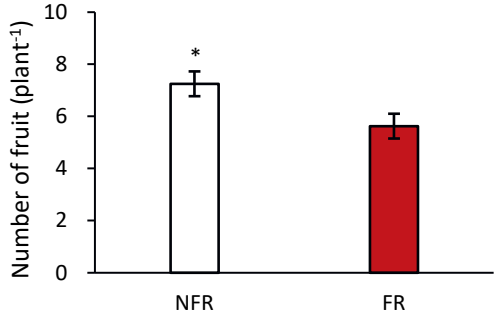

Figure S1. The number of fruits per plant in a high light intensity climate chamber experiment. Sweet pepper plants 'Gialte' were pruned to have 8 main shoots and 12 flowers per plant. 'NFR' indicates treatment without additional far-red (FR), which consisted of 252.3 ± 4.7 µmol m^{-2} s^{-1} photosynthetic active radiation (PAR, 400-700nm) and 2.3 ± 0.2 µmol m^{-2} s^{-1} FR (700-800nm), with a PSS of 0.88; 'FR' indicates the treatment with additional FR, which consisted of 252.2 ± 4.2 µmol m^{-2} s^{-1} of PAR and 122.5 ± 0.4 µmol m^{-2} s^{-1} of FR, with a PSS of 0.74. The light spectrum of PAR, photoperiod and other climate conditions were the same as NAA and yucasin experiments in Chapter 3. Each treatment had four statistical replicates, where each replicate consisted of 6 plants. Independent sample t-test was used at *P*=0.05, and '*' indicates significant differences between two treatments.

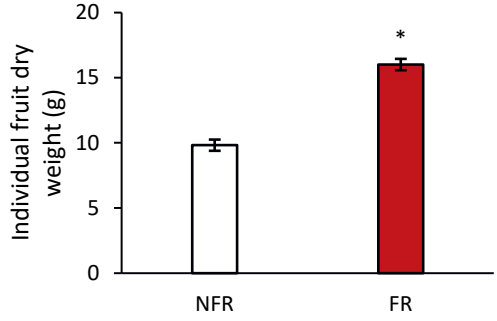

Figure S2. The individual fruit dry weight, when only one fruit was kept per plant (sweet pepper 'Gialte') in a climate chamber. Plants were decapitated after anthesis to ensure fruit set. Fruits were harvested ripe when they turned fully yellow. 'NFR' indicates treatment without additional far-red (FR), 'FR' indicates the treatment with additional FR. Photosynthetic active radiation (PAR, 400-700nm) in 'NFR' and 'FR' were 109.7 ± 5.5 µmol m^{-2} s^{-1}, 109.4 ± 4.8 µmol m^{-2} s^{-1} respectively. PAR consists of only blue and red light, with a blue: red ratio of 1:10. Additional FR (700-800 nm) was 58.6 ± 2.8 µmol m^{-2} s^{-1}, resulting in PSS of 0.88 and 0.77 in treatment 'NFR' and 'FR' respectively. Other experimental aspects were the same as NAA and yucasin experiments in Chapter 3. Each treatment had four statistical replicates, where each replicate consisted of 6 plants. Independent sample t-test was used at *P*=0.05, and '*' indicates significant differences between two treatments.

Summary

Fruit set indicates the initiation of growth and development of the female flower part (gynoecium) into a fruit, and the cessation of this process is termed as abortion. Fruit set or abortion influences the plant reproduction success in nature, and crop yield in agriculture. In many important crops, such as apple, pear, citrus, melon and peppers, mature fruits are only produced from a small portion of (female) flowers. **Chapter 1** first introduces the significance of fruit set in scientific and societal context. Fruit set, as the earliest stage of fruit growth, is sensitive to the environment. Various external stresses can affect fruit set, usually through physiological changes in hormones and carbohydrates. Among hormones, auxin and gibberellins promote fruit set, whereas ethylene and abscisic acid inhibit fruit set. Factors constraining carbohydrate supply from source to sink (new fruit) influence fruit set, where invertases and a few other components play an important role. Fruit set can also be influenced by the growth of other organs on the same plant, through competition for resources and/or correlative inhibition by hormone signaling. Light spectrum is an important environmental factor, however, its effect on fruit set has barely been studied. Using sweet pepper (*Capsicum annuum* L.) as a model species, this thesis aims to investigate how red: far-red ratio (R:FR) and blue:red ratio (B:R) of light can influence fruit set, and the underlying mechanisms regarding plant growth and morphology, carbohydrates and hormones.

Chapter 2 investigated whether R:FR influences fruit set in sweet pepper. In a climate chamber experiment, four light treatments were applied to plants grown under 130 µmol m^{-2} s^{-1} of red-rich LED light. Treatments consisted of different intensities of far-red (FR; 0, 50, 100 µmol m^{-2} s^{-1}) applied throughout the day or applied at the end of day (EOD; 30 minutes, 30 µmol m^{-2} s^{-1}). These treatments resulted in phytochrome photostationary state (PSS) values of 0.88, 0.77. 0.70 and EOD 0.16 respectively. This study demonstrated that additional FR reduced fruit set in sweet pepper, regardless of whether FR light was provided during the whole day or only at the end of the day. This effect cannot be explained by source strength, as additional FR enhanced source strength (using plant biomass as a measure for source strength). Therefore, a few other potential reasons for FR-reduced fruit set were discussed, including apical dominance, considering the vigorous growth of stems and the increased fraction of dry matter partitioned to stems at additional FR.

Following the discussion in the previous chapter, **Chapter 3** investigated whether apical dominance mediates FR-reduced fruit set. Several climate chamber experiments were conducted, where plants were grown under white or red-rich LED light, with or without additional FR. Additional FR was found to enhance apical

dominance: it increased auxin levels in the apices of dominant shoots, and caused a greater difference in internode length and apical auxin levels between dominant and subordinate shoots. Additional FR reduced fruit set in intact plants but not in decapitated plants, suggesting a crucial role of shoot apices in this effect. However, reducing basipetal auxin transport in the stems with NPA (N-1-naphthylphthalamic acid) did not influence FR-reduced fruit set, although auxin levels in the stem were largely reduced. Moreover, applying the synthetic auxin NAA (1-naphthaleneacetic acid) on decapitated apices did not influence fruit abortion. Whilst, applying the auxin biosynthesis inhibitor yucasin to shoot apices reduced fruit abortion regardless of the light conditions, accompanied by slight shoot growth retardation. Based on these findings, it was concluded that the basipetal auxin stream of apical dominance does not mediate FR-reduced fruit set. This study also found FR-reduced fruit set was associated with reduced sucrose accumulation and reduced invertase activity in flowers. Thus, an alternative hypothesis was put forward proposing that the high auxin level in shoot apices at additional FR enhances competition for assimilates between apices and flowers, which limits assimilate import into flowers and consequently reduces fruit set.

Considering a very high B:R reduces PSS as low R:FR does, **Chapter 4** explored the effects of B:R on fruit set. Plants were grown at B:R of 1:10, 1:3, 1:1 or 9:1 with a total photosynthetic photon flux density of 200 µmol m^{-2} s^{-1}, resulting in a PSS of 0.88, 0.88, 0.86 and 0.72, respectively. Only plants grown at the highest B:R (9:1) showed a reduced fruit set compared to the other three treatments. This response matched with the changes in PSS, suggesting the B:R effect on fruit set was likely controlled by phytochrome signaling. Reduced fruit set was associated with a reduced starch content in flowers, which may result from a reduced source strength and a drop in sucrose synthase activity in flowers. Flowers in the low fruit set treatment (B:R of 9:1) presented a low auxin, high salicylic acid and high *cis*-Zeatin type cytokinin (cZs) level; and maintained a high abscisic acid and ethylene level after anthesis. It was concluded that both the reduced starch content and the altered hormonal balance in flowers play a role in triggering fruit abortion at the high B:R of 9:1.

Additional FR was found to reduce fruit set in previous chapters in climate chamber experiments, whereas literature showed that additional FR promotes sweet pepper yield in greenhouse cultivation. Therefore, **Chapter 5** investigated whether additional FR still reduces fruit set when sweet pepper plants are grown as in commercial greenhouse production, as well as the effect of additional FR on sweet pepper yield. In a 24-week winter cultivation (Oct 2022 – Mar 2023), FR was added to 190 µmol m^{-2} s^{-1} of supplementary PAR (photosynthetic active radiation) in 4 treatments: 0, 50 or 100 µmol m^{-2} s^{-1} FR throughout the whole generative growth, or 100 µmol m^{-2} s^{-1} FR for only four weeks. Yield increased linearly with the cumulative

amount of FR in supplementary light. A yield component analysis revealed that increased fruit dry weight was mainly associated with increased total plant dry weight (source strength), where additional FR only caused a marginal increase in the fraction of dry matter partitioned to fruits. Additional FR increased light use efficiency (plant biomass per unit of incident supplementary PAR), but reduced radiation use efficiency (plant dry weight per unit of incident supplementary PAR plus FR). This indicates that FR was less efficient than PAR for biomass production. Additional FR enhanced fruit set percentage, which was in line with literature but in contrast with previous chapters. The difference between climate room and greenhouse experiments in the FR effect on fruit set was briefly discussed in this chapter, and further elaborated in Chapter 6. Moreover, both long-term and short-term FR application elevated fruit set peaks, resulting in stronger fruit set fluctuations over time.

Chapter 6 summarized and discussed the findings from Chapter 2 to 5. First, the possible physiological mechanisms underlying FR-reduced fruit set (Chapters 2 and 3) were described, in terms of assimilate unloading, and the role of hormones other than auxin. Then, the effect of high B:R (Chapter 4) and FR on fruit set (Chapters 2 and 3) was compared, where the similarities and differences between these two effects were discussed. Afterwards, the possible factors influencing the FR effect on fruit set were discussed by comparing climate chamber studies (Chapters 2 and 3) with the greenhouse study (Chapter 5). Background light spectrum and intensity could be a relevant factor, and the effect of additional FR to reduce fruit set may depend on the PSS range. Furthermore, a potential difference in source: sink threshold for fruit set, a potential difference in local light conditions within the canopy, and different plant architecture may play a role. The findings in this thesis suggest that the translation of knowledge achieved in one experimental set-up to another requires caution. Then, I discussed what could be the natural logic of a low fruit set, and how the findings in this thesis could be relevant for commercial practice. Lastly, I brought up perspectives for future research, which would improve the understanding of the physiological mechanisms of fruit set in response to light spectrum, and could contribute to fruit set regulation with light spectrum in practice.

Acknowledgments

Looking back to the last few years, I always feel I am so lucky to get this far. The PhD journey was not like climbing one big mountain, instead, it was filled with countless ups and downs. Nevertheless, it was a brilliant trip, because I met so many nice people to share the joy during the ups, and I have received so much support during the downs.

First of all, I want to give big thanks to my supervisors. **Ep**, I still remember the day clearly, when I was still doing my master's thesis, and I told you that I was interested in doing a PhD in our group. I was surprised that you seemed happy to know that, and afterwards, you discussed the possibility with Leo on the same day. Later, we developed the research ideas together, and with your support, I applied and successfully received a scholarship for my PhD study. I am very grateful for what happened back then and the opportunity you provided. During the PhD project, communication with you is always efficient, sufficient, straightforward, and enlightening, which is one of the most important reasons that I could finish my PhD in time. **Leo**, you always give essential help and input for big decisions when I need them the most. Your creativity often inspires me. At the last phase of my PhD, you dedicated a lot of time to reviewing my chapters, and checking on my status, which was very kind and caring of you. I appreciate it very much, and it helped me through the tough writing phase. **Remko**, thank you for joining this project. Your perspectives on fundamental plant physiology are highly appreciated, which played a key role in shaping my thesis, especially for Chapter 3. Without your contribution, I really doubt if we would publish it in such a high-impact factor journal. I am also grateful that you critically reviewed the part of my thesis where you did not directly participate.

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Doing a PhD is challenging, both mentally and physically. I want to thank all my colleagues and friends who provided their generous support in this process. I would like to start with my paranymphs. **Wannida**, you are always so kind, gentle, and caring, with a lot of smiles on your face, bringing extra warmth to me and other colleagues in this not-so-warm country. We started our PhD together and will finish our PhD at a similar time. Thank you for fighting with me shoulder by shoulder. I would like you to know that you have a lot of strength under your softness, never lose faith in yourself, and keep fighting! **Linqi**, thanks for your willingness to fly across the sea to witness this important moment with me. We have been friends since junior high school, which has been 16 years, time flies! It is so precious that we are still in close contact and keep encouraging each other during the hardships of PhD life. Let's keep going together, and our childhood dreams will come true. **Balta**, you are always so cheerful and positive! Your hospitality is impressive! I will always remember your hand-made pizza and your being considerate about the various dietary requests of the guests. Thank you for always generously sharing your ideas. I am very grateful that you shared the information about plant sugars summer school in France, which was a very fruitful experience for me. Cooperating with you was always pleasant. **Elena**, **Paul**, **Ying** and **Yongran**, thank you for generously sharing your knowledge and research experience with me. I want to give special thanks to Paul, thank you for your help with language, and for thinking along with me and trying to make sense of my complicated results. **Hua** and **Xiaohan**, thank you for your mental support. You are always so caring, and our friendship gave me a lot of courage. **Kim (Wenqing)**, **Ningyi** and **Xixi**, you can easily calm my mind even if I was anxious about the uncertainties in life. Talking to you is really soothing and refreshing, thank you. Facing my various requests about developing research ideas, methodologies, language, writing, tough harvesting and so on, I received so much support from previous and present colleagues in HPP. Here I want to express my sincere gratitude to **Alejandro**, **Anastasia**, **Cristy**, **Dália**, **Dianfan**, **Diego**, **Dorthe**, **Elias**, **Ernst**, **Hendrik**, **Ingeborg**, **Jiayu**, **Jordan**, **Julian**, **Katharina** (Huntenburg), **Lucas**, **Margarethe**, **Maria**, **Marijke**, **Martina**, **Mehdi**, **Mexx**, **Miaomiao**, **Michele**, **Na**, **Naixin**, **Nik**, **Rachel** (Schipper), **Samikshya**, **Sharath**, **Siok Hiang**, **Stephan** (David), **Willy**, **Wim** (van Ieperen), **Xin**,

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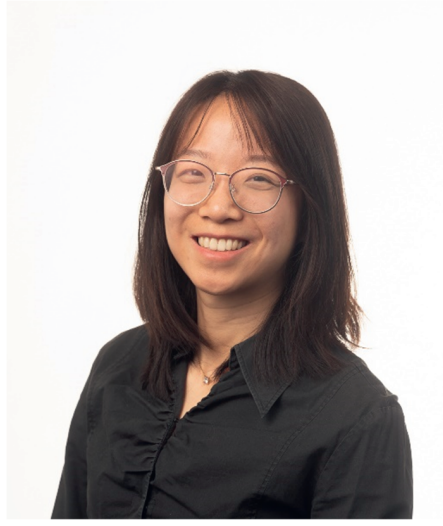
Coming to an end, it seems my PhD research left me with more questions than answers, which frustrated me sometimes. But because of so many pleasant memories with people around me, I felt this journey was never lonely. I learned so much from all of you, which I will carry with me to face new challenges in the future. Thank you all, and I wish you a lot of happiness in the future!

Spring & Autumn (Artwork by Jiaxing)

About the author



Sijia Chen was born in January 1995 in Hubei province, China. With her parents, she moved to Guangzhou when she was 3 years old and lived there for 15 years. Guangzhou, also called as 'city of flowers', holds a tradition of New Year flower markets, where many people will buy all sorts of festive ornamental plants and decorations during lunar new year. Thanks to the subtropical climates in Guangzhou, she enjoyed a high diversity of ornamentals, fruits, and vegetables, which raised her interest in plants. She pursued her interest and earned her bachelor's degree in biological sciences as one of the top students at Nankai University, Tianjin, China. For her bachelor thesis, she investigated the optimal hormonal recipes for *in vitro* propagation of '*Clerodendranthus spicatus*', which is a traditional medicinal plant in China for improving the urinary system. During her bachelor's study, she found the idea of 'plant factory' especially fascinating. Following her interests again, she came to Wageningen to learn more about plant sciences in the context of high-tech greenhouses and vertical farming. She achieved her master's degree at Wageningen University after a master's thesis about the heat inhibition of flowering in chrysanthemum and a research practice as a preliminary study for her PhD project. In November 2019, she started her PhD study focusing on light spectrum effects on sweet pepper fruit set and yield. In her spare time, she likes gardening, badminton, anime, and cooking. Gardening is one of her biggest hobbies. She grows plants at the windowsill, in a homemade mini vertical farm, and outside of her apartment – wherever it is possible. She dreams of having a big garden and producing her own vegetables in the future, as taking care of plants, and seeing them thriving is one of the most joyful things to her. She submitted her thesis in March 2024 and is currently working on a postdoc project about basil transpiration until the end of 2024. In the future, she will continue to follow her passion, and she is looking forward to new chapters on plant research, either in academia or in industries.

Contact: scarlettchen927@gmail.com

List of publications

Chen, S., Marcelis, L. F., & Heuvelink, E. (2022). **Far-red radiation increases flower and fruit abortion in sweet pepper (*Capsicum annuum* L.).** *Scientia Horticulturae*, 305, 111386. https://doi.org/10.1016/j.scienta.2022.111386.

Chen, S., Marcelis, L. F., Offringa, R., Kohlen, W., & Heuvelink, E. (2024). **Far-red light-enhanced apical dominance stimulates flower and fruit abortion in sweet pepper.** *Plant Physiology*, kiae088. https://doi.org/10.1093/plphys/kiae088.

Conference presentations

Oral presentation at IX International Symposium on Light in Horticulture (online, Sweden, 2021): Regulation of fruit set by light quality - A case study on pepper.

Poster presentation at International Symposium on Plant Photobiology (online, United States, 2021): Regulation of fruit set by light quality - A case study on pepper.

Oral presentation at International Symposium on Advances in Vertical Farming (Angers, France, 2022): The effect of far-red in the fruit set of pepper - What is the role of apical dominance?

Oral presentation at International Symposium on New Technologies for Sustainable Greenhouse Systems (Cancún, Mexico, 2023): Sweet pepper in winter greenhouse - How does additional FR influence the fruit set and yield?

Oral presentation (Fast talk) at International Workshop on Vertical Farming (Bologna, Italy, 2024): How blue: red light ratios influence fruit set and plant growth of sweet pepper?

Awards

ISHS Young Minds Award for Best Oral Presentation at IX International Symposium on Light in Horticulture (online Sweden, 2021).

ISHS Young Minds Award for Best Oral Presentation at International Symposium on Advances in Vertical Farming (Angers, France, 2022).

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review/project proposal (9 ECTS)

- Unravelling the mechanisms of light spectrum effects on fruit set: a case study in sweet pepper

Post-graduate courses (7.9 ECTS)

- Linear models; PE&RC (2020)
- Introduction to R and R studio; PE&RC (2021)
- Transcription factors and transcriptional regulation; EPS (2021)
- Annual meeting experimental plant sciences; EPS (2021)
- Environmental signalling in plants; Institute of Environmental Biology of Utrecht University (2022)
- Plant sugars: plant sugar metabolism, transport and signalling in a challenging environment; Institut Jean-Pierre Bourgin, Institute of Plant Sciences Paris-Saclay (2022)
- Plant hormones; EPS (2023)
- Bioinformatic introduction course; EPS (2023)

Invited review of journal manuscripts (2 ECTS)

- Environmental and experimental botany: the effect of adding FR light on crop biomass (2022)
- Scientia Horticulturae: the effect of salicylic acid on plant resistance (2023)

Deficiency, refresh, brush-up courses (0.3 ECTS)

- Advanced statistics; WUR-MAT (2020)

Competence, skills and career-oriented activities (1.6 ECTS)

- Presenting with Impact; WGS (2020)
- Efficient writing strategy; WGS (2020)
- Project and Time Management; WGS (2020)

- Scientific Artwork, Data visualisation and Infographics with Adobe Illustrator; WUR Library (2021)
- Writing propositions for your PhD; WGS (2022)
- Reviewing a Scientific Manuscript; WGS (2022)
- Career Orientation; WGS (2023)

Scientific integrity/ethics in science activity (0.9 ECTS)

- Ethics in plant and environmental sciences; WGS (2020)
- Scientific integrity; WGS (2020)

PE&RC Annual meetings, seminars and PE&RC weekend/retreat (1.5 ECTS)

- PE&RC Weekend for first years (2020)
- PE&RC Midterm retreat (2022)

Discussion groups/local seminars or scientific meetings (6 ECTS)

- Frontier literature in plant physiology (2020-2023)

International symposia, workshops and conferences (11 ECTS)

- ISHS IX International symposium on light in horticulture; oral presentation; online (2021)
- International symposium on plant photobiology; poster presentation; online (2021)
- 31st International horticultural congress; oral presentation; Angers, France (2022)
- The GreenSys: international symposium on new technologies for sustainable greenhouse systems; oral presentation; Cancún, México (2023)
- The 3rd International workshop on vertical farming; oral presentation; Bologna, Italy (2024)

Societally relevant exposure (0.7 ECTS)

- Young mind award for oral presentation & research summary of the presentation at Lighysym (2021)
- Experimental photo published in magazine Focus (2022)
- Young mind award for oral presentation at IHC (2022)
- Oral presentation included in webinar ISHS talks on vertical farming (2023)

Committee work (4 ECTS)

- PE&RC PhD Council buddy system (2021-2023)
- PE&RC PhD Council career committee (2021-2023)

Lecturing/supervision of practicals/tutorials (4.2 ECTS)

- Crop ecology (2020, 2022)
- Greenhouse technology (2020, 2022, 2023)
- Advanced methods for plant-climate research in controlled environments (2021, 2022)
- Crop, physiology and environment (2021, 2023)
- Research methods in crop science (2022)
- Quantitative aspects of crop production (2023, 2024)

BSc/MSc thesis supervision (6 ECTS)

- The role of apical dominance in the reduced fruit set under additional far red in sweet pepper
- Additional far-red radiation increases fruit sink strength and affects fruit quality in sweet pepper
- The role of auxin in reduced fruit set under additional far-red in sweet pepper
- The role of sugar and increased light intensity in reduced fruit set under additional far-red in sweet pepper *Capsicum annuum L*.
- Does red:blue light ratio determine fruit set in sweet pepper
- The effect of far-red light on photosynthesis in greenhouse cultivation of sweet pepper
- Supplementary far-red lighting increases fruit sink strength of sweet peppers cultivated in greenhouses
- Effect of additional far-red light on light interception of sweet pepper plants in greenhouse
- Effect of pre-harvest far-red light on post-harvest quality of sweet pepper fruits
- Literature study on the effect of light spectrum on sugar metabolism with a practical comparison of RNA isolation methods

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