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Research Article

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 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 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.

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% to 80% (Wubs et al. 2009). Flower and fruit abortion can be induced by various environmental signals, such as far-red radiation (FR; 700 to 800 nm). Generally, FR is an important signal for plants (Demotes-Mainard et al. 2016). Plants perceive FR with the phytochrome light receptors, which are activated by red light (R; 600 to 700 nm) 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 (Chen et al. 2022). However, the mechanism of this phenomenon is still unknown.

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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 and Whitelaw 2001). Ethylene promotes fruit abscission, while auxin hinders this process and reduces the sensitivity of the abscission zone to ethylene (Taylor and 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 and 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 (Chen et al. 2022).

Apical dominance has been mainly studied in the outgrowth of lateral buds, where the controlling role of auxin can be direct or indirect (Domagalska and 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 indole-3-acetic acid (IAA) (Bangerth 1989, 2000). Parthenocarpic fruits were even formed in tomato (*Solanum lycopersicum* L.) and pea (*Pisum sativum* L.) when apical dominance was released (Rodrigo and García-Martínez 1998; Serrani et al. 2010). 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. 2023). 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 (Morris and Arthur 1987; Patrick and Steains 1987; Bangerth 1989). 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, 1-naphthaleneacetic acid (NAA) 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 (SuSy) 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 N-1-naphthylphthalamic acid (NPA), (3) synthetic auxin NAA 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.

Results

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-wk-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 d 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. 1, B and C; Supplementary Fig. S1). Additional FR had less influence on the unbalanced growth of the first and second layer of internodes (internodes 1 and 2), which was probably related to the developmental stages of internodes when light treatments started (Supplementary Fig. S2). 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. S1). This effect of FR was also found in our other experiments (Supplementary Fig. S3). All these findings suggest that additional FR enhanced the dominance of the larger apical shoots over the smaller apical shoots and over lateral shoots.

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 four main shoots potentially carrying eight flowers (Fig. 2A). Additional FR was added 11 d 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. S4), and a higher dry mass accumulation and partitioning to stems (Supplementary Fig. S5). 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. S6). 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 nondecapitated 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.

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. 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.

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 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 nondecapitated plants was very low: despite the consistent trends with previous experiments that additional FR reduced fruit number, the effect

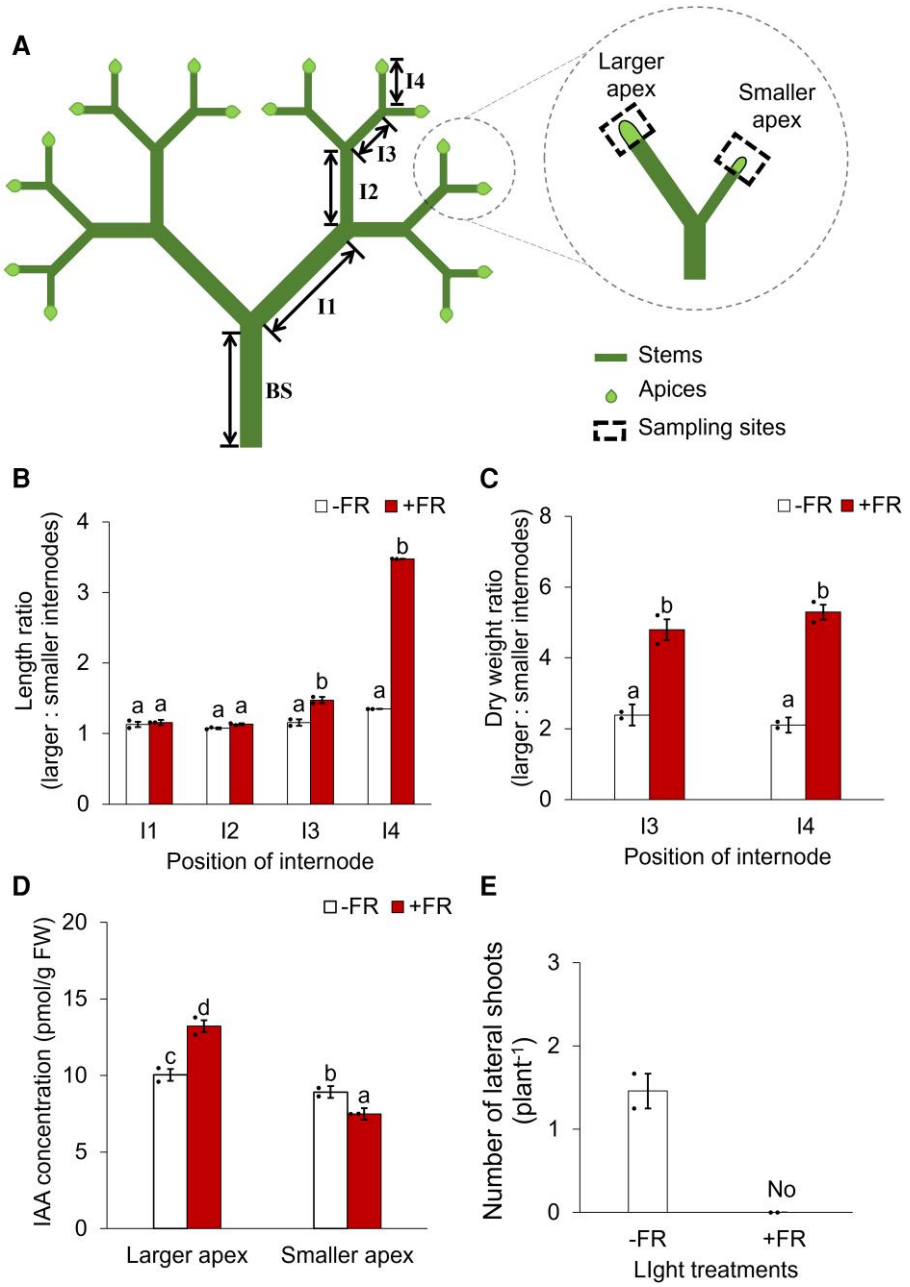

Figure 1. FR stimulates dominance from apical shoots in pepper plants. **A)** Plant shoot architecture during the vegetative growth experiment. All flowers were removed. Leaves are not shown. BS = bottom stem, the stem before splitting; I1 to I3 = internode 1 to 3; I4 = internode 4, and the apex above. Plants were cultivated with or without 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 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 two statistical replicates, each based on six plants. 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 $P = 0.10$.

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

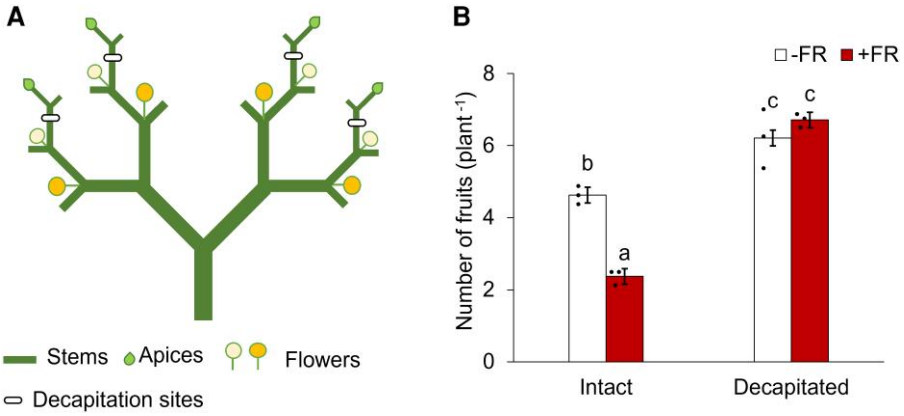

Figure 2. FR has no influence on fruit abortion in decapitated pepper plants. **A)** Plant shoot architecture during decapitation experiment. Plants were cultivated with or without FR. Plants were pruned to have four main shoots carrying eight flowers. Leaves are not shown. Decapitation was performed 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 three statistical replicates, each based on eight plants. 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 $P = 0.10$.

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. S3) indicating that the NAA application was effective in partially restoring apical dominance with respect to shoot branching.

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 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.

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. S7). Additional FR increased the total soluble sugar and nonstructural carbohydrates to different extents in different tissues (Supplementary Fig. S8). 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 d before and 7 d 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. S9), which suggests a suppression on fruit growth by additional FR.

We examined the enzyme activity of multiple groups of invertases and SuSys, 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

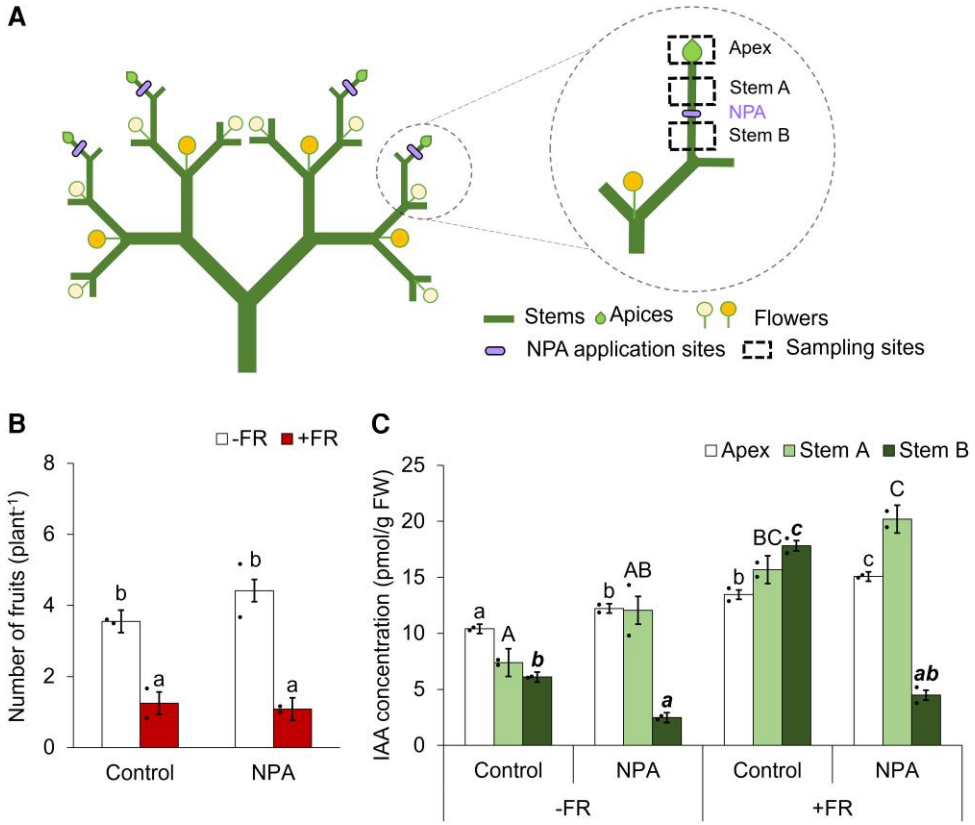

Figure 3. NPA application reduces auxin basipetal transport but does not influence fruit abortion. **A)** Plant shoot architecture in the NPA experiment. Plants were pruned to have four main shoots carrying 12 flowers. Leaves are not shown. Plants were cultivated with or without FR. NPA (5 mg/g) was 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 in apex, stem A, and stem B. FW stands for fresh weight. Mean values were derived from two statistical replicates, each based on six plants. Dots indicate individual data of each statistical replicate. ANOVA based on split-plot design was performed for (**B**) and (**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.

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 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. S10).

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. S7). Additional FR also led to a higher ethylene emission rate in flowers on day 1 to 3 after anthesis, but not anymore on day 4 to 7 after anthesis (Supplementary Fig. S11). 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. S7).

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 and 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. S12 and S13). Thus, compromised pollination or fertilization is unlikely to explain FR-stimulated abortion.

Discussion

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

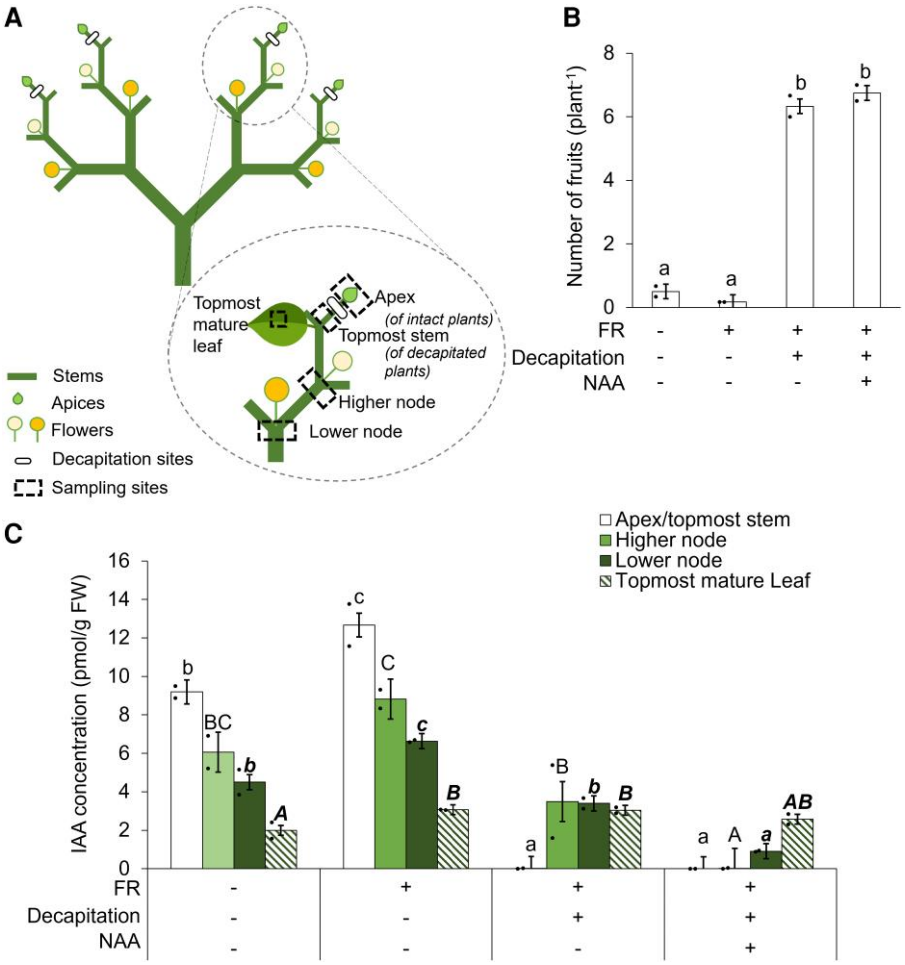

Figure 4. NAA application to decapitated shoots does not promote fruit abortion. **A)** Plant shoot architecture in NAA experiment. Plants were pruned to have four main shoots carrying eight flowers. Plants were cultivated with or without FR. NAA (1% w/w) was applied in lanolin paste on the cutting surface after decapitation, while sole lanolin was applied to the control group. Decapitation and NAA application were performed on the same day when the upper layer of flowers reached anthesis. Afterwards, NAA was renewed every week for two 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 and 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 in and plant apex/topmost stem, lower node, higher node, and leaf. FW stands for fresh weight. Mean values were derived from two statistical replicates, each based on six plants. 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.

plant developmental processes. In our previous study, we showed a negative effect of FR on fruit set in sweet pepper (*C. annuum* L.) (Chen et al. 2022). 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. S3). 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 (*P. sativum* L.) system with two cotyledonary shoots: IAA transport from the subordinate shoot was

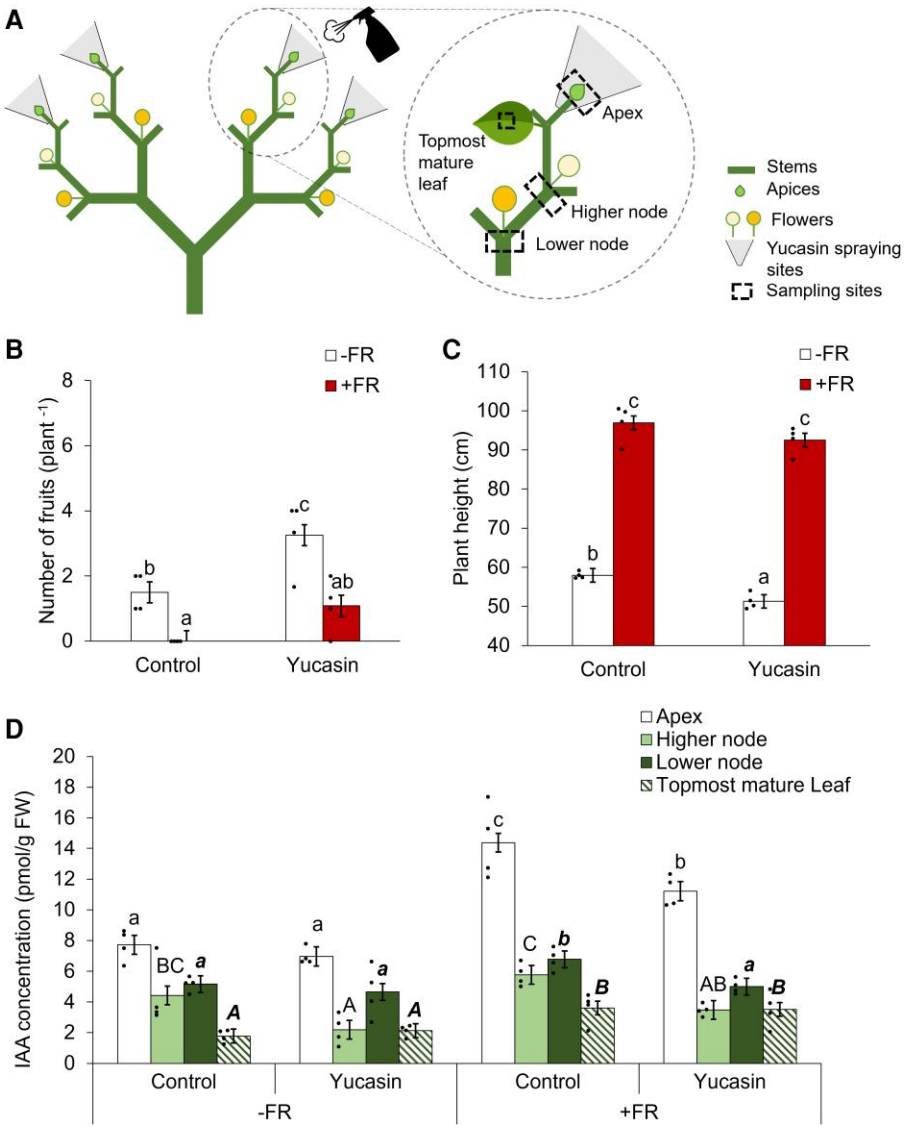

Figure 5. Inhibition of auxin biosynthesis in shoot apices reduces fruit abortion independent of FR. **A)** Plant shoot architecture upon treatment with the auxin biosynthesis inhibitor yucasin. Plants were cultivated with or without FR. Plants were pruned to have four main shoots carrying eight flowers. Yucasin solution (50 mM for first application, and 25 mM for second to fourth 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 and 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 in apex, higher node, lower node, and leaf. FW stands for fresh weight. Mean values were derived from four statistical replicates, each based on three plants. Dots indicate individual data of each statistical replicate. ANOVA based on split-plot design was performed for (**B**) to (**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.

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: Walker and Bennett 2018; Kotov et al. 2021; Beveridge et al. 2023). Based on this, we suggest that the enhanced unbalanced growth between two dichotomous shoots of a plant (Fig. 1, B and 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, 2000). Additional FR did not influence fruit abortion in decapitated plants (Fig. 2), suggesting shoot apices are the key mediators of the FR effect

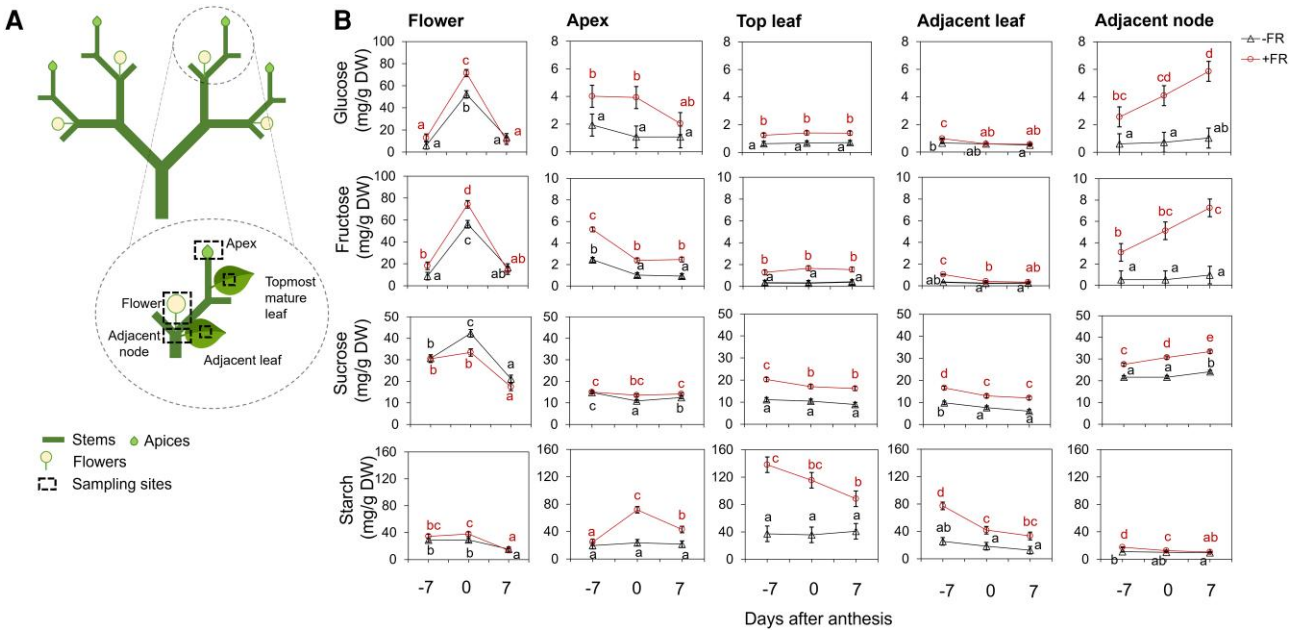

Figure 6. Additional FR leads to higher sugar content except for sucrose content in flowers. **A)** Plant shoot structures when sampling tissues for sugar and hormone measurements. Plants were pruned to have four main shoots, each carrying one flower. Plants were cultivated with or without FR. Sampled 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 four statistical replicates, each based on two 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 $P = 0.10$.

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.

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.

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

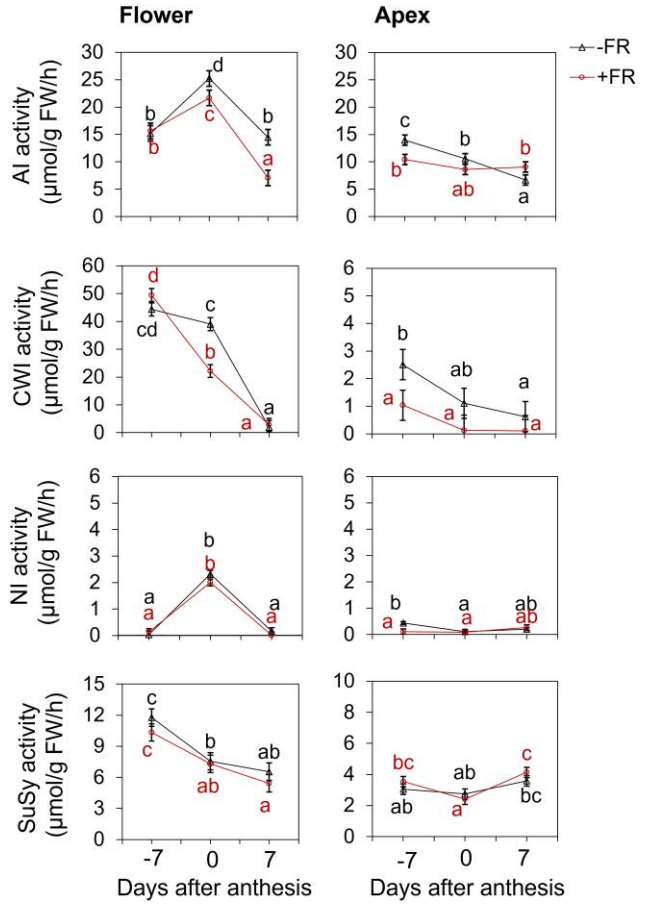

Figure 7. Effect of additional FR on the activity of sucrose cleavage enzymes. Plants were cultivated with or without FR. The activity of soluble AI, cell wall invertase (CWI), NI, and SuSy in flowers and apex on day 7 before anthesis, at anthesis, and on day 7 after anthesis. FW stands for fresh weight. Samples were collected within the last hour of light period. Mean values were derived from four statistical replicates, each based on two 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 $P = 0.10$.

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 (Ji et al. 2019; Kalaitzoglou et al. 2019; Chen et al. 2022). 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 (Morris and Arthur 1987; Patrick and Steains 1987; Bangerth 1989). 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 redistribution 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 (*S. lycopersicum* L.), lettuce (*Lactuca sativa* L.), and tobacco (*Nicotiana tabacum* L.) (Kasperbauer et al. 1970; Courbier et al. 2020; Zou et al. 2023). 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. S9).

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 and 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 SuSy and invertases in pepper flowers (Aloni et al. 1997). SuSy and invertases (especially CWI 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 and Tang 1999). CWI seem to have a more important role than SuSy 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 SuSy in our samples, which was not influenced by additional FR. Under additional FR, the lower activity of soluble AIs and CWI 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 AI 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 and 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 to 3 d 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 and 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.

Conclusions

Additional 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).

Materials and methods

Plant materials and growth conditions

Seeds of sweet pepper (*C. 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 wk 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 FR (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 four times a day with the same nutrient solution.

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 to 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 to 5 d 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.

Light treatments

In all experiments, 6-wk-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. S14). 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 1 wk before anthesis and 2 wk after anthesis (reviewed in Wubs et al. 2009). All the light treatments started at least 1 wk before the first anthesis and ended at least 2 wk 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.

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 d. 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.

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 Feb 5 to March 26, 2020, at Wageningen, Netherlands (51°N, 5°E). The glasshouse was divided into six compartments by white plastic sheets, where three replicates of

Table 1. Light conditions in various experiments

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;	−FR*	19	43	38	192.2 ± 2.3	4.1 ± 0.1	0.86	16
NPA EXP	+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;	−FR	10	0	90	122.4 ± 1.4	0.7 ± 0.2	0.88	14
Yucasin EXP	+FR				124.9 ± 2.1	56.2 ± 1.8	0.78	

See Supplementary Fig. S14 for Light spectra. PAR indicates photosynthetic active radiation (400 to 700 nm), where 400 to 500 nm was considered as blue light, 500 to 600 nm as green light, and 600 to 700 nm as red light. PPFD stands for photosynthetic photon flux density within the range of PAR. Light in the range of 700 to 800 nm was considered as FR. PSS = phytochrome photostationary state (calculated based on Sager et al. 1988). The light intensity was expressed as the mean ± SD 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. S14. Photoperiod of acclimation light was the same as the light treatments in the respective experiment.

two light conditions "−FR" and "+FR" were distributed over these compartments randomly. The light treatments started around 1 wk before the first anthesis and lasted for 44 d.

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 10 g slow-release fertilizer (Osmocote Exact Standard 8 to 9 M, 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 nondecapitated plants. Plants were rotated weekly within one compartment. All 16 plants per compartment were used for final morphological measurements.

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 2 wk before the first anthesis and lasted for 52 d. In each compartment, we divided plants into two subplots, six plants were treated with NPA 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.

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 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 third 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 3 wk. Light treatments started around 2 wk before the first anthesis and lasted for 53 d until final measurements and collection of plant tissues.

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 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.

Morphological observations and measurements

Anthesis, flower and fruit abortion were observed weekly. The reproductive organ abortion in our experiments happened mostly (>90%) 1 wk 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 h. Fruits with a diameter larger than 2 cm were cut open to count the number of seeds.

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 five 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 h, 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 d before anthesis; at anthesis (when flower petals just opened), and 7 d after anthesis (Supplementary Fig. S15). Flowers from spare plants were used for pollen viability test and pollen quantification as described in Supplementary Fig. S12.

IAA extraction and quantification

To measure the concentration of free auxin (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 10 s and placed on an orbital shaker in darkness at 4 °C overnight. The samples were then centrifuged at 10,000 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. S7; and the quantification of ethylene emission is described in Supplementary Fig. S11.

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 5 mL 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 8,500 rcf at 4 °C for 5, and 1 mL 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 21,300 rcf at 4 °C for 10 min. The samples from flowers were diluted 10 times, while the other samples were diluted five times with Milli-Q water for quantification of glucose, fructose, and sucrose.

The remaining pellet after extraction was washed three 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 8,500 rcf at 4 °C for 5 min. The supernatant was centrifuged at 21,300 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 45 mM 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).

Enzyme assay

The methods to determine the activity of invertases and SuSys 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 21,300 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 AI 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 NI. The activity of SuSy 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 SuSy 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 AI were centrifuged at 21,300 rcf shortly before the colorimetric determination.

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.

Supplementary data

The following materials are available in the online version of this article.

Supplementary Figure S1. Sweet pepper plants in the vegetative growth experiment.

Supplementary Figure S2. The length of stem and internodes of sweet pepper plants grown with or without additional FR.

Supplementary Figure S3. Outgrowth of lateral buds in various experiments.

Supplementary Figure S4. Plant height in various experiments.

Supplementary Figure S5. Plant total aboveground dry weight, and the partitioning of dry weight to stem, leaf, and fruit in various experiments.

Supplementary Figure S6. The pedicel length of flowers and young fruits at the end of decapitation experiment.

Supplementary Figure S7. Hormonal profiles of flowers before anthesis, at anthesis, and after anthesis.

Supplementary Figure S8. Effect of additional FR on total soluble sugar (TSS) and nonstructural carbohydrates (NSC) based on results in Fig. 6.

Supplementary Figure S9. The sample size of flower (young fruit) on day 7 after anthesis, for enzyme assay, carbohydrate analysis, and hormonal analysis.

Supplementary Figure S10. Protein content of flowers and apex samples used in Fig. 7.

Supplementary Figure S11. Ethylene emission rate of flowers and leaves grown with or without FR.

Supplementary Figure S12. Effect of additional FR on pollen number and pollen viability of sweet pepper flowers.

Supplementary Figure S13. Seed number of all available fruits (when fruit width >2 cm) in various experiments.

Supplementary Figure S14. Light spectra used in various experiments.

Supplementary Figure S15. Three developmental stages of flowers collected for carbohydrate and hormone analysis.

Supplementary Table S1. Nutrient solution recipe.

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Author contributions

S.C., E.H., L.M., and R.O. designed the research. S.C. conducted experiments in climate rooms and performed plant tissue sampling. E.H. supervised the study. W.K. conducted hormone measurements and supported their interpretation. S.C. analyzed the data. S.C. interpreted the findings with input from E.H., L.M., and R.O. L.M. secured funding. S.C. drafted the manuscript and all co-authors made substantial contributions to improve the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest statement. None declared.

Data availability

The data underlying this article are available in the article and in its online supplementary material.

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