Plant Growth Regul (2007) 51:53–61
DOI 10.1007/s10725-006-9147-x

ORIGINAL PAPER

Interaction of red to far red light ratio and ethylene in regulating stem elongation of *Helianthus annuus*

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Received: 8 June 2006 / Accepted: 30 October 2006 / Published online: 30 November 2006

Abstract Sunflower (*Helianthus annuus* L.) stems showed increased elongation under two types of vegetative shade: canopy shade (low red to far red [R/FR] ratio) and neighbouring proximity shade (FR enrichment). Hypocotyls also elongated more under narrow-band FR light than under narrow-band R light. Ethylene levels were determined in actively elongating 7-day-old hypocotyls and 17-day-old internodes under three R/FR ratios. Ethylene levels were lower in both sunflower hypocotyls and internodes when the R/FR ratio was reduced. Both FR enrichment of normal R/FR ratio and narrow-band FR light with very low light irradiance resulted in reduction in ethylene levels in 7-day-old hypocotyls. Further, in application experiments, sunflower stems grown under low R/FR ratio were more sensitive to ethephon and less sensitive to aminoethoxyvinylglycine (AVG) than stems grown under high R/FR ratio. Low R/FR ratio appears to initiate reduction in ethylene levels in sunflower seedlings, allowing maximum stem elongation. These results, and findings of other authors, suggest that various plant species may have developed different ways of regulating stem elongation and ethylene levels in response to low R/FR ratio.

Keywords Ethylene · Light quality · Narrow-band light · Stem elongation

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Introduction

Plants grown under canopy shade or in the shade of neighbouring, proximate vegetation are subjected to a lower red to far red (R/FR) ratio (Smith 2000). This lower R/FR ratio usually results from increased FR light irradiance reflected from adjacent leaves and/or a combination of increased FR light irradiance reflected from adjacent leaves and decreased R and blue (B) light irradiances due to absorption by canopy or taller neighbouring plants (Ballare et al. 1990; Ballare 1999; Franklin and Whitelam 2005). Plants grown under lower R/FR ratio conditions normally have etiolated stems and often exhibit reduced leaf development (Smith 2000; Franklin and Whitelam 2005; Vandenbussche et al. 2005).

Ethylene is often shown to inhibit stem elongation (Abeles et al. 1992), although in a few cases a growth-stimulatory role for endogenous ethylene has been reported (Raskin and Kende 1984; Jackson 1985; Rijnders et al. 1997). These apparently contradictory results can be explained by reports showing that ethylene, like auxin,

demonstrates a biphasic dose-response curve. Lower doses producing stimulation of various growth and developmental events, but higher doses producing the inhibitory responses, see Zobel and Roberts (1978), Reid et al. (1985), and Lee and Reid (1997). Interaction between ethylene and phytochrome was first reported in etiolated pea (*Pisum sativum*) seedlings, where short-term exposure to broad-band R and FR lights decreased ethylene evolution and increased plumular growth, with R light inducing double the plumular growth with half the ethylene content observed under FR light (Goeschl et al. 1967). In excised apical segments of etiolated rice (*Oriza sativa*), coleoptiles produced more ethylene when exposed to short-term FR light and less ethylene when exposed to short-term R light (Imaseki et al. 1971). Further, FR light reversed the inhibition of ethylene evolution observed in rice coleoptiles grown under R light (Imaseki et al. 1971).

Increased ethylene levels under low R/FR ratio has been reported for sorghum (*Sorghum bicolor*) plants containing a null mutation in the gene encoding phytochrome B (*phyB-1*) (Finlayson et al. 1998). These plants exhibited a constitutive phenotype similar to plants grown in shade and had higher ethylene levels under dim, FR enriched light [R/FR ratio = 0.75] (Finlayson et al. 1998, 1999). In a different study using mature tobacco (*Nicotiana tabacum*) plants, application of ethylene in low concentration resulted in increased stem elongation under FR enriched light with a low R/FR ratio of 0.2 (Pierik et al. 2003). Ethylene-insensitive transgenic tobacco plants failed to elongate to the same extent as wild type plants in response to a low R/FR ratio (Pierik et al. 2003).

Plants sense changes in light environment via photoreceptors, namely phytochromes, cryptochromes and phototropins (Smith 2000). Phytochromes can sense changes in R and B light irradiances, whereas cryptochromes appear to be involved in B light sensing (Franklin and Whitelam 2005). In *Arabidopsis thaliana*, five phytochrome and two cryptochrome photoreceptors have been identified and interactions between them have been shown (Vandenbussche et al. 2005). The phytochrome B (phyB) has been specifically implemented in R/FR ratio responses (Smith 2000) and Finlayson et al. (1998, 1999) studies shown a clear connection between phyB and ethylene.

In this study, the interaction of R and FR lights with ethylene in regulating stem elongation in young sunflower (*Helianthus annuus* L.) seedlings was investigated to better understand the role of ethylene in stem elongation under low R/FR ratio.

Materials and methods

Plants and experimental system:

Sunflower seeds (6946, Pioneer Seeds, USA) were imbibed under running water (25°C) in dark conditions for 24 h. Germinated seeds were then planted in soil mix (2 parts peat moss, 1–perlite, 1–vermiculate and 0.25–terra green) and transferred to growth chambers (Model PGR15, Conviron, Manitoba) equipped with fluorescent (Sylvania cool white 160 W) and incandescent lights (Philips 60 W). Temperature was maintained at 20°C during 16 h of light and reduced to 16°C during an 8 h dark period. Sunflower seedlings were watered daily with 0.25% strength Hoagland's solution (Hoagland and Arnon 1950).

Sunflower seedlings were harvested on day 7 or day 17 following planting. Day 7 was chosen for hypocotyl collection, as this is the midpoint for elongation, between hook opening (day 4) and initiation of internode growth with cessation of hypocotyl elongation (day 10). Day 17 was chosen for internode collection because rapid elongation of the internode occurs at this time, but the second leaf pair has not yet appeared. The hypocotyls (below the cotyledons and 5 mm above the roots) and internodes (first internodes between the cotyledons and first pair of leaves) were measured for length.

Combinations of fluorescent and incandescent light sources were used to alter the R/FR ratios and distance between lights and sunflower seedlings was adjusted to maintain PAR. Both R/FR ratio and PAR values were measured with a LI-COR LI-1800/22 (LI-COR Inc., Lincoln, Nebraska, USA) quantum sensor. For narrow-band

and FR light enrichment experiments, LED units (Quantum Inc., USA) producing narrow bandwidth light with a peak at 670 nm (R light), 735 nm (FR light) and 460 nm (B light) were utilized. FR light enrichment experiments were conducted in growth chambers (Model E7/2, Conviron, Manitoba) equipped with fluorescent (Sylvania cool white 100 W) and incandescent lights (Philips 60 W). Narrow-band FR, R and B light experiments were conducted in a dark room with 16 h light and 8 h dark photoperiod and constant temperature (20°C). Details on experimental light conditions are presented in Table 1.

The Tukey's ANOVA test for analysis of significance (at $P \leq 0.05$) and Spearman rank correlation test were run on SPSS software Version 14.

Measurement of ethylene evolution

Ethylene evolution by the entire 7-day-old hypocotyl or the entire 17-day-old internode was measured by incubating the tissue in a 3 ml syringe (1.5 ml volume) for 15 min. A 1 ml gas sample was collected and injected into a Photovac 10Splus GC (Photovac Inc., Markham, Ontario) with a photoionization detector and a 40/60 Carbopack B column (Supelco Canada, Oakville, Ontario).

Chemicals

The ethylene biosynthesis inhibitor, aminoethoxyvinylglycine (AVG, from Sigma Inc., Oakville, Ontario, Canada) was applied at concentrations of 10^{-2}, 10^{-3} and 10^{-4} M. The ethylene-releasing chemical, ethephon (2-chloroethylphosphonic acid, from Sigma Inc., Oakville, Ontario, Canada) was prepared on the day of use at concentrations of 10^{-3}, 10^{-4} and 10^{-5} M. For experiments involving 7-day-old hypocotyls, sunflower seedlings were sprayed to drip-off on days 4, 5 and 6 after planting. For experiments involving 17-day-old internodes, sunflower seedlings were sprayed on days 10, 12, 14 and 16 following planting.

Results

R/FR ratio interaction with ethylene in regulation of stem elongation (experiment #1):

Low R/FR ratio significantly promoted elongation of 7-day-old sunflower hypocotyls and 17-day-old sunflower internodes (Fig. 1a), whereas high R/FR ratio significantly reduced elongation of both hypocotyls and internodes, with more pronounced effect on internode elongation (Fig. 1a), all compared to normal R/FR ratio

Table 1 Experimental light conditions utilized in three experiments

Experiment #1	
High R/FR ratio of 4.63 (172 μmol m^{-2} s^{-1})	Fluorescent lighting only: very high R and B lights, FR light barely detectable
Normal R/FR ratio 1.31 (172 μmol m^{-2} s^{-1})	Decrease in fluorescent lighting plus addition of incandescent lighting: decreases in R and B lights and increase in FR light
Low R/FR ratio of 0.82 (172 μmol m^{-2} s^{-1})	Further decrease in fluorescent lighting and increase in incandescent lighting: further decreases in R and B lights and increase in FR light
Experiment #2	
R light (3 μmol m^{-2} s^{-1})	Narrow-band R (670 nm) light in dark from LED source
B light (3 μmol m^{-2} s^{-1})	Narrow-band B (460 nm) light in dark from LED source
FR light (3 μmol m^{-2} s^{-1})	Narrow-band FR (735 nm) light in dark from LED source
Experiment #3	
Normal R/FR ratio of 1.24 (158 μmol m^{-2} s^{-1})	R, B and FR wavelengths from fluorescent and incandescent light sources
Very low R/FR ratio of 0.21 (158 μmol m^{-2} s^{-1})	R, B and FR wavelengths from fluorescent and incandescent light sources plus addition of FR light emitting LED source

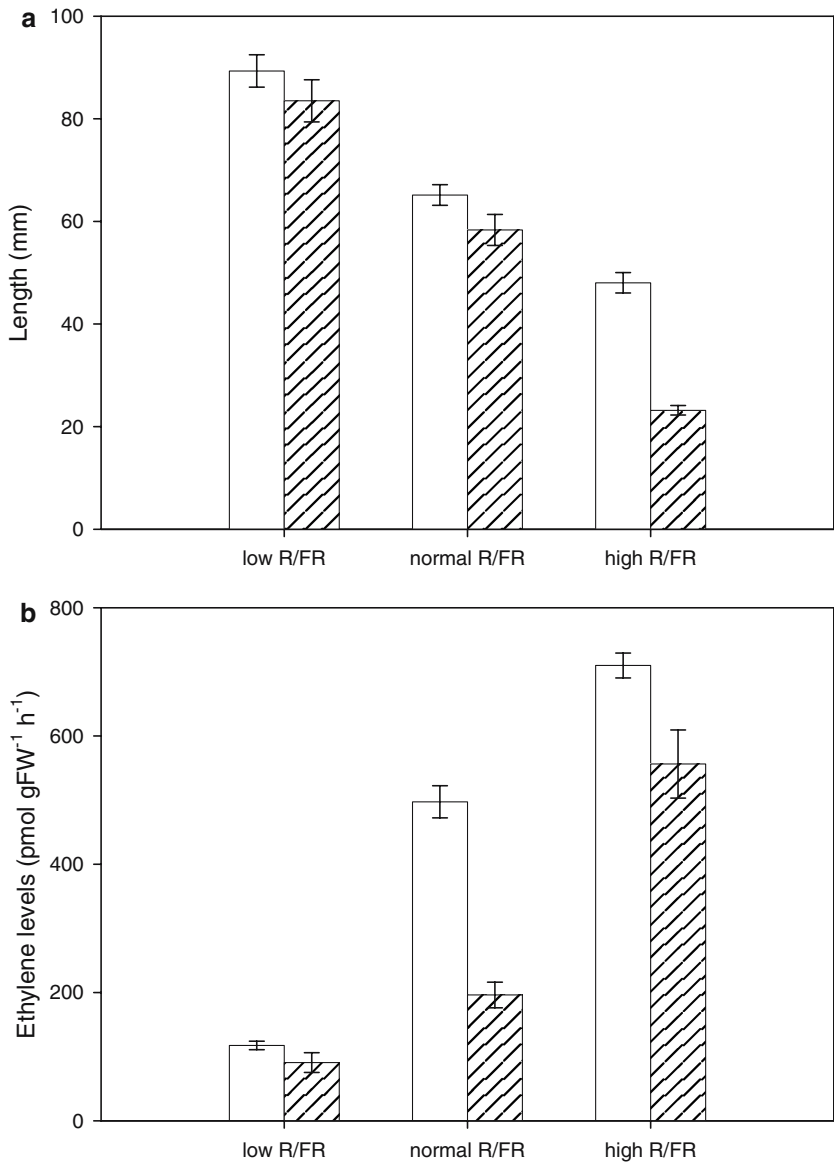

Fig. 1 Lengths (**a**) and ethylene levels (**b**) of 7-day- old hypocotyls (*open bars*) and 17-day-old internodes (*dashed bars*) grown under varying R/FR ratios (low: 0.82, normal: 1.31 and high: 4.63) and fixed (*low*) PAR of 172 μmol m^{-2} s^{-1}. The *error bars* indicate one SE of the mean

at same PAR irradiance (Table 1). Both 7-day-old hypocotyls and 17-day-old internodes exhibited significant reduction in ethylene levels (pmol gFW^{-1} h^{-1}) as R/FR ratio decreased (Fig. 1b). While ethylene levels under low and high R/FR ratios were similar between hypocotyls and internodes, 7-day-old hypocotyls under normal R/FR ratio produced twice as much ethylene as 17-day-old internodes (Fig. 1b). The largest change in ethylene levels for hypocotyls occurred between low and normal R/FR ratios, and for internodes between normal and high R/FR ratios (Fig. 1b).

Treatments of ethephon, which releases ethylene in plant cells following application, at concentrations of 10^{-4} and 10^{-5} M, and AVG, an inhibitor of ACC synthase, at concentrations of 10^{-3} and 10^{-4} M, were mostly ineffective in altering sunflower hypocotyl or internode growth (data not shown). Application of ethephon at 10^{-3} M inhibited 7-day-old hypocotyl elongation under low (ca 20%) and normal (ca 10%), but not under

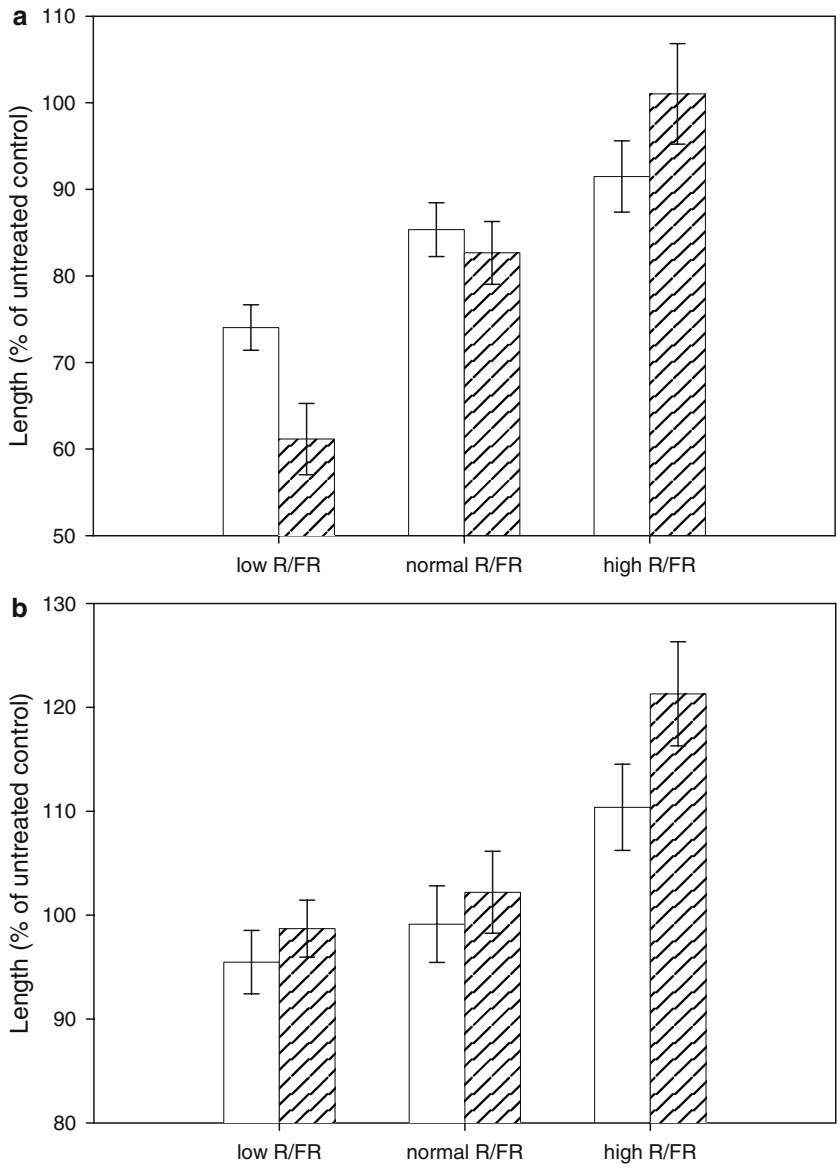

Fig. 2 Lengths (*% of untreated control*) of 7-day-old hypocotyls (*open bars*) and 17-day-old internodes (*dashed bars*) grown under varying R/FR ratios (low: 0.82, normal: 1.31 and high: 4.63) and fixed (*low*) PAR of 172 μmol m^{-2} s^{-1}. The plants were treated with either ethephon at 10^{-3} M (**a**) or AVG at 10^{-2} M (**b**) on days 4, 5 and 6 (*hypocotyl*) or days 10, 12, 14 and 16 (*internode*). The control value was set at 100% for each light quality treatment and tissue. The *error bars* indicate one SE of the mean

high R/FR ratio (Fig. 2a). A similar trend was obtained for 17-day-old internodes, although internodes were more sensitive to ethephon application, i.e. inhibition of elongation by ca 40% under low R/FR ratio and ca 20% under normal R/FR ratio conditions (Fig. 2a). Applied AVG at 10^{-2} M had no effect on stem elongation under low and normal R/FR ratios, but promoted elongation under high R/FR ratio of ca 10% for hypocotyls and ca 20% for internodes (Fig. 2b).

Narrow-band light regulation of stem elongation and ethylene levels (experiment #2):

Seven-day-old sunflower hypocotyls grown under monochromatic FR light were ca 20% shorter than dark-grown hypocotyls and ca 20% taller than R or B light-grown hypocotyls (Fig. 3a) at the same PAR irradiance (Table 1). A similar trend was obtained for fresh and dry

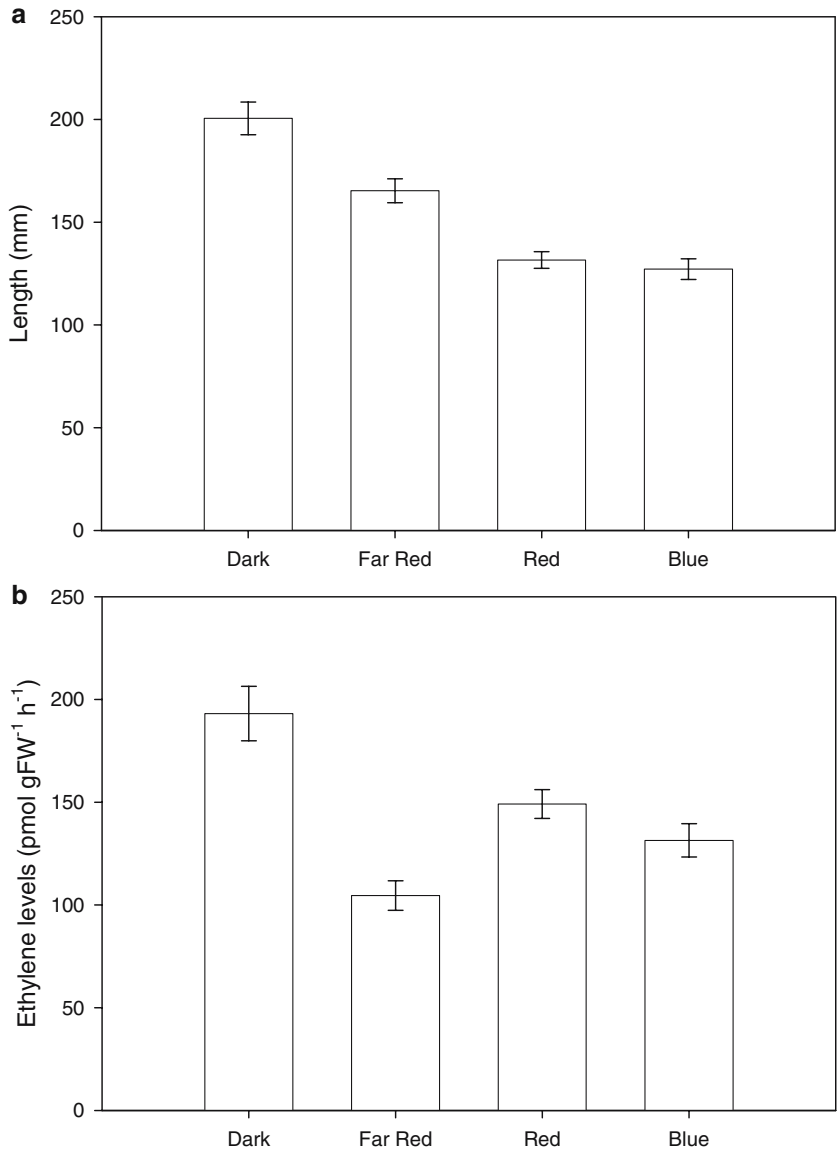

Fig. 3 Hypocotyl lengths (**a**) and ethylene levels (**b**) of 7-day-old sunflower seedlings grown under dark and narrow-band red, blue, far red lights with fixed (*very low*) PAR of 3 μmol m^{-2} s^{-1}. The *error bars* indicate one SE of the mean

biomass (data not shown). Hypocotyls grown under far red light produced half the ethylene of dark-grown hypocotyls and on average ca 30–40% less ethylene than R or B light-grown hypocotyls (Fig. 3b). There was no significant difference in length or ethylene levels between R and B light-grown hypocotyls (Fig. 3).

FR enrichment regulation of stem elongation and ethylene levels (experiment #3)

Seven-day-old sunflower hypocotyls grown under very low R/FR ratio were taller than hypocotyls grown under normal R/FR ratio by ca 30–40% (Fig. 4a) at the same PAR irradiance (Table 1). Hypocotyls grown under very low R/FR ratio also accumulated larger fresh and dry biomass (data not shown), but produced marginally (yet significantly) less ethylene than hypocotyls grown under a normal R/FR ratio (Fig. 4b).

Discussion

Short, slow growing *H. annuus* (sunflower) seedlings grown in the proximity of taller, faster

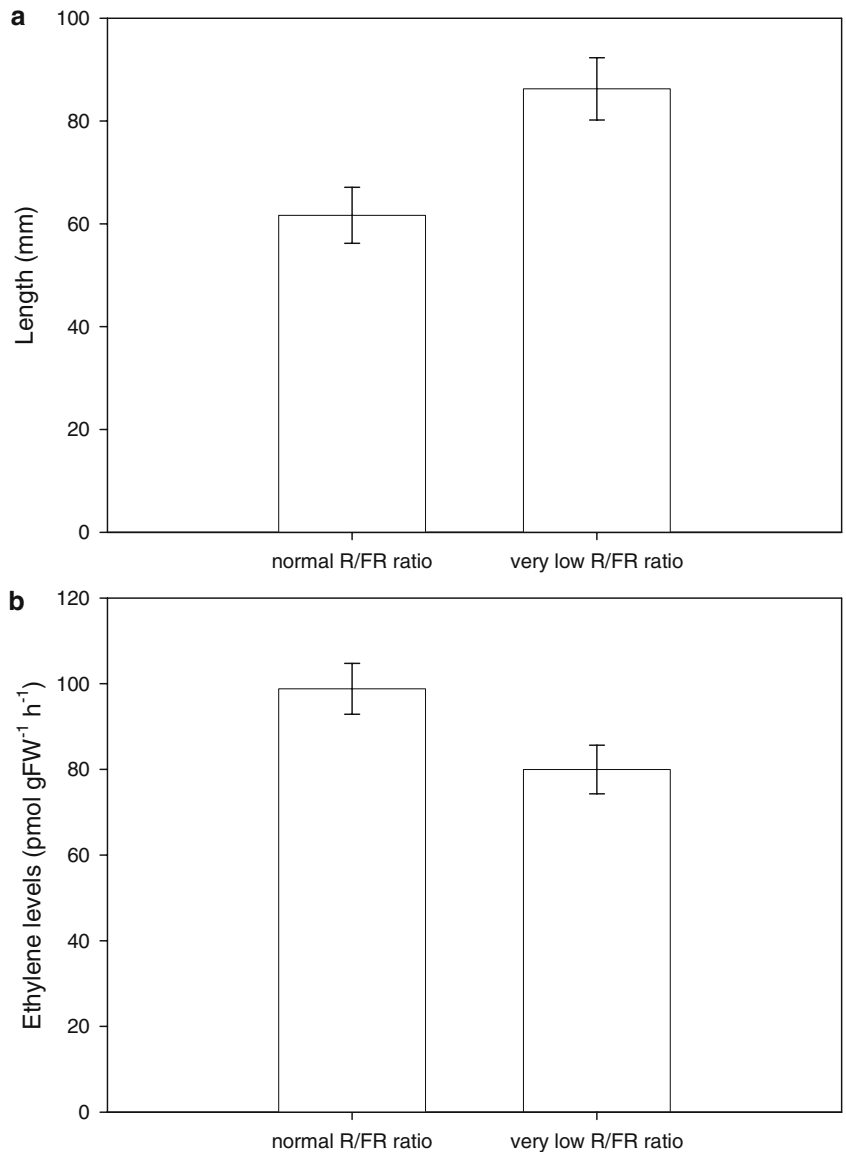

Fig. 4 Hypocotyl lengths (**a**) and ethylene levels (**b**) of 7-day-old sunflower seedlings grown under normal R/FR ratio of 1.24 and very low R/FR ratio of 0.21 with fixed (*low*) PAR of 158 μmol m^{-2} s^{-1}. The *error bars* indicate one SE of the mean

growing, neighbouring sunflower or other plants are subjected to reduced R/FR ratio. This reduced (low) R/FR ratio can result from increased FR light enrichment due to reflection of FR light from adjacent leaves of taller plants or from combined FR light enrichment and reduction in R and B light irradiances due to absorption of R and B light by canopy leaves of taller plants. As expected, seven-day-old sunflower hypocotyls responded with increased elongation to both types of low R/FR ratio present in natural field conditions (Figs. 1a and 4a; Smith 2000; Franklin and Whitelam 2005; Vandenbussche et al. 2005). Further, hypocotyls grown under narrow band FR light and very low light irradiance (3 μmol m^{-2} s^{-1}) were significantly taller than hypocotyls under R or B light with the same irradiance level (Fig. 3a). Internodes of 17-day-old sunflower seedlings responded to decreased R/FR ratio with increased elongation and were twice as sensitive (i.e. exhibited higher de-etiolation) as 7-day-old hypocotyls to abnormally high R/FR ratio (Fig. 1a).

Using the Spearman rank correlation test, a positive and significant (at $P = 0.01$) correlation was determined between R/FR ratio, stem

elongation and ethylene levels in both 7-day-old hypocotyls and 17-day-old internodes. As the R/FR ratio decreases, stem elongation increases and ethylene levels decrease (Fig. 1). This was further confirmed with hormone and inhibitor application experiments (see Fig. 2). Application of ethephon at 10^{-3} M to sunflower seedlings caused more inhibition of stem elongation under low R/FR ratio compared to high R/FR ratio, especially for internodes (Fig. 2a). Application of AVG at 10^{-2} M to hypocotyls and internodes resulted in increased stem elongation under high R/FR ratio with internodes being more responsive, but was not effective in altering elongation under normal or low R/FR ratio conditions (Fig. 2b). These results suggest that decreased R/FR ratio activates increased stem elongation by means of, at least partially, a reduction in ethylene levels. The fact that FR light with very low irradiance and FR enrichment of normal R/FR ratio both resulted in decreased ethylene levels and increased hypocotyl elongation, further supports this hypothesis.

The growth inhibitory role of ethylene in response to numerous environmental stressors is well known (reviewed in Abeles et al. 1992). However, as several studies have indicated a positive correlation between growth and ethylene levels under short-term exposure to FR (Imaseki et al. 1971) or under reduced R/FR ratio conditions (Finlayson et al. 1998; 1999; Pierik et al. 2003), the results obtained in this study were unexpected. Although contrary to predictions, it is possible that diverse plant species have developed different hormonal networks for regulating stem elongation under low R/FR ratio conditions. As an example, an ecotype of *Stellaria longipes* from an open alpine habitat (1D) and another from a shaded, prairie grassland habitat (7B) exhibited similar increases in stem elongation under low light irradiance, but ethylene levels were significantly lower under low light irradiance for only the alpine (1D) ecotype (Kurepin et al. 2006b). Study of the effect of R and white lights on ethylene levels in leaves of fourteen different plant species showed inhibition, stimulation or no effect on ethylene levels (Michalczuk and Rudnicki 1993). In that study, Michalczuk and Runicki (1993) reported that *H. annuus* and *Phaseolus multiflorus* were the only two species among the 14 tested that exhibited increased ethylene levels (by 86 and 78%, respectively) in response to R light treatment. Also, following incubation of *H. annuus, P. multiflorus* and *Avena sativa* leaves in 1 mM 1-aminocyclopropane-1-carboxylic acid (ACC; immediate precursor of ethylene) solution, exposure to R light resulted in conversion of ACC to ethylene, whereas R light inhibited ACC conversion to ethylene in eleven other species (Michalczuk and Rudnicki 1993). These and our previous findings suggest that one should be careful in generalizing when considering the response of diverse species while examining light and ethylene interaction in regulating plant growth.

Although phytochrome levels were not investigated in this study and phytochrome genes in sunflower have not been cloned yet, it is highly likely that in sunflower, as in other higher plant species (Franklin and Whitelam 2005; Vandenbussche et al. 2005), phyB is responsible for FR light sensing. If so, and since phyB-ethylene connection has been shown (Finlayson et al. 1998, 1999; Smith 2000; Franklin and Whitelam 2005; Vandenbussche et al. 2005) it appears that phyB down-regulates ethylene levels in sunflower stems.

While these results suggest a direct involvement of light-mediated changes in ethylene levels and stem elongation, it is highly unlikely that ethylene is the only factor regulating stem elongation under low R/FR ratio. Other plant hormones have also been shown to be involved in increased stem elongation under low R/FR ratio (Vandenbussche et al. 2005). In *S. longipes*, for example, not only are there significant differences in ethylene levels between two ecotypes in response to low light irradiance, but also significant differences in gibberellin (GA) metabolism and perception (Kurepin et al. 2006a). Further, interaction between ethylene and GAs in regulation of sunflower hypocotyl elongation under normal light conditions has been previously reported (Pearce et al. 1991).

To conclude, *H. annuus* 7-day-old hypocotyls and 17-day-old internodes exhibited increased elongation, less ethylene levels, more responsiveness to the application of ethephon and less

responsiveness to the application of AVG with decreased R/FR. Enrichment of normal R/FR ratio with FR light or narrow-band FR light alone promoted 7-day-old hypocotyl elongation and had less ethylene than under normal R/FR ratio or narrow-band R light. These results suggest that ethylene acts as a negative growth regulator of stem elongation in sunflower plants under canopy shade or neighbor proximity shade.

Acknowledgements We would like to thank Ms. Bonnie Smith and Mr. Ken Girard for excellent greenhouse assistance. This work was funded by a NSERC (Canada) grant to DMR.

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