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Canopy studies on ethylene-insensitive tobacco identify ethylene as a novel element in blue light and plant–plant signalling

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Summary

Plants growing at high densities express shade avoidance traits as a response to the presence of neighbours. Enhanced shoot elongation is one of the best researched shade avoidance components and increases light capture in dense stands. We show here that also leaf movements, leading to a more vertical leaf orientation (hyponasty), may be crucial in the early phase of competition. The initiation of shade avoidance responses is classically attributed to the action of phytochrome photoreceptors that sense red:far-red (R:FR) ratios in light reflected by neighbours, but also other signals may be involved. It was recently shown that ethylene-insensitive, transgenic (Tetr) tobacco plants, which are insensitive to the gaseous plant hormone ethylene, have reduced shade avoidance responses to neighbours. Here, we report that this is not related to a reduced response to low R:FR ratio, but that Tetr tobacco plants are unresponsive to a reduced photon fluence rate of blue light, which normally suppresses growth inhibition in wild-type (WT) plants. In addition to these light signals, ethylene levels in the canopy atmosphere increased to concentrations that could induce shade avoidance responses in WT plants. Together, these data show that neighbour detection signals other than the R:FR ratio are more important than previously anticipated and argue for a particularly important role for ethylene in determining plant responses to neighbours.

Keywords: blue light, ethylene, growth, photoreceptors, R:FR ratio, shade avoidance.

Introduction

Plant growth at high plant densities is restricted by competition for light. Many plant species attempt to escape from the associated low-light conditions through morphological responses constituting the 'shade avoidance syndrome'. Shade avoidance consists, among others, of enhanced stem and petiole elongation, a more vertical orientation of the leaves (hyponasty) and apical dominance (Ballaré, 1999; Smith, 2000). Interestingly, plants can already sense and respond to neighbours well before the canopy closes and light becomes limiting (Ballaré *et al.*, 1987, 1990). This highly sensitive detection of neighbours is ascribed to the perception of a lowered red:far-red (R:FR) light ratio by the phytochrome system. This ratio decreases upon light reflection by, or transmittance through, plant tissue, because of selective absorption of R light by chlorophyll (Ballaré *et al.*, 1987, 1990; Holmes and Smith, 1975; Smith, 2000). In addition, plants can sense changes in the total amount of light and that of blue light through specific blue light photoreceptors, such as cryptochromes and phototropins (Casal, 2000; Lin, 2000). Even though Ballaré *et al.* (1991b, 1999) have implicated signals other than R:FR ratio (such as the reduced light quantity) in shade avoidance, this ratio is still thought to be the predominant neighbour detection signal eliciting shade avoidance responses in dense canopies (Aphalo *et al.*, 1999; Botto and Smith, 2002; Gilbert *et al.*, 2001; Schlichting and Smith, 2002).

Recent work on transgenic tobacco plants that are insensitive to the gaseous plant hormone ethylene shed new light on this paradigm of plant neighbour detection. These

ethylene-insensitive, transgenic (Tetr; Knoester *et al.*, 1998) plants appeared to have reduced shade avoidance responses to neighbours in crowded canopies. Both increases in leaf angle and stem elongation are reduced in this genotype when grown at high plant densities. Consequently, these plants are very weak competitors when competing for light with normal wild-type (WT) neighbours (Pierik *et al.*, 2003). These findings clearly implicate ethylene in the detection of neighbouring plants. However, it is as yet unknown if the hormone is an essential downstream component in signalling cascades of neighbour detection systems such as the phytochrome family of photoreceptors or even other photoreceptors.

Here, we present data on the differential involvement of ethylene sensing in the responses to R:FR and blue light signals in tobacco. We used the comparison of WT and Tetr canopy growth as a tool to increase the mechanistic understanding of plant sensing of and responsiveness to neighbours. This enabled us to relate environmental signals to plant morphological changes and competitive success. The results show that responses to neighbours are mediated via a multisignal mode of neighbour detection with a crucial role for ethylene as both an essential signal transduction component and a putative atmospheric signal.

Results

Shade avoidance responses during competition coincide with changes in canopy signals

Wild-type and Tetr tobacco plants were grown in high-density monocultures and monitored through time in order to relate morphological responses to micro-environmental signals present in the canopies.

Leaf angles to the horizontal increased as a response to high plant density and resulted in almost vertical leaves at the end of the experiment. These changes of leaf angles occurred earlier and faster in WT than in Tetr plants (Figure 1a). Leaf angles stayed essentially unchanged and thus horizontal in individually grown, non-competing plants (Figure 1b) throughout the experiment. Stem elongation was accelerated by the presence of neighbours (compare Figure 1c,d) and was faster in WT than in Tetr plants (Figure 1c,d). The major differences in stem length between WT and Tetr developed somewhat earlier in competing than in non-competing plants. Shoot biomass accumulation was severely reduced in Tetr plants competing with WT (Figure 1e), showing the disadvantage of the reduced shade avoidance properties mentioned above, whereas the two genotypes had identical biomass when grown in the absence of competition (Figure 1f). Similar results were obtained with a different and independent Tetr line (Tetr20; Knoester *et al.*, 1998), confirming that Tetr's phenotype is not an artefact caused by the genetic transformation of the plants (Pierik *et al.*, 2003).

The leaf area index (LAI: m^2 leaf area m^{-2} soil surface area) increased with time in the dense canopies as the result of plant growth (Figure 2). In these canopies, we measured R:FR light ratio, photosynthetically active radiation (PAR), blue photon fluence rate and ethylene concentration and all the light parameters decreased within the very same time frame (Figure 3), concomitant with the increase in LAI. Remarkably, atmospheric within-canopy ethylene concentrations (Figure 3d) were elevated as compared to ambient concentrations (approximately 25 nl l^{-1} in the canopy versus approximately 7 nl l^{-1} as the ambient concentration in the greenhouse).

Ethylene is differentially involved in plant responses to the various canopy signals

As all canopy signals changed at the same time, these changes did not allow an analysis of the relative importance of these signals in determining the shade avoidance responses in WT and Tetr plants. Therefore, the effects of the separate light signals on leaf angle and stem length of single-grown WT and Tetr plants were measured.

Artificially lowering the R:FR ratio to levels that are comparable to those found in the dense stands induced shade avoidance responses in isolated WT and Tetr plants. Tetr's stem elongation was reduced, but its hyponastic response was similar to that of WT (Figure 4a,b). Apart from the similar amplitude (Figure 4a), also the kinetics of the hyponastic response in Tetr plants were as rapid as in WT (data not shown). This result did not correspond with Tetr's reduced hyponastic response to true neighbours (Figure 1), and therefore we investigated the responsiveness to the other canopy signals measured.

The amount of light decreased steeply in the developing canopies and a comparably low photon fluence rate (30 μmol m^{-2} sec^{-1}) also induced enhanced elongation and hyponasty in individually grown WT plants (Figure 4c,d). However, Tetr plants showed no stem elongation and hyponastic response at all to this shade treatment (Figure 4c,d), although stem elongation does seem to increase in low PAR but this effect was not significant. Prolonged treatment for several days also did not lead to further responses of Tetr plants (data not shown). Such a shade treatment of course leads to a low photon fluence rate of blue light, which was also observed in the dense canopies (Figure 3a,c). As plants carry specific photoreceptors for blue light, we investigated the responsiveness of tobacco plants to the absence of blue light at normal total photon fluence rate (100 μmol m^{-2} sec^{-1}), obtained with a light source that emits no-blue light (low pressure sodium (SOX) light). This no-blue light treatment induced hyponasty and enhanced elongation in WT, but comparable to the low PAR treatment,

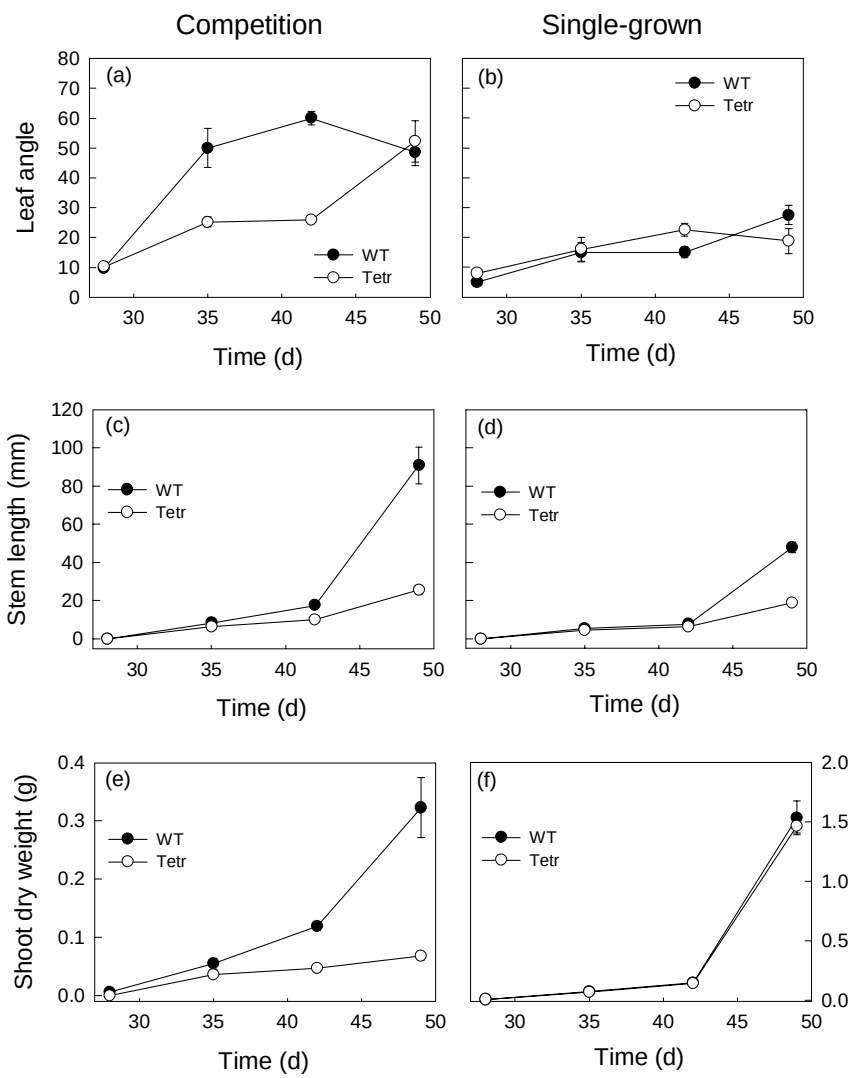

Figure 1. Transgenic, ethylene-insensitive (Tetr) tobacco plants have reduced shade avoidance responses and competitive ability in dense canopies with wild-type (WT) neighbours.
Time courses of leaf angles to the horizontal of the youngest fully developed leaf (a,b), stem length (c,d) and shoot DW (e,f) of WT (●) and Tetr (○) tobacco grown in crowded 1 : 1 mixtures (a,c,e) or individually without competition at all (b,d,f).
Symbols represent means of three plot replicates or six individually grown plants; error bars (when larger than symbol size) indicate SE.

not at all in Tetr plants (Figure 4e,f), and leaf angles even seemed to decrease slightly in Tetr plants but this was not significant (Figure 4e). To check if the hyponastic response of WT in the no-blue light treatment was really because of the lack of blue light and not caused by any other property of the light source used, we subsequently added 10 μmol m^{-2} sec^{-1} of blue light to the SOX light background. This prevented the hyponastic response occuring in WT and could even bring hyponastic leaves (induced by the absence of blue light) back to the normal, non-hyponastic position (Figure 4g). This shows clearly that low levels of blue light induce hyponasty and that this process requires ethylene action.

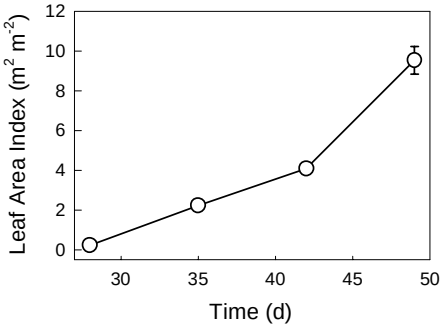

Figure 2. Time course of LAI of crowded 1 : 1 WT:Tetr mixed canopies. Data are means of three plot replicates ± SE.

Ethylene induces shade avoidance traits at concentrations found in dense canopies

As ethylene concentrations were elevated in the canopies, the effects of these enhanced ethylene concentrations on morphology were investigated as well. It appeared that application of ethylene also induced shade avoidance responses in WT but of course not in Tetr plants, confirming the insensitivity of the latter genotype to ethylene (Figure 5a,b). Even when ethylene concentrations were

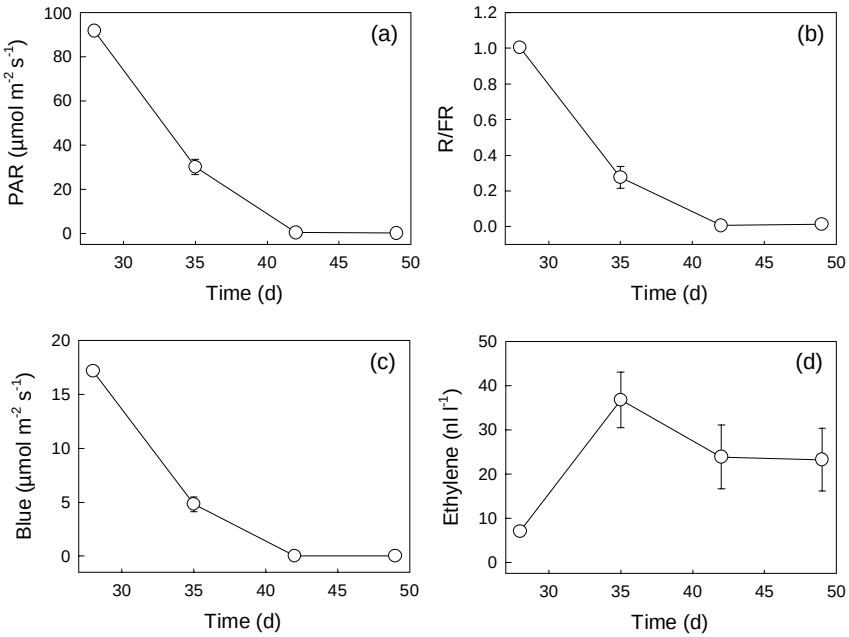

Figure 3. Time course of light quality and quantity in horizontally reflected light and atmospheric ethylene concentrations in crowded 1 : 1 WT:Tetr mixed canopies.
(a) Total photon fluence rate (PAR; 400–700 nm).
(b) R:FR ratio (655–665 nm/725–735 nm).
(c) Photon fluence rate of blue light (400–500 nm).
(d) Ethylene concentration (nl l^{-1}). Symbols represent means ($n = 3$); error bars (when larger than symbol size) represent SE.

increased up to 1 µl l^{-1} (which is approximately 40-fold higher than that observed in the canopies), Tetr plants still showed no response at all. The dashed line in Figure 5 represents the ethylene levels measured in the canopies, showing that the canopy ethylene levels are probably high enough to induce shade avoidance responses in WT.

Discussion

Developing dense canopies of ethylene-sensing (WT) and Tetr tobacco plants showed a steeply increasing LAI with time (Figure 2). As a result, light quality and quantity (R:FR ratio, blue photon fluence rate and PAR levels) and ethylene concentrations changed in a comparable way during canopy development (Figure 3). Detailed measurements in *Datura ferox* canopies revealed that R and blue light are depleted in identical amounts by a given LAI (Ballaré *et al.*, 1991b), which was also found by Yanovsky *et al.* (1995) for wheat canopies. This is not surprising, as R and blue light are both specifically absorbed by chlorophyll. Still, the R:FR ratio may change prior to the depletion of R light, because of FR light reflection by neighbouring plants (Ballaré *et al.*, 1991b), but many more measurements in the low LAI range would have been required in order to register this very early and subtle change in R:FR. Interestingly, ethylene concentrations within the canopy atmospheres reached values of approximately 0.025 µl l^{-1} (Figure 3d), which is a fourfold increase of the normal background concentration in the greenhouse. We know of only one other experiment where ethylene concentrations in a dense canopy were determined, in which considerably elevated ethylene concentrations (approximately 0.08 µl l^{-1}) were also found (Heilman *et al.*, 1971).

Concomitant with the changes of canopy signals described, the plants showed shade avoidance responses to their neighbours, consisting of enhanced shoot elongation and more vertical leaf angles (hyponasty). These shade avoidance responses were delayed in the Tetr genotype, which consequently was suppressed by WT neighbours (Figure 1; Pierik *et al.*, 2003). WT plants showed the hyponastic response as soon as neighbour detection signals originated in the canopies, whereas Tetr plants still remained irresponsive. Thereafter (time point 3 in Figure 1), clear differences in shoot DW were recorded between competing WT and Tetr plants, and also stem length started to differentiate between the two genotypes. Thus, the growth (biomass) differences were preceded by differences in hyponasty, but not by differences in stem elongation. This indicates that, in the early phase of competition (LAI $\leq$ 2 in the present experiment), the hyponastic response may be the predominant strategy to avoid shading by neighbours, whereas later on (LAI $\geq$ 4 in the present experiment), stem elongation may be the most important determinant. The reduced stem elongation of Tetr plants may have been the result of a weakened response to canopy signals and of a reduced availability of photosynthetically active light caused by dominating WT neighbours in the canopy. In a recent paper, we showed, however, that Tetr plants really have a reduced stem elongation response to canopy signals as the stem elongation response was also reduced in a Tetr monoculture where the plants were not selectively suppressed (Pierik *et al.*, 2003). This reduced elongation response of Tetr

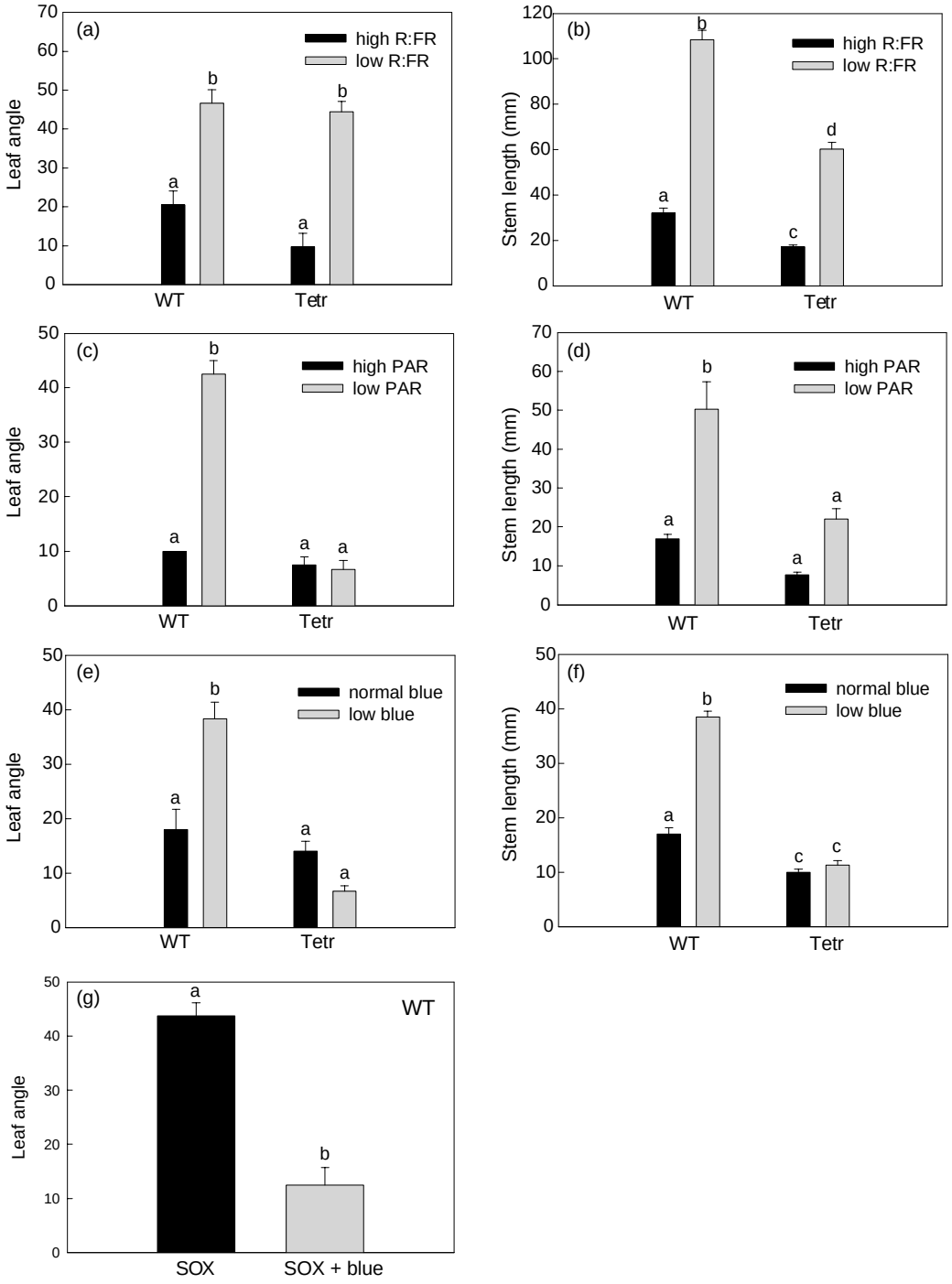

Figure 4. Leaf angles (a,c,e,g) and stem length (b,d,f) of WT and Tetr tobacco after 1 (leaf angle) or 11 (stem length) days at different light conditions.
(a, b) High (6.26) or low (0.14) R:FR ratio. At the start of treatment, plants had leaf angle values of 27.7 ± 4.9 (WT, high R:FR), 18.6 ± 4.9 (Tetr, high R:FR), 26.2 ± 4.8 (WT, low R:FR) and 20.5 ± 4.1 (Tetr, low R:FR) and stem length values of 5.4 ± 0.2 (WT, high R:FR), 4.1 ± 0.2 (Tetr, high R:FR), 6.0 ± 0.2 (WT, low R:FR) and 4.1 ± 0.3 (Tetr, low R:FR) mm.
(c, d) High (120 μmol m^{-2} sec^{-1}) or low (30 μmol m^{-2} sec^{-1}) PAR levels. At the start of treatment, plants had leaf angle values of 10.0 ± 0.0 (WT, high PAR), 7.5 ± 1.4 (Tetr, high PAR), 11.2 ± 1.2 (WT, low PAR) and 9.6 ± 1.9 (Tetr, low PAR) and stem length values of 3.7 ± 0.2 (WT, high PAR), 2.2 ± 0.4 (Tetr, high PAR), 3.5 ± 0.6 (WT, low PAR) and 2.3 ± 0.3 (Tetr, low PAR) mm.
(e, f) With or without blue light. At the start of treatment, plants had leaf angle values of 18.3 ± 3.3 (WT, white), 15 ± 3.6 (Tetr, white), 20 ± 2.7 (WT, SOX no blue) and 16 ± 4 (Tetr, SOX no blue) and stem length values of 5.6 ± 0.2 (WT, white), 4.5 ± 0.2 (Tetr, white), 5.4 ± 0.4 (WT, SOX no blue) and 4.6 ± 0.4 (Tetr, SOX no blue) mm.
(g) Indicates WT plants in SOX light just before and 8 h after addition of 10 μmol m^{-2} sec^{-1} blue light by means of blue LEDs.
All data are means ($n = 4$–6) ± SE. Different letters indicate statistically significant differences ($P < 0.05$).

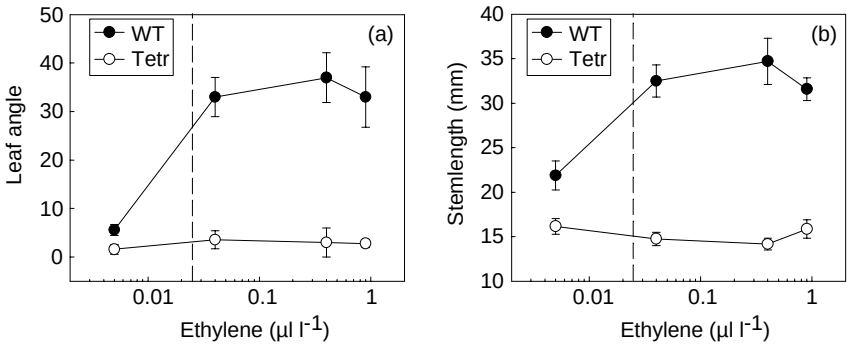

Figure 5. Angle to the horizontal of the largest leaf ((a), after 1 day of treatment) and stem length ((b), after 7 days of treatment) of WT (●) and Tetr (○) tobacco at increasing exogenous ethylene concentrations. The vertical dashed line in each graph approximates the ethylene concentration measured in the dense canopies. Experiments were started with 5-week-old plants ensuring that plants were beyond the rosette stage and thus stem formation had commenced. Symbols represent means of six plants; error bars (when larger than symbol size) represent SE. Note that 0.005 µl l^{-1} ethylene is approximately the ambient atmospheric concentration.

plants is consistent with the stimulatory effects of ethylene in hypocotyls of light-grown *Arabidopsis* (Smalle *et al.*, 1997), which also explains the somewhat reduced stem elongation of Tetr plants in the absence of competition (Figure 1d). The similar time course of changes in the putative neighbour detection signals makes it impossible to disentangle the relative importance of these signals in determining the quantitatively different responses of WT and Tetr plants to neighbours in dense canopies. We therefore investigated the potential of these canopy signals (low R:FR, low PAR, low blue and elevated ethylene) to induce shade avoidance responses in single-grown WT and Tetr plants separately.

Figures 5 and 6 show that each of these signals can induce shade avoidance responses in WT plants, viz. enhanced stem elongation and hyponasty. Of these signals,

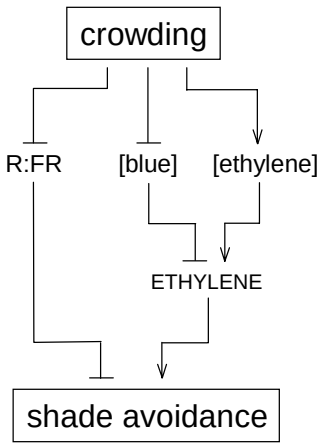

Figure 6. Diagram describing the action of the different canopy signals to induce shade avoidance responses (hyponasty and stem elongation). Crowding indicates a dense canopy. 'R:FR', '[blue]' and '[ethylene]' indicate the R:FR light ratio, blue light intensity and ethylene concentration in the dense canopy. 'ETHYLENE' represents ethylene signalling, and 'shade avoidance' indicates stem elongation and hyponastic (shade avoidance) responses. Arrows represent stimulation, horizontal bars inhibition.

the lowered R:FR ratio is often considered to be the key player inducing shade avoidance responses in dense stands (Ballaré and Scopel, 1997; Ballaré *et al.*, 1990; Holmes and Smith, 1975; Smith and Whitelam, 1997). Yet, an artificially lowered R:FR ratio could also induce enhanced stem elongation and hyponasty in Tetr plants (Figure 4), whereas these responses were reduced in Tetr plants in actual canopies (Figure 1). This is one of the very few unambiguous indications that the R:FR signal alone cannot fully account for the differences observed between shade avoidance responses of WT and Tetr plants in dense stands.

Low PAR and low blue light induced shade avoidance responses in a comparable way, as was found earlier for hypocotyl elongation of WT cucumber (Ballaré *et al.*, 1991a) and stem elongation of tobacco (Casal and Sánchez, 1994). In contrast to the low R:FR ratio, low PAR and more specifically low blue light could not significantly induce hyponasty and stem elongation in Tetr plants (Figure 4), suggesting that ethylene is an important component of blue light-mediated stem elongation and hyponasty. Alternatively, it can be proposed that hyponasty is 'normally' suppressed by blue light and stimulated by ethylene, with the actual petiole angle being the net result of these opposing forces. The hyponastic response to low fluence rates of blue light would then represent a loss of this suppression by blue light, facilitating ethylene-mediated hyponasty. This interpretation would explain the absence of a hyponastic response to low fluence rates of blue light in the Tetr plants. It does, however, not completely apply to the R:FR effects. Although low R:FR-induced hyponasty may very well reflect a loss of high R:FR-mediated suppression of hyponasty, this effect was also observed in the Tetr plants. This unambiguously shows that there also has to be a mechanism that leads to hyponasty that is independent of ethylene. We therefore conclude that the regulation of hyponasty by R:FR ratio and blue light involves distinct

mechanisms with distinct hormonal interactions, as the first does not involve ethylene (Figure 4a), whereas the latter cannot proceed without it (Figure 4e). To our knowledge, blue-light mediated hyponasty and the requirement of ethylene for this process were previously unknown. Also, whilst blue light effects on shoot elongation are well documented (e.g. Ballaré *et al.*, 1991a; Casal, 1994; Kigel and Cosgrove, 1991; Yanovsky *et al.*, 1995), the involvement of ethylene in this process was yet unknown.

The critical role for ethylene in blue light-mediated shade avoidance corresponds with the reduced shade avoidance responses of Tetr plants to neighbours and suggests that blue light reduction in a canopy may be a far more important signal than previously thought. This contention is not fully supported by findings of Ballaré and Scopel (1997), who found for dense plant canopies that normal leaf angle responses were displayed by an *Arabidopsis* cryptochrome 1 mutant (*hy4*, also referred to as *cry1*). These data may suggest that blue light is not the canopy signal that induces leaf angle responses in *Arabidopsis*. Alternatively, other blue light receptors (e.g. other cryptochromes, phototropins) may regulate hyponasty in *Arabidopsis* and probably also in other species.

Together with the light signals described, also ethylene accumulating between the crowded plants may 'warn' plants about the presence of neighbours, as it will reduce the diffusion gradient from the plant to the atmosphere leading to increased internal levels. Interestingly, ethylene has also been shown to be one of the volatiles produced by plants upon herbivore attack, which may 'warn' still unaffected neighbour plants for an upcoming attack (Dicke *et al.*, 2003; Tscharntke *et al.*, 2001). Although it may be questionable if ethylene accumulation in the canopy would really be an effective and reliable neighbour detection signal, very low levels of ethylene are already sufficient to induce shade avoidance responses (Figure 5). This intriguing suggestion calls for further testing under natural field conditions, which would be a very interesting and promising avenue of research to be taken by ecologists and agronomists.

A model summarising the action of the several neighbour detection signals described here is presented in Figure 6. It can be derived from this scheme that shade avoidance can be induced by either of the three canopy signals represented (reduced R:FR ratio, reduced blue light and increased ethylene concentrations). Yet, both ethylene accumulation and blue light reduction strictly require ethylene action in order to lead to shade avoidance responses, whereas a low R:FR ratio can induce these responses independently of ethylene action (Figure 6). Not included in Figure 6, however, are putative interactions between these signalling cascades as they may be deduced from the literature. The production of ethylene is strongly inhibited by active phytochrome (Imaseki *et al.*, 1971; Vangronsveld *et al.*, 1988), and thus enhanced upon phytochrome de-activation by a lowered R:FR ratio (Finlayson *et al.*, 1998, 1999). The elevated ethylene levels found for the dense tobacco canopies may therefore be the result of such increased ethylene production rates induced by low R:FR ratios, combined with entrapment within the canopy. The effects of blue light signalling on ethylene production are, to our knowledge, unknown. Interaction and interdependence of blue light photoreceptors and the phytochrome photoreceptors are known from several studies (Casal and Boccalandro, 1995; Neff and Chory, 1998; Quail, 2002). Blue light responses may require phytochrome action as for example in the case of reduced hypocotyl elongation by blue light in etiolated *Arabidopsis* seedlings (Ahmad and Cashmore, 1997), whereas phytochrome-mediated shoot elongation responses to low R:FR ratio may be stimulated by blue light, as was found by Casal and Smith (1988) for *Sinapis alba*. The combination of several signals acting together seems to be the most likely explanation for the reduced, but not absent, hyponastic and stem elongation responses to neighbours in Tetr plants. Such an integrative multiple signalling mechanism for neighbour detection is likely to be more robust and reliable than one single (R:FR) signal.

Experimental procedures

Plant growth

For the competition, low PAR and ethylene experiments, WT and Tetr (Knoester *et al.*, 1998) tobacco plants (*Nicotiana tabacum* cv. Samsun NN) were sown on moist sand covered with polyethylene sheets (16 h light, 100 μmol m^{-2} sec^{-1}; 8 h dark; 20°C). Seedlings were transplanted after 9 days to cylindrical pots (78 cm^3) or competition plots (see below for details) with moist sand and covered again with the polyethylene sheets. The sheets were removed 1 week later, and plants were subsequently watered daily with full-strength Hoagland's nutrient solution, thereby preventing nutrient limitations and competition for nutrients.

For the blue light and R:FR experiments, WT and Tetr plants were sown on moist filter paper in Petri dishes. Seedlings were transplanted to pots containing a 1 : 1 mixture of sand and autoclaved potting soil after 8 days and covered with polyethylene sheets (16 h light, 175 μmol m^{-2} sec^{-1} (General Electric 65W/35); 8 h dark; 23°C). The sheets were removed 1 week later, and plants subsequently received full-strength Hoagland's nutrient solution every other day.

Characterisation of growth and signals in dense canopies

Plant growth in high densities was measured in order to track the changes in morphology as a response to neighbours and to relate these changes to the micro-environmental signals that were present in these canopies. In this way, we could check to what extent the changes in these signals matched the changes in plant morphology.

WT and Tetr plants were grown in a greenhouse (Nijmegen, the Netherlands) in mono- and mixed cultures in rectangular

even-spaced plots at a plant density of 1111 plants m^{-2} (Pierik *et al.*, 2003). Separate WT and Tetr plants were grown individually in large pots (1680 cm^3) to study growth of non-competing plants. Plot plants and individually grown plants were harvested with 1-week intervals starting 4 weeks after sowing. We determined the angle to the horizontal of the youngest fully developed leaf, stem length and shoot biomass. Three replicate plots of each type and six individually grown plants of each genotype were harvested per time point. Micro-environmental parameters within the canopy were measured the day before harvesting. Light quality and quantity were determined with a Licor 1800 spectroradiometer (Licor Inc., Lincoln, NB, USA) with a remote cosine receptor attached to it that was placed in the canopy. The receptor was held both horizontally and vertically in order to record reflected and transmitted light. PAR levels were determined as the light intensity from 400 to 700 nm, blue light as the photon fluence rate between 400 and 500 nm and the R:FR ratio was determined as the photon fluence rate between 655 and 665 nm (R)/photon fluence rate between 725 and 735 nm (FR). Ethylene concentrations were determined with a high-sensitivity, laser-driven photo-acoustic detection system (Voesenek *et al.*, 1990) in 1 ml air samples that were withdrawn from the canopies with syringes. Sampling was performed with a minimal disturbance of the canopy atmosphere so that the sample was representative for the canopy gas composition.

Light treatments

Once the canopy signals had been described and related to plant morphological changes in these canopies, the signals (R:FR, blue light, total light and ethylene) were tested for their effectiveness to induce shade avoidance responses in isolated, i.e. non-competing, plants. In order to do so, 5-week-old plants of the two genotypes were placed in either a high versus low R:FR ratio, high versus low PAR or a high versus low blue light environment, and leaf angles were measured 24 h later. Stem length was measured after 12 days of treatment.

In the R:FR experiment (Figure 7a), plants were placed in 120 μmol m^{-2} sec^{-1} PAR fluorescent light (General Electric F75W/33) with either a high R:FR ratio (655–665 nm/725–735 nm = 6.26) resulting from this fluorescent white light source or a low R:FR (0.14) ratio obtained by addition of FR light (Osram Haloline Halogen R7s 500W, filtered through black acrylic material (Black 901 Crylex; A.S.H. Plastics, Wolverhampton, UK)) to the white light background. In the PAR experiment (Figure 7b), plants

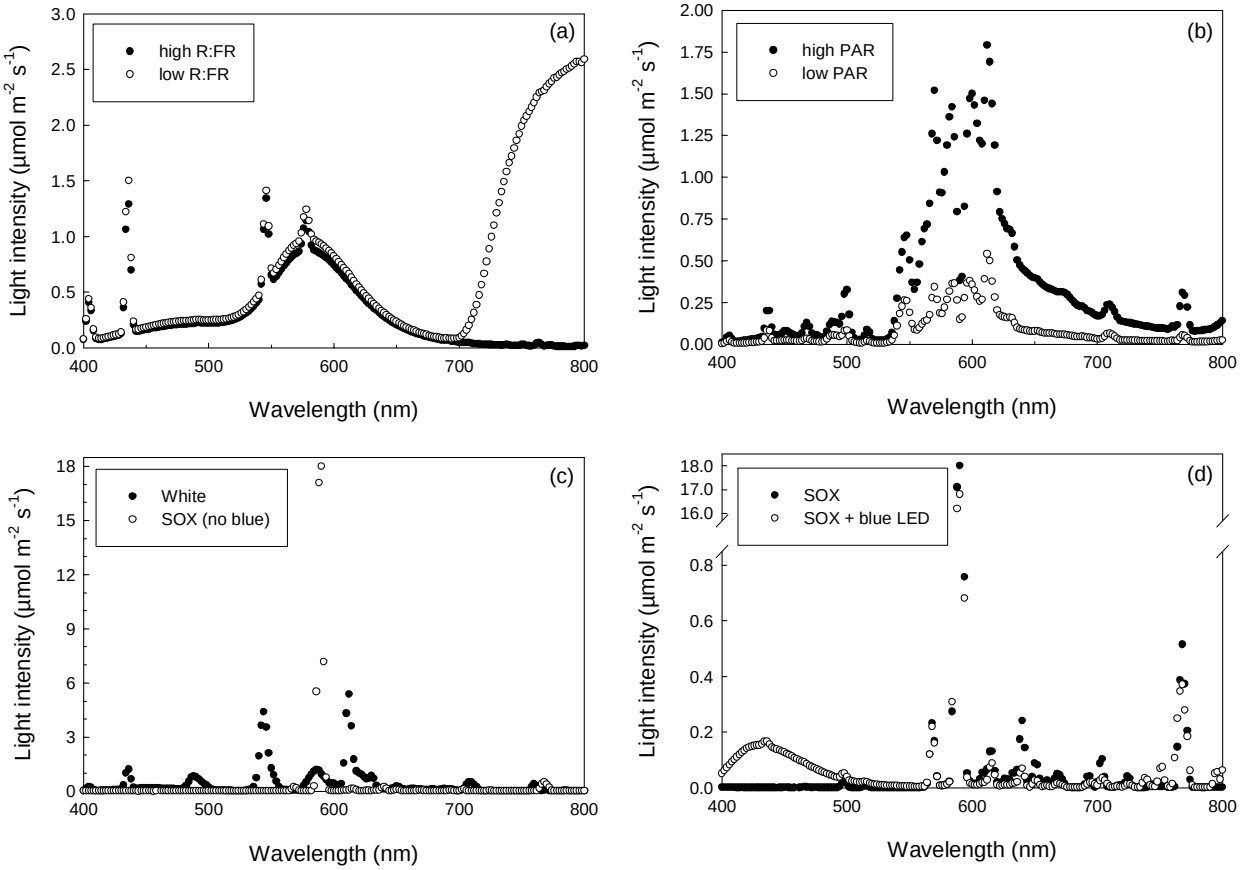

Figure 7. Light spectra from the experimental set-up where a control (●) and a treatment (○) environments were compared.
Experiments on growth and morphology of WT and Tetr plants in (a) high and low R:FR ratio (PAR = 120 μmol m^{-2} sec^{-1}; high R:FR = 6.26; low R:FR = 0.14).
(b) High and low light (high PAR = 120 μmol m^{-2} sec^{-1} with R:FR = 2.8 and blue light = 7.8 μmol m^{-2} sec^{-1}; low PAR = 30 μmol m^{-2} sec^{-1} with R:FR = 3.1 and blue light = 2.6 μmol m^{-2} sec^{-1}).
(c) High and no blue light (white: PAR = 105 μmol m^{-2} sec^{-1} with R:FR = 10 and blue light = 26 μmol m^{-2} sec^{-1}; SOX: 105 μmol m^{-2} sec^{-1} with R:FR = 1.3 and blue light = 0.1 μmol m^{-2} sec^{-1}).
(d) SOX light versus SOX light plus blue addition (SOX as for (c); SOX + blue: PAR = 106 μmol m^{-2} sec^{-1} with R:FR = 1.3 and blue light = 10.1 μmol m^{-2} sec^{-1}).

were grown at either a high or a low photon fluence rate. The high photon fluence rate was 100 μmol m^{-2} sec^{-1} PAR (400–700 nm), containing 7.8 μmol m^{-2} sec^{-1} blue light (400–500 nm) with an R:FR ratio (655–665 nm/725–735 nm) of 2.7. The low photon fluence rate was 30 μmol m^{-2} sec^{-1} PAR, containing 2.6 μmol m^{-2} sec^{-1} blue light also with an R:FR ratio of 2.7. The low-light treatment was obtained by placing a neutral filter (five layers of ULS 10; Ludvig Svensson, the Netherlands) between the plants and the lamps (Philips SON-T 600W; 16 h light, 8 h dark; 21°C).

In the blue light experiment (Figure 7c,d), plants were grown at 105 μmol m^{-2} sec^{-1} continuous light generated by SOX lamps (Osram 135W SOX) and containing less than 0.2 μmol m^{-2} sec^{-1} blue light (400–500 nm) or in a normal continuous white light control (135 μmol m^{-2} sec^{-1} (General Electric, Polylux F36W/835), containing 24 μmol m^{-2} sec^{-1} blue light). Leaf angles of the fifth leaf were recorded 1 day after start of the no-blue light treatment and also after 11 days of treatment when stem lengths were measured as well. To check the reversibility of the leaf angle response, 10–12 μmol m^{-2} sec^{-1} of blue light (428 and 430 nm LEDs; Farnell, Leeds, UK) was added to four individual plants that had been in the no-blue (SOX) light environment for 1 day. Results of the separate light treatments were statistically analysed in two-way ANOVA designs with light treatment and genotype as fixed variables.

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