

# Plant neighbor detection through touching leaf tips precedes phytochrome signals

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Edited by Janet Braam, Rice University, Houston, TX, and accepted by the Editorial Board July 24, 2012 (received for review March 30, 2012)

Plants in dense vegetation compete for resources, including light, and optimize their growth based on neighbor detection cues. The best studied of such behaviors is the shade-avoidance syndrome that positions leaves in optimally lit zones of a vegetation. Although proximate vegetation is known to be sensed through a reduced ratio between red and far-red light, we show here through computational modeling and manipulative experiments that leaves of the rosette species *Arabidopsis thaliana* first need to move upward to generate sufficient light reflection potential for subsequent occurrence and perception of a reduced red to far-red ratio. This early hyponastic leaf growth response is not induced by known neighbor detection cues under both climate chamber and natural sunlight conditions, and we identify a unique way for plants to detect future competitors through touching of leaf tips. This signal occurs before light signals and appears to be the earliest means of above-ground plant-plant signaling in horizontally growing rosette plants.

competition | phenotypic plasticity | thigmomorphogenesis | canopy development

**P**lant growth in dense vegetations is dominated by a fierce battle over resources. In competition for light, small size inequalities can have major effects on light capture and thus competitive power (1–3). Therefore, it is essential for plants in dense stands to timely respond to neighbor detection cues and adjust growth to that of their competitors. Reduction of the red (R):far-red (FR) ratio, signaled through phytochromes and caused by horizontal FR reflection from neighboring plants, is seen as the earliest above-ground detection signal of neighbors. The R:FR is decreased even before true shading occurs through overlap of leaves, and induces shade-avoidance responses such as upward leaf movement (hyponasty) and stem elongation that secure light capture during subsequent plant competition (reviewed in refs. 4–6). Accordingly, low R:FR can induce shade-avoidance responses in plants grown without neighbors (7, 8), and plants blinded to FR-enrichment in a dense canopy fail to respond to neighbors at an early stage of competition (9). Although some additional above-ground neighbor detection signals are known, e.g., blue light depletion (10, 11) and volatile ethylene accumulation (11), none of these acts as early as a decrease in the reflected R:FR.

The paradigm of decreased R:FR as the earliest neighbor-detection signal in competition for light has been a breakthrough in mechanistic plant competition research. Interestingly, this paradigm is based on research on stem-forming forbs and trees (12–14), but has not been studied in plants that lack an appreciable height growth before competition in the vegetative life stage, e.g., rosette species such as *Arabidopsis thaliana* and many other species. Here we study early neighbor detection in dense stands of *Arabidopsis*. Hyponasty appears to be the earliest shade-avoidance response and occurs exclusively in leaves that touch neighboring leaf tips, before a physiologically meaningful decrease in R:FR. Using a combination of manipulative experiments and computational modeling, we introduce physical touch as a signal in neighbor detection that is required to establish sufficient FR reflection to generate a low R:FR light cue in rosette canopies under both artificial and natural light quality conditions.

## Results

### Early Neighbor-Induced Hyponasty Precedes a Functional Decrease in R:FR

To study early neighbor detection in a rosette species, we studied above-ground competition with neighboring plants in the plant model *Arabidopsis thaliana*. Plants were grown at a density of 2,066 plants/m<sup>2</sup> in individual pots to exclude below-ground interactions. Over time (expressed through leaf area index (LAI): leaf area/soil area), high density-grown plants developed a typical shade-avoidance phenotype (Fig. 1A). At an LAI of 0.65, plants growing in canopy setup started to show increased petiole angles (hyponasty) compared with single-grown control plants (Fig. 1B), whereas enhanced petiole elongation of canopy-grown plants only occurred from an LAI of 1.27 onward (Fig. 1B). The R:FR in the light reflected from leaves within the canopy decreased most rapidly between LAI 0.5 and 1.0, and only decreased below 1.0 at LAI >2, when leaves were fully overlapping (Fig. 1B). At the LAI (0.65) when hyponasty first occurred, the R:FR in the reflected light had only decreased to 1.7. Interestingly, microarray analysis on petioles from canopy plants at LAI 1.0 showed no induced expression of genes known to be involved in shade avoidance (Dataset 1 and Table S1). Accordingly, a quantitative RT-PCR time course throughout canopy development indicated that expression of the low R:FR-inducible marker genes *ATHB2* and *XTH15* (15, 16) was not induced at the onset of hyponasty (LAI 0.65). These genes were only up-regulated after petiole elongation occurred in canopy plants at an LAI >1.27, which corresponded with a R:FR <1.3 (Fig. 1B and C). In conclusion, hyponastic growth is the earliest shade-avoidance response in *Arabidopsis* canopies and occurs already after a relatively small decrease in R:FR (from 2.1 to 1.7) and before shade-avoidance marker genes are induced.

### Neighbor-Induced Hyponasty Is Not Induced by Known Neighbor Detection Signals

Because the first neighbor response occurred after a relatively small decrease in R:FR, we studied whether the decrease in R:FR from 2.1 (standard light) to ~1.7 (LAI = 0.65) would be sufficient to induce the hyponastic response measured at LAI 0.65. We observed that even a slightly stronger reduction in R:FR (from 2.1 to 1.5) was insufficient to induce hyponasty in individually grown plants without neighbors, and that only when the R:FR ratio was further reduced to 1.2 or lower was hyponasty observed (Fig. 2A).

Author contributions: M.d.W., W.K., and R.P. designed research; M.d.W., W.K., J.B.E., M.H.V.-v.E., P.G., and R.P. performed research; J.B.E. contributed new reagents/analytic tools; M.d.W., W.K., and J.B.E. analyzed data; and M.d.W., W.K., L.A.C.J.V., and R.P. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission. J.B. is a guest editor invited by the Editorial Board.

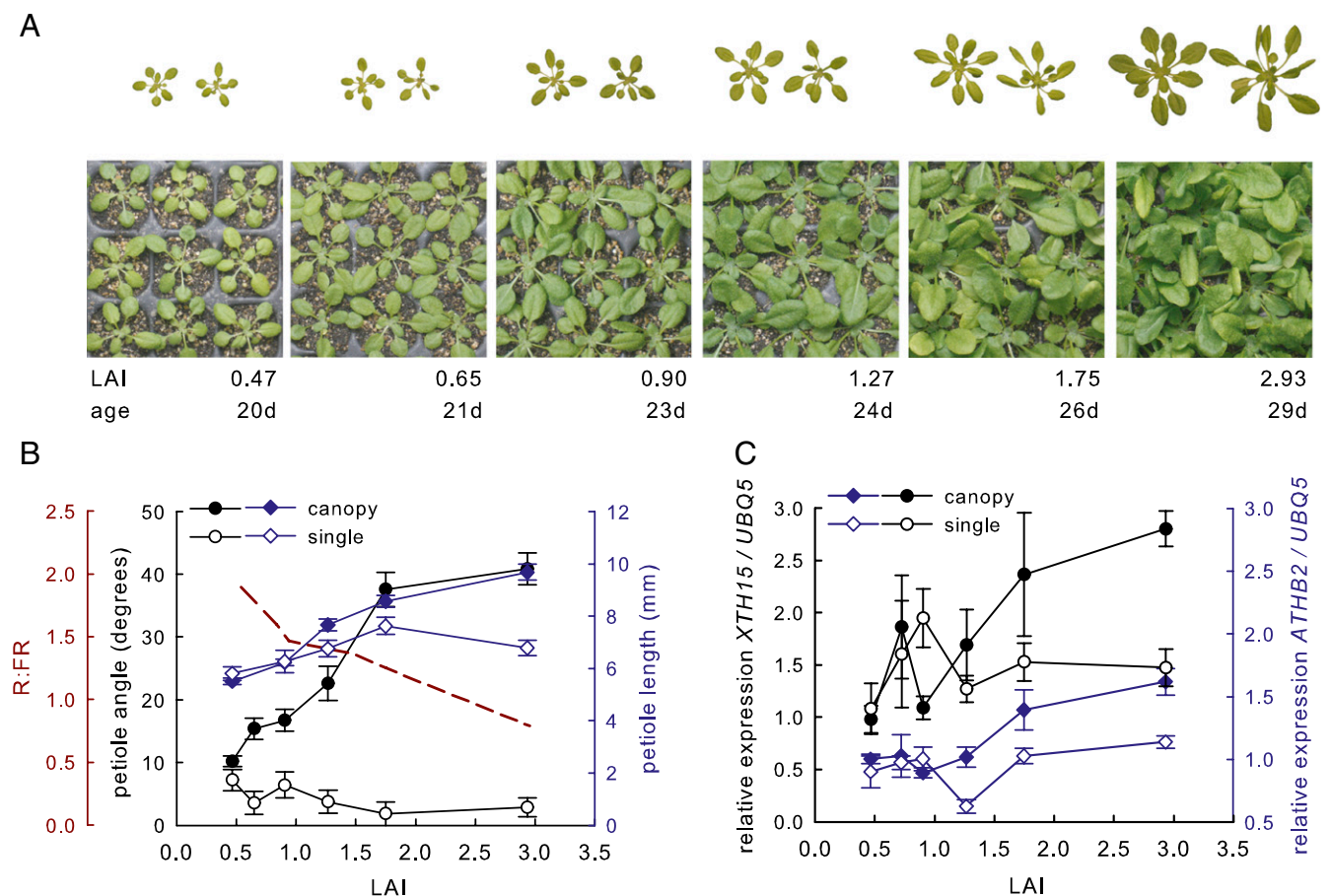
Data deposition: The microarray data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, [www.ncbi.nlm.nih.gov/geo](http://www.ncbi.nlm.nih.gov/geo) (accession no. GSE39010).

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This article contains supporting information online at [www.pnas.org/lookup/suppl/doi:10.1073/pnas.1205437109/-DCSupplemental](http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1205437109/-DCSupplemental).



**Fig. 1.** Shade-avoidance traits in a developing *Arabidopsis* canopy. (A) Representative photographs of the inner nine plants of a canopy at different LAIs with above each canopy photo two individual rosettes of the same age, of which the left is always a single-grown, and the right is a canopy-grown plant. (B) R:FR of reflected light inside the canopy, petiole angles (left y axis, black lines) and lengths (right y axis, blue lines) of the third-youngest leaves of canopy-grown (filled symbols) and single-grown (open symbols) plants ( $n = 27$  from three individual canopies for canopy plants,  $n = 9$  for single-grown plants). Petiole angles from canopy plants are significantly different from single plants from LAI = 0.65 onward; petiole lengths from LAI = 1.27 onward (Student's  $t$  test,  $P < 0.05$ ). (C) *XTH15* (left y axis, black lines) and *ATHB2* (right y axis, blue lines) relative expression (quantitative RT-PCR) in canopy-grown (filled symbols) and single-grown (open symbols) plants ( $n = 4$ ). Expression from both genes is significantly induced in canopy plants at LAI = 2.9 (Student's  $t$  test,  $P < 0.05$ ). Data represent means  $\pm$  SE. Data for single plants are plotted against canopy LAI and represent plants of the same age as their equivalent in the canopy at a specific LAI.

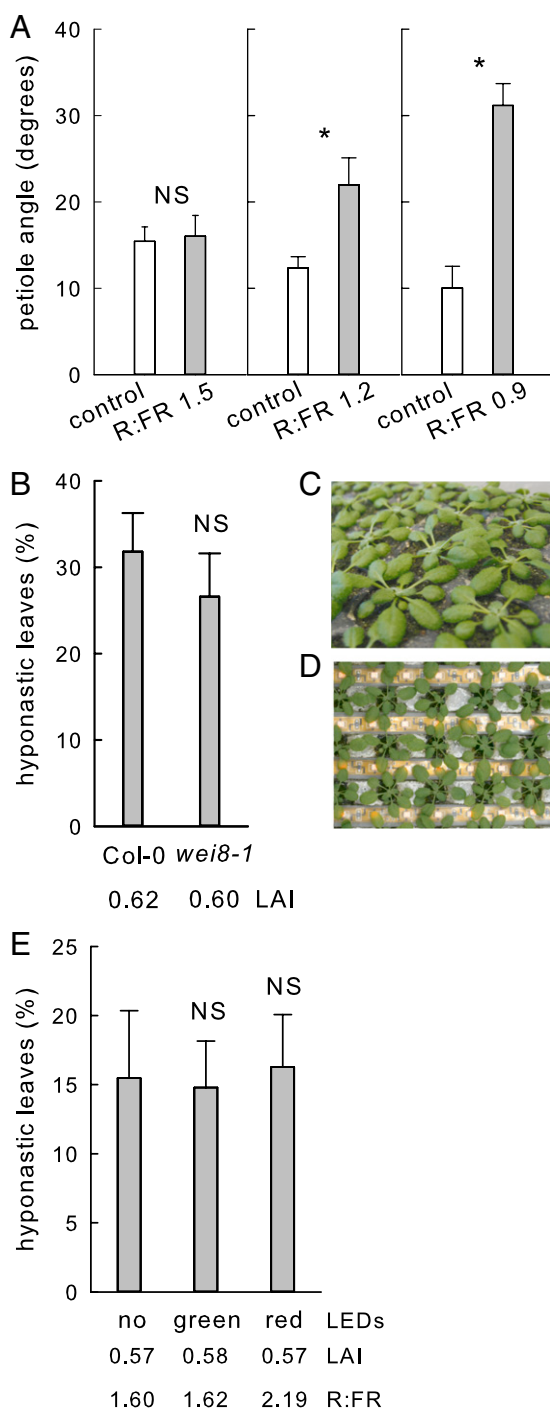
Such strong R:FR reductions occurred only at LAI >1.5 when petiole elongation was also induced (Fig. 1B). This finding corresponds with the induction of the shade-avoidance marker genes (Fig. 1C) that were only up-regulated at LAIs at which the R:FR was below 1.3, and suggests that the R:FR in the canopy is not responsible for the early hyponastic response to neighbors.

To verify that the early hyponastic response is not induced by a decrease in low R:FR, we used the *wei8-1* mutant, which lacks functional TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1 (TAA1) that acts in the auxin biosynthetic pathway required for shade-avoidance responses to low R:FR (17, 18). Consequently, *wei8-1* does not show increased leaf angles in response to low R:FR (Fig. S1). Interestingly, this mutant showed a wild-type early hyponastic response to neighbors in high density (Fig. 2B and C), because a similar percentage of all leaves were hyponastic (petiole angle  $>15^\circ$ ) in *wei8-1* and wild-type canopies. As final proof, we mounted R-emitting wide-angle light-emitting diode (LED) lights between the dense plants (Fig. 2D), which at LAI = 0.6 restored the R:FR from 1.6 (control canopies) to 2.1 (background light). Elevated R:FR, however, did not reduce the number of hyponastic leaves at LAI 0.6, which was 15–20% in all canopies (Fig. 2E). Experiments on ethylene, low light, and blue light intensity showed that these alternative signals do not induce hyponasty in the

early canopy environment (Fig. S2), confirming that early hyponasty in response to proximate vegetation in *Arabidopsis* stands is induced by a unique signal.

**Early Hyponasty Is Induced by Touching of Neighboring Leaves.** We observed that the first leaves to become hyponastic were always those that were touching leaves from neighboring plants. Instead of one leaf overgrowing the leaf of another plant, both leaves would move upward (Fig. 3*A*). Accordingly, all hyponastic leaves (petiole angle  $>15^\circ$ ) at LAI 0.6 were found to be touching a leaf from a neighboring plant (Fig. 3*D*), suggesting that the early hyponastic response in canopies is induced through touching of leaves. Indeed, when two single-grown plants were placed next to each other with the leaf tips from the two different plants facing each other, a similar hyponastic response could be induced (Fig. 3*B*). To exclude the influence of a R:FR signal in this touch-induced hyponasty, we placed transparent tags at the end of leaf tips in the soil next to a single plant. Over time, the petioles grew upward when touching the tags (Fig. 3 *C* and *E*) and remained elevated after subsequent removal of the tags. This response was only induced in touching leaves and not in systemic ones (Fig. 3 *A–C* and *E*), and started after  $\sim 4$  h of touch (Fig. S3*A*). Furthermore, repeated touching of leaf tips with half-hour or 1-h intervals did not result in

de Wit et al.



**Fig. 2.** FR- and neighbor-induced hyponasty. (A) Petiole angles after 6 h of R:FR 1.5, R:FR 1.2, and R:FR 0.9 ( $n = 10$ ). (B) Percentage from total leaves that are hyponastic in a Col-0 canopy and in a *wei8-1* canopy. (C) Representative photograph of hyponastic *wei8-1* canopy and (D) of a canopy with supplemental R LEDs. (E) Percentage from total leaves that are hyponastic in a canopy without LEDs, with green supplemental LEDs, and with red supplemental LEDs. Data represent means  $\pm$  SE. Asterisk indicates significant difference; NS, not significant (one-way ANOVA,  $P < 0.05$ ,  $n = 18$  plants from two individual canopies).

elevated petioles, suggesting that the touch stimulus has to be continuous to induce hyponasty (Fig. S3B).

Mechanostimulation has been associated with the induction of so-called *TOUCH* genes (19). We tested if these genes are also induced in the touch treatments applied by the tags but found no significant

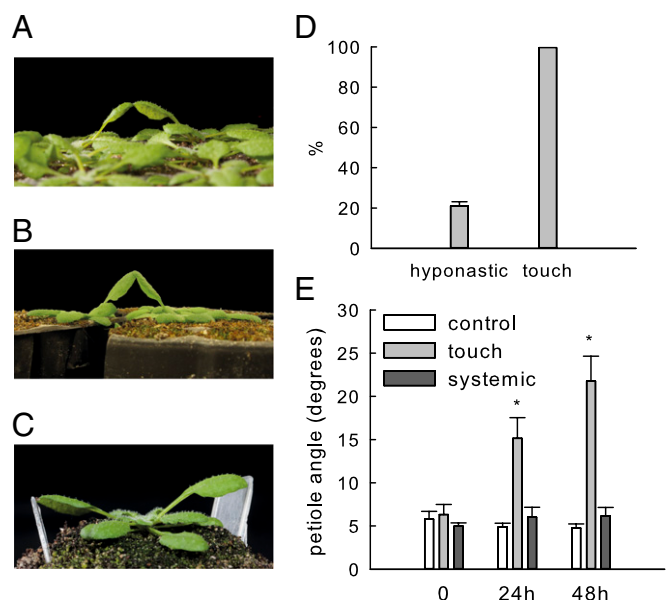
induction of these genes (Fig. S4 A and B). Furthermore, the microarray data from canopy plants at LAI 1.0 did not point toward a known regulatory pathway for touch responses (Dataset 1 and Table S1). Gene ontology (GO) analysis showed an overrepresentation of several defense-related processes and brassinosteroid (BR) signaling (Table S1). The defense hormone jasmonic acid (JA) has recently been associated with touch-induced morphogenesis (20). However, jasmonic acid mutants showed a wild-type response to continuous touching, as did mutants in various other hormonal pathways and shade-avoidance mutants (Fig. S4 C–F). The BR signaling mutant *brl1-1* seemed to show a somewhat reduced response, although it was still able to show touch-induced hyponasty (Fig. S4F).

Together, these data show that mild, continuous physical touching of leaves can explain the early R:FR-independent hyponasty in *Arabidopsis* canopies, of which future studies should identify the molecular mechanisms. This suggests that touch is an important early neighbor detection signal that precedes the R:FR signal in dense stands of rosette species.

#### Hyponasty Is Required for a Low R:FR Signal in *Arabidopsis* Canopies.

Next, we studied the functionality of the early hyponastic response in the *Arabidopsis* canopies. Elevating the leaves before elongation would ensure that growth is directed in a mostly vertical direction toward the light. A similar sequence of responses has been shown in the rosette species *Rumex palustris*, where hyponasty is a prerequisite for submergence-induced petiole elongation (21). Hyponasty, however, was not required for low R:FR-induced elongation, because petioles of single-grown plants that were fixed in a horizontal position still elongated upon low R:FR (Fig. S5).

We furthermore hypothesized that the touch-induced changes in canopy structure from the predominantly flat architecture of rosette plants toward a more vertical canopy orientation increase the horizontal FR reflection by the elevated leaves toward neighboring plants. This enhanced FR reflection consequently



**Fig. 3.** Touch-induced hyponasty. Representative photographs of leaves hyponastic through touch in (A) canopy-grown plants, (B) two single-grown plants with touching leaf tips, and (C) a single-grown plant touching a transparent tag. (D) Percentage of total leaves in canopy that is hyponastic, and percentage of hyponastic leaves that is being touched by other leaves. (E) Petiole angles after 24 h and 48 h from leaves of plants touching a transparent tag (touch) and untouched leaves from the same plants (systemic) or control plants ( $n = 10$ ). Data represent means  $\pm$  SE. Asterisk represents significant difference (one-way ANOVA,  $P < 0.05$ ).



lowers the R:FR perceived by neighbors and could trigger subsequent shade-avoidance responses. We used a 3D virtual plant model based on phenotypic plant parameters from true canopies (Fig. 1) at different LAIs and reflectance properties (22) to virtually increase the leaf angles at fixed LAI values and study how the vertical leaf orientation affects the R:FR within the canopy. The model reproduced realistic virtual representations of the canopy and simulated the R:FR on all leaves individually, confirming the relatively small decrease in R:FR when hyponasty was first observed at LAI 0.65 (Fig. 4A and B and Fig. S6A and B). We then manipulated the virtual leaf angles at LAI 0.65 to test the effect of leaf angles on the R:FR reflected onto neighboring leaves. These simulations showed that high petiole angles that create a vertical canopy arrangement can bring down the R:FR in the canopy by ~50% (Fig. 4C), confirming that a decrease

in the R:FR in dense stands of rosette plants depends largely on a hyponastic orientation of the leaves.

The virtual canopy model further allowed us to study if these results would still apply under natural R:FR conditions by using a light source with R:FR = 1.2, representing natural sunlight (23), as input. First we confirmed that the R:FR at LAI = 0.65 would not induce a hyponastic response in natural light conditions. The decrease in R:FR at LAI = 0.65 as calculated by the model under these natural light conditions (from 1.2 to 1.0) could not induce elevated leaf angles in plants grown permanently at R:FR = 1.2 (Fig. S6C and D), indicating that the early hyponastic response is R:FR-independent irrespective of the background R:FR. When we manipulated the leaf angles in the model with background R:FR = 1.2 we again found a 50% decrease in R:FR perceived by the leaves, as in the simulations with background R:FR = 2.1 (Fig. 4C). Previously, the R:FR has been described to decrease to ~0.6 at an LAI of 0.6 in stands of two stem-forming species in natural sunlight (9). In the current *Arabidopsis* canopies, the petiole angles had to be virtually manipulated to >65° to reach this R:FR at LAI 0.65.

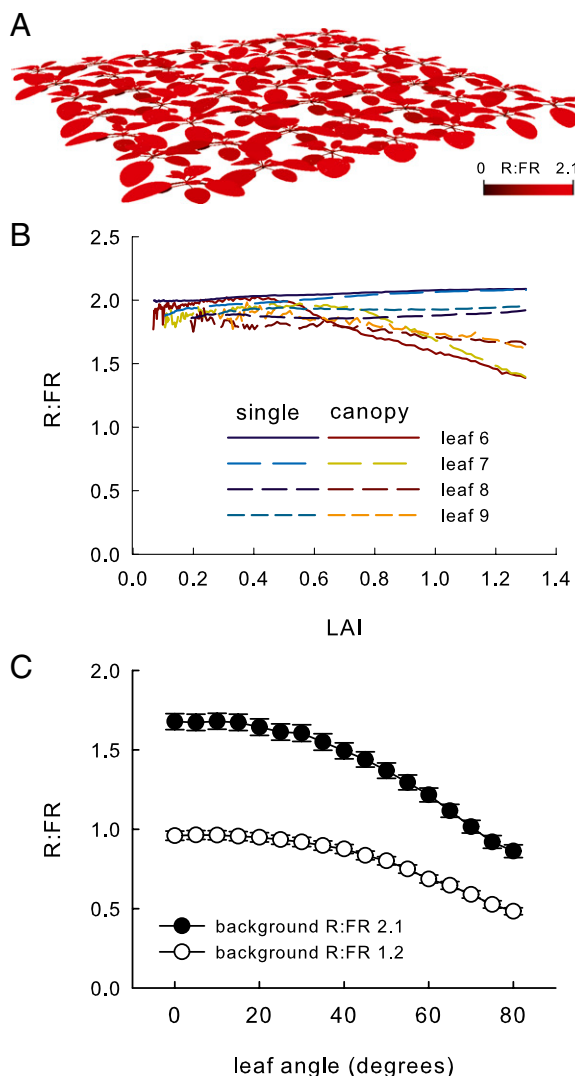
In conclusion, these results illustrate that a R:FR signal that is sufficiently low to induce a shade-avoidance response can only be generated in a vertical canopy structure, which in dense stands of the rosette species *Arabidopsis* is brought about by early touch-induced hyponasty.

## Discussion

In dense vegetation, it is crucial for plants to perceive early signals from surrounding vegetation to respond adequately to imminent shading. A decrease in R:FR has long been established as the earliest cue to sense the presence of proximate neighbors, because it can be perceived before actual shading takes place through light reflected from neighboring leaves. Here, we provide evidence for leaf touching as a neighbor detection signal in competition for light that induces a hyponastic response that is necessary to generate a low R:FR signal in a dense stand of rosette plants characterized by horizontally growing leaves.

Hyponastic growth in dense *Arabidopsis* stands occurred well before enhanced petiole elongation in response to neighboring plants (Fig. 1). These response kinetics resemble the submergence response of the semiaquatic plant *Rumex palustris* (24). Enhanced petiole elongation during submergence in this species occurs only when an angle of 40–50° was reached (21), thereby providing the plant with a mechanism to direct its growth investment such that it contributes to surviving the submergence stress by restoring aerial contact of the shoots. Different from the submergence example, low R:FR-induced petiole elongation still occurred when petioles were kept horizontally. However, a computational modeling approach of our canopy setup showed that more vertically oriented hyponastic leaves are essential to create a low R:FR signal before actual leaf shading occurs, thus presenting hyponastic leaf growth in canopies to be required for subsequent petiole elongation in the rosette species *Arabidopsis*. This also explains why in their seminal paper on early neighbor detection, Ballaré et al. (9) found R:FR reductions in dense stands of the two stem-forming species *Datura ferox* (fierce thornapple) and *Sinapis alba* (white mustard) that through modeling could only be approached in the *Arabidopsis* canopies at a similar LAI when petiole angles were virtually manipulated to ~65°. This finding shows the importance of a vertical growth structure for the generation of a low R:FR signal that can be perceived by neighbors, which lacks in rosette canopies before a neighbor-induced hyponastic response.

The early, R:FR-independent hyponastic response observed here in dense *Arabidopsis* stands appear to be induced by touching neighboring leaves. Responses to mechanical stimulation are called thigmomorphogenic responses and typically include strengthening of the exposed tissue by decreased elongation growth and an increased diameter (as reviewed in ref. 25). Mechanostimulation of



**Fig. 4.** Simulations of a developing *Arabidopsis* canopy. (A) Virtual representation of a canopy at LAI 0.65. The brightness of the color corresponds to the R:FR on the leaves. (B) R:FR output of virtual canopy model for lamina of leaves 6–9 from canopy-grown and single-grown plants. These are the most responsive leaves and include the leaf measured in Fig. 1 (leaf 8). Data for single plants are plotted against canopy LAI and represent plants of the same age as their equivalent in the canopy at a specific LAI. Model output for all leaves is given in Fig. S6. (C) Average R:FR output for all lamina of a virtual canopy plant at LAI = 0.65 with manipulated leaf angles in background light with R:FR 2.1 or 1.2. Data represent means from nine simulated inner canopy or single-grown plants.

plant tissues is associated with the induction of *TOUCH* (*TCH1-4*) genes that encode different physiological regulators such as the XTH22 protein and calmodulins (19). Interestingly, we found no induction of the four main *TOUCH* genes in the mild treatment that mimicked touch from a neighboring plant (Fig. S4 A and B). Recently, the hormone JA has been implicated in thigmomorphogenesis (20), but JA mutants showed a wild-type hyponastic response to continuous touch (Fig. S4 E and F). The microarray data indicated that BR may be involved (Table S1), and the BR signaling mutant *br1-1* indeed seemed to show a somewhat reduced hyponastic response to touch (Fig. S4 F). Because the situation in dense stands with two leaves pushing each other upward is particular in that the mechanical force is mild, comes from both sides, and increases with time rather than being static, it seems plausible that at least parts of the regulatory pathway are different from established touch-signaling components. This difference would also be key to competitive success, because the classic response to mechanical stimulation of decreased elongation growth would reduce competitive performance in dense stands (26). Some examples of growth induction by mechanical stimulation, other than touch during competition, have been described previously. For example, hypocotyl elongation can be stimulated by mild vibration in seedlings of various species, including *Arabidopsis* (27, 28). This finding shows that a very light touch could indeed positively affect cell elongation, which is also driving hyponastic growth (29), although the mechanisms through which growth is stimulated remain to be elucidated.

We conclude that the earliest response to neighbors in a dense stand of *Arabidopsis* is induced by touching of leaves. Thigmomorphogenesis is likely to occur in most dense stands of wild plants and crops, but touch-induced neighbor responses might be a particularly important signal for stands of horizontally growing rosette plants that generate less FR reflection than stem-forming plants at an early stage of competition.

## Materials and Methods

**Plant Growth and Measurements.** Wild-type Columbia-0 (Col-0), *wei8-1* (17), and *pif4pif5*, *pif7-1*, *rot3-1*, *pin3-3*, *etr1-4*, *aos*, *jar1-1*, *coi1-1*, *npr1-1*, and *br1-1* (30–39) were stratified (dark, 4 °C, 3 d) and subsequently grown on soil with additional nutrients (40) in a growth room [9-h light period (200  $\mu\text{mol m}^{-2}\text{s}^{-1}$  PAR; Philips HPI-T Plus, 400 W), 20 °C, 70% RH]. After 10 d, seedlings were transferred to individual 70-mL pots or to tree trays with individual pots of 19 mL (2.2  $\times$  2.2  $\times$  6.2 cm) to create dense stands (2,066 plants/m<sup>2</sup>) of 7  $\times$  7 plants (2.2 cm between the nearest neighboring plants). In canopy development time-series, two different canopies were harvested and analyzed at each time point. Only the inner nine plants were measured; the outer rows served to minimize edge effects. To synchronize graphs between genotypes and treatments, plant development in canopies was plotted against leaf LAI (cm<sup>2</sup> leaf area/cm<sup>2</sup> soil area available per plant), which increased with time, a standard way to represent competition intensity (10, 13). Single plants were plotted against the LAI of canopies and represent control of precisely the same age as the corresponding plants in a canopy. Plants of 24 d old were used for all experiments with individually grown plants, which is the age at which canopy-grown plants show a strong increase in leaf angle. For touch experiments, three transparent tags (polycyclical olefin) were placed in the soil such that three leaf tips were just touching the tag. The third-youngest leaf was measured for hyponasty and elongation from pictures using ImageJ (41) (measurements on individual plants), a digital caliper and a protractor (for canopy plants). Leaf areas were measured with a Li-3100 Area Meter (LI-COR) to calculate LAI. Lamina shape was assessed for modeling by measuring blade width as a function of distance to the blade tip, in intervals of 5 mm along the midrib of the blade, on five randomly chosen leaves. Hyponasty kinetics were determined in continuous light from time-lapse images as in Millenaar (40) using a computer-controlled moving digital camera that takes pictures at regular intervals from the side, and ImageJ to record angles of target petioles.

**Light Manipulations and Recordings.** R:FR ratio manipulations occurred through supplemental FR LEDs (730 nm; Philips Green Power) in a control white-light background of 110  $\mu\text{mol}$  photosynthetically active radiation (PAR) m<sup>-2</sup>s<sup>-1</sup>. Low blue light was created with a blue light-absorbing filter (LEE 010 Medium Yellow), leaving 90  $\mu\text{mol}$  m<sup>-2</sup>s<sup>-1</sup> PAR after filtering. Control plants were exposed to the same light intensity. Light quality inside

canopies was manipulated with narrow strips of red light-emitting LEDs (620–635 nm), or green (520–530 nm) light as a control group, that were placed in between the rows of plants. Measurements of reflected light inside canopies were taken with a LI-COR 1800 spectroradiometer using a glass fiber with cosine corrector. Representative scans of the light spectrum in control light and in a hyponastic canopy of LAI 2.54 are shown in Fig. S7. The R:FR was calculated from the irradiance within the 654- to 664-nm waveband for R and 724–734 nm for FR. Blue light was calculated as the irradiance within a 400- to 500-nm waveband. Every reading was taken four times and the average calculated.

**Ethylene Production.** Ethylene production was measured on freshly harvested shoot tissue (0.2–0.3 g fresh weight) after 20 min of headspace accumulation in a syringe, as described previously (40), using a GC955 gas chromatograph (Synspec).

**Simulations.** A functional-structural plant model (42) was built to simulate *Arabidopsis* canopies (2,066 plants/m<sup>2</sup>) and the R:FR ratio within this canopy (using the simulation platform GroIMP and its radiation model) (43), that used leaf appearance rate, blade extension rate, petiole extension rate, blade shape, blade size, petiole size, and phyllotactic angle as input (most parameter values were obtained from dedicated experiments, though some were from literature) (44); the complete model is available upon request. In simulations used to calculate R:FR in a developing canopy, leaf hyponastic angle was taken as input and calibrated from our experimental data. In simulations used to assess the effect of canopy structure on canopy R:FR, leaf hyponastic angle of all leaves was increased from 0° to 80° in steps of 5°, in static canopies at LAI = 0.65. Canopy development was simulated by arranging 49 individual plants in a regular 7  $\times$  7 grid similar to the experimental setup. The middle 3  $\times$  3 plants were used for analysis to avoid border effects. Simulations of single plants were performed nine times to reach the same sample size. Orientation in the x-y plane of simulated plants was chosen at random. The radiation model simulated light rays emitted from a virtual light source above the simulated plant(s) and traced them through the canopy, taking into account differential scattering and absorption by the plant organs of red and far-red light as experimentally determined for *Arabidopsis* plants.

**RNA Isolation and Real-Time RT-PCR.** Petioles of the third-youngest leaf were harvested separately and snap-frozen for storage at –80 °C. Petioles from at least three individual plants were pooled into one sample and homogenized, and total RNA was extracted using the Qiagen RNeasy Plant Mini Kit with on-column DNase treatment. cDNA was synthesized by 100 units of SuperScript III reverse transcriptase (Invitrogen) with random hexamers at 50 °C in a reaction volume of 20  $\mu\text{L}$ . Real-time RT-PCR was performed in a BioRad MyIQ single-color real-time PCR detection system using SYBR Green Supermix (BioRad). Gene-specific primers (Table S2) were designed using the Primer3Plus software (45) and the 2<sup>– $\Delta\Delta\text{Ct}$</sup>  method was used to calculate relative gene expression (46) with *UBQ5* as internal standard.

**Microarray Analysis.** Total RNA was extracted from homogenized material for three biological replicates of pooled petioles using the RNeasy Plant Mini Kit with on-column DNA digestion (Qiagen) following the manufacturer's instructions. cDNA synthesis, cRNA synthesis, and hybridization to ATH1 Affymetrix *Arabidopsis* Gene Chips were executed by ServiceXS (authorized service provider, Affymetrix). The robust multiarray analysis algorithm (47) was used to normalize expression values, and the empirical Bayes method (48) and Benjamini and Hochberg multiple-testing correction (49) were used to assess differential expression in the Bioconductor packages in R ([www.bioconductor.org](http://www.bioconductor.org)). Genes with a threshold B value (empirical Bayes log odds of differential expression) of 2 were considered differentially expressed. GO analysis was done with the BiNGO plug-in of Cytoscape (50). Data are available at the NCBI gene expression and hybridization array data repository, Gene Expression Omnibus database ([www.ncbi.nlm.nih.gov/geo](http://www.ncbi.nlm.nih.gov/geo); accession no. GSE39010).

**Statistics.** Data were analyzed through ANOVA or Student's *t* test in the R statistical environment (51).

**ACKNOWLEDGMENTS.** We thank Ronald Leito, Martijn van Zanten, Rob Welschen, and Lot Gommers for technical assistance. We also thank Christian Fankhauser for the time that M.d.W. spent finishing the manuscript at the University of Lausanne. This research was funded by The Netherlands Organization for Scientific Research (NWO) Grants 021.001.030 (to M.d.W.) and 818.01.003 (to R.P. and W.K.).

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