

Letters

Coordinated and rapid whole-plant systemic stomatal responses

Stomata play a pivotal role in protecting plants from immediate or long-term damage associated with changes in environmental conditions or pathogen attack (Melotto *et al.*, 2006; Cutler *et al.*, 2010; Vahisalu *et al.*, 2010; Woolfenden *et al.*, 2018; Lawson & Vialet-Chabrand, 2019). They can respond rapidly, by closing or opening their aperture within minutes, or more slowly over hours (Melotto *et al.*, 2006; Cutler *et al.*, 2010; Vahisalu *et al.*, 2010; Raven, 2014; Guzel Deger *et al.*, 2015; Chen *et al.*, 2017; Woolfenden *et al.*, 2018; Zhang *et al.*, 2018; Lawson & Vialet-Chabrand, 2019). Rapid changes in stomatal aperture were reported to occur in treated leaves of plants in response to changes in air humidity, CO₂ concentration, light stress, or pathogen attack (Melotto *et al.*, 2006; Vahisalu *et al.*, 2010; Raven, 2014; Guzel Deger *et al.*, 2015; Chen *et al.*, 2017; Devireddy *et al.*, 2018; Zhang *et al.*, 2018). Remarkably, stomatal responses to light stress, initiated at a single treated leaf, were recently shown to trigger a systemic signal that caused the activation of a rapid whole-plant stomatal closure response in almost all untreated systemic leaves of the plant (Devireddy *et al.*, 2018). This rapid systemic response was shown to be mediated by the reactive oxygen species (ROS) wave and to require abscisic acid (ABA; Miller *et al.*, 2009; Mittler *et al.*, 2011; Devireddy *et al.*, 2018). It is unclear, however, whether this response is unique to light stress, or whether it represents a more general systemic response involved in the acclimation of plants to other environmental conditions. To address this question we subjected a single *Arabidopsis thaliana* leaf to heat stress, dark-to-light transition, or wounding and measured changes in stomatal aperture at 0 and 10 or 15 min in the local leaf, as well as in different untreated systemic leaves (Fig. 1a–d; Supporting Information Methods S1). While heat stress and dark-to-light transition initiated a rapid local and systemic stomatal opening responses, wounding induced rapid local and systemic stomatal closure responses, similar to the light stress-induced local and systemic responses previously reported (Devireddy *et al.*, 2018). Taken together, the results shown in Fig. 1(a–d) reveal that rapid whole-plant systemic stomatal responses (opening or closure) are a general acclimation response of plants triggered by different stimuli.

The plant tissues conducting the propagation of rapid systemic responses to wounding and other environmental stimuli have recently been the subject of intense investigation, with increasing evidence pointing to the plant vascular system, and in particular the phloem network, as a key mediator (Kangasjarvi *et al.*, 2009; Choudhury *et al.*, 2018; Nguyen *et al.*, 2018; Toyota *et al.*, 2018; Kollist *et al.*, 2019). To determine whether systemic stomatal responses are mediated via a signal that propagates through the

plant vascular system, we measured the kinetics of the stomatal closure response to excess light stress in local and different systemic leaves (Fig. 1e). Interestingly, although the local (treated) leaf is primarily connected to only two to three systemic leaves via the vascular phloem system (Toyota *et al.*, 2018), the rapid systemic stomatal response occurred at an almost similar rate in all systemic leaves measured (with the exception of faster kinetics observed in younger leaves; S4–6), suggesting that the systemic signal mediating rapid systemic stomatal responses to excess light stress is not limited in its transport to vascular phloem cells. Nevertheless, as shown in Fig. 1(f–h), in contrast to the rate of stomatal closure in local leaves, which was similar between stomata located at different parts of the leaf (i.e. stomata in areas A–C; Fig. 1g), the rate of stomatal closure in the different systemic leaves was faster in stomata closest to the midvein (stomata in areas A) and slower in stomata at the periphery of the leaf (stomata in areas B and C; Fig. 1h). The findings presented in Fig. 1(e,f–h) point to a possibility that two different signals are involved in mediating systemic stomatal responses, one that travels through, or is associated with, the vascular system (e.g. ABA; Schachtman & Goodger, 2008; Kangasjarvi *et al.*, 2009; Gorecka *et al.*, 2014; Yoshida & Fernie, 2018), and another that travels rapidly through the entire plant (e.g. the ROS wave; Miller *et al.*, 2009; Mittler *et al.*, 2011). At least when it comes to wounding, it was recently shown that although systemic wound responses were primarily mediated through the phloem and involve glutamic acid signaling, locally applied glutamic acid triggered a whole-plant glutamate receptor-like-dependent systemic calcium wave (Toyota *et al.*, 2018). Excess light and wounding may therefore trigger several different rapid systemic signals (e.g. calcium, ROS electric and/or hydraulic waves) that interact and control different aspects of the plant systemic responses, such as transcript expression, metabolite accumulation and stomatal responses (Gorecka *et al.*, 2014; Devireddy *et al.*, 2018; Yoshida & Fernie, 2018; Kollist *et al.*, 2019; Zandalinas *et al.*, 2019; Fig. 1a–h). It is possible, for example, that the rapid signal that travels throughout the entire plant (ROS/hydraulic/electric/calcium wave) triggers ABA production in the vascular system of systemic leaves and that this ABA (produced by the vascular system of systemic leaves) reaches the stomata of systemic leaves and signals their closure (Fig. 1e–h).

Three of the major players thought to be involved in the root-to-shoot, or leaf-to-leaf, systemic response of plants to different environmental stimuli are ABA, jasmonic acid (JA) and salicylic acid (SA) (Schachtman & Goodger, 2008; Kangasjarvi *et al.*, 2009; Gorecka *et al.*, 2014; Devireddy *et al.*, 2018; Yoshida & Fernie, 2018; David *et al.*, 2019; Förster *et al.*, 2019; Kollist *et al.*, 2019). Although the systemic leaf-to-leaf stomatal response to excess light was previously shown to depend on local light-induced ABA accumulation, which leads to ROS production and the initiation of the ROS wave, the role of open stomata 1 (OST1) in this response

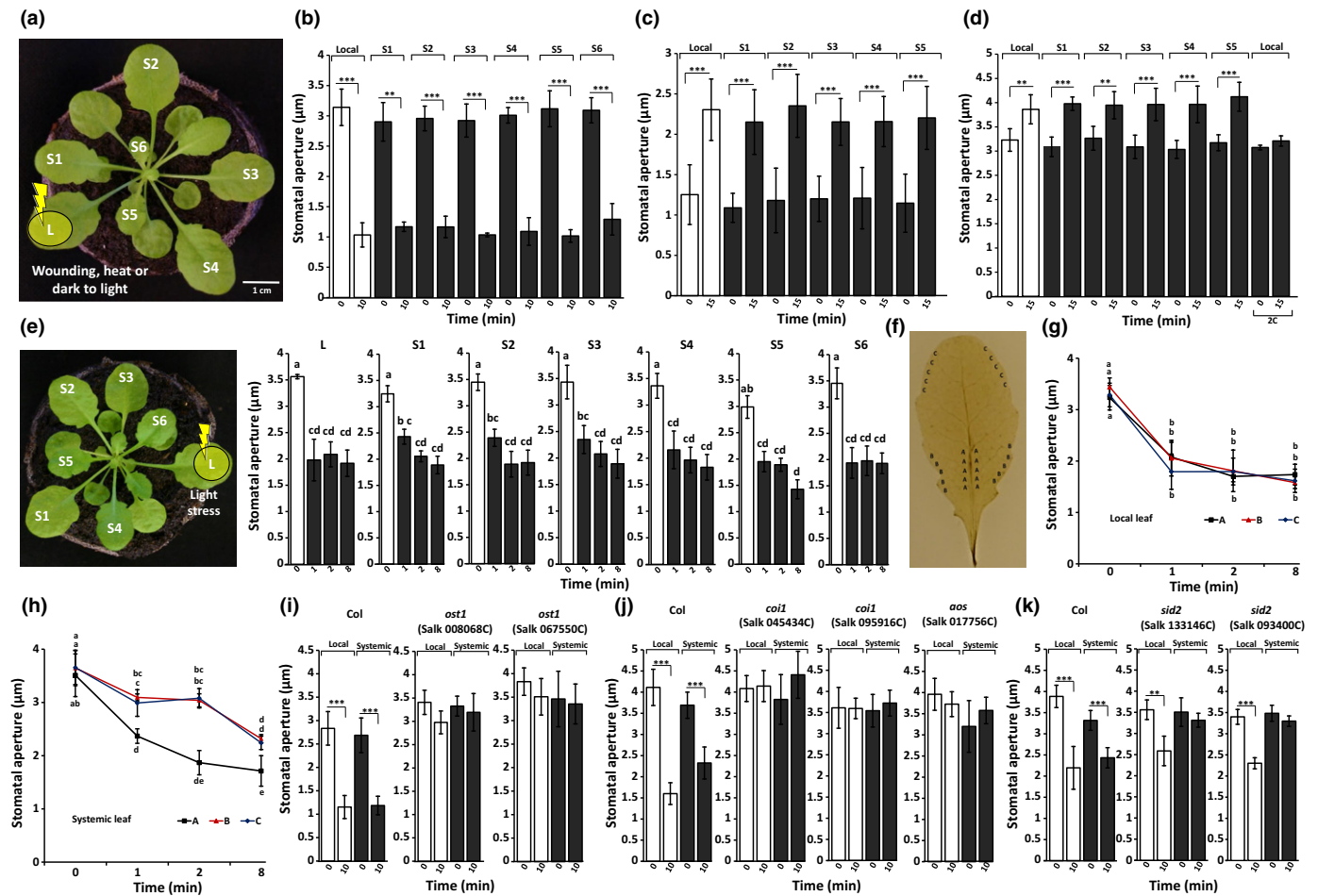


Fig. 1 Activation of systemic stomatal responses in Arabidopsis in response to wounding, transition from dark to light, heat stress and excess light. (a) The experimental design used to measure systemic stomatal responses to the different stimuli. (b–d) Systemic stomatal responses at 10 min after wounding (b), 15 min after transition of the treated leaf from dark to light (c), and 15 min after heat stress (d). The heat stress experimental system was calibrated such that the temperature of the treated leaf was 38°C (17°C above ambient) and the temperature of any of the systemic leaves did not exceed 2°C above ambient. In control experiments it was found that a 2°C rise in leaf temperature did not affect stomata aperture significantly (d). (e) Experimental design used to measure the kinetics of the systemic stomatal response to light stress (left) and measurements of local and systemic stomatal responses at 1, 2, and 8 min in the different leaves (right; S4–S6 are leaves younger than S1–S3 or leaf L). (f) A representative leaf image showing the position of the different stomata groups (A–C) measured in local or systemic leaves. (g, h) Graphs showing the kinetics of stomatal responses in the different areas of local (g) or systemic (h) leaves. (i–k) Local and systemic stomatal responses measured 10 min after the application of light stress to a local leaf in wild-type and different mutants impaired in abscisic acid (i), jasmonic acid (j) and salicylic acid (k) signaling. Statistical analyses in (b–d) and (i–k) were done with two-tailed Student's *t*-test (\pm SE, $n = 500$ stomata from 10 different plants: ***, $P < 0.001$; **, $P < 0.01$). Statistical analyses in (e, g, h) were done with ANOVA followed by Dunnett's *post hoc* test ($n = 500$ stomata from 10 different plants).

was not determined (Devireddy *et al.*, 2018). Here it is shown that rapid local and systemic stomatal responses of Arabidopsis to excess light are dependent on the function of the serine/threonine protein kinase OST1, demonstrating that this systemic response could be dependent on ABA-derived ROS production via OST1-respiratory burst oxidase protein D (OST1-RBOHD)-mediated signaling, or ABA-derived OST1–SLAC1 interactions (Fig. 1i; Devireddy *et al.*, 2018). As shown in Fig. 1(j), the rapid excess light-induced local and systemic stomatal closure responses of Arabidopsis were further suppressed in coronatine insensitive 1 (*coi1*) and allene oxide synthase (*aos*) mutants, demonstrating that JA is involved in this response. In contrast to ABA and JA, which were required for stomatal closure in local and systemic leaves, suppression of SA biosynthesis in the SA induction deficient 2 (*sid2*) mutant only

affected stomatal responses in systemic leaves (Fig. 1k), potentially suggesting that systemic stomatal responses to light stress could be associated with pathogen-induced stomatal closure pathways (Chen *et al.*, 2017; Devireddy *et al.*, 2018; David *et al.*, 2019; Kollist *et al.*, 2019). The findings presented in Fig. 1(i–k), suggest that JA and ABA could interact during local and systemic stomatal responses to excess light. Because rapid systemic responses to excess light or wounding depend on ROS and calcium signaling (Devireddy *et al.*, 2018; Toyota *et al.*, 2018; Kollist *et al.*, 2019), it is possible that JA- and ABA-regulated calcium and ROS concentrations mediate systemic stomatal responses via OST1–SLAC1 (Murata *et al.*, 2001), and/or calcineurin B-like protein (CBL)–CBL-interacting protein kinase (CIPK)–GORK modulation (Förster *et al.*, 2019).

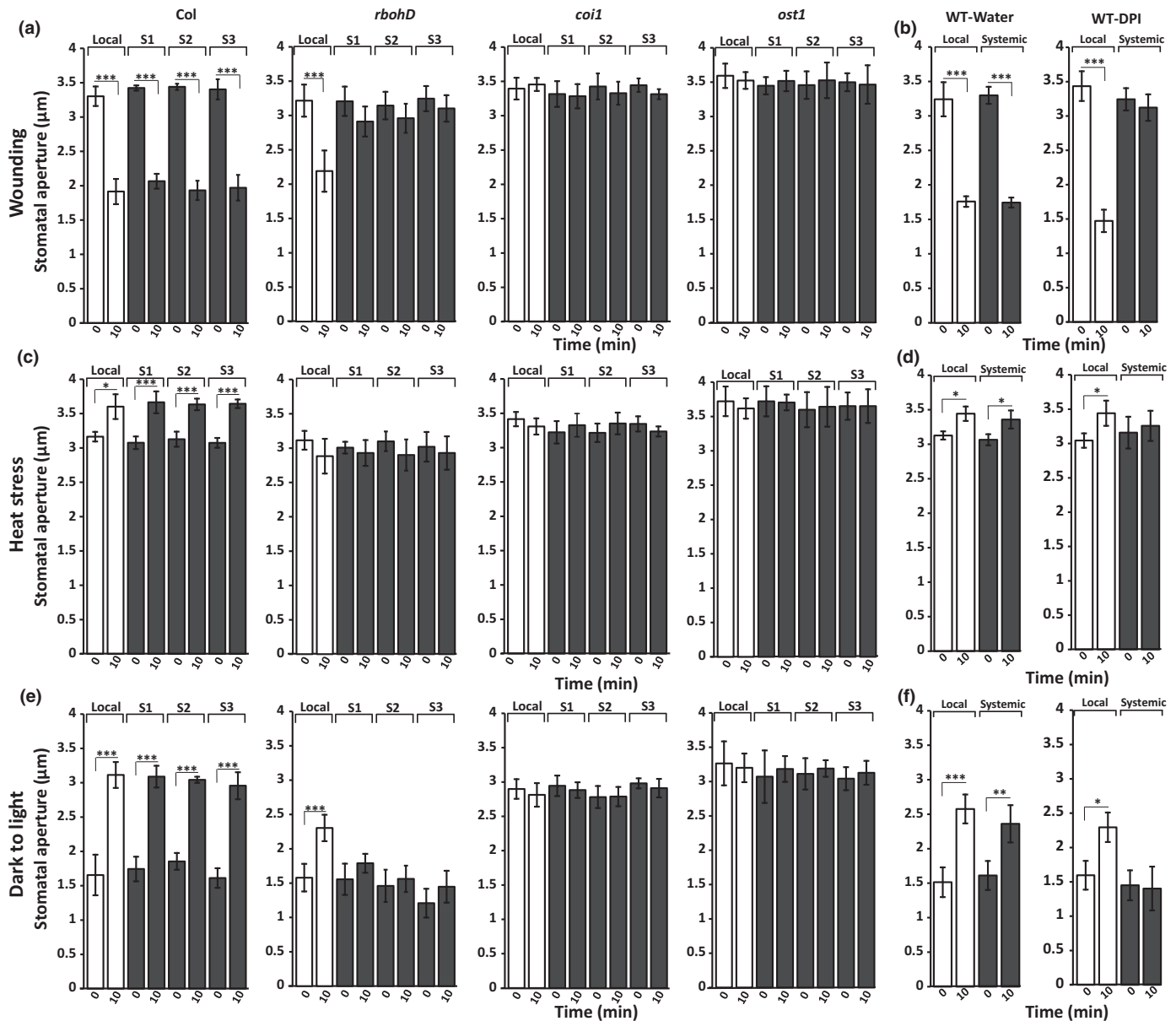


Fig. 2 Open stomata 1 (OST1), coronatine insensitive1 (COI1) and respiratory burst oxidase protein D (RBOHD) are involved in the systemic stomatal response of Arabidopsis to wounding, transition from dark to light, and heat stress. (a) Local and systemic stomatal responses of wild-type Arabidopsis plants, *coi1* (SALK_045434C), *ost1* (SALK_067550C) and *rbohD* mutants subjected to wounding for 10 min. (b) Inhibition of systemic stomatal responses to wounding by diphenyleneiodonium (DPI). (c, d) As (a, b), but for heat stress. (e, f) As (a, b), but for dark-to-light transition. All treatments were applied to the local leaf only. The experimental design for (a), (c), and (e) is shown in Fig. 1(a), and the experimental design for (b), (d), and (f) is shown in Supporting Information Fig. S1. Statistical analyses in (a–f) were done using two-tailed Student's *t*-test (\pm SE, $n = 500$ stomata from 10 different plants: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$).

The involvement of ROS, JA and ABA in regulating systemic stomatal responses to excess light (Fig. 1i–k; Devireddy *et al.*, 2018;) prompted us to test whether these plant hormones are also involved in the regulation of stomatal responses to wounding, dark-to-light transition and heat (Fig. 1a–d). Rapid systemic, but not local, stomatal closure responses to wounding required ROS production via RBOHD (Fig. 2a) and ROS production/redox alterations along the systemic path of the signal (inhibited by the application 50 µM diphenyleneiodonium (DPI), an RBOH, ROS and redox modulator/inhibitor (Riganti *et al.*, 2004; Devireddy

et al., 2018), to the midpoint between the local and systemic leaves; Figs 2b, S1). By contrast, rapid local and systemic responses to wounding required JA signaling via COI1, as well as signaling through OST1 (Fig. 2a). Rapid local and systemic stomatal opening responses to heat stress required ROS production via RBOHD (Fig. 2c), ROS production/redox alterations along the systemic path (inhibited by DPI; Figs 2d, S1), JA signaling through COI1, and signaling through OST1 (Fig. 2c). Rapid systemic, but not local, stomatal opening response to a dark-to-light transition required ROS production via RBOHD (Fig. 2e) and ROS

production/redox alterations along the systemic path (inhibited by DPI application; Figs 2f, S1). By contrast, rapid local and systemic stomatal opening responses to a dark-to-light transition did not occur in *ost1* and *coi1* mutants (Fig. 2e). The results presented in Fig. 2 suggest that local wounding and dark-to-light transition stomatal responses did not require RBOHD, and that systemic stomatal responses to all treatments require ROS production/redox alterations along the path of the systemic signal. Although ABA and JA appear to be required for both local and systemic stomatal responses to all treatments, the role of COI1 and OST1 in these responses is not entirely clear, because *coi1* and *ost1* display a constitutively open stomata phenotype that might not be further affected by wounding, heat stress or dark-to-light transition. Further studies are therefore required to decipher the involvement of JA and ABA in coordinating systemic stomatal responses to different stresses.

Rapid alterations in stomatal aperture were previously shown to regulate leaf temperature, rates of CO₂ exchange, water vapor loss and responses to pathogen infection (Melotto *et al.*, 2006; Vahisalu *et al.*, 2010; Raven, 2014; Guzel Deger *et al.*, 2015; Chen *et al.*, 2017; Devireddy *et al.*, 2018; Yoshida & Fernie, 2018; Zhang *et al.*, 2018; Kollist *et al.*, 2019). Here, it is shown that rapid alterations in stomatal aperture (opening or closing) induced by different environmental stimuli in a local leaf trigger a ROS-dependent systemic signal that alters the stomatal aperture in multiple systemic untreated leaves within minutes (Figs 1, 2). Much like multiple coral reef sponge cells responding in unison to a local stimulus, plants can therefore mount a systemic cell (guard cell) movement response that is mediated by an intricate balance of hormone signaling, calcium and ROS concentrations, electric signals and ion channel activation. This type of whole-plant systemic stomatal response most likely evolved to augment the fitness of land plants and enable them to rapidly acclimate to changes in their environment, helping to cement their role as the key energy converters sustaining life on Earth.



Acknowledgements



We are grateful to the Arabidopsis Biological Resource Center (ABRC) and the Nottingham Arabidopsis Stock Centre (NASC) for providing the T-DNA insertion lines used in this study. This work was supported by funding from the National Science Foundation (IOS-1353886, IOS-1932639, MCB-1936590) and the University of Missouri. We apologize to all authors of papers not mentioned in this manuscript owing to space limitations.

Author contributions

ARD and JA performed experiments and analyzed the data. RM designed all experiments, analyzed the data and wrote the manuscript.

ORCID

Amith R. Devireddy  <https://orcid.org/0000-0003-3541-5161>
Ron Mittler  <https://orcid.org/0000-0003-3192-7450>

Amith R. Devireddy^{1,2} , Jimmie Arbogast³ and
Ron Mittler^{1,2*} 

¹Division of Plant Sciences, College of Agriculture Food and Natural Resources, University of Missouri, 1201 Rollins St., Columbia, MO 65201, USA;

²Interdisciplinary Plant Group, Christopher S. Bond Life Sciences Center, University of Missouri, 1201 Rollins St., Columbia, MO 65211, USA;

³Department of Biological Sciences, College of Science, University of North Texas, 1155 Union Circle #305220, Denton, TX 76203-5017, USA

(*Author for correspondence: tel +1 940 293 7170; email mittler@missouri.edu)

References

- Chen D, Cao Y, Li H, Kim D, Ahsan N, Thelen J, Stacey G. 2017. Extracellular ATP elicits DORN1-mediated RBOHD phosphorylation to regulate stomatal aperture. *Nature Communications* 8: 2265.
- Choudhury FK, Devireddy AR, Azad RK, Shulaev V, Mittler R. 2018. Local and systemic metabolic responses during light-induced rapid systemic signaling. *Plant Physiology* 178: 1461–1472.
- Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR. 2010. Abscisic acid: emergence of a core signaling network. *Annual Review of Plant Biology* 61: 651–679.
- David L, Harmon AC, Chen S. 2019. Plant immune responses – from guard cells and local responses to systemic defense against bacterial pathogens. *Plant Signaling & Behavior* 14: e1588667.
- Devireddy AR, Zandalinas SI, Gomez-Cadenas A, Blumwald E, Mittler R. 2018. Coordinating the overall stomatal response of plants: rapid leaf-to-leaf communication during light stress. *Science Signaling* 11: 1126.
- Förster S, Schmidt LK, Kopic E, Anschutz U, Huang S, Schlucking K, Koster P, Waadt R, Larrieu A, Batistic O *et al.* 2019. Wounding-induced stomatal closure requires jasmonate-mediated activation of GORK K(+) channels by a Ca(2+) sensor-kinase CBL1-CIPK5 complex. *Developmental Cell* 48: 87–99.
- Gorecka M, Alvarez-Fernandez R, Slattery K, McAusland L, Davey PA, Karpinski S, Lawson T, Mullineaux PM. 2014. Abscisic acid signalling determines susceptibility of bundle sheath cells to photoinhibition in high light-exposed Arabidopsis leaves. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 369: 20130234.
- Guzel Deger A, Scherzer S, Nuhkat M, Kedzińska J, Kollist H, Brosche M, Unyayar S, Boudsocq M, Hedrich R, Roelfsema MR. 2015. Guard cell SLAC1-type anion channels mediate flagellin-induced stomatal closure. *New Phytologist* 208: 162–173.
- Kangasjarvi S, Nurmi M, Tikkanen M, Aro EM. 2009. Cell-specific mechanisms and systemic signalling as emerging themes in light acclimation of C₃ plants. *Plant, Cell & Environment* 32: 1230–1240.
- Kollist H, Zandalinas SI, Sengupta S, Nuhkat M, Kangasjarvi J, Mittler R. 2019. Rapid responses to abiotic stress: priming the landscape for the signal transduction network. *Trends in Plant Science* 24: 25–37.
- Lawson T, Vialet-Chabrand S. 2019. Speedy stomata, photosynthesis and plant water use efficiency. *New Phytologist* 221: 93–98.
- Melotto M, Underwood W, Koczan J, Nomura K, He SY. 2006. Plant stomata function in innate immunity against bacterial invasion. *Cell* 126: 969–980.
- Miller G, Schlauch K, Tam R, Cortes D, Torres MA, Shulaev V, Dangl JL, Mittler R. 2009. The plant NADPH oxidase RBOHD mediates rapid systemic signaling in response to diverse stimuli. *Science Signaling* 2: ra45.
- Mittler R, Vanderauwera S, Suzuki N, Miller G, Tognetti VB, Vandepoel K, Gollery M, Shulaev V, Van Breusegem F. 2011. ROS signaling: the new wave? *Trends in Plant Science* 16: 300–309.
- Murata Y, Pei ZM, Mori IC, Schroeder J. 2001. Abscisic acid activation of plasma membrane Ca(2+) channels in guard cells requires cytosolic NAD(P)H and is

- differentially disrupted upstream and downstream of reactive oxygen species production in *abi1-1* and *abi2-1* protein phosphatase 2C mutants. *Plant Cell* 13: 2513–2523.
- Nguyen CT, Kurenda A, Stolz S, Chetelat A, Farmer EE. 2018. Identification of cell populations necessary for leaf-to-leaf electrical signaling in a wounded plant. *Proceedings of the National Academy of Sciences, USA* 115: 10178–10183.
- Raven JA. 2014. Speedy small stomata? *Journal of Experimental Botany* 65: 1415–1424.
- Riganti C, Gazzano E, Polimeni M, Costamagna C, Bosia A, Ghigo D. 2004. Diphenyleioidonium inhibits the cell redox metabolism and induces oxidative stress. *Journal of Biological Chemistry* 279: 47726–47731.
- Schachtman DP, Goodger JQ. 2008. Chemical root to shoot signaling under drought. *Trends in Plant Science* 13: 281–287.
- Toyota M, Spencer D, Sawai-Toyota S, Jiaqi W, Zhang T, Koo AJ, Howe GA, Gilroy S. 2018. Glutamate triggers long-distance, calcium-based plant defense signaling. *Science* 361: 1112–1115.
- Vahisalu T, Puzorjova I, Brosche M, Valk E, Lepiku M, Moldau H, Pechter P, Wang YS, Lindgren O, Salojarvi J *et al.* 2010. Ozone-triggered rapid stomatal response involves the production of reactive oxygen species, and is controlled by SLAC1 and OST1. *The Plant Journal* 62: 442–453.
- Woolfenden HC, Baillie AL, Gray JE, Hobbs JK, Morris RJ, Fleming AJ. 2018. Models and mechanisms of stomatal mechanics. *Trends in Plant Science* 23: 822–832.
- Yoshida T, Fernie AR. 2018. Remote control of transpiration via ABA. *Trends in Plant Science* 23: 755–758.
- Zandalinas SI, Sengupta S, Burks D, Azad RK, Mittler R. 2019. Identification and characterization of a core set of ROS wave-associated transcripts involved in the systemic acquired acclimation response of Arabidopsis to excess light. *The Plant Journal* 98: 126–141.
- Zhang J, De-Oliveira-Ceciliato P, Takahashi Y, Schulze S, Dubeaux G, Hauser F, Azoulay-Shemer T, Toldsepp K, Kollist H, Rappel WJ *et al.* 2018. Insights into the molecular mechanisms of CO₂-mediated regulation of stomatal movements. *Current Biology* 28: R1356–R1363.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 The experimental procedure used to block the systemic signal using DPI (50 μM, 15 min) and to measure stomatal responses.

Methods S1 Description of experiments.

Please note: Wiley Blackwell are not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.

Key words: abiotic stress, heat, light, rapid, stomata, systemic.

Received, 2 August 2019; accepted, 20 August 2019.



About New Phytologist

- *New Phytologist* is an electronic (online-only) journal owned by the New Phytologist Trust, a **not-for-profit organization** dedicated to the promotion of plant science, facilitating projects from symposia to free access for our Tansley reviews and Tansley insights.
- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as ready' via *Early View* – our average time to decision is <26 days. There are **no page or colour charges** and a PDF version will be provided for each article.
- The journal is available online at Wiley Online Library. Visit **www.newphytologist.com** to search the articles and register for table of contents email alerts.
- If you have any questions, do get in touch with Central Office (np-centraloffice@lancaster.ac.uk) or, if it is more convenient, our USA Office (np-usaoffice@lancaster.ac.uk)
- For submission instructions, subscription and all the latest information visit **www.newphytologist.com**

See also the Commentary on this article by McLachlan, 225: 5–6.