A light-dependent molecular link between competition cues and defense responses in plants

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ABSTRACT

One of the principal internal signals controlling plant growth and defense is jasmonate (JA), a potent growth inhibitor that is simultaneously a central regulator of plant immunity to herbivores and pathogens. When shade-intolerant plants perceive the proximity of competitors using the photoreceptor phytochrome B (phyB), they accelerate growth and down-regulate JA responses. However, the mechanisms by which photoreceptors relay light cues to the JA signaling pathway are not understood. Here we identify a sulfotransferase (ST2a) that is strongly up-regulated by plant proximity perceived by phyB via the phyB-Phytochrome Interacting Factor (PIF) signaling module. By catalyzing the formation of a sulfated JA derivative, ST2a acts to degrade bioactive forms of JA and represents a direct molecular link between photoreceptors and hormone signaling in plants. The enzyme provides a molecular mechanism for prioritizing shade avoidance over defense under close plant competition.

RESULTS and DISCUSSION

Growth responses to competition with other plants (*1*) and defense responses to the attack of consumer organisms (*2*) are two paradigmatic examples of adaptive phenotypic plasticity in plants. However, the mechanistic and functional links between these responses are not well understood. JAs are potent growth inhibitors (*3*) and regulators of cell division (*4, 5*), and their role in balancing growth and defense is evolutionarily conserved in land plants, from bryophytes (*6*) to angiosperms (*7*). When shade-intolerant plants perceive a high risk of competition for light with neighboring individuals, they activate the shade-avoidance syndrome (SAS), which allows them to position their leaves in well-illuminated areas of the canopy. Under these competitive conditions, plants also often attenuate the expression of JA-mediated defense responses against pathogens and herbivore (*8*). This attenuation of defense presumably allows the plant to efficiently focus its resources and developmental decisions on escaping shade, sacrificing plant parts that are unlikely to contribute to resource capture.

Plants perceive the proximity of competitors using photoreceptors. Low ratios of red (R) to far-red (FR) radiation (R:FR ratio), which indicate a high risk of competition, result in partial inactivation of the photoreceptor phyB, which in turn promotes growth-related hormonal pathways (*9*), and attenuates signaling mechanisms involved in the activation of defense responses, such as the JA and salicylic acid signaling pathways (*8*). The attenuation of defense

50 responses triggered by supplemental FR radiation or mutations in the *PHYB* gene is not merely
51 a consequence of redirecting resources to growth (i.e., a simple reflection of an energetic
52 tradeoff between growth and defense)(*10, 11*). Attenuation of JA responses under low R:FR
53 ratios has been associated with FR-induced changes in the balance between DELLA and JA-ZIM-
54 domain (JAZ) repressor proteins (*12, 13*), and decreased stability of key transcription factors,
55 such as MYC2 (*13*). However, the specific molecular links between the phyB and JA signaling
56 pathways have not been demonstrated.

57 Most JA-upregulated genes and metabolites are down-regulated by low R:FR ratios or phyB
58 inactivation (*8, 11*). In order to identify signaling elements that could be involved in the
59 suppression of defense in plants exposed to competition cues, we searched for genes with an
60 inverse pattern of regulation (i.e., JA-related genes showing increased expression under
61 conditions that inactivate phyB). In a microarray experiment, we found a small cluster of
62 approximately 100 genes that were positively regulated by JA whose expression was promoted
63 by phyB inactivation; this cluster included various *JAZ* genes, and a gene of the
64 sulfotransferase family annotated as *ST2a/SOT15* (Fig. S1A). Moerover, in an analysis of
65 expression patterns of JA-related genes using publicly-available microarray databases, we
66 discovered that this *ST2a* gene was consistently and strongly upregulated by low R:FR ratios
67 (Fig. S1B). Experimentally, we confirmed a strong upregulation of *ST2a* mRNA by FR radiation
68 treatments that mimicked the effects of plant proximity and mild suppression by UV-B
69 radiation (Fig. S1C), suggesting that this gene could indeed be involved in relaying information
70 on the canopy light conditions to the JA signaling pathway. Chromatin immunoprecipitation
71 sequencing (ChIP-seq) results indicate that *ST2a* is among the direct targets of Phytochrome
72 Interacting Factors (PIFs) (*14*). PIFs are growth-promoting transcription factors (*15*), and PIF4,
73 PIF5, and PIF7 have been shown to link phyB inactivation with growth responses to shade
74 signals (*16, 17*). We tested a *pif4 pif5 pif7* triple knock out mutant (*18*) and found that *ST2a*
75 mRNA was upregulated by tissue damage just like in Col-0, but the transcriptional response of
76 *ST2a* to supplemental FR radiation was completely lost (Fig. S1D). These results demonstrate
77 that low R:FR ratios upregulate the transcription of *ST2a* via the phyB-PIF transcription
78 module.

79 *ST2a* belongs to a family of 21 sulfotransferase-encoding genes in Arabidopsis (*19, 20*), and
80 shows sequence similarity to proteins in many dicotyledonous species (Fig. S2). In vitro, the
81 ST2a protein catalyzes the sulfation of 12-hydroxy JA (OH-JA) to form JA sulfate (HSO_4-JA) (*21*).
82 First described in the late 1800s (*22*), sulfation consists of the transfer of a sulfate residue to a

83 hydroxyl or amino group. In mammalian systems, sulfation represents an important pathway
84 for the biotransformation of hormones, neurotransmitters, and numerous xenobiotics (*23, 24*),
85 which in most cases results in increased water solubility and decreased biological activity.
86 Based largely on their activities and substrate specificities in vitro, sulfotransferases have also
87 been proposed to be important in the regulation of hormonal signaling in plants (*19, 20*). For
88 example, in Arabidopsis, a tyrosylprotein-sulfotransferase was reported to regulate the activity
89 of hormone peptides involved in the control of cell proliferation (*25*). However, there is very
90 little evidence from functional genetic studies that sulfotransferases are involved in the
91 adaptive modulation of phytohormonal pathways, and no information about their ecological
92 role in the regulation of plant responses to environmental cues.

93 We reasoned that a plausible physiological role for the activation of *ST2a* transcription via the
94 phyB-PIF module could be the attenuation of JA-mediated responses in plants exposed to
95 competition signals (low R:FR ratios). To test this hypothesis, we treated Arabidopsis plants
96 with methyl JA (MeJA) and evaluated the formation of JA-related metabolites and
97 accompanying changes in the expression of genes related to JA metabolism. Plants were kept
98 under either white light (Amb light treatment) or white light supplemented with FR radiation
99 (FR treatment). MeJA treatment induced rapid (≤30 min) increases in concentrations of JA and
100 the bioactive conjugate JA-Ile, which were followed by increases in further metabolites,
101 including OH-JA, OH-JA-Ile, and later-on by a marked increase in COOH-JA-Ile and HSO_4-JA (Fig.
102 1). Addition of FR to ambient light significantly decreased the abundance of JA and JA-Ile, and
103 their oxidized derivatives. In contrast, the pool of HSO_4-JA was significantly increased by
104 supplemental FR. The increase in HSO_4-JA concentration under FR correlated well with a
105 dramatic increase in *ST2a* mRNA level (Fig. S3). Genes encoding other enzymes involved in the
106 metabolism of JAs, such as *IAR3*, *ILL6*, and *CYP94B3* were also up-regulated by FR but to a
107 much lesser extent than *ST2a* (Fig. 1, Fig. S3). In summary, low R:FR ratios decreased the pools
108 of JA and JA-Ile, and this effect coincided with a massive up-regulation of *ST2a* gene
109 expression.

110 In the field, shade treatments, compared to full sunlight, have been reported to attenuate the
111 JA burst induced by insect herbivory, which correlates with attenuated production of defense
112 metabolites (*26*). Are the effects of shade on JA accumulation mediated by phyB inactivation
113 and functionally connected with *ST2a* gene expression? To address this question, we first
114 isolated and characterized two *ST2a* null alleles (*st2a-1* and *st2a-2*), and demonstrated that
115 both knock-out mutants produced only trace levels of HSO_4-JA (Fig. S4). The mutant carrying

116 the *st2a-1* allele was used for further functional characterization. In Col-0 plants, supplemental
117 FR reduced the JA burst induced by wounding, and this effect correlated with an increase in
118 the pool of HSO_4-JA (Fig. 2). Importantly, in *st2a-1* plants, the effect of FR attenuating the JA
119 response burst was completely missing (Fig. 2). OH-JA was reduced by FR in Col-0, and it was
120 more abundant in *st2a-1* than in wild type plants (Fig. S5). Within the resolution of our
121 sampling, the levels of JA-Ile were very low and variable, and most of the JA-Ile conjugates
122 were present in the carboxylated form (COOH-JA-Ile) 4 h after wounding. The concentrations
123 of the sum of JA-Ile conjugates was significantly lower in FR plants than in plants of the
124 ambient light treatment (Fig. S5), and significantly higher in *st2a-1* than in Col-0 plants at 4 h.
125 Overaccumulation of COOH-JA-Ile has also been reported in lines lacking JOX/JAOs (*27*), the
126 enzymes responsible for generating OH-JA (the putative substrate of ST2a). Thus, genetic
127 ablation of *JAO/JOX* or *ST2a* results in increased flux through JA-Ile metabolism and catabolite
128 accumulation. Because *ST2a* transcription in response to FR supplementation was minimal in
129 the *pif4 pif5 pif7* triple mutant (Fig. S1D), we used this mutant as a complementary genetic
130 tool to manipulate *ST2a* mRNA levels. Plants of *pif4 pif5 pif7* accumulated HSO_4-JA in response
131 to wounding, but failed to do so in response to supplemental FR (Fig. S6A). Furthermore, when
132 *ST2a* expression was physiologically manipulated in Col-0 plants using various combinations of
133 FR and wounding treatments, the variation in the HSO_4-JA content at 4 h after wounding was
134 largely explained by the variation in the levels of *ST2a* mRNA detected by qPCR (Fig. S6B).
135 Finally, when we overexpressed *ST2a* under a strong promoter, we obtained JA metabolite
136 profiles that were qualitatively similar to those generated in response to FR radiation (HSO_4-JA
137 was greatly increased, with a concomitant reduction in OH-JA, JA, and oxidized JA-Ile
138 conjugates; Fig S7). Neither wounding nor supplemental FR radiation affected the abundance
139 of transcripts of S*T2b*, a gene closely related to *ST2a* (Fig. S2), and a *st2b* null mutant showed
140 normal levels of HSO_4-JA (Fig. S8). These results provide compelling evidence that ST2a is the
141 sole sulfotransferase responsible for the generation of HSO_4-JA in Arabidopsis, and that the
142 increased sulfation of OH-JA under low R:FR ratios is functionally connected with the
143 transcriptional upregulation of the *ST2a* gene mediated by the phyB-PIF module.
144 Pharmacological experiments indicate that exogenous applications of OH-JA, the preferred
145 ST2a substrate in vitro, can enhance some JA-Ile triggered responses in Arabidopsis (*27*) and
146 induce the expression of certain genes, including *ST2a* (*21*). Therefore, sulfation of OH-JA by
147 ST2a may be critical for the generation of a genuinely inactive metabolite, channeling JA
148 molecules away from bioactive pools.

149 To define the functionality of ST2a, we measured JA-response markers in plants exposed to
150 mechanical wounding under contrasting light conditions. Genes involved in JA biosynthesis
151 (*LOX2*), JA signaling (*MYC2*), and JA response (*VSP2*) were regulated as expected in Col-0, with
152 FR repressing the response to wounding (Fig.3A). In the *st2a-1* null mutant, the basal
153 expression of these genes was higher than in Col-0, and the suppressing effect of FR radiation
154 completely disappeared (Fig. 3A). RNAseq analysis of samples from wounded rosettes revealed
155 a statistically significant overlap between the genes downregulated by FR in Col-0 plants and
156 those upregulated by the *st2a-1* mutation under FR radiation. The group of overlapping genes
157 was significantly enriched in the GO terms "Response to JA" and "JA biosynthetic process" (Fig.
158 3B and Data File S1). Consistent with the pattern of expression of JA biosynthetic genes in Col-
159 0 and *st2a-1*, we found that FR reduced the accumulation of *cis*-12-oxo-phytodienoic acid (*cis*-
160 OPDA) in Col-0, particularly at high rates of FR supplementation, but this effect of FR was less
161 marked in *st2a-1* plants (Fig. S9). Glucosinolates (GS) are important defense compounds in
162 Arabidopsis, which are often regulated by JA (*28*) (Fig. S10). In Col-0, the accumulation of these
163 JA-dependent compounds was attenuated when plants were exposed to supplemental FR
164 radiation (Fig. 3C), as expected (*29*). In contrast, in *st2a-1* plants, FR failed to inhibit GS
165 accumulation (Fig. 3C). Collectively, these data (Fig. 3) indicate that the sulfation reaction
166 catalyzed by ST2a plays a central role suppressing JA-dependent responses in plants
167 undergoing shade avoidance.

168 To investigate the functional role of changes in JA metabolism caused by ST2a activity, we
169 tested the *st2a-1* null mutant in bioassays with larvae of *Spodoptera littoralis* (a chewing
170 insect) and *Botrytis cinerea* (a necrotrophic pathogen). In Col-0, supplemental FR radiation
171 caused increased growth of *S. littoralis* caterpillars that fed on the plants, and increased the
172 size of necrotic lesions generated by *B. cinerea* (Fig. 4A). These FR effects were missing in
173 plants of *st2a-1*, which correlated strongly with the lack of effect of FR reducing the
174 concentration of JA (Fig. 2), JA marker gene transcripts, and defense compounds (Fig. 3).
175 Furthermore, the *pif4 pif5 pif7* triple mutant, which did not upregulate the transcription of
176 *ST2a* in response to supplemental FR, was significantly more resistant to *B. cinerea* than Col-0
177 under low R:FR ratios (Fig. 4A). These data provide compelling empirical support for a
178 functional connection between *ST2a* transcription, increased JA catabolism, and reduced
179 defense under low R:FR ratios.

180 Rosettes of the *st2a-1* null mutant appeared similar to those of Col-0 under ambient light, and
181 they showed normal morphological responses to supplemental FR radiation (leaf hyponasty

182 and petiole elongation) (Fig. 4B). However, when plants were treated with MeJA, the shade-
183 avoidance response to supplemental FR radiation was significantly attenuated in *st2a-1*; in
184 contrast, Col-0 plants were capable of reconfiguring their morphology, and showed a normal
185 response to FR even under MeJA elicitation (Fig. 4B and Fig. S11). Taken together, these results
186 suggest that the key function of the sulfotransferase ST2a under low R:FR ratios is to facilitate
187 the inactivation of JA, thereby allowing the plant to express its full repertoire of shade-
188 avoidance responses and maximize its competitive ability in crowded stands.

189 Failure to respond to competition signals with a rapid reconfiguration of shoot architecture
190 and leaf traits carries a disproportionate fitness penalty for plants competing for light in fast
191 growing stands. Under these conditions, suppression of the 'growth brake' (*3, 4*) imposed by JA
192 could be a key determinant of success, even if it comes at the cost of attenuating defense
193 responses. Our results demonstrate the molecular mechanism that links neighbor perception
194 via phyB with the attenuation of JA signaling (Fig. 4C), and provide a compelling example of the
195 role of sulfotransferases in the adaptive modulation of hormonal metabolism in plants. This
196 phyB-dependent sulfation mechanism generates a metabolic sink for bioactive JA, and allows
197 the plant to refocus its strategy on rapid growth when the perceived risk of competition for
198 light is strong.

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SUPPLEMENTARY MATERIALS

The file includes:

Materials and Methods

Figs. S1 to S12

Tables S1 and S2

References

Data Files S1 and S2

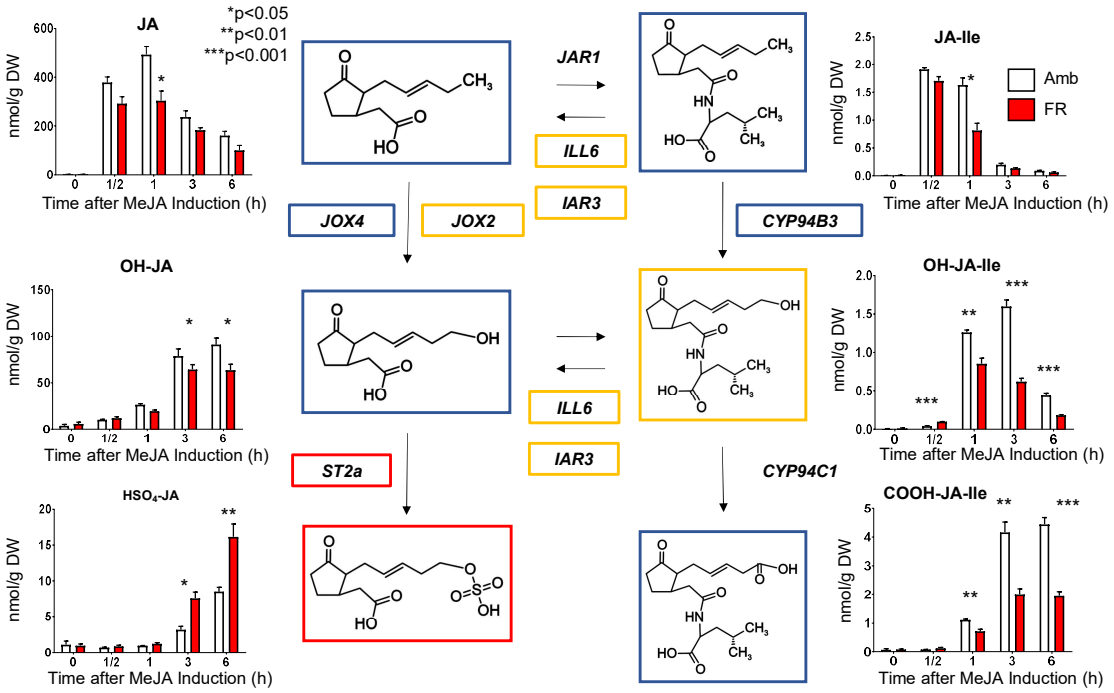


Fig. 1. FR supplementation reduces the pool of bioactive JAs and increases *ST2a* transcription and JA sulfation. Col-0 Arabidopsis plants were sprayed with 200 µM MeJA and harvested at the indicated time points for measurements of JA pools and gene expression. The color of the box outline indicates the direction of the FR effect: Blue = downregulation; Yellow = transient upregulation; Red = upregulation; unboxed genes were not significantly regulated by FR. Metabolic map adapted from Wasternack and Feussner (*30*). The bar charts show quantitative data for metabolite concentrations (thin bars indicate 1 SE; n = 3 biological replicates). For gene expression data, see Fig. S3.

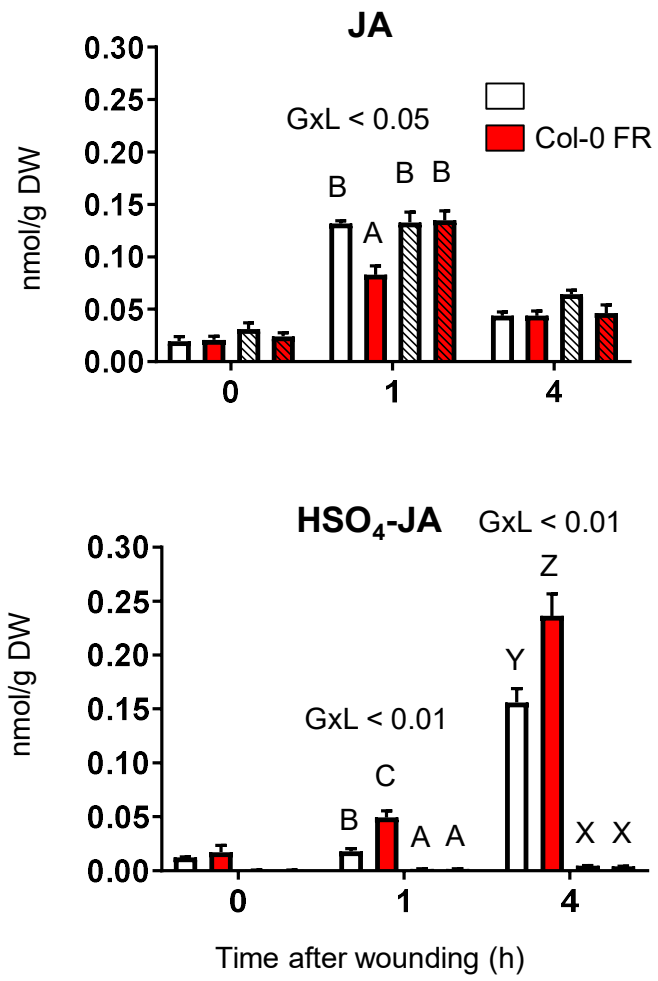
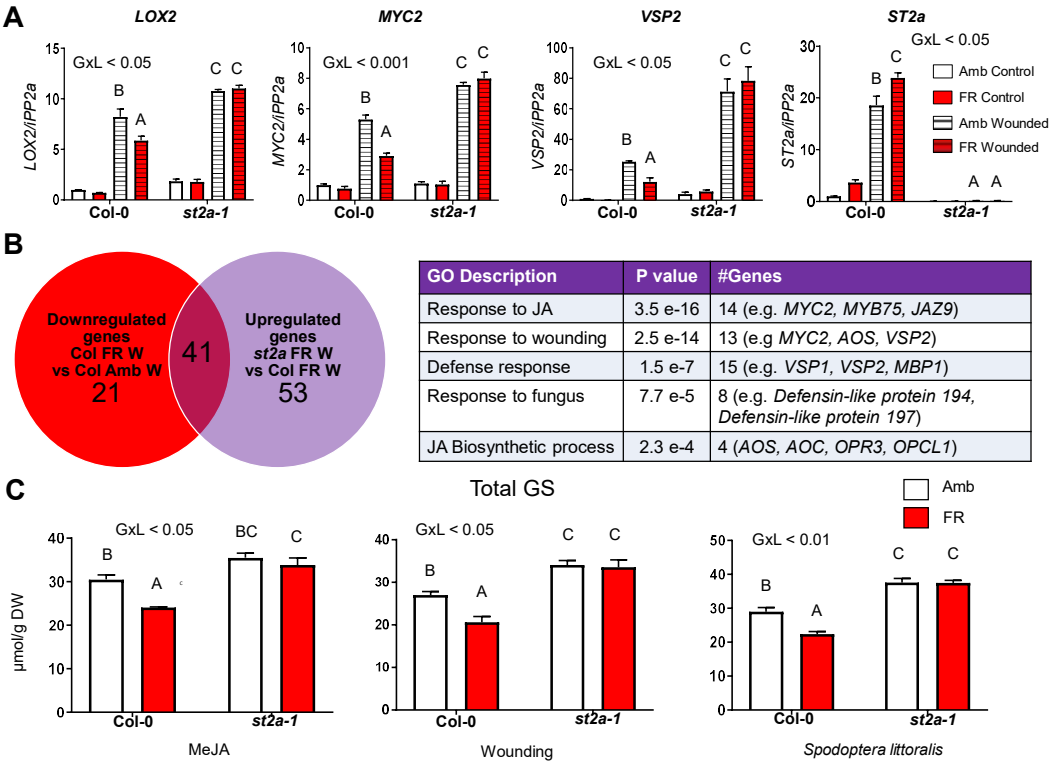


Fig. 2. FR attenuates the JA burst triggered by mechanical wounding and increases the concentration of HSO_4-JA in a *st2a*-dependent manner. Significant genotype x light (GxL) interaction terms are indicted. For each time point, different letters indicate significant differences between means ($P < 0.05$); thin bars indicate 1 SE (n = 6 biological replicates). DW = dry weight. For additional jasmonate pools, see Fig. S5.



GO Description	P value	#Genes
Response to JA	3.5 e-16	14 (e.g. *MYC2, MYB75, JAZ9*)
Response to wounding	2.5 e-14	13 (e.g *MYC2, AOS, VSP2*)
Defense response	1.5 e-7	15 (e.g. *VSP1, VSP2, MBP1*)
Response to fungus	7.7 e-5	8 (e.g. *Defensin-like protein 194, Defensin-like protein 197*)
JA Biosynthetic process	2.3 e-4	4 (*AOS, AOC, OPR3, OPCL1*)

Fig. 3. FR downregulates gene and metabolite markers of jasmonate signaling in an *ST2a*-dependent manner. (A) qPCR results for selected markers of JA synthesis, signaling and response. (B) Summary of RNAseq results demonstrating a significant overlap between the genes downregulated by FR in Col-0 plants and those upregulated by the *st2a-1* mutation in wounded plants. The table shows the GO categories overrepresented in the set of overlapping genes (for details on analysis see Data File S1). (C) Suppression by FR of glucosinolate accumulation in plants treated with MeJA, mechanical wounding or insect herbivory (*Spodoptera littoralis*) was missing in a *st2a-1* null mutant. For specific data on 4MSOB and I3M in wounded plants, see Fig. S10 B). For induced plants, the significance of the genotype x light (GxL) interaction term is indicated in panels A and C. Different letters indicate significant ($P < 0.05$) differences between means; thin bars indicate 1 SE (n = 6 biological replicates for glucosinolate data o 3 for transcriptomic data).

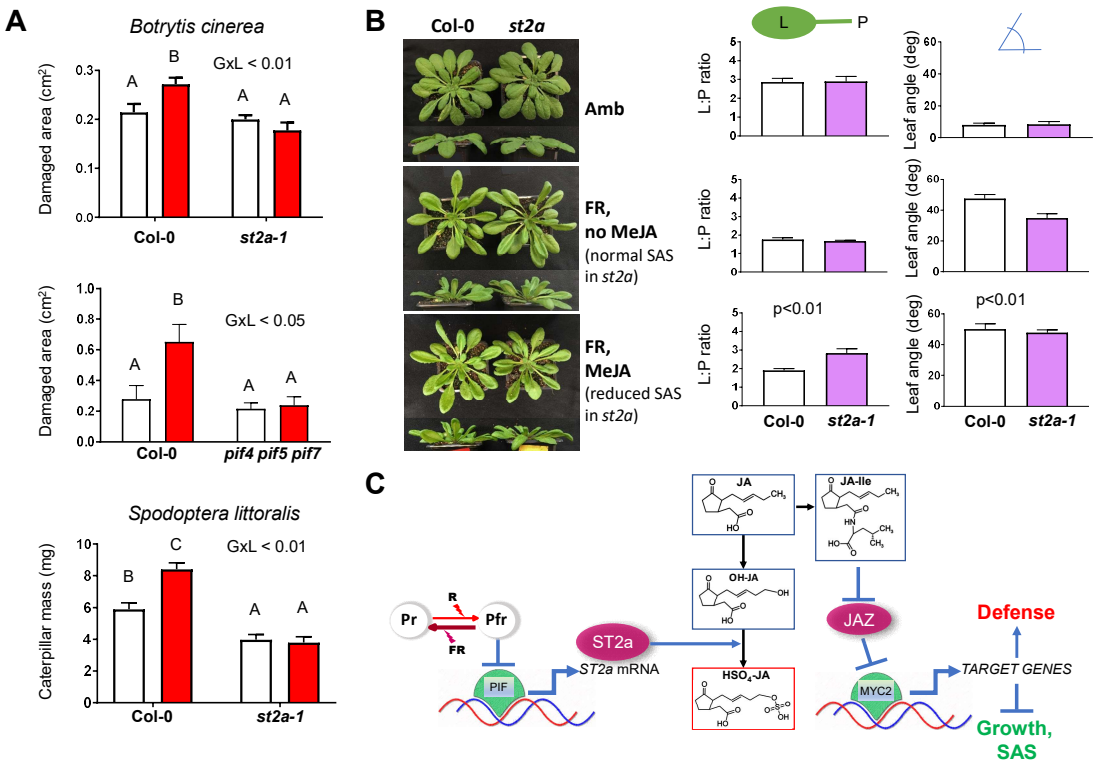


Fig. 4. Sulfotransferase ST2a is key in regulating the growth/defense balance in response to changes in the R:FR ratio. (A) Under FR supplementation, plants that do not upregulate *ST2a* expression are better defended than their Col-0 counterparts. Bioassays were carried out comparing Col-0 and *st2a-1* rosettes using larvae of *Spodoptera littoralis* (upper panel) and inoculations with *Botrytis cinerea* spore suspensions (middle panel), and also comparing Col-0 and *pif4pif5pif7* triple mutants inoculated with *B. cinerea* spore suspensions (lower panel). The significance of the genotype x light interaction term (GxL) is indicated for each factorial experiment; different letters indicate significant ($p < 0.05$) differences between means. (B) *st2a-1* rosettes display normal phenotypes under control conditions but, compared with Col-0 rosettes, they display impaired shade-avoidance responses when exposed to low doses of MeJA (100 µM). **, $p<0.01$; for full dataset, see Fig. S10. (C) Conceptual model linking the perception of low R:FR ratios via phyB with the modulation of jasmonate metabolism and signaling through regulation of *ST2a* transcription via the phyB-PIF transcription module. Pr, inactive form of phytochrome; Pfr, active form of phytochrome.