Review

Special Issue: Specificity of plant–enemy interactions

Evolution of jasmonate and salicylate signal crosstalk

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The evolution of land plants approximately 470 million years ago created a new adaptive zone for natural enemies (attackers) of plants. In response to attack, plants evolved highly effective, inducible defense systems. Two plant hormones modulating inducible defenses are salicylic acid (SA) and jasmonic acid (JA). Current thinking is that SA induces resistance against biotrophic pathogens and some phloem feeding insects and JA induces resistance against necrotrophic pathogens, some phloem feeding insects and chewing herbivores. Signaling crosstalk between SA and JA commonly manifests as a reciprocal antagonism and may be adaptive, but this remains speculative. We examine evidence for and against adaptive explanations for antagonistic crosstalk, trace its phylogenetic origins and provide a hypothesis-testing framework for future research on the adaptive significance of SA–JA crosstalk.

Attack, hormonal signaling and plant defense

Sessile organisms, such as terrestrial green plants, are subject to pervasive attack by diverse attackers. These attackers include microbial pathogens (e.g. viruses, bacteria and fungi), macroscopic herbivores and parasites (e.g. parasitic plants and arthropods) and browsing herbivores (e.g. ungulates). The vast majority of attackers are relatively specialized in terms of the number of host species that they utilize (specialists), and a minority are less restricted in host range (generalists) [1,2]. Over the past 470 million years [3], plants have evolved effective inducible defense systems [4] to cope with attack by these diverse and abundant enemies. Yet, the specific match between particular attackers and plant defense traits, and whether attackers have the upper hand in these interactions, is poorly understood [5]. The specificity of plant–attacker interactions, from both sides of the equation, has important implications for understanding the evolution of resistance in plants and the evolution of virulence in the enemies [6].

Plants have to balance the costs and potential benefits of investing in defense in an environment where enemy attack is variable. On the one hand, defenses are costly to produce; in the absence of enemies, deploying defenses reduces plant fitness [7]. Because they are costly to produce, natural selection is presumed to favor the evolution of inducibility, meaning that these defenses are only produced in the presence of attack. On the other hand, having an immediate impact on an attacker could be paramount to deterring further attacks. Plants generally strike a balance and maintain constitutive and inducible defenses. However, individual plants are likely to be attacked by more than one organism. Microbial pathogens, which are typically endophagous and single-celled, require vastly different defenses than macroscopic herbivores, which may even move among plant individuals while feeding. Among herbivores, different defenses are required for different guilds. For example, defense traits that are effective against aphids, which feed on plant phloem, are distinct from those that are effective against caterpillars, which typically defoliate plants [8]. Characterization of the specificity of the plant response is a focus of intense research among ecologists and plant scientists [5,9,10]. Of particular interest in this review is whether adaptive tailoring of the response occurs, or if tailoring is a byproduct of manipulation by enemies.

Despite the caveats discussed above, the inducible plant defense system can be generally divided into two branches – one effective primarily against biotrophic (feeding on living tissue) pathogens and one against herbivores and necrotrophic (feeding on dead tissue) pathogens [11]. Inducible defenses are incredibly diverse and include morphological structures such as trichomes, fast-killing toxins such as alkaloids, digestibility reducers such as proteinase inhibitors and indirect defenses such as extrafloral nectaries and plant volatiles that can recruit other insects that deter herbivores [1,12–14]. Several plant hormones regulate the production of downstream resistance molecules in each branch. The SA pathway is primarily induced by and effective in mediating resistance against biotrophic pathogens and the JA pathway is primarily induced by and effective in mediating resistance against herbivores and necrotrophic pathogens [9]. This is an overly simplistic view of the complex repertoire of plant hormones that probably play a role in mediating inducible defenses, including abscisic acid (ABA), auxin, brassinosteroids, cytokinins, ethylene (ET) and gibberellic acid [15]. Interestingly, evidence from several distantly related plant species suggests that there can be evolutionarily conserved SA- and JA-signaling crosstalk resulting in reciprocal antagonism between the SA and JA signaling pathways [9]. The adaptive significance of this crosstalk, if any, is the focus of this review.

The dynamics and genetic bases of SA–JA crosstalk, including the reciprocal antagonism often observed as a

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result, has mainly been dissected in the model plant *Arabidopsis thaliana* (*Arabidopsis*) [16–18]. The genetic basis of the reciprocal antagonism is extremely complex and an overview is presented below, in the context of the evolution of each of the major genetic players. Here we focus on SA and JA; however, ET is a critical third player from the perspective of understanding how plants prioritize and tailor their responses to diverse attackers and a brief focus on its role in mediating crosstalk is warranted. SA is typically prioritized over JA in *Arabidopsis* [19]. However, plants use ET to fine tune defenses by prioritizing JA induction over SA in response to multiple attackers [20]. ET also modifies the effect of a key protein (*NPR1*; *NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES 1*) involved in SA suppression of JA. In *Arabidopsis*, NPR1 is necessary for expression of SA-responsive genes and for repression of JA by SA. However, when ET is present, NPR1 function is no longer required for SA suppression of JA [20,21], suggesting that ET signaling acts to suppress JA in the presence of SA by bypassing NPR1. Many other plant hormones are also important in mediating the crosstalk, but the genetic bases of this crosstalk are less well studied. Recent approaches that examine genetic interaction networks in *Arabidopsis* have been fruitful for identifying candidate loci to be studied in detail for their potential role in defense signaling crosstalk [22].

The SA–JA crosstalk that often results in reciprocal antagonism between these two pathways has been interpreted as being an adaptive plant strategy, representing a cost-saving measure given that phenotypically different enemies are susceptible to distinct defense strategies. However, specific defenses that induce resistance to one attacker may render the plant more susceptible to another if alternative defenses are repressed by crosstalk [23]. We first focus on the phylogenetic distribution of crosstalk, candidate loci underlying crosstalk and the nature of the evidence used to assay for crosstalk. We then evaluate adaptive and nonadaptive evidence for the SA–JA reciprocal antagonism and illuminate a research path that integrates phylogenetic, genetic and ecological approaches towards testing explicit hypotheses on the origins and adaptive value of signal crosstalk. We end with a discussion of SA–JA signal interactions as a mechanism that generates specificity in plant–attacker interactions.

Distribution of SA–JA reciprocal antagonism

Although the SA–JA antagonism is clearly present in many plant species, an open question is whether there is a common genetic basis to this crosstalk and if so, whether the trait is conserved across all plants. Similarly, although it can be a reciprocal antagonism, the strength of the downregulation from each side of the SA and JA equation is not identical and may not be antagonistic across plants.

We searched for all studies that tested for antagonisms in SA–JA signaling (Table 1). A paper was included as presenting evidence for SA–JA antagonism if there was a genetic or biochemical measure widely believed to be regulated by the jasmonate and salicylate pathways, or if one pathway was genetically manipulated and a response was measured in the other. Our survey included papers that measured JA, SA or their derivatives, gene expression or chemical end-products known to be regulated by one of the pathways. In some studies, one pathway was elicited and had direct effects on the other pathway. In other studies, SA–JA antagonism was seen when induction of one pathway reduced the response to elicitation of the other pathway. We did not include studies that only found antagonisms in resistance to bioassay organisms if there was not evidence that the antagonism was due to SA–JA crosstalk. From the well-studied systems including *Arabidopsis*, tomato (*Solanum lycopersicum*) and tobacco (*Nicotiana* spp.), a subset of studies is included to highlight the ecological conditions under which antagonism can occur. There are systems that show conditionality in the antagonism and these were scored as having SA–JA antagonism for the purposes of Table 1 and are discussed in the text. It is important to point out that although there are a growing number of studies using biological inducers, most of the evidence for antagonism is based on treating plants with exogenous SA and JA, either singly or in combination. In most cases, there have not been studies that test whether there is a common genetic basis or a correlated gene expression phenotype that underlies the gross SA–JA antagonism reported across plants. Therefore, the results of this survey and our inferences on trait evolution need to be interpreted with caution because of the inherent limitations of screening for SA–JA antagonism using chemical elicitors and the lack of direct evidence for a common mechanism. The evolutionary interpretations below and our interpretations are hypotheses to be tested.

The pathways that produce both hormones at the center of this story have ancient origins. SA is produced downstream of isochorismate synthase (ICS), which occurs in many green and red algae as well as in bacteria, and may have a plastid origin in plants [24]. By contrast, jasmonates are end-products of the ancient octadecanoid (C18) oxylipin pathway. Oxylipins are bioactive lipid derivatives that are used as signaling molecules in plants, animals, fungi [25], as well as in several marine algae species [26]. An allene oxide synthase (AOS) homolog (the second enzyme in the octadecanoid biosynthetic pathway) has been discovered in the moss *Physcomitrella patens* [27,28], and distant structural homologs to AOS have been putatively identified in three metazoan lineages [29]. The specific compounds JA and methyl JA also have been detected in *P. patens* [27,30–32], as well as in ferns [33], suggesting that JA production arose at least in the common ancestor of mosses, ferns and seed plants (Figure 1).

Despite the ancient origins of each hormone, the antagonism between SA and JA may have more recent origins. SA–JA antagonism has been reported in a total of 17 plant species, including 11 crop plants and six wild species (Table 1). Ancestral state reconstruction [34] indicates that SA–JA antagonism evolved at least at the base of angiosperms, but possibly before the split of gymnosperms and angiosperms (Figure 1; using data from Table 1). The presence of orthologs of genes known to be involved in the SA–JA antagonism including NPR1, WRKY70 (WRKY DNA-binding protein 70), GRX480 (Glutaredoxin 480), ERF1 (ETHYLENE RESPONSE FACTOR 1), MYC2 (JASMONATE INSENSITIVE 1, JIN1), ORA59 (OCTADECANOID-RESPONSIVE

Table 1. Evidence for SA–JA antagonism across plant species

Plant species	Method of SA elicitation	Method of JA elicitation	SA pathway inducibility measurement	JA pathway inducibility measurement	Bioassay result	Refs
Arabidopsis thaliana	*Pieris brassicae* eggs/egg extracts	–	SA induced	Ten insect-induced JA regulated transcripts decrease	Decreased resistance to *Spodoptera littoralis*	[44]
Arabidopsis thaliana	SA	–	–	Peroxidase, polyphenol oxidase, chitinase, glucosinolates decrease	Decreased resistance to *Spodoptera exigua*	[84]
Arabidopsis thaliana	SA	Pathogens: *Alternaria brassicola*, *Botrytis cinerea*, insects: *Frankliniella occidentalis*, *Pieris rapae*	–	PDF 1.2 (PLANT DEFENSIN 1.2) decreases	–	[59]
Arabidopsis thaliana	*Hyaloperonospora parasitica*	MeJA	–	PDF1.2 expression decreases	–	[59]
Arabidopsis thaliana	*Pseudomonas syringae*	MeJA	–	–	Decreased resistance to *Trichoplusia ni*	[10]
Arabidopsis thaliana	Mutant plants with elevated or suppressed SA	–	–	–	*Trichoplusia ni* resistance decreased as SA expression increased	[65]
Arabidopsis thaliana	SA	MeJA	–	Genome wide effects	–	[85]
Arabidopsis thaliana	Cucumber mosaic virus		–	JA inducible transcripts decrease	–	[45]
Solanum lycopersicum (tomato)	SA	JA and systemin	–	Proteinase inhibitors decrease	–	[86]
Solanum lycopersicum	BTH	JA	PR4 (PATHOGENESIS RELATED 4) transcripts downregulated	Oxidative enzymes decrease	Decreased resistance to *Spodoptera exigua* and *Trichoplusia ni*	[53,55]
Solanum lycopersicum	*Botrytis cinerea*	–	SA induced	Proteinase inhibitor transcripts decrease	*B. cinerea* disease increased	[47]
Solanum lycopersicum	Parasitic plant *Cuscuta pentagona* and SA deficient plants	–	SA induced	JA and herbivore induced plant volatiles decrease	*Spodoptera exigua* performance not affected	[48]
Solanum lycopersicum cv. cerasiforme (wild tomato)	BTH	JA	–	Polyphenol oxidase activity decrease	*Spodoptera exigua* performance not affected	[52]
Oryza sativa (rice)	Mechanical damage	–	–	Increased JA correlates with decreased SA		[70]
Nicotiana tabacum (tobacco)	Tobacco mosaic virus inoculation	–	–	JA and nicotine decrease	Decreased resistance to *Manduca sexta*	[46]
Nicotiana tabacum	Genetically reduced SA production	–	–	JA, nicotine, polyphenol oxidase increase	Increased resistance to *Heliothis virescens*	[87]
Nicotiana attenuata	–	Fatty acid–amino acid conjugates from *Spodoptera exigua* oral secretion	SA decreases	–	–	[80]
Hordeum vulgare (barley)	SA	–	–	13-Hydroxyoctadecatri(di)enoic (JA suppressor) increase	–	[88]

Cucumis sativus (cucumber)	BTH	JA	Reduced chitinase levels on dual-elicited plants	–	*Colletotrichum orbiculare* disease severity lower on dual elicited plants	[58]
Pisum sativum (pea)	SA	Wounding, JA	–	JA, polyphenol oxidase downregulated	–	[51]
Phaseolus lunatus (lima bean)	Whitefly, SA	JA	–	JA, volatiles	Predatory mite attraction reduced	[49]
Gossypium hirsutum (cotton)	*Phenacoccus solenopsis* (mealy bugs)	–	SA-induced volatiles and upregulation of SA-dependent transcripts	Gossypol and other transcripts downregulated	–	[50]
Sorghum bicolor (sorghum)	SA	MeJA	Some SA transcripts downregulated	some JA transcripts downregulated	–	[56]
Ginkgo biloba	Transgenic suppression of SA	–		JA, OPDA levels decrease	–	[72]
Brassica carinata (Ethiopian mustard)	*Sclerotinia sclerotiorum* (white mold)	*Sclerotinia sclerotiorum*	JA transcripts upregulated after SA transcripts downregulated	SA transcripts upregulated only after JA transcripts are downregulated	–	[89]
Brassica nigra (black mustard)	SA applied to roots	–	–	JA downregulated in roots	–	[66]
Brassica oleracea (cabbage)	SA applied to roots	–	–	JA downregulated in roots	–	[66]
Brassica napus (oilseed rape)	SA	Mechanical wounding, Methyl jasmonate	–	Myrosinase-associated protein downregulated in dual-elicited plants	–	[90]
Asclepias tuberosa (butterfly milkweed)	–	*Danaus plexippus* (monarch) herbivory	SA decreases	JA upregulated	–	A.A. Agrawal, unpublished

Abbreviations: BTH, benzothiadiazole; JA, jasmonic acid; SA, salicylic acid.

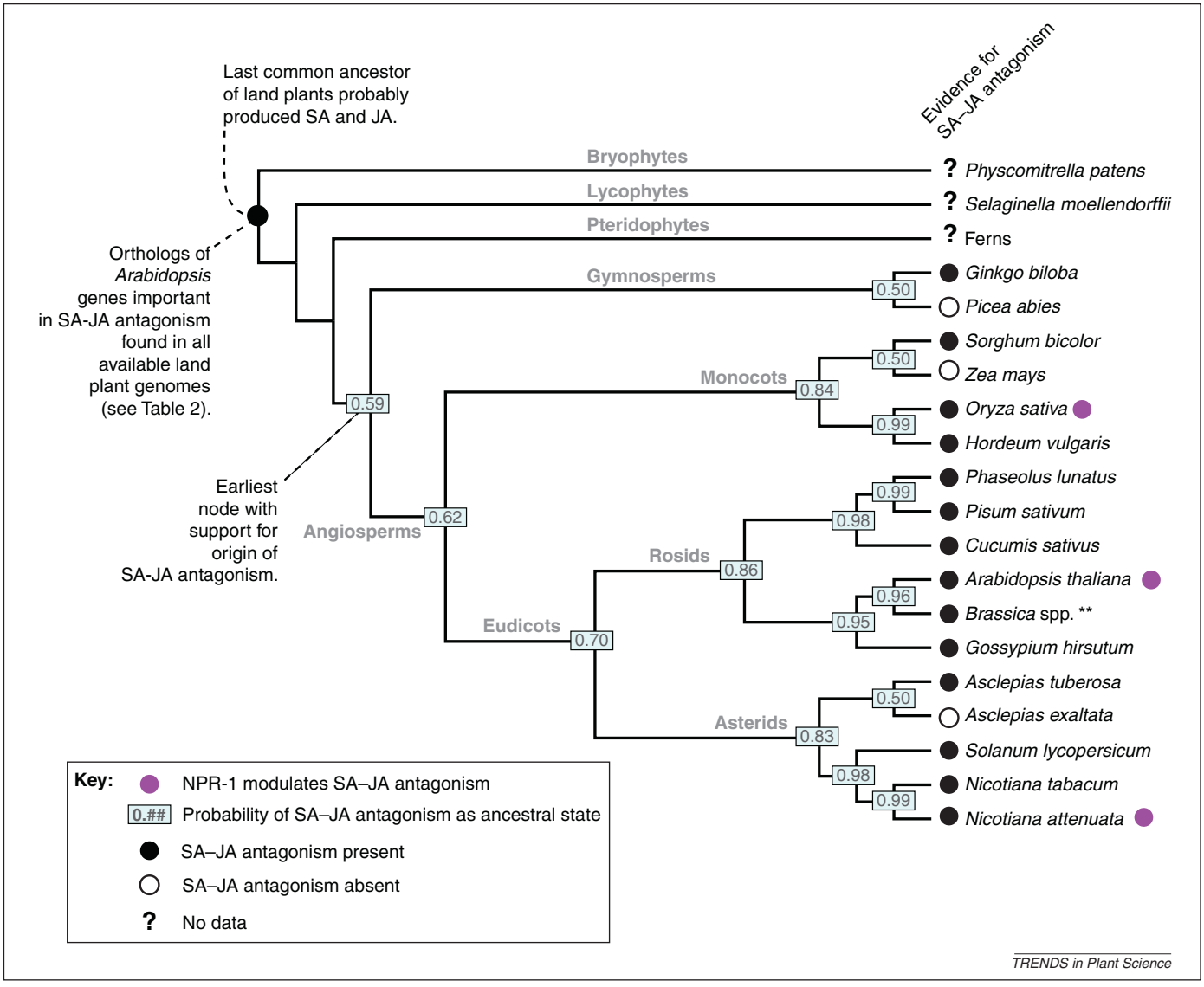

Figure 1. Phylogeny of green plants showing putative and reconstructed ancestral states for key aspects of SA–JA antagonism. Topology is based on that published on the Angiosperm Phylogeny Website [96]. Antagonism between SA and JA signaling has only been investigated in seed plants, and only sparsely among gymnosperms (see Table 1 for details and references). The ancestral state of the antagonism was inferred using the *ace* function in the R library APE [34] using maximum likelihood with branch lengths set to 1. Node labels are probabilities (between 0 and 1) of trait presence given equal gain/loss transition probabilities. The antagonism was probably present in the ancestor of all angiosperms, and in the ancestor of all seed plants, but whether the antagonism is present in the gymnosperms is equivocal given poor taxon sampling. To our knowledge, there are no data addressing the existence of SA–JA antagonism in sister taxa of seed plants. This is despite the occurrence of close genetic orthologs of many genes known to affect the antagonism in angiosperms (Table 2). BLASTs of *Arabidopsis thaliana* genes (Table 2) were conducted using blastp searches against the following taxa: *Physcomitrella patens* (NCBI taxon id: 3218), *Selaginella moellendorffii* (taxon id: 88036), *Sorghum bicolor* (taxon id: 4558), *Zea mays* (taxon id: 4577), *Oryza sativa* var. Japonica (taxon id: 39947), *Solanum lycopersicum* (taxon id: 4081), as well as an expressed sequence tag database for the fern *Pteridium aquilinum*. For all genes, a hit was found to all taxa (except *P. aquilinum*) with an *e*-value $< e^{-10}$. Hits (*e*-value $< e^{-6}$) to *P. aquilinum* were found for AtGRX480 and AtMPK4 using blastn searches against the nonhuman, nonrodent EST database; additional genes in this fern were possibly missed due to low coverage of the *P. aquilinum* transcriptome. Because of extensive gene and genome duplications across plants, BLAST results convey conservation of gene families, members of which were inherited by the ancestor of all land plants, although the vast majority of hits from the taxa above represent reciprocal best blast hits back to the *Arabidopsis thaliana* genes used as queries. Thus, in principle, the genetic machinery underpinning the SA–JA antagonism was available early on in the evolution of land plants. This in itself is not evidence of SA–JA antagonism. An NPR1 ortholog in *Oryza sativa* modulates the SA–JA antagonism, which is similar to NPR1 in *Arabidopsis*, suggesting this aspect of the antagonism may have been present before the split between monocots and eudicots. More extensive taxon sampling is required before evaluating the evolution of this function for NPR1 across plants. **Four *Brassica* species (*B. carinata, B. nigra, B. oleracea* and *B. napus*) all exhibit SA–JA antagonism (Table 1).

ARABIDOPSIS AP2/ERF 59), JAZ1-JAZ3 (JASMONATE ZIM-DOMAIN) are predicted, based on reciprocal best blastp searches using *Arabidopsis* proteins as subjects (Table 2), to have been present in the first land plants, after this lineage split with green algae. This suggests that many regulatory features of SA–JA crosstalk have diverse and potentially ancient roles in the cell. An ortholog of the canonical crosstalk regulator NPR1 was probably present in the ancestor of all land plants, indicating that the potential for this gene to mediate SA–JA antagonism exists in all species in which the antagonism has been found (Table 1, Figure 1). NPR1 exhibits unique roles in SA–JA crosstalk in different extant plant species. Unlike in *Arabidopsis*, tobacco (*Nicotiana attenuata*) NPR1 acts as a negative regulator of signal crosstalk in the presence of herbivory by preventing SA from suppressing JA-responsive defenses [35]. In this study, herbivory induced SA and JA, as well as NPR1 gene expression [35]. This functioned to prevent SA from repressing JA defenses against the herbivore, thus prioritizing JA over SA.

Table 2. Phylogenetic distribution of orthologs to *Arabidopsis thaliana* genes important in the JA–SA antagonism

AT gene symbol	*Arabidopsis thaliana*	*Solanum lycopersicum*	*Sorghum bicolor*	*Oryza sativa*	*Zea Mays*	*Selaginella moellendorffii*	*Physcomitrella patens*	Role in JA–SA crosstalk in *Arabidopsis*	Refs
GRX480	NP_174170.1	NP_001233988.1	XP_002440249.1	NP_001043812.1	NP_001147414.1	XP_002988222.1	XP_001770429.1	This SA- and NPR1-induced glutaredoxin represses JA-responsive PDF1.2 in a TGA-transcription factor-dependent manner.	[91]
ERF1	NP_188965.1	NP_001234695.1	XP_002463464.1	NP_001051973.1	NP_001170395.1	XP_002967934.1	XP_001779786.1	This ET- and JA-responsive factor suppresses MYC2-dependent JA responses. ERF1 is suppressed by NPR1.	[91,92]
MYC2	NP_174541.1	AAF04917.1	XP_002467448.1	NP_001065478.1	AAD15818.1	XP_002987548.1	XP_001754025.1	This JA-induced transcription factor is inhibited by ET/JA-mediated ERF1 expression.	[92]
NPR1	NP_176610.1	NP_001234558.1	XP_002455011.1	NP_001042286.1	NP_001152107.1	XP_002992598.1	XP_001778211.1	SA suppression of JA in *Arabidopsis* is NPR1-dependent.	[93]
ORA59	NP_172106.1	NP_001234695.1	XP_002461637.1	NP_001051973.1	NP_001170395.1	XP_002966804.1	XP_001779786.1	This ET- and JA-responsive transcription factor is necessary for preventing SA suppression of JA in the presence of ET.	[20]
WRKY70	NP_191199.1	NP_001234530.1	XP_002441930.1	NP_001055192.1	NP_001147748.1	XP_002961829.1	XP_001778254.1	NPR1-mediated suppression of JA is controlled by WRKY70 and downstream TGA transcription factors.	[94]
MPK4	NP_192046.1	NP_001234660.1	XP_002467591.1	NP_001061028.2	NP_001105239.1	XP_002976336.1	XP_001763232.1	This MAP kinase is a negative regulator of SA and a positive regulator of JA by suppressing the SA activators/JA repressors PAD4 and EDS1.	[95]
JAZ1	NP_564075.1	NP_001234883.1	XP_002465159.1	NP_001060268.1	NP_001150658.1	XP_002984538.1	XP_001785097.1	JAZ proteins mediate JA crosstalk with a variety of other pathways, including SA, ET, Auxin and Gibberellin.	[39]
JAZ2	NP_565096.1	NP_001234883.1	XP_002461012.1	NP_001050322.1	NP_001148852.1	XP_002984538.1	XP_001785091.1	In the absence of JA, JAZ proteins repress the JA-responsive TFs EIN3/EIL1, which suppresses SA synthesis through effects on ICS (SID2).	[39]
JAZ3	NP_566590.1	NP_001234373.1	XP_002462352.1	NP_001063121.1	NP_001141029.1	XP_002975031.1	XP_001754769.1	JAZ3 (JAI3) was the first JAZ protein identified to repress the JA-responsive transcription factor, MYC2.	[39]

Functional data, based on gene expression and/or other studies, show that NPR1 modulates SA–JA antagonism in rice, *Arabidopsis* and tomato, suggesting that this subfunction for NPR1 may have ancient origins in the common ancestor of monocots and eudicots. Another *Arabidopsis* gene involved in crosstalk, WRKY70, is present as a ortholog in rice (*Oryza sativa* WRKY13). These orthologs positively regulate SA-induced and negatively regulate JA-induced responses [36]. Although OsWRKY13 is not a one-to-one ortholog of WRKY70 in *Arabidopsis*, the central role of a WRKY transcription factor in modulating between SA- and JA-dependent responses is important in both species. In addition, a microarray analysis on OsWRKY13-overexpression rice lines found suites of SA- and JA-regulated genes displaying reciprocal antagonism [37]. These are patterns similar to those found in *Arabidopsis*. Among their many functions, JAZ proteins repress the JA-responsive ethylene-signaling genes EIN3 (ETHYLENE INSENSITIVE 3)/EIL1 (ETHYLENE INSENSITIVE 3 LIKE1), which when expressed lead to suppression of SA synthesis [38]. Upon activation of the JA receptor COI1 (CORONATINE INSTENSITIVE 1), JAZ repressor proteins are degraded, allowing for the activation of JA-responsive signaling cascades [39]. JAZ-mediated repression and derepression appears to be important in mediating not only SA–JA crosstalk but also JA–ET, JA–Gibberellin and JA–auxin signal interactions [39]. This suggests that signal crosstalk may be a fundamental attribute of plant genetic networks [22,40] and may be commonly achieved through JAZ-mediated repression [39]. Orthologs of JAZ proteins identified in *Arabidopsis* have been discovered in *P. patens* and other early diverging land plants [41] (and this study). Together, the role of NPR1, WRKY and JAZ genes in regulating SA–JA and SA–JA–ET crosstalk from rice to eudicots suggests a generally conserved core genetic architecture to defense signaling in flowering plants. Nonetheless, the presence/absence of these genes is not sufficient evidence of any SA–JA antagonism. Although the antagonism frequently occurs, it also appears to be absent in several lineages: *Picea abies* (Pinaceae) (J. Arnerup, PhD thesis, Swedish University of Agricultural Sciences, 2011), *Zea mays* (Poaceae) [42] and *Asclepias exaltata* (Apocynaceae) (Table 1, Figure 1). For two closely related milkweed species studied in the same experiment, *Asclepias tuberosa* showed the antagonism whereas *A. exaltata* did not (A.A. Agrawal, personal communication).

Antagonisms are common when chemical elicitors are the inducing agents [43] and when one pathway is genetically suppressed (ginkgo, *Arabidopsis*, tomato, tobacco) (Table 1). There is also widespread evidence that an antagonism occurs following induction by a biological agent. An extensive range of inducers in *Arabidopsis* has been investigated and the antagonism has been found following infection by bacteria, virus and fungi, leaf damage by thrips (Thysanoptera) and lepidopteran larvae, and oviposition of lepidopteran eggs [16,44,10,45]. Virus infection reduces induction of the JA pathway in tobacco [46]. In tomato, the antagonism occurs following infection by a parasitic plant and a fungus [47,48]. In lima bean (*Phaseolus lunatus*) and cotton plants (*Gossypium hirsutum*), SA induction by whiteflies and mealybugs decreased sensitivity to JA [49,50]. Finally, in milkweed plants (*A. tuberosa*) monarch caterpillar feeding increased JA levels and decreased SA (A.A. Agrawal, personal communication).

Does antagonism at the level of gene expression or hormone levels translate into a change in actual resistance level? In a small subset of these examples antagonism is inferred based on monitoring readouts of an end-product such as gossypol levels in cotton [50], polyphenol oxidase activity in pea (*Pisum sativum*) [51] and volatiles in cultivated tomato (*Solanum lycopersicum*) [48]. There are few examples that also include a bioassay to test for an antagonism, and when they do, an antagonism at the level of gene expression sometimes resulted in reduced resistance and sometimes it did not. Specifically, in *Arabidopsis*, cultivated tomato and tobacco, the antagonism has been shown to decrease resistance to a future attacker, yet in wild tomato there was no effect [52]. In the cases where the antagonism occurred following a biological inducer and resulted in decreased resistance, the inducing agent was usually a generalist attacker (whiteflies, aphids, *Pseudomonas syringae*; Table 1). Very few papers examined SA–JA antagonism in a field setting [53,35] and we found no study that measured the consequences of the antagonism for plant fitness.

Although SA induction frequently suppresses JA induction, and plants have long been hypothesized to prioritize SA over JA induction, there are seven species in which JA responses were associated with the suppression of SA induction [54–58]. The sequence in which SA and JA are added exogenously in experiments influences the strength of the reciprocal antagonism [20], and the timing [59] or dosage [60] of hormone application is important for realization of the antagonism [59]. In some cases, SA and JA pathways are each upregulated by one attacker species, but their induction is not simultaneous. For example, following infection by *Fusarium* spp., a hemibiotrophic fungal pathogen, both the SA and JA pathways are induced after infection, but SA is important in establishing resistance early on, and JA is important in facilitating resistance during later time points [61]. Thus, although both the SA and JA pathways are induced by the same pathogen, the responses are temporally disconnected. Screens across genotypes of *Arabidopsis* revealed variation in priming of the SA and JA pathways that manifested as coinduction of SA and JA when a fungal species was used as the inducer [62]. However, these genotype-specific effects were only in the context of actual pathogen attack and were not observed when hormones were applied to plants [59]. All of this work points to the fact that the antagonism is highly context-dependent, both in terms of what is used to elicit SA and JA, the timing of the elicitation, and possibly with respect to genetic variation underlying the antagonism. The suppression of SA by JA is either triggered by a biological inducer (*Arabidopsis*, milkweed and *Brassica*), or follows after chemical or genetic manipulation of the SA pathway (tomato, millet, tobacco, cucumber). For example, the jasmonate mimic coronatine produced by *Pseudomonas syringae* activates the JA pathway and suppresses the SA pathway in *Arabidopsis* [63].

Within-plant factors including the timing, concentration and location of induction influence whether crosstalk and an antagonism occurs. Effects of timing have been shown with elicitor studies that temporally manipulate the sequence of application, and with studies showing that endogenous JA and SA levels change inversely with each other [59,64]. Most studies only test for local but not systemic interactions. However, systemic antagonism in *Arabidopsis* is induced by *Pseudomonas* infection [10,65], and insect eggs and egg extracts only induced antagonism locally [44]. Root SA elicitation decreased JA inducibility within the root but did not reduce JA inducibility in shoot tissues [66]. We know little about how intensity of induction [55,67,68] and factors such as plant sectoriality and phenology influence signal antagonism. Because these aspects of plant form and growth influence hormone induction *per se* [69], they will probably influence the interaction between hormonal pathways.

Adaptive and nonadaptive hypotheses for the antagonism

Is the SA–JA antagonism an artifact of complex signaling?

Plants have a limited number of hormone signal molecules, which by chance may sometimes interact to affect gene expression positively or negatively. In this scenario, different environmental conditions such as the location and timing of attacked generate specificity in the antagonism. Although this is possible, the existence of conserved genes (e.g. NPR1), conserved across several distantly related plant taxa that regulate SA–JA interactions in diverse taxa (e.g. rice, tobacco, *Arabidopsis*) makes this hypothesis unlikely.

Is the SA–JA antagonism an ancient constraint found in plants and animals?

Lipid-derived, jasmonate-like animal hormones such as prostaglandins are inhibited in animals by aspirin (i.e. acetylsalicylic acid). Because a similar antagonism is also widespread in plants (Figure 1), it may represent an ancient evolutionary constraint [70]. In addition, several genes that underpin crosstalk regulation in *Arabidopsis* have close homologs in the moss *P. patens* and the lycophyte *Selaginella moellendorffii*, in addition to several angiosperms (Table 2, Figure 1). This indicates that the genetic machinery to express and regulate crosstalk is widely conserved to this day and was probably ancestral to all land plants. However, gene presence/absence does not imply functional conservation. Because there is variation in whether the antagonism is expressed even between closely related taxa (Figure 1), expression of SA–JA antagonism is not an unbreakable constraint.

Is SA–JA antagonism due to resource allocation costs of induction?

There are fitness costs associated with the induction of SA and JA defenses in the absence of a natural enemy attack [71]. Thus, the SA–JA antagonism could be viewed as either a limitation of or adaptation to a resource-limited environment. There are at least two scenarios to consider whereby JA and SA pathways either regulate different defense products or the same defense products. When crosstalk limits production of a product, antagonisms in the induction of each may prevent simultaneous induction [72]. When the crosstalk limits production of a product regulated by only one pathway, signal crosstalk can be a means of maintaining production of one product instead of another. The effect of elicitor concentration and exposure time on whether the antagonism is found supports this hypothesis [43].

Resource allocation costs are probably partially responsible for shaping the patterns of induction following attack, but several lines of evidence suggest they are not likely to be the only factor. The SA and JA pathways do not utilize the same precursors or components for their signal transduction pathways, which makes specific resource limitation less likely to be the explanation at that level. However, more importantly, strict competition for precursors, such as amino acids, should result in downregulation of many plant functions, not only particular JA or SA regulated genes. Therefore, costs alone do not explain the apparent specificity in the antagonism: decreased inducibility of the jasmonate pathway following light limitation is due to specific hormonal modulation [73], not simply reduced resource availability. Similarly, decreasing nitrogen availability actually increased the expression of the jasmonate pathway due to altered interactions between jasmonate and ethylene. Thus, decreasing nutrient levels can even increase defense expression, evidence against strict resource mediated SA–JA crosstalk [74].

Is SA–JA antagonism a means for the plant to adaptively tailor its responses to different enemies and also a target for manipulation by enemies?

Downstream defenses that are modulated by the SA and JA pathways affect pathogens and herbivores, and each attacker may be affected by a different subset of these defense products. Thus, the adaptive tailoring hypothesis predicts that the plant should induce the components of each pathway that are most effective against the current attacker. This implies some degree of specificity on the plant's part – if the plant is tailoring its defense response adaptively then different enemies must be recognized as distinct by the plant [5,75]. Many of the patterns described above almost make specificity axiomatic, such as the general asymmetry of SA and JA suppression, the important role of other hormones, the effects of the pattern of damage on the expression of crosstalk, and the effect of other enemies present on the plant [76].

A major unanswered question is whether crosstalk is adaptive for the plant [42]. If crosstalk tailors the plant's response to a particular attacker this specificity should increase the plant's resistance to that attacker. However, the selective advantage of manipulating crosstalk from the perspective of a particular attacker must be high. Thus, the specificity of response is a complex phenotype mediated by plant and attacker. The elicitors present in, for example, the saliva or accessory gland secretions from a particular herbivore species that is attacking a plant often determine the specificity of these responses in the plant [10,77]. Manipulation of hormonally regulated pathways may be a mechanism by which enemies can suppress induced

defenses in biochemically divergent plants [78]. Given that the SA–JA antagonism appears to be phylogenetically widespread and ancient, this method of manipulating the host plant has been available for a long time and may work against a wide diversity of plants [78]. Consistent with this hypothesis, generalist enemies have been found to induce SA–JA crosstalk in a way that benefits them [10,79–81].

Thus, experiments are required to understand who benefits (the plant, the plant's attacker or neither?) and yet few studies explicitly connect the plant's specific response to an effect on plant resistance [82] or performance. We propose that testing the adaptive value of specificity will require experiments that incorporate a 'neutral' inducer such as mechanical damage or pure hormonal application as controls. The effects of 'neutral' induction and induction in response to the biological organism can then be compared. For specificity to be adaptive for the plant, the plant's response to the neutral and biological inducer must differ and this tailoring must benefit the plant. For example, evidence was found against adaptive specificity when chemical elicitation caused a similar pattern of crosstalk as biological induction [59]. If the attacker benefits, it may be manipulating the plant to its benefit. Alternatively, the response may not be adaptive for the plant or the attacker. Consistent with the adaptive tailoring hypothesis is that there is extensive variation in patterns of induction across plant diversity, which is a prerequisite for, but not yet evidence of, adaptation. In summary, critical data on the consequences of SA–JA antagonism for plants in the field are too scant to address this adaptive tailoring hypothesis at present.

Box 1. What experiments are needed to effectively test the adaptive significance of SA–JA antagonism?

Constraints hypothesis

(i) Do simple phylogenetic constraints explain SA–JA antagonism? Analysis of phylogenetic distribution of the SA–JA antagonism using common elicitors would illuminate repeated losses and gains. SA–JA antagonism is widespread across plants, but evidence is missing from early diverging lineages.
(ii) Do the same pathways exist across plants for modulating the antagonism? This can be tested by measuring patterns of gene expression in candidate SA, JA and crosstalk modulator loci in dual elicitation experiments across plant diversity in a common environment [9]. If orthologous loci show common patterns of expression during dual elicitation, it is unlikely to be adaptive tailoring and more likely to be a constraint.

Resource allocation costs of induction hypothesis

(i) **Resource limitation**. Isotope tracer studies measuring flux of resources and precursors between the pathways would directly demonstrate resource diversion [43,83]. Resource limitation could also be tested by manipulating resource availability and addressing if SA–JA antagonism is weaker in resource-rich conditions.
(ii) **Cost of single versus dual elicitation**. Is inducing both pathways more costly than inducing one? Plant fitness should be measured following single and dual elicitation.

Adaptive tailoring hypothesis

(i) Biological elicitors result in varied expression levels and patterns of loci involved in the antagonism relative to chemical elicitors or mechanical elicitation. Greater variance and distinct patterns in the antagonism across attacker species would support the tailoring hypothesis. If chemical and biological inducers show similar patterns, this would not support adaptive tailoring. Higher resistance and plant performance following biological induction of SA–JA antagonism compared with chemical elicitation would be evidence for adaptive tailoring.
(ii) Is genetic variation in the antagonism adaptive? Genotypes varying in the antagonism could be placed into environments varying in attacker composition. In environments with one attacker, the antagonism is more likely to benefit the plant compared with environments with multiple enemies (unless the antagonism is induced to the benefit of the attacker). Artificial selection experiments and forcing induction of the alternative pathway could reveal how natural selection shapes the antagonism.

A prospectus on future experiments

Our most important conclusion is that in order to test the various hypotheses proposed above: (i) measurements of the SA–JA reciprocal antagonism in the form of gene expression and biochemical activity must be coupled with pathogen and herbivore bioassays and simultaneous measurements of plant fitness, and (ii) that these experiments must be conducted in ecologically relevant settings and across plant diversity. From an evolutionary perspective, future experiments should attempt to test if the SA–JA antagonism arose in a reciprocal manner or sequentially with unidirectional antagonisms arising separately. Future genome sequencing of plant species where there is no evidence for the antagonism could reveal if, and perhaps how, SA–JA antagonism was lost or if there are other conditions under which the antagonism is expressed. Researchers should focus on understanding if indeed SA–JA reciprocal antagonism arose once and if there is a common genetic basis to this phenomenon across the plants in which it occurs. Specific recommendations are given in Box 1.

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