Jasmonate response decay and defense metabolite accumulation contributes to age-regulated dynamics of plant insect resistance

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Immunity deteriorates with age in animals but comparatively little is known about the temporal regulation of plant resistance to herbivores. The phytohormone jasmonate (JA) is a key regulator of plant insect defense. Here, we show that the JA response decays progressively in Arabidopsis. We show that this decay is regulated by the miR156-targeted SQUAMOSA PROMOTER BINDING PROTEIN-LIKE9 (SPL9) group of proteins, which can interact with JA ZIM-domain (JAZ) proteins, including JAZ3. As SPL9 levels gradually increase, JAZ3 accumulates and the JA response is attenuated. We provide evidence that this pathway contributes to insect resistance in young plants. Interestingly however, despite the decay in JA response, older plants are still comparatively more resistant to both the lepidopteran generalist Helicoverpa armigera and the specialist Plutella xylostella, along with increased accumulation of glucosinolates. We propose a model whereby constitutive accumulation of defense compounds plays a role in compensating for age-related JA-response attenuation during plant maturation.
As sessile organisms, plants have evolved complex defense systems against herbivores for successful survival and reproduction. Induced defense response refers to immune responses elicited by specific stimuli whereas constitutive defense refers to the accumulation of insecticidal components in plant tissues during the course of normal growth and development. Plants of *Arabidopsis thaliana* produce glucosinolates (GLSs), which function as defense metabolites against insect herbivores and pathogens.

In plants, JA is the major defense hormone in activating defense reactions against herbivorous insects and necrotrrophic pathogens, and in *Arabidopsis* most responses are regulated by the JA-amino acid conjugate jasmonyl-L-isoleucine (JA-Ile). JA ZIM-domain (JAZ) proteins, which are the repressors of JA signalling, have two conserved domains: the N-terminal ZIM domain and the C-terminal Jas domain. The Jas domain is a protein–protein interaction surface required for binding to either transcription factors such as MYC2, or CORONATINE INSENSITIVE1 (COI1), a component of the ubiquitin E3 ligase SCF^COI1_ and the JA-Ile receptor.

In normal conditions, the protein–protein interaction surface required for binding to either transcription factors such as MYC2, or CORONATINE INSENSITIVE1 (COI1), a component of the ubiquitin E3 ligase SCF^COI1_ and the JA-Ile receptor appears dysfunctional. In plants, miR156 functions as an important regulator of developmental processes. To test if JA response was altered according to plant age, we first analysed the endogenous levels of JA and the bioactive JA-Ile. Although higher in young than in old plants, the JA and JA-Ile contents were generally low in untreated plants. Upon *H. armigera* damage, their levels were evidently elevated, but the resultant JA-Ile contents did not show a significant difference between the young and the old plants (Fig. 1c). We then monitored the expression of JA-inducible genes, including LIPXYGENASE 2 (*LOX2/ATG4S1400*), VEGETATIVE STORAGE PROTEIN 2 (*VSP2/ATG24770*) and TYROSIINE AMINOTRANSFERASE (*TAT1/ATG23600*). After application of methyl-JA (MeJA), expression of JA-responsive genes in aerial tissues was induced to a higher degree in young plants compared with old plants (Fig. 1d). When the newly initiated leaves (new leaves) were analysed, the results were similar; young plants exhibited a more pronounced JA response than old plants (Supplementary Fig. 2a).

The JA signalling pathway plays important roles, not only in plant defense against herbivorous insects, but also in a wide range of developmental processes. To test if JA response was altered according to plant age, we first analysed the endogenous levels of JA and the bioactive JA-Ile. Although higher in young than in old plants, the JA and JA-Ile contents were generally low in untreated plants. Upon *H. armigera* damage, their levels were evidently elevated, but the resultant JA-Ile contents did not show a significant difference between the young and the old plants (Fig. 1c). We then monitored the expression of JA-inducible genes, including LIPXYGENASE 2 (*LOX2/ATG4S1400*), VEGETATIVE STORAGE PROTEIN 2 (*VSP2/ATG24770*) and TYROSIINE AMINOTRANSFERASE (*TAT1/ATG23600*). After application of methyl-JA (MeJA), expression of JA-responsive genes in aerial tissues was induced to a higher degree in young plants compared with old plants (Fig. 1d). When the newly initiated leaves (new leaves) were analysed, the results were similar; young plants exhibited a more pronounced JA response than old plants (Supplementary Fig. 2a).

**Plant defense involves a metabolic cost, where a tradeoff occurs between defense and growth.** In plants, defense is a common phenomenon. In plants, immunity is also associated with age, as old plants may display increased resistance to pathogens, which is referred to as age-related resistance. For plant-herbivore interactions, the Plant Vigour Hypothesis is based on observations that many herbivores attack young and vigorous plants more frequently than old and mature plants. However, the molecular mechanisms underlying ARR and the Plant Vigour Hypothesis remain elusive.

In plants, miR156 functions as an important regulator of age-dependent development through targeting a group of transcription factors called SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) trans. The level of miR156 is high during the juvenile stage and steadily decreases during later plant growth and development, leading to a progressive increase in the level of SPLs, which regulate a broad range of processes including flowering, secondary metabolite production, trichome initiation, vernalization and shoot regeneration.

The phytohormone gibberellin (GA) plays an important role in regulating diverse aspects of plant growth and development. Similar to JAZ proteins in the JA signalling pathway, DELLAs, a group of GRAS family proteins, function as repressors to control GA signal output. When the level of bioactive GAs increases, DELLAs are subject to ubiquitination and degradation. Recent investigations revealed that GA signalling cross-talks with both the JA response and the miR156-SPL mediated aging pathways. DELLAs directly bind to SPL proteins and interfere with their transcriptional activity, and they also interact with JAZ proteins or MYC2 to modulate the JA response.

Here we demonstrate that the JA response declines with plant age. We show that this attenuated JA response is primarily regulated by the miR156-targeted SPL proteins that can interact with certain JAZ proteins, and appears to be independent of the GA signalling pathway. We propose a model whereby accumulation of GLS in older plants accumulates for attenuated JA response.

**Results**

**The JA response declines with plant age.** Cotton bollworm (*Helicoverpa armigera*) is a generalist lepidopteran pest that can live on a wide range of plants including *Arabidopsis*. When *H. armigera* larvae were placed on 14- and 26-day-old *Arabidopsis* plants for three days, respectively, the larvae on young plants grew faster than those on old plants. Another lepidopteran, Diamondback moth (*Plutella xylostella*), is a specialist herbivore living on plants of Brassicaceae, and *Arabidopsis thaliana* can be used as a model host. When *P. xylostella* larvae were tested, similar results were obtained: young plants provided quicker larval growth than old plants. Nevertheless, Diamondback moth (*Plutella xylostella*), is a specialist herbivore living on plants of Brassicaceae, and *Arabidopsis thaliana* can be used as a model host.

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In plants, miR156 functions as an important regulator of age-dependent development through targeting a group of transcription factors called SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL). The level of miR156 is high during the juvenile stage and steadily decreases during later plant growth and development, leading to a progressive increase in the level of SPLs, which regulate a broad range of processes including flowering, secondary metabolite production, trichome initiation, vernalization and shoot regeneration.

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effect of the miR156-regulated SPLs on plant resistance. For *H. armigera*, the SPL9:rSPL9 and 35S:MIR156 leaves provided larvae a higher weight increase, whereas the 35S:MIR156 leaves retarded larval growth. For *P. xylostella*, the larvae also grew faster on SPL9:rSPL9 leaves than on wild-type or 35S:MIR156 leaves (Fig. 2a and Supplementary Fig. 3a). These data suggest that, although functioning as an aging cue, the miR156-targeted SPLs repress plant defense against insects, contrary to the observation that the old plants had higher insect resistance than young plants (Fig. 1b).

To test whether SPL9 affected plant resistance to insects by interfering with JA signalling, 35S:MIR156 and SPL9:rSPL9 were introduced into the JA-insensitive mutant coi1-2, respectively. The SPL9 transcript level was elevated in SPL9:rSPL9 coi1-2 and reduced in 35S:MIR156 coi1-2 (Supplementary Fig. 4), as expected. Insect feeding assays showed that both *H. armigera* and *P. xylostella* larvae grew faster on coi1-2 than on the wild-type leaves (Fig. 2a,b), as reported for *Pieris rapae*, another lepidopteran herbivore42. However, the negative effect of SPL9 on insect resistance was abolished in the coi1-2 background (Fig. 2b), suggesting that JA signalling was involved in the repression of defense by SPL9, although a possible indirect influence due to different developmental or physiological states because of the changed level of SPLs could not be excluded. Consistent with their insect resistance, the 35S:MIR156 plants exhibited a higher JA response than the wild-type plants, whereas the SPL9:rSPL9 and 35S:MIM156 plants were less sensitive to the MeJA treatment (Fig. 2c, Supplementary Figs 3b,c and 5).

To analyse the effect of SPL9 on JA response more directly, we employed an inducible expression system. In SPL9:rSPL9-GR plants, rSPL9 was fused to the hormone-binding domain of the rat glucocorticoid receptor43 and expressed under the 35S promoter. Treating the plant with dexamethasone (DEX) leads to translocation of rSPL9-GR fusion protein from cytoplasm to nucleus and a phenotype similar to that of the rSPL9 expressing 35S:MIM156 plants20. We found that, in the absence of DEX, the response to MeJA was similar between the SPL9:rSPL9-GR and the wild-type plants (Fig. 3a). Upon DEX-application, the

Figure 1 | Insect resistance enhances and JA response attenuates with plant age. (a) Diagrams of young (14-day-old, 14D) and old/adult (26-day-old, 26D) plants of *Arabidopsis thaliana* (Col-0) grown in LD. Black arrows indicate the newly initiated leaves (new leaves, ~3 mm in width), and red arrows indicate the rapidly expanding leaves. (b) Weight increase of *H. armigera* and *P. xylostella* larvae fed with rapidly expanding leaves harvested from the indicated plants for 3 days, both gained less weight from the old (26D) plants. Data are means ± s.d. (n = 25). Asterisk indicates significant difference from the 14D group (Student’s t-test, *P < 0.05*). Embedded in the column is the image of the *H. armigera* larva after feeding. Scale bar, 1 cm. (c) Analysis of JA and JA-Ile contents in young (14D) and old (26D) plants by UPLC-MS. Plants were challenged by *H. armigera* third instar larvae for 12 h and the rapidly expanding leaves were collected for analysis. Intact plants were used as control. Data are means ± s.d. (n = 3). (d,e) Expression of LOX2, VSP2 and TAT1 in young (14D) and old (26D) plants in LD. Transcript levels were detected by qRT-PCR. Data were analysed by multiple comparisons (Tukey test) followed by two-way ANOVA (*P < 0.05, **P ≤ 0.01, ***P ≤ 0.001). Error bars represent ± s.d. (n = 3). (d) Gene expressions in total aerial tissues of young (14D) and old (26D) plants 4 h post-MeJA treatment, the JA response attenuated with plant age. The expression in the 14D control plants (−JA) was set to 1. (e) Gene expressions in rapidly expanding leaves of the young (14D) and the old (26D) plants 2 h post-wounding (W) treatment, the wounding response attenuated with plant age. The expression in the 14D intact plants (CK) was set to 1. ANOVA, analysis of variance; UPLC-MS, ultra performance liquid chromatography-mass spectrometry.
**SPL9** can interact with **JAZ** proteins. SPL9 has dual molecular functions, acting as transcriptional activator20,21 or as a signalling modulator through binding other factors22,29. Quantitative real-time PCR (qRT-PCR) showed that the elevated SPL9 level did not upregulate **JAZ** genes at the transcriptional level (Supplementary Fig. 7). We then asked whether SPL9 attenuates the JA response through interacting with known JA signalling factors, such as COI1, MYC2 and JAZ, at the protein level. In yeast two-hybrid assays SPL9 had no obvious interactions with COI1 and MYC2 (Fig. 4a), but a direct interaction was observed between SPL9 and a number of **JAZ** proteins, including **JAZ1**, **JAZ3**, **JAZ4**, **JAZ6**, **JAZ10** and **JAZ11**. **SPL2**, a homologue of SPL9, interacted with **JAZ1**, **JAZ4** and **JAZ9**. In contrast, **SPL3**, which belongs to a separate SPL group and harbours the SBP DNA-binding domain only44, did not bind to any of the **JAZ** proteins tested (Fig. 4b). Yeast two-hybrid and pull-down assays further demonstrated that **JAZ3** (**JAZ** without the Jas motif-containing C-terminal) interacted with SPL9, whereas removal of the ZIM domain-containing N-terminal of **JAZ3** (**JAZ3**N) abolished the binding activity (Fig. 4c,d and Supplementary Fig. 8).

**SPL9** promotes **JAZ3** accumulation. As plants grow, **SPL9** transcript abundance is gradually elevated as **miR156** levels decline20. To examine the change of **SPL9** protein abundance during plant age, we used **SPL9**:**GFP** (**SPL9** plants where a green fluorescent protein (GFP):**SPL9** fusion was expressed from its endogenous promoter). New leaves (#1–2, #2–4 and #5–6) of **SPL9**:**GFP** (**SPL9** plants or 35S:miR156 (35S:miR156) or **SPL9** (35S:**SPL9**) Plants (30-day-old in SD) were treated with 50 μM MeJA (+ JA) or ethanol (− JA) as control, and 4 h later the transcript levels in new leaves of the indicated plants were detected by qRT-PCR. The expression in the wild-type was set to 1. Data were analysed by multiple comparisons (Tukey test) followed by two-way ANOVA (*P<0.05, **P<0.01). Error bars represent ± s.d. (n = 3). ANOVA, analysis of variance.

**SPL9**r**SPL9-GR** plants became less sensitive to MeJA, as the degree of induction of all three **JAZ**-responsive genes tested was reduced (Fig. 3b). When the DEX treated plants were used for feeding assays, both **H. armigera** and **P. xylostella** larvae fed with the indicated plant leaves, both insects gained higher weight from the **SPL9**r**SPL9** plants. Leaves from the ~30-day-old plants in SD were used in feeding. Data are means ± s.d. (n = 25), asterisks indicate a significant difference from the wild-type (WT) group (Student’s t-test, *P<0.05, **P<0.01).** (c) **LOX2** and **VSP2** expressions in plants over-expressing miR156 (35S:miR156) or **SPL9** (35S:**SPL9**) Plants (30-day-old in SD) were treated with 50 μM MeJA (+ JA) or ethanol (− JA) as control, and 4 h later the transcript levels in new leaves of the indicated plants were detected by qRT-PCR. The expression in the wild-type was set to 1. Data were analysed by multiple comparisons (Tukey test) followed by two-way ANOVA (*P<0.05, **P<0.01). Error bars represent ± s.d. (n = 3). ANOVA, analysis of variance.

**Figure 2 | SPL9 negatively regulates plant resistance to insect and JA response.** (a,b) Weight increase of **H. armigera** and **P. xylostella** larvae fed with the indicated plant leaves, both insects gained higher weight from the **SPL9**:**SPL9** plants. Leaves from the ~30-day-old plants in SD were used in feeding. Data are means ± s.d. (n = 25), asterisks indicate a significant difference from the wild-type (WT) group (Student’s t-test, *P<0.05, **P<0.01).** (c) **LOX2** and **VSP2** expressions in plants over-expressing miR156 (35S:miR156) or **SPL9** (35S:**SPL9**) Plants (30-day-old in SD) were treated with 50 μM MeJA (+ JA) or ethanol (− JA) as control, and 4 h later the transcript levels in new leaves of the indicated plants were detected by qRT-PCR. The expression in the wild-type was set to 1. Data were analysed by multiple comparisons (Tukey test) followed by two-way ANOVA (*P<0.05, **P<0.01). Error bars represent ± s.d. (n = 3). ANOVA, analysis of variance.
**Figure 3 | Translocation of SPL9 into nucleus by DEX treatment dampens JA response.** The wild-type and SPL9::SPL9-GR plants (12D in LD) were first sprayed with ethanol (control) (a) or 10 μM DEX (b), and after 12 h the plants were treated with 50 μM MeJA (+ JA) or ethanol (− JA). Four hours later the transcript levels in the first pair of leaves were detected by qRT-PCR. The expression in the wild-type free from DEX and JA was set to 1. Data were analysed by multiple comparisons (Tukey test) followed by two-way ANOVA (*P < 0.05, **P < 0.01, ***P < 0.001). Error bars represent ± s.d. (n = 3). ANOVA, analysis of variance.

**Figure 4 | SPL proteins can interact with JAZ proteins.** Yeast two-hybrid assay. SPLs were fused to GAL4 DNA-binding domain (BD), MYC2, COI1, JAZ3, JAZ3ΔN and JAZ3ΔC was fused to GAL4 activation domain (AD), respectively. Interactions were examined with 10 mM (for SPL2 and SPL3) or 15 mM (for SPL9) 3-amino-1,2,4-triazole. Schematic diagrams of truncated versions of JAZ3 are shown in c, blue box indicates ZIM domain and yellow box indicates Jas domain. SPL9 and SPL2, but not SPL3, interacted with JAZs (a, b), and the N-terminal of JAZ3 was responsible for binding to SPL9 (c). (d) Pull-down assay of JAZ3-SPL9 binding. Recombinant HIS-SPL9 protein was incubated with total proteins of the tobacco leaf expressing either JAZ3ΔN-HA or JAZ3ΔC-HA driven by the 35S promoter. Anti-HA antibody was used to detect the truncated fusion proteins of JAZ3 before (Crude) or after (Pull-down) immunoprecipitation. KD, kilodalton.
Figure 5 | SPL9 promotes JAZ3 protein accumulation. (a) Images of wild-type and SPL9::GFP-SPL9 plants in LD at indicated days post germination (DPG). White arrows indicate new leaves (leaf #1–2, 3–4 and 5–6). (b–g) Protein levels of SPL9 and JAZ3 in new leaves. KD, kilodalton. (b) SPL9::GFP fusion protein in new leaves as described in a were detected by anti-GFP antibody, the protein level increased with plant age. (c–g) JAZ3::HA fusion protein became insensitive to the high level of GA 47. When the irreversible repression of MeJA-inducible DELLAs is achieved, GA then causes the plant to lose JA signalling activity, but the activity of GA-related functions remains intact. (f) JAZ3::HA level was increased in the SPL9::GFP background. The first pair of leaves from 35S::JAZ3::HA (JAZ3) and 35S::JAZ3::HA 35S::MIR156 (JAZ3 MIR156) plants were collected for analysis. (g) JAZ3::HA level was similar among leaves (leaf #1–2, 3–4 and 5–6) harvested from 35S::JAZ3::HA, 35S::JAZ3::HA and 35S::JAZ3::HA plants at the indicated DPG. The JAZ3::HA fusion protein, but not its truncated versions, exhibited the age-dependent accumulation. (d) JAZ3::HA level was decreased in the 35S::MIR156 background. The first pair of leaves from 35S::JAZ3::HA (JAZ3) and 35S::JAZ3::HA 35S::MIR156 (JAZ3 MIR156) plants were collected for analysis. (e) JAZ3::HA level was similar among leaves (leaf #1–2, 3–4 and 5–6) harvested from 35S::JAZ3::HA 35S::MIR156 plants at the indicated DPG. (f) JAZ3::HA level was increased in the SPL9::GFP-SPL9 background. The first pair of leaves from 35S::JAZ3::HA and 35S::JAZ3::HA 35S::MIR156 plants, which was uncoupled from SPL9.
protein level) or paclobutrazol (PAC, to block GA biosynthesis). Immunoblot assays revealed that neither GA nor PAC treatments blocked the JAZ3 protein accumulation in old plants (Fig. 6d). Together, these data suggest that altered GA levels do not reverse the progressive attenuation of JA response with plant age.

High levels of JAZ3 attenuate plant insect resistance. To analyse the role of JAZ3 in insect resistance, we chose two 35S:JAZ3-HA lines, among which 35S:JAZ3-HA-2 had a much higher level of JAZ3-HA than 35S:JAZ3-HA-1 (Supplementary Fig. 15a). As expected, JA response was inversely correlated to the level of JAZ3: the 35S:JAZ3-HA-2 plant exhibited less sensitivity to MeJA.
treatment than the wild-type and the 35S:JAZ3-HA-1 plants (Supplementary Fig. 15b,c). Insect feeding assays further showed that *H. armigera* larvae fed 35S:JAZ3-HA-2 leaves gained significantly more weight than those fed wild-type or 35S:JAZ3-HA-1 leaves (Supplementary Fig. 15d), indicating that elevated accumulation of JAZ3 protein reduced plant resistance to insects.

**Glucosinolate accumulation strengthens insect resistance.** The above results suggest that the miR156-SPL-JAZ module is responsible for the age-dependent decay of JA responsiveness. However, in feeding assays, old plants were more resistant to insects than young plants (Fig. 1b and Supplementary Fig. 1). There are of course many other factors than JA that affect plant defense. Plant secondary metabolites can act as phytoalexins against herbivores and pathogens, and in *Arabidopsis* GLSs serve as major defensive compounds to deter generalist insects. We then performed both gas-chromatography-mass spectrometry and liquid chromatography-MS (LC-MS) to analyse GLSs in leaf extracts. Among the seven classes of GLSs in the wild-type rosettes, 4-methylsulfinylbutyl glucosinolate (4MSOB) was the major component amounting to 50% of the total (Supplementary Fig. 16), consistent with a previous report. When the aerial parts were analysed, total GLSs were more abundant in old plants than in young plants (Supplementary Table 1). To minimize the impact of leaf conditions we used rapidly expanding leaves (Fig. 1a) to detect the GLSs, and the results were consistent: the content of total GLSs gradually increased with plant age and the difference in insect resistance between young and old plants was significant (Fig. 7a and Supplementary Table 2).

The cytochrome P450 monoxygenases CYP79B2 and CYP79B3 are key enzymes in the biosynthesis of indole related GLSs. In *cyp79b2 cyp79b3* double mutant the aliphatic GLS content was largely unaffected but the amount of indole related GLSs was greatly reduced (Supplementary Fig. 17a,b and Supplementary Table 3), as reported. However, probably because the indole-type GLSs constitute a very low proportion of total leaf GLSs, the *cyp79b2 cyp79b3* double mutant had only a mild effect on *H. armigera* larva growth (Supplementary Fig. 17c).

Two R2R3-MYB transcription factors, MYB28 and MYB29, were shown to regulate aliphatic GLS biosynthesis and in the *myb28 myb29* double mutant aliphatic GLS were barely detectable. We found that, despite a great reduction in this double mutant, the amount of residual GLSs still increased with age (Fig. 7a, Supplementary Table 2). Feeding assays showed that bollworm larvae grew much faster on *myb28 myb29* mutant leaves than on wild-type leaves, consistent with the report that generalist herbivores are typically sensitive to GLSs. For the parts were analysed, total GLSs were more abundant in old plants than old wild-type leaves, consistent with the report that GLSs constitute a very low proportion of total leaf GLSs, the *cyp79b2 cyp79b3* double mutant had only a mild effect on *H. armigera* larva growth (Supplementary Fig. 17c).

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**Discussion**

Plants encounter attacks from varied populations of insect herbivores at different developmental stages. In this investigation, we report an age-related temporal change of JA response, which is highly active in the early stage of plant life and declines along with plant growth. In contrast to this tendency, defense compounds like GLSs accumulate cumulatively, contributing to the insect resistance in adult plants (Fig. 8a). Fast turn-over of JAZ proteins holds the key to JA signal output and our data suggests that SPL9 can stabilize JAZ3, possibly through protein–protein interaction. We propose that the increased amount of SPL proteins in adult plants results in elevated accumulation of JAZ proteins, which in turn dampen JA responses (Fig. 8b). In human the miR181-DUSP6 module is responsible for desensitization of T cell receptor-triggered immune signalling cascade. Our finding that the miR156-targeted SPLs regulate age-dependent decline of JA-induced defense in plant resembles this miR156-a/6 (DUSP6)-mediated immunosenescence in human.

We found that DELLA proteins, which repress GA signalling, do not impair the general tendency of the age-related JA response.
deterioration. However, DELLAs do interact with JAZ and this interaction prevents JAZ from suppression of JA-responsive genes. Here we demonstrate that SPL9 stabilizes JAZ, likely by preventing degradation through the COI1-mediated pathway which we propose results in higher JAZ activities that repress JA responses.

Figure 7 | Increased accumulation of GLSs in old plant enhances insect resistance. GLSs content was detected by LC-MS (a,c,f,h). Error bars represent ± s.d. (n = 3). (a) GLSs content in the wild-type (Col-0) and myb28 myb29. Rapidly expanding leaves of 16-, 20- and 24-day-old plants in LD were used. Asterisk indicates significant difference from the 16-day-old plant (Student’s t-test, *P < 0.05). (b) Weight increase of H. armigera larvae fed with leaves described in a. Error bars represent ± s.d. (n = 25). asterisk indicates significant difference from the 16D group (Student’s t-test, *P < 0.05). (c) GLSs content in rapidly expanding leaves of 20D (20D) or 40-day-old (40D) plants of different genotypes in SD. Asterisk indicates significant difference from the 20D group (Student’s t-test, *P < 0.05). (d) Weight increase of H. armigera larvae fed with leaves described in c. Error bars represent ± s.d. (n = 25), asterisk indicates significant difference from the 20D group (Student’s t-test, *P < 0.05, **P < 0.01). (e) Negative relation between GLSs content and larval growth. X axis represents GLSs content in the plant leaves as in c and y axis indicates larval weight increase in d. Weight increase of H. armigera larvae fed with leaves of 20-day-old plant in SD of the wild type (WT), 35S::MIR156, SPL9::rSPL9 and coi1-2 shown as red, purple, blue and yellow hollow triangles, respectively, and those with 40-day-old plant leaves as the respective solid triangles. (f) GLSs decline in leaves during senescence. The first two leaves at vigorous stage (Vigour) from the 14-day-old plants or at senescent (Senescence) stage from the 28-day-old plants in LD were used. Asterisk indicates significant difference from vigour stage (Student’s t-test, *P < 0.05). (g,h) Possible mobilization of GLSs from the early leaves to new leaves. The first to fifth leaves from the 20-day-old plant in LD were removed or wounded, four days later the rest four leaves from the plant (cut), the same set of leaves from wounded plants (wounded) and the intact plant (intact) (g) were harvested for GLSs detection (h). White arrows indicate leaves collected for analysis and red triangles refer to wounded leaves. Asterisk indicates significant difference (Student’s t-test, *P < 0.05, **P < 0.01).
We propose that in the early stages after germination, when plants have little biomass and potentially an insufficient amount of defense compounds, the active JA response is required to endure or resist herbivores and possibly other pathogens. As defense metabolites accumulate with age, they may provide a higher level of basal or constitutive resistance, and thus may alleviate the burden on JA-mediated active defense. While providing plant with high resistance to herbivores and certain types of pathogens, JA induces plant growth by interfering with auxin and GA signals. The age-dependent decay of JA signal could be a strategy of plant to ensure successful development, in which the SPL proteins contribute to the balance between defense and the growth.

Various secondary metabolites act as defense compounds to protect plants from herbivores and pathogens. Interestingly, both induced and constitutive defenses may utilize the same defense compounds. For example, gossypol and related sesquiterpene aldehydes are constitutively synthesized and stored in cotton plants, but their production is also induced by elicitation. Although JA did not affect the tendency of stored GLSs in cotton plants, but their production is also induced by sesquiterpene aldehydes are constitutively synthesized and both induced and constitutive defenses may utilize the same defense. Finally, although GLSs play a predominant role in the active JA response, they also play a role in promoting the biosynthesis of GLSs, as shown here (Fig. 7c) and reported previously.

Remobilization of nutrients such as potassium, phosphorus and nitrogen from leaf to leaf during the vegetative stage or from leaves to seeds during the reproductive stage is common in plants. Besides nutrients, plants also redistribute secondary compounds. In Arabidopsis GLSs can be transported from leaf to seed, and the nitrate transporters of GTR1 and GTR2 are responsible for this translocation. In this investigation, the possibility of GLSs translocation from senescent to new leaves reflects a conceivable strategy of plant to save energy through re-use of previously synthesized defense metabolites (rather than discard them being discarded as waste). Export of substances from senescent leaves may contribute to plant constitutive defense. Finally, although GLSs play a predominant role in defense against generalist herbivores in Arabidopsis, the resistance to P. xylostella, a specialist insensitive to GLSs, still increased with plant age. Besides GLSs, changes of other secondary metabolites, nutritional components and structural or mechanical factors may also augment insect resistance and compensate for decayed JA response in adult plant, which deserve further investigation.

**Methods**

**Plant materials and treatments.** Plants of Arabidopsis thaliana (ecotype Col-0 or Ler-0) were grown at 22 °C in long-day (LD, 16 h light/8 h dark) or short-day (SD, 8 h light/16 h dark) conditions, as indicated. 35S:MIR156, 35S:MIM156, 35S:MIR9, 35S:MIR9-GFP, 35S:MIR9-C0, SPL9:rSPL9-GR, 35S:MIM156, 35S:MIR9, and SPL9 were cloned into pCAMBIA1300. For overexpression of miR156 and SPL9, the coding regions of miR156 and SPL9 were fused to the 35S promoter in and PAC treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis. For GA3 and MeJA treatments, samples were collected at an indicated time for analysis.
(1 unit per ml; Fermentas) and used for preparing the first strand cDNA (Invi-
tron). qRT-PCR was performed and S18 ([AAtg99800]) was used as internal control. Biological replicates with technical duplicates (2–3 biological, 10–20 technical) were used to test the significance of the effects. Nucleotide primers used in this investigation are listed in Supplementary Table 7.

**Yeast two- and three-hybrid assay.** For yeast two-hybrid assay, a series of SPLs was introduced into the pGBK7 (Clontech), as described^{2}, and COI1, JAZ1, JAZ2, JAZ4, JAZ6, JAZ7; JAZ9, JAZ10, JAZ11, JAZ12 and JAZ3 (JAZ, JAZ3C and JAZ3N) were introduced into pGADT7 (Clontech), respectively. The MYC2/ pGADT7 construct was as described^{34}. Plasmids were transferred into yeast strain AH109 (Clontech) by the LiCl-polyethylene glycol method. Transformants were selected on SD-Leu-Trp plates. The interactions were tested on SD-Leu-Trp-His or SD-Aden-Leu-Trp-His plates with 3-amino-1,2,4-triazole, incubating for 3–4 days at 30 °C. At least 10 individual clones were analysed. For yeast three-hybrid assay, JAZ3 and SPL9 were inserted into pBAG1 (Clontech), forming a JAZ3-SPL9/ pBAG1 construct. The yeast strain AH109 was co-transformed with a JAZ5-SPL9/ pBAG1 and a COI1/pGADT7 construct and plated on SD-Leu-Trp selective dropout medium. Colonies were transferred to the appropriate selective dropout liquid medium (SD-Leu-Trp-His) with 40 μM coronatine (Sigma-Aldrich) and different concentrations of methionine. SPL9 expression from the pBAG1 construct was controlled by the Pgal4,5 promoter, and the SPL9 level was decreasing along with the increasing concentrations of Met.

**Pull-down and immunoblot analyses.** Proteins were extracted from leaves by an extraction buffer (50 mM MHEPES, 10 mM EDTA, 50 mM NaCl, 10% glycerol, 1% polyvinylpolypyrrolidone, 2 mM DTT, 1 mM phenylmethanesulfonyl fluoride, 10 mM MG-132, and 1× protease inhibitor cocktail, pH 7.5). Immuneblotting was performed by loading proteins onto a 10% SDS–polyacrylamide gel electrophoresis gel (80 μg proteins per lane). After electrophoresis, the proteins were electro-

**Glucosinolates extraction and detection.** GLS were analysed as described^{43-49}. Plant materials, immediately after harvest, were frozen in liquid nitrogen and preserved at –80 °C. In 10 mg of fresh weight (FW) dry matter, 0.75 mL of 80% methanol (3 mL containing 0.05–0.1 μM of an internal standard (sinigrin)) and incubated under 60 °C for 1 h at shaking. Samples were then cooled to 4 °C and centrifuged at 4000 g. The supernatant was loaded onto an anion-exchange column (DEAE-Sephadex A-25). After washing with 4 mL sodium acetate (20 mM) the GLS was capped and treated overnight with 10 μL of arylsulfatase (Helix Pomatia Type H-1, Sigma) to convert the GLS to their desulfated derivatives, which were eluted from the column with double-distilled water.

Samples were separated on a 6120 Quadrupole LC-MS system (Agilent) fitted with a 150 μm × 2 mm reversed-phase column (synchros RP-18, 250 × 4.6 mm i.d. 5 μm particle size, Thermo Scientific), using a water (Solvent A)-acetonitrile (Solvent B) gradient at a flow rate of 1 mL min \(^{-1}\). The 32 min run at ambient room temperature consisted of 1.5% B (1 min), 1.5–5.0% B (5 min), 5.0–7.0% B (2 min), 7.0–21.0% B (10 min), 21.0–29.0% B (5 min), 29.0–43.0% B (7 min), 43.0–93.0% B (0.5 min), a 4-min hold at 93.0%, 93.0–1.5% B (0.5 min) and a 7-min hold at 1.5% B. The following parameters were used to obtain positive ionisation: dry gas flow at 12.0 mL min \(^{-1}\), spray voltage 4000 V, drying gas temperature 350 °C, mass range 50–1500, fragmentor 70, threshold 150. Eluent was monitored by diode array detection between 190 and 600 nm (2 nm interval). Desulfated GLS were identified by comparison of retention time and MS data to those previously reported, and quantified by A229 nm relative to the internal standard.

**Data availability.** The authors declare that all data supporting the findings of this study are available within the article and its Supplementary Information files or are available from the corresponding author upon request.

**References.**

1. Gatehouse, J. A. Plant resistance towards insect herbivores: a dynamic interaction. *New Phytol.* 156, 145–169 (2002).
2. Manzaneda, A. J., Prasad, K. V. & Mitchell-Olds, T. Variation and fitness costs for tolerance to different types of herbivore damage in *Boccea stricta* genotypes with contrasting glucosinolate structures. *New Phytol.* 188, 464–477 (2010).
3. Koo, A. J. & Howe, G. A. The wound hormone jasmonate. *Phytochemistry* 70, 1571–1580 (2009).
4. Lorenzo, O., Chico, J. M., Sanchez-Serrano, J. I. & Solano, R. JASMONATE-INSENSITIVE1 encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in Arabidopsis. *Plant Cell* 16, 1938–1950 (2004).
5. Chin, A. et al. The JAZ family of repressors is the missing link in jasmonate signalling. *Nature* 448, 666–664 (2007).
6. Thines, B. et al. JAZ repressor proteins are targets of the SCF(CO1) complex during jasmonate signalling. *Nature* 448, 661–665 (2007).
7. Yan, J. et al. The Arabidopsis CORONATINE INSENSITIVE1 protein is a jasmonate receptor. *Plant Cell* 21, 2220–2236 (2009).
8. Katsir, L., Schmiller, A. L., Stawick, P. E., He, S. Y. & Howe, G. A. COI1 is a critical component of a receptor for jasmonate and the bacterial virulence factor coronatine. *Proc. Natl Acad. Sci. USA* 105, 7100–7105 (2008).
9. Withers, J. et al. Transcription factor-dependent nuclear localization of a transcriptional repressor in jasmonate hormone signaling. *Proc. Natl Acad. Sci. USA* 109, 20148–20153 (2012).
10. Sheard, L. B. et al. Jasmonate perception by inositol-phosphate-potentiated COI1-JAZ co-receptor. *Nature* 468, 400–405 (2010).
11. Melotto, M. et al. A critical role of two positively charged amino acids in the Jas motif of Arabidopsis JAZ proteins in mediating coronatine- and jasmonoyl isoleucine-dependent interactions with the COI1 F-box protein. *Plant J.* 55, 979–988 (2008).
12. Chung, H. S. et al. Regulation and function of Arabidopsis JASMONATE ZIM-domain genes in response to wounding and herbivory. *Plant Physiol.* 146, 952–964 (2008).
13. Zhai, Q. et al. Phosphorylation-coupled proteolysis of the transcription factor MYC2 is important for jasmonate-signaled plant immunity. *PLoS Genet.* 9, e1003422 (2013).
14. Chico, J. M. et al. Repression of Jasmonate-dependent defenses by shade involves differential regulation of protein stability of MYC transcription factors and their JAZ repressors in Arabidopsis. *Plant Cell* 26, 1967–1980 (2014).
15. Huot, B., Yao, J., Montgomery, B. L. & He, S. Y. Growth-defense tradeoffs in plants: a balancing act to optimize fitness. *Mol. Plant* 7, 1267–1287 (2014).
16. Goronyz, J. I. & Weyand, C. M. Understanding immunosuppression to improve responses to vaccines. *Nat. Immunol.* 14, 428–436 (2013).
17. Kus, J. V., Zaton, K., Sarkar, R. & Cameron, R. K. Age-related resistance in Arabidopsis is a developmentally regulated defense response to Pseudomonas syringae. *Plant Cell* 14, 479–490 (2002).
18. Price, P. W. The plant vigor hypothesis and herbivore attack. *Oikos* 62, 244–251 (1991).
19. Wu, G. & Poethig, R. S. Temporal regulation of shoot development in *Arabidopsis thaliana* by miR156 and its target SPL3. Development 133, 3539–3547 (2006).
20. Wang, J. W., Czech, B. & Weigel, D. miR156-regulated SPL transcription factors define an endogenous flowering pathway in *Arabidopsis thaliana*. *Cell* 138, 738–749 (2009).
21. Yamaguchi, A. et al. The microRNA-regulated SBP-Box transcription factor SPL5 is a direct upstream activator of LEAFY, FRUITFULL, and APETALA1. *Dev. Cell* 17, 268–278 (2009).
22. Gou, J. Y., Filipjes, F. F., Liu, C. J., Weigel, D. & Wang, J. W. Negative regulation of anthocyanin biosynthesis in *Arabidopsis* by a miR156-targeted SPL transcription factor. *Plant Cell* 23, 1512–1522 (2011).
23. Yu, Z. X. et al. Progressive regulation of sesquiterpene biosynthesis in *Arabidopsis* and *Patchouli* (Pogostemon cablin) by the miR156-targeted SPL transcription factors. *Mol. Plant* 8, 98–110 (2015).
24. Wu, G. et al. The sequential action of miR156 and miR172 regulates developmental timing in *Arabidopsis*. *Cell* 138, 750–759 (2009).
25. Xue, X. Y. et al. Interaction between two timing microRNAs controls trichome distribution in *Arabidopsis*. *PloS Genet.* 10, e1004266 (2014).
26. Yu, N. et al. Temporal control of trichome distribution by microRNA156-targeted SPL genes in *Arabidopsis thaliana*. *Plant Cell* 22, 2323–2333 (2010).
27. Bergonzini, S. et al. Mechanisms of age-dependent response to winter temperature in perennial flowering of *Arabis alpina*. *Science* 340, 1094–1097 (2013).
28. Zhang, H. C. M. et al. Molecular basis of age-dependent vernalization in *Cardamine flexuosa*. *Science* 340, 1097–1100 (2013).
29. Zhang, T. Q. et al. An intrinsic microRNA timer regulates progressive decline in shoot regenerative capacity in plants. *Plant Cell* 27, 349–360 (2015).
30. Sun, T. P. The molecular mechanism and evolution of the GA-GID1-DELLA signaling module in plants. *Curr. Biol.* 21, R338–R345 (2011).
31. Shan, C. M. et al. Control of cotton fibre elongation by a homeodomain transcription factor GhHOX3. *Nat. Commun.* 5, 5519 (2014).
32. Yu, S. et al. Giberellin regulates the *Arabidopsis* floral transition through miR156-targeted SQUAMOSA PROMOTER BINDING-LIKE transcription factors. *Plant Cell* 24, 3320–3332 (2012).
33. Hou, X., Lee, L. Y., Xia, K., Yan, Y. & Yu, H. DELLAs modulate jasmonate signaling via competitive binding to JAZs. *Dev. Cell* 19, 884–894 (2010).
34. Hong, G. J., Xue, X. Y., Mao, Y. B., Wang, L. J. & Chen, X. Y. *Arabidopsis MYC2* interacts with DELLAs proteins in regulating sesquiterpene synthase gene expression. *Plant Cell* 24, 2635–2648 (2012).
35. Mao, Y. B. et al. Silencing a cotton bollworm *P450* monoxygenase gene by plant-mediated RNAi impairs larval tolerance of *Gossypol*. *Nat. Biotechnol.* 25, 1307–1313 (2007).
36. Tao, X. Y., Xue, X. Y., Huang, Y. P., Chen, X. Y. & Mao, Y. B. Gossypol-enhanced *P450* gene pool contributes to cotton bollworm tolerance to a pyrethroid insecticide. *Mol. Ecol.* 21, 4371–4385 (2012).
37. Barker, J., Poppy, G. & Payne, C. The sequential action of miR156 and miR172 regulates developmental timing in *Arabidopsis*. *Plant J.* 33, 3355–3352 (2012).
38. Li, G. et al. Decline in miR-181a expression with age impairs T cell receptor sensitivity by increasing DUSP6 activity. *Nat. Med.* 18, 1518–1524 (2012).
39. Chen, S. et al. *CYP79F1* and *CYP79F2* have distinct functions in the biosynthesis of aliphatic glucosinolates in *Arabidopsis*. *Plant J.* 33, 923–937 (2003).
40. Schweizer, F. et al. *Arabidopsis* basic helix-loop-helix transcription factors MYC2, MYC3, and MYC4 regulate glucosinolate biosynthesis, insect performance, and feeding behavior. *Plant Cell* 25, 3117–3132 (2013).
41. Bak, S. & Feyereisen, R. The involvement of two P450 enzymes, CYP38B1 and CYP38A1, in auxin homeostasis and glucosinolate biosynthesis. *Plant Physiol.* 127, 108–118 (2001).
42. Shin, C. et al. *CYP79F1* and *CYP79F2* have distinct functions in the biosynthesis of aliphatic glucosinolates in *Arabidopsis*. *Plant J.* 33, 923–937 (2003).
43. Hult, A. K., Vrij, R. & Celenza, J. L. *Arabidopsis* cytochrome P450s that catalyze the first step of tryptophan-dependent indole-3-acetic acid biosynthesis. *Proc. Natl Acad. Sci. USA* 97, 2379–2384 (2000).
44. Nour-Eldin, H. H. et al. NRT1/PTR transporters are essential for translocation of glucosinolate defence compounds to seeds. *Nature* 488, 531–534 (2012).
45. Li, G. et al. Decline in miR-181a expression with age impairs T cell receptor sensitivity by increasing DUSP6 activity. *Nat. Med.* 18, 1518–1524 (2012).
46. Chen, Q. et al. The basic helix-loop-helix transcription factor MYC2 directly represses PLETHORA expression during jasmonate-mediated modulation of the root stem cell niche in *Arabidopsis*. *Plant Physiol.* 163, 3355–3352 (2014).
47. Wittstock, U. & Gershenzon, J. Constitutive plant toxins and their role in defense against herbivores and pathogens. *Curr. Opin. Plant Biol.* 5, 300–307 (2002).
48. Liu, C., Steinbeck, P. & Chen, X. Y. Expression pattern of genes encoding farnesyl diphosphate synthase and sesquiterpene cyclase in cotton suspension-cultured cells treated with fungal elicitors. *Mol. Plant Microbe Interact.* 12, 1095–1104 (1999).
49. Badenes-Perez, F. R., Reichelt, M., Gershenzon, J. & Heckel, D. G. Interaction of glucosinolate content of *Arabidopsis thaliana* mutant lines and feeding and oviposition by generalist and specialist lepidopterans. *Phytochemistry* 86, 36–43 (2013).
50. Schultz, S., Munier-Jolain, N., Jeudy, C., Burstin, J. & Salon, C. Dynamics of exogenous nitrogen partitioning and nitrogen remobilization from vegetative organs in pea revealed by 15N in vivo labeling throughout seed filling. *Plant Physiol.* 137, 1463–1473 (2005).
51. Schwaab, R. et al. Specific effects of microRNAs on the plant transcriptome. *Dev. Cell* 8, 517–527 (2005).
52. Wang, J. W., Schwab, R., Czech, B., Mica, E. & Weigel, D. Dual effects of miR156-targeted SPL genes and CYP79B1/CLH1 on plastochron length and organ size in *Arabidopsis thaliana*. *Plant Cell* 20, 1231–1243 (2008).
53. Xu, L. et al. The SCF(CO11) ubiquitin-ligase complexes are required for jasmonate response in *Arabidopsis*. *Plant Cell* 14, 1919–1935 (2002).
54. Yang, D. H., Hetttenhausen, I. T., Baldwin, I. T. & Wu, J. Silencing *Nicotiana attenuata* calcium-dependent protein kinases, CDPK4 and CDPK5, strongly up-regulates wound- and herbivory-induced jasmonic acid accumulations. *Plant Physiol.* 159, 1591–1607 (2012).

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Author contributions
Y.-B.M. and X.-Y.C. designed the research and wrote the article. Y.-B.M. performed most of the experiments. Y.-Q.L., D.-Y.C., F.-Y.C. and G.-J.H. performed part of the experiments. L.-J.W. and X.F. assisted in LC-MS analysis. J.-W.W. assisted in data analysis.

Additional information
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