The differential response of cold-experienced Arabidopsis thaliana to larval herbivory benefits an insect generalist, but not a specialist

Jana Oberländer1,2, Vivien Lortzing3, Monika Hilker3 and Reinhard Kunze1*

Abstract

Background: In native environments plants frequently experience simultaneous or sequential unfavourable abiotic and biotic stresses. The plant’s response to combined stresses is usually not the sum of the individual responses. Here we investigated the impact of cold on plant defense against subsequent herbivory by a generalist and specialist insect.

Results: We determined transcriptional responses of Arabidopsis thaliana to low temperature stress (4 °C) and subsequent larval feeding damage by the lepidopteran herbivores Mamestra brassicae (generalist), Pieris brassicae (specialist) or artificial wounding. Furthermore, we compared the performance of larvae feeding upon cold-experienced or untreated plants. Prior experience of cold strongly affected the plant’s transcriptional anti-herbivore and wounding response. Feeding by P. brassicae, M. brassicae and artificial wounding induced transcriptional changes of 1975, 1695, and 2239 genes, respectively. Of these, 125, 360, and 681 genes were differentially regulated when cold preceded the tissue damage. Overall, prior experience of cold mostly reduced the transcriptional response of genes to damage. The percentage of damage-responsive genes, which showed attenuated transcriptional regulation when cold preceded the tissue damage, was highest in M. brassicae damaged plants (98%), intermediate in artificially damaged plants (89%), and lowest in P. brassicae damaged plants (69%). Consistently, the generalist M. brassicae performed better on cold-treated than on untreated plants, whereas the performance of the specialist P. brassicae did not differ.

Conclusions: The transcriptional defense response of Arabidopsis leaves to feeding by herbivorous insects and artificial wounding is attenuated by a prior exposure of the plant to cold. This attenuation correlates with improved performance of the generalist herbivore M. brassicae, but not the specialist P. brassicae, a herbivore of the same feeding guild.

Keywords: Plant stress response, Herbivore, Defense, Larval feeding, Pieris brassicae, Mamestra brassicae

Background

Plants have evolved a plethora of mechanisms to cope with abiotic or biotic environmental stress (e.g. [1–4]). Attack by herbivorous insects is a major threat for plants as it can lead to rapid loss of leaf material and thus reduced photosynthetic capacity, often causing severe yield and fitness loss [5–7].

Plant defense responses induced by herbivore attack represent a strategy, which is mobilized only on demand [8, 9]. Inducible defense responses are associated with transcriptional regulation of many genes and shifts in phytohormone levels. Intensively studied key regulators of wounding and herbivore defense responses are the phytohormones jasmonic acid (JA), abscisic acid (ABA), salicylic acid (SA) and ethylene (ET), which are the backbone of the plant immune signaling network [10–15]. Fine-tuning of defense responses to different herbivores is achieved by crosstalk of these signaling pathways and
may involve additional plant hormonal regulators like auxins, gibberellins, and brassinosteroids [13, 16].

In natural environments, plants are frequently exposed to simultaneously or consecutively occurring environmental stresses. Combined stresses typically provoke distinct transcriptome reprogramming and plant reactions, which are not simply due to additive effects of the single stresses [17–23]. In case of consecutively occurring environmental stress, plants can “memorize” a past stressful event and benefit from this memory by preparing themselves for a more effective response to upcoming stress. This process has been termed “priming” of a stress response by a past stress experience (reviewed in [24–27]).

Studies on priming of plant responses to insect herbivory especially focused on herbivore-related priming factors, which reliably indicate future herbivory [28]. For example, plant volatiles induced by herbivory and perceived by as yet undamaged plant tissue have been shown to serve as a reliable factor in preparing a plant for improved anti-herbivore defense [29–32]. Furthermore, insect egg depositions on leaves that indicate an upcoming larval herbivory have been shown to prepare a plant for more effective defense against the hatching larvae [30]. Previous exposure of plants to herbivory-induced volatiles or to insect egg depositions are known to alter the transcriptional response to herbivory [33–39].

So far, only a few recent studies addressed the influence of an herbivory-unrelated, abiotic stress – especially drought – on the plant’s response to subsequent herbivory by including transcriptional and/or metabolic analysis (e.g. [40–42]). However, stressful conditions such as cold often precede plant attack by herbivorous insects, which usually need warm temperatures for their activities. A study by Firtzlaff et al. [43] examined how exposure of Arabidopsis thaliana to mild cold affects plant defense against later herbivory by the specialist Pieris brassicae. The study showed that a significant subset of cold-regulated genes maintained altered transcript levels even after 1 day of deacclimation. Larval feeding, which started 1 day after deacclimation, induced a different transcriptome in the previously cold-exposed than in previously untreated plants and showed a weakened response of defense genes. However, larval performance of the specialist P. brassicae was similar on cold-experienced and untreated plants [43]. These findings are in accordance with some other studies, which also revealed that host plants with attenuated plant defense capacity did not affect the extent of feeding damage inflicted by a specialized herbivorous insect [44] nor the herbivore’s performance [45].

Generalist and specialist herbivorous insects are known to exhibit different tolerances to plant defenses [46]. However, it is unknown as yet whether they are differentially affected by changes in plant defense that are due to prior exposure of plants to abiotic stress. Here we addressed the questions of whether a generalist insect herbivore shows different sensitivity to cold-mediated changes of feeding-induced host plant defense than a specialist, and if so, which transcriptional differences between cold-treated plants fed on by a generalist or a specialist insect may explain these ecological effects. As in our previous study [43], we used the butterflies P. brassicae and Mamestra brassicae and the Brassicaceae A. thaliana as host plant. Pieris brassicae is specialized on glucosinolate-containing host plants [47], mostly from the Brassicaceae family. Like other Pieridae species it possesses highly specific enzymes for detoxification of the glucosinolates [48–50], which are typical secondary metabolites of the Brassicaceae. As generalist, we studied Mamestra brassicae, a moth whose larvae are polyphagous on over 70 plant species in 22 plant families, but exhibit a preference for Brassica crops [51]. In contrast to P. brassicae, M. brassicae detoxifies glucosinolates by general oxidizing enzymes (reviewed by [52]). Both lepidopteran species are active in Europe from early spring to late autumn [53, 54]. They may produce two to three generations per season until they hibernate in the soil as pupae. In the natural habitats of M. brassicae and P. brassicae, which largely overlap with that of A. thaliana (GBIF Secretariat: GBIF Backbone Taxonomy. Accessed via www.gbif.org/species/1920506 and www.gbif.org/species/3052436 on 01 June 2019), in spring and in autumn a succession of cold days followed by a warm period is common.

In a first approach, we compared performance of M. brassicae and P. brassicae on A. thaliana plants previously exposed to mild cold. We found that M. brassicae showed improved performance on cold-experienced plants, whereas P. brassicae larval performance was the same on cold-experienced and control plants, thus confirming our previous results with this latter species [43]. To elucidate the transcriptional basis of these different ecological effects, we compared the transcriptomes of cold-experienced plants exposed to feeding by the specialist, the generalist or to artificial wounding. Including the artificial wounding treatment allowed disentangling insect species-specific effects from wounding effects on the cold stress-reprogrammed plant transcriptome. We found that transcriptional responses of previously cold-exposed plants to specialist feeding, generalist feeding and artificial wounding differed.

Prior cold experience led to differential regulation of 360 M. brassicae feeding damage-responsive genes. In 98% of these the transcriptional response to feeding damage was attenuated. In contrast, the respective fraction of genes was smaller in artificially wounded (681 genes, 84% with attenuated response) and in P. brassicae
feeding-damaged plants (125 genes, 69% with attenuated response). These transcriptional changes in conjunction with the larval performance data indicate that the generalist benefits from the cold-mediated attenuation of feeding-induced gene de-regulation, whereas the specialist does not.

Results
Generalist and specialist herbivores show different performances on cold-stressed and control plants
We exposed *A. thaliana* plants to cold (4 °C) for 5 days. After a deacclimation phase (20 °C) of 1 day, larvae of the generalist *M. brassicae* and the specialist *P. brassicae* were allowed feeding upon the previously cold-treated plants or on control plants. The weight of these larvae on previously cold-treated (Fig. 1: P + T P and P + T M) and untreated (Fig. 1: T P and T M) plants and the extent of leaf damage inflicted by the larvae were compared.

Weight gain and total weight of *P. brassicae* larvae, their leaf area consumption (Fig. 2) and the relative growth rate (RGR) of the larvae (Additional file 1: Figure S1) did not differ on previously cold-treated compared to untreated plants. In contrast, on previously cold-treated plants *M. brassicae* larvae consumed more leaf tissue, gained more weight and were heavier on these plants after a four- and six-day-feeding period than on untreated plants (Fig. 2). Accordingly, the RGR of the larvae was higher on cold-treated plants (Additional file 1: Figure S1). This observation suggests that the cold treatment alters either the metabolic status of the plants or their physiological reaction to leaf tissue damage in a way that is beneficial for the larval development of the generalist herbivore *M. brassicae*, but without consequences for the development of the specialist *P. brassicae*.

Transcriptional response of *Arabidopsis* to feeding damage and artificial wounding
To investigate whether *Arabidopsis* plants respond differently to leaf damage by *P. brassicae* and *M. brassicae* larvae and to artificial wounding, we analyzed the transcriptomes in leaves from plants grown at 20 °C (Fig. 1, samples T P, T M, T W and C2). A Principal Component Analysis (PCA) based on gene expression values of the differently treated plants revealed that the patterns of plants exposed to *P. brassicae* feeding, *M. brassicae* feeding and artificial wounding were clearly separated from untreated control samples. However, the patterns of the treated samples partially overlapped with each other, indicating that expression of a fraction of genes is similarly regulated in the treated samples (Fig. 3a).

Overall, 1975, 1695, and 2239 differentially expressed genes (DEGs) were identified that showed ≥2-fold expression change after 2 days feeding damage by *P. brassicae* (T P vs C2), *M. brassicae* (T M vs C2) or wounding (T W vs C2), respectively (Fig. 3b, Additional file 2: Table S1). As the majority of these genes responded qualitatively and quantitatively similarly to the three damage types (Additional file 1: Figure S2), the magnitude of the plant's transcriptional response to herbivory or artificial wounding was similar. However, feeding damage by the generalist *M. brassicae* resulted in a larger fraction of upregulated genes (62% of 1695 genes in T M vs C2; Fig. 3b) than by the specialist *P. brassicae* (43% of 1975 genes in T P vs C2, Fig. 3b). In total, 507 DEGs were regulated in all three sample types (central intersection in Fig. 3b). 176 DEGs specifically responded to larval feeding by either species but not to artificial wounding (Fig. 3b; intersection of T P vs C2 and T M vs C2 but not T W vs C2), and 639, 700 and 767 genes were uniquely regulated upon *P. brassicae* feeding, *M. brassicae* feeding and artificial wounding, respectively. In the intersections, almost all DEGs (94–99%) were regulated in the same direction (Fig. 3b).

To disentangle common and unique regulated processes, an enrichment analysis of biological process-Gene Ontology (GO) terms was conducted (Fig. 3d).
Among the 255 genes downregulated by all three damage types (Fig. 3b; central intersection), 13 GO terms were significantly enriched, which associate predominantly with responses to light, transcription, and growth. Among the 245 commonly upregulated genes, 28 GO terms were enriched, including several defense-related processes, such as response to and regulation of jasmonic acid, glucosinolate metabolism and response to insects, herbivores, bacteria, and fungi. These defense-related processes include many well described wounding- and feeding-responsive genes, i.e. JAZ10, VSP1, VSP2, LOX2, CYP79B2, CYP79B3, IGMT1.

Among the DEGs that were specifically responding to *P. brassicae*-feeding, two GO terms associated with abiotic stress were enriched in the 265 upregulated genes (“response to ABA”, “response to water deprivation”) and four GO terms were enriched in the downregulated genes, including the biological process “response to salicylic acid”.

Many *M. brassicae* feeding-specific upregulated genes fall into GO terms related to transcription and defense, including processes like “DNA replication initiation”, “response to jasmonic acid” and “response to salicylic acid”, whereas the six GO terms overrepresented among *M. brassicae*-specific downregulated genes are associated with development and growth.

Artificial wounding-specific responses were overall more generic. Out of 401 upregulated genes only one GO term (“secondary metabolic process”) consisting of 19 genes was weakly enriched. The eight GO terms associated with downregulated genes ranged from protein folding to “defense response to bacterium” to “response to abiotic stress”.

Feeding by *P. brassicae* evoked only a weak upregulation of two indole-glucosinolate biosynthesis genes, CYP79B2 and CYP79B3, the indole-glucosinolate O-methyltransferases IGMT1 and IGMT5 [55, 56] and the nitrile specifier gene NSP3 (Additional file 2: Table S1). Feeding by *M. brassicae* induced a stronger and more complex transcriptional response in the glucosinolate pathway. In addition to the *P. brassicae*-induced genes, MYBS1, NSPI, CYP81F2, CYP81F4 and IGMT2 were upregulated. Yet, the strongest effects on the glucosinolate system, upregulation of indole-glucosinolate synthesis
genes and nitrile specifier protein genes and downregulation of aliphatic glucosinolate synthesis genes, was observed upon artificial wounding of the leaf.

In total, 1648 genes were responsive to at least two damage types. The vast majority of these genes were regulated in the same direction, only 29 genes were regulated in opposite directions (intersections in Fig. 3b). The genes with highest regulation differences (15- to 21-fold difference) between at least two treatments were ARGAH2, IAA29, SLAH3, DREB26, and PXMT1 (Fig. 3c). ARGAH2, one of two arginase proteins known in Arabidopsis, is involved in defense responses, as its expression is inducible by methyl jasmonate treatment [57]; this gene was clearly downregulated only by P. brassicae feeding, but not by M. brassicae damage nor by artificial wounding.

JA is a major signaling molecule involved in response to wounding and defense against chewing herbivores [58–60]. Concordantly, artificially wounded leaves showed upregulation of most of the genes involved in JA biosynthesis (i.e. LOX2, AOS, AOC1 to AOC4, OPR3), JA homeostasis and turnover (i.e. JAZ2, JAZ9, JAZ10, IAR3, ILC6, CYP94B3) [61–64] and JA signaling (i.e. VSP1, VSP2) [58]. The JA-responsive defensin PDF1.2a [65] was upregulated as well in artificially wounded leaves. Furthermore, several JA-responsive genes
involved in biosynthesis (i.e. CYP81F4, IGMT5) [55, 66] and metabolism of glucosinolates (i.e. PYK10, NSP1) [67, 68] were upregulated.

Prior cold treatment affects the transcriptional response to tissue damage

To analyze the influence of a preceding cold stress on the transcriptional response to artificial wounding or feeding by a generalist or specialist herbivore, transcriptome analyses of leaf material from plants subjected to the treatments described in the Methods section and in Fig. 1 were performed.

A principle component analysis of gene expression values revealed a clear separation of the C2 control plant transcriptome from that of the other plant treatments, except for the transcriptome of M. brassicaceae feeding-damaged plants (T_M), whose 95% confidence interval overlapped slightly with that of the C2 control (Fig. 4a). The cold-treated plants (P2) showed a transcriptome shift relative to the C2 control, displayed in the first principle component (PC1), which accounts for ~25% of sample variances in all three sample groups. This indicates that deacclimation was not yet completed at the time of sampling. Subsequent feeding damage by P. brassicaceae (P + T_P) or M. brassicaceae (P + T_M) led to a separation of the transcriptome from that of P2 plants, whereas artificial wounding (P + T_W) did not. This suggests that a prior cold treatment results in a different plant transcriptional response to continuous two-day larval feeding damage than to discontinuous artificial wounding. Moreover, the T- and P + T-induced transcriptomes differed also in a species-specific manner, indicating that Arabidopsis can distinguish between damage by P. brassicaceae or M. brassicaceae (Additional file 1: Figure S3).

In cold-treated plants, the total number of regulated genes ranged from 1367 in M. brassicaceae-damaged leaves to 2341 in P. brassicaceae-damaged leaves to 3293 in artificially wounded leaves relative to untreated and undamaged control plants (Fig. 4b, d; P + T vs C2; the regulated genes are listed in Additional file 2: Table S1). Following prior cold stress, 446, 793, and 2439 genes were differentially regulated compared to untreated plants upon P. brassicaceae or M. brassicaceae feeding or artificial wounding, respectively (Fig. 4b, d; P + T vs T). Thus, the total number of genes which were differentially expressed due to prior cold stress was higher in artificially wounded than in larval feeding-damaged plants. In general, roughly equal fractions of DEGs were up- or downregulated in P + T_P, P + T_M, and P + T_W plants compared to the respective T plants.

Of particular interest are those genes that were differentially regulated in cold-treated and damaged plants relative to damaged plants (P + T vs T) and also in untreated and damaged (T vs C2) and/or cold-treated and damaged plants (P + T vs C2) relative to control plants (Fig. 4b, colored intersections in Venn diagrams). These gene sets comprise 284, 490, and 1768 genes in P. brassicaceae feeding-, M. brassicaceae feeding- and wounding-damaged leaves, respectively (Additional file 2: Table S1). The 80, 270, and 465 genes in the intersection of T vs C2 and P + T vs T, but not P + T vs C2 (Fig. 4b, underlined numbers) were regulated by tissue damage, however, the magnitude of the transcriptional response to damage was diminished when the plants had previously experienced cold. In contrast, genes exclusively occurring in the overlapping intersection of P + T vs T and P + T vs C2 were regulated only upon sequential experience of cold and tissue damage by feeding or wounding, but not by damage of untreated control plants. The intersections of T vs C2, P + T vs T and P + T vs C2 consist of genes that respond to feeding or wounding, and this response was significantly different when plants had been exposed to a prior cold phase.

We further investigated whether genes were specifically or commonly regulated by the three cold / damage combinations. Upon prior cold treatment, 46 DEGs were commonly regulated (40 up, 6 down), i.e. their transcriptional response was independent of the insect species and type of wounding (larval feeding, artificial damage) (Fig. 4c, Additional file 2: Table S1). Additionally, 15 genes were differentially regulated (7 up, 8 down) after cold exposure and subsequent feeding damage by both herbivore species, but not after cold exposure and subsequent artificial wounding (Fig. 4c).

The prior cold treatment also affected the magnitude of the transcriptional response to subsequent tissue damage. In Pieris-damaged leaves, the cold pre-treatment caused a significant intensification of damage-induced up- or downregulation in 39 of the 125 damage-induced genes (31%), whereas in the remaining genes the magnitude of regulation was diminished or even turned into opposite regulation (Fig. 4e; Additional file 2: Table S1). In artificially wounded local leaves, regulation of 84% of the damage-induced genes was attenuated. In leaves damaged by M. brassicaceae, almost all (98%) feeding-induced genes exhibited attenuated regulation upon prior cold treatment. Only 2% of the feeding-induced genes exhibited intensified expression changes in cold pre-treated plants (Fig. 4e). These results show that a cold phase attenuated the transcriptional response to subsequent leaf damage in the majority of damage-induced genes. However, the degree of attenuation was dependent on the type of damage.

Leaf tissue damage affects the cold deacclimation process

To investigate whether leaf tissue damage by larval feeding and artificial wounding has an impact on gene
expression during deacclimation, we compared the transcriptomes of cold-treated plants during deacclimation with or without experience of tissue damage. First, we compared the transcriptome of plants at the end of the cold-period (Fig. 1; P1 plants) with that of plants after 3 days of deacclimation (Fig. 1; P2 plants). In the P2 plants we found more than 1500 newly regulated genes with 25 significantly enriched biological process GO terms, indicating that deacclimation also involves activation of cellular processes (Fig. 5a). Eleven GO terms are enriched
only for downregulated genes, nine terms only for upregulated genes, and six terms are enriched for up- and downregulated genes (Fig. 5b). Interestingly, the downregulated terms include the ‘glucosinolate biosynthesis process’. A closer look reveals that in this category especially genes with function in aliphatic glucosinolate biosynthesis were downregulated, like MAM3, CYP79F1, CYP79F2, SOT18, IPMI1, IPMI2 and CYP83A1. Noticeably, with the exception of MAM3, none of these genes were differentially regulated after 5 days cold in P1 plants.

It was therefore interesting to investigate how larval feeding or wounding affects this cold deacclimation response, especially with respect to the genes involved in glucosinolate biosynthesis. Overall, the regulation of 14–19% of all 2588 DEGs in deacclimating P2 plants was attenuated when feeding or wounding occurred (Fig. 5c, Additional file 2: Table S1), resulting in a faster decay of the cold deacclimation response. However, feeding damage by *P. brassicae* larvae resulted in higher expression of five of the seven above mentioned aliphatic glucosinolate biosynthesis genes (MAM3, CYP79F1, CYP79F2, SOT18 and IPMI1) in P + T compared to deacclimating P2 plants (expression of IPMI2 and CYP83A1 is not altered). In contrast, feeding by *M. brassicae* larvae increased the expression of only two of the seven genes (IPMI1 and CYP79F1). Wounding alone did not increase the transcription level of any of the seven genes.

Stress- and stress combination-dependent transcriptional regulation of biological processes

The transcriptome analyses revealed that (i) a preceding cold phase leads to a modified transcriptional response of feeding- or wounding-regulated genes (Fig. 4e) and (ii) leaf damage by feeding or wounding modifies the transcription profile of cold-regulated genes during deacclimation (Fig. 5c). This raised the question which biological process GO terms contributed to the overall transcriptional status of P + T plants. We thus determined the enriched GO terms (Fig. 6) among the genes differentially regulated solely by cold treatment (blue characters in Figs. 4b and 6), by damage (green characters in Figs. 4b and 6), by cold or damage (red characters in Figs. 4b and 6) and by the combination of prior cold and subsequent damage (orange characters in Figs. 4b and 6), respectively. Enhanced gene regulation in many biological process GO terms was triggered almost exclusively by the single stresses cold (P2), damage (T), or the combined stressors cold + damage (P + T). Other GO terms, though, were enriched in cold exposed plants but also after damage (P2 or T). For example, leaf damage exclusively contributed to upregulation of the ‘response to JA’ process. In contrast, in the process ‘response to wounding’ some genes were induced by cold or damage, while other genes were upregulated only by damage.

Of all regulated genes in P + T plants, 13% (*Mamestra*), 22% (*Pieris*) and 39% (Wounding) only changed in expression if a cold treatment preceded the tissue damage (Fig. 4b, orange characters). These genes can be considered as primable for improved damage-triggered induction by prior cold exposure.
Damage of *A. thaliana* leaves by *P. brassicae*, *M. brassicae* and artificial wounding resulted in significant transcriptional changes in 187, 173, and 235 defense- or glucosinolate synthesis-related genes (Additional file 2: Table S1). Of these, 30% (*Pieris*), 43% (*Mamestra*) and 30% (Wounding) were upregulated. Remarkably, 70% of the defense- or glucosinolate synthesis-related genes upregulated by *P. brassicae* feeding were also upregulated.
by *M. brassicae* feeding, whereas 75% of the genes downregulated upon *P. brassicae* feeding were not regulated by *M. brassicae* feeding. When preceded by cold, the transcriptional response of only 4% of these *P. brassicae* feeding-induced genes was attenuated by ≥2-fold. In contrast, responses of much greater fractions of genes deregulated by *M. brassicae* feeding (29%) or wounding (32%) were attenuated by a factor of 2 or more, when the plants had previously been exposed to cold.

Thus, drought or cold stress preceding tissue damage apparently affects similar biological processes of *A. thaliana*, but not necessarily the same genes.

**Discussion**

**Similarities and differences in *A. thaliana* transcriptional response to leaf damage by *P. brassicae* feeding, *M. brassicae* feeding and artificial wounding**

After a prior cold treatment of *Arabidopsis* plants, larvae of the generalist herbivore *M. brassicae* performed better than on untreated plants whereas larvae of the specialist *P. brassicae* did not benefit, indicating that the cold treatment induced changes in the plant that promoted larval development of *M. brassicae*, but not of the specialist *P. brassicae*. It is conceivable that after a cold phase the plant’s metabolic status or response to differences in leaf tissue damage patterns is altered.

For each of the three leaf damage scenarios approximately two thirds of all regulated genes were also regulated in one or both of the other two damage types. Remarkably, >98% of these genes were regulated in the same direction, only 29 genes showed opposite regulation upon different damage types (Fig. 3b). Several of the genes with the largest regulation differences are known to be involved in plant responses to phytopathogens. For example, *argah2* mutants show increased susceptibility to pathogens inducing clubroot disease [69, 70]. SLAH3 is an anion channel expressed in guard cells and involved in stomatal immunity by closure of guard cells in response to pathogen attack [71]. DREB26 is responsive to infection by the necrotrophic fungus *Botrytis cinerea* and to various abiotic stresses as well [72]. PXMT1 is a target of miR163, a microRNA which promotes in a light-dependent manner seed germination and primary root length [73] and modulates defense responses against bacterial pathogens [74]. The Aux/IAA protein IAA29 is a transcription factor acting as repressor of the auxin signaling pathway [75].

Several studies addressed the hypothesis that highly specialized herbivores are more tolerant towards defenses of their host plants than generalists (reviewed by [46]). However, plant defense responses are multifaceted and not by default more effective against generalists than specialists. Thus, to identify plant responses specifically induced or suppressed by generalist and specialist herbivores, a treatment like artificial wounding can provide a baseline for changes at the molecular level [46, 76]. Here, 23–30% of genes transcriptionally responding to leaf damage by the specialist *P. brassicae*, the generalist *M. brassicae* or artificial wounding were shared (Fig. 3b, central intersection), including upregulation of JA-responsive defense-related genes. This shows that part of the responses to feeding damage overlapped with the reaction to artificial wounding. The majority of wound-responding genes could not be assigned to a distinct, significantly regulated process, indicating a generic “panic” response of *A. thaliana* to artificial wounding [77].

Artificial wounding resulted in upregulation of many JA biosynthesis genes. Most of these genes were also upregulated to very similar levels in response to feeding by *M. brassicae* larvae, whereas transcriptional induction was attenuated or lacking upon feeding by *P. brassicae* larvae (Additional file 2: Table S1). Interestingly though, many SA-responsive genes are downregulated in response to *P. brassicae* feeding. Salicylic acid can act antagonistically to JA-mediated plant defense responses [12, 14, 60, 78], but it can also positively modulate the plant defense against herbivores [35, 79]. We found that after two days feeding by 10 *P. brassicae* larvae eight SA-associated WRKY transcription factors are downregulated. The SA-responsive factors WRKY38, WRKY60 and WRKY70 are only downregulated upon *P. brassicae* feeding, but not upon *M. brassicae* feeding or artificial wounding. It will thus be interesting to investigate whether *P. brassicae* oral secretions negatively affect the plant’s SA-response pathway towards a diminished herbivore defense [80–82].

Strikingly, opposite to *P. brassicae* feeding, *M. brassicae* feeding was accompanied by more up- than down-regulated SA-response genes, and seven of the eight WRKY genes downregulated upon *P. brassicae* feeding were not responding to *M. brassicae* feeding. It is tempting to speculate that, in contrast to *P. brassicae*, *M. brassicae* oral secretions do not dampen the plant’s SA-response pathway. Moreover, *M. brassicae* feeding induced in *Arabidopsis* leaves a stronger and more complex transcriptional response of glucosinolate biosynthesis-associated genes. Striking is the upregulation of *MYB51*, a regulator of indole glucosinolate biosynthesis [83], the nitrile specifier protein NSP1 [84], the P450 monooxygenases *CYP81F2* [85] and *CYP81F4* [66] and the indole glucosinolate methyltransferase *IGMT2* [55]. Elevated expression of plant specifier proteins has been found to promote *A. thaliana’s* defense against *P. rapae* larvae, a close relative of *P. brassicae*, as it deters *P. rapae* from egg deposition on the plants. In addition, the endoparasitoid *Cotesia rubecula*, which prefers *P. rapae* larvae as hosts, is more attracted to *P. rapae*-infested plants overexpressing specifier proteins than to
P. rapae-infested Col-0 wild-type plants. In contrast to Col-0, the specifier overexpressors accumulate mainly simple nitriles from glucosinolate hydrolysis [86]. CYP81F2 encodes a P450 monoxygenase involved in 4MI3G (4-methoxyindol-3-ylmethylglucosinolate) synthesis and antifungal defense [85].

Studies of plant interactions with the lepidopteran generalist Spodoptera littoralis and with specialists (including P. rapae and P. brassicae) revealed that application of larval oral secretion of these insects results in suppression of plant defense gene expression [87, 88]. Among the genes with attenuated expression in our study the protease inhibitor DR4 and extracellular lipase 3 EXL3 showed a more pronounced attenuation upon P. brassicae than upon M. brassicae feeding (Additional file 2: Table S1). These genes are also suppressed upon feeding by S. littoralis [87]. It is thus conceivable that the expression attenuation we observed was caused by oral secretions of the herbivores. It is known that plants can distinguish between damage by different herbivores and by artificial wounding [89] because their oral secretions contain species-specific herbivore-associated molecular patterns (HAMPs) that enable plants to modulate their defense responses (reviewed in [90–92]). It will be interesting to investigate in the future whether the observed differences between the expression patterns upon P. brassicae or M. brassicae feeding depend on such HAMPs.

Prior low temperature exposure causes attenuated regulation of genes responsive to leaf damage

The comparison of expression changes in herbivory- or wounding-responsive genes in plants, which had previously experienced 5 days at 4 °C, revealed similarities, but also striking damage type-dependent differences in transcriptional reprogramming. Among the 46 genes that were regulated after each of the three damage types, several were reported to function in stress responses. The flavonol monoxygenase 1 (FMO1) is known to be essential for the establishment of systemic acquired resistance (SAR) and therefore systemic defenses against pathogens like Pseudomonas syringae [93]. UGT72E2 and UGT72E3 are involved in glucosylation of monoglignols, which results in increased content of coniferin, syringin, and other phenylpropanoids [94–97]. ALLENE OXIDE CYCLASE1 (AOC1), a key enzyme in JA biosynthesis, is known to be rapidly responding to cold stress ([98, 99], reviewed by [100]).

Fifteen genes were differentially regulated only upon leaf damage by either of the two herbivores but not upon artificial wounding. Among the eight commonly downregulated genes is a terpene synthase (TPS03), which is known to be inducible by wounding and herbivory [101]. The transcription factors RAP2.9 and ZAT10 function as regulators in biotic and abiotic stress responses as well as in stress combinations [102–104]. ORA59 is involved in JA/ET synergistic regulation and important for pathogen defense via PDF1.2 activation [15, 105]. Commonly upregulated genes (7) include LOX5, a member of the 9-lipoxygenases involved in pathogen defense [106] and PIL1, a transcription factor known to be cold- and high light-stress responsive with functions in shade avoidance. It is also JA responsive in a COI-dependent manner [107–109]. The gene ST2A displayed increased expression in previously cold-experienced plants responding to P. brassicae feeding, while the response to M. brassicae was opposite. ST2A, one of 18 sulfotransferases in Arabidopsis, is involved in JA metabolism by sulfating 11-OH-JA and 12-OH-JA [110].

Common for all three types of tissue damage was that a smaller fraction of damage-responsive genes was more strongly up- or downregulated, whereas in the majority of them the transcriptional response was attenuated after a prior cold treatment. The difference in the fractions of genes with altered regulation between P. brassicae and M. brassicae is striking, though. In leaves damaged by P. brassicae larvae 31% of the genes are more intensely and 69% more weakly regulated. In contrast, upon herbivory by M. brassicae larvae, only 2% of the damage-responsive genes are more intensely regulated whereas in 98% of these genes the expression change is lower than in plants that were not exposed to cold.

Feeding and wounding promote a decline of the cold acclimation status

Cold acclimation and subsequent deacclimation are known to be accompanied by extensive transcriptomic and metabolomic reorganization. Not only acclimation but also deacclimation is an active and tightly regulated process, which involves metabolic changes in lipid and cell wall components, downregulation of protein synthesis, and transcriptional reprogramming of jasmonate, brassinosteroid and other hormonal pathways [111, 112]. Pagter et al. [111] found that the deacclimation-associated responses of A. thaliana Col-0 proceed most rapidly during the first 12 h after shifting 4 °C-acclimated plants to 20 °C. However, deacclimation is only in part a reversion of cold acclimation, and even after 24 h the plant metabolism and transcriptome have not yet fully reverted to the non-acclimated status [111]. It is thus conceivable that after 24 h of deacclimation the plant response to herbivore attack differs from that of untreated plants, but it is not predictable whether the prior cold treatment results in an unspecific or herbivore-specific, improved or compromised defense.

Although the cold deacclimation response is considered to be rapid and mainly passive [112, 113], more than 1500 genes were newly regulated 3 days after
terminating the plant’s exposure to cold. Similar results were obtained in an earlier study by Firtzlaff et al. [43]. Conspicuously, among the newly regulated genes the GO term ‘glucosinolate biosynthesis process’ is downregulated. A weaker expression of these genes could imply a reduced aliphatic glucosinolate content in P2 plants and therefore provide advantageous conditions for the larvae of the generalist herbivore species, *M. brassicae*. Performance of this generalist species is negatively affected by aliphatic glucosinolates [114, 115]. In contrast, the specialist *P. brassicae* is well known to effectively detoxify glucosinolates (e.g. [48]).

In addition, the differences in the transcriptional response of cold-treated Arabidopsis plants to feeding by the two herbivores support the notion that the generalist *M. brassicae*, but not the specialist *P. brassicae*, benefits from a cold phase prior to hatching of the larvae. For instance, AOS (allene oxide synthase), a key gene in JA biosynthesis [116], the antifungal/antimicrobial defense thionin gene THI2.1 [117], and the indolic glucosinolate synthesis genes CYP81F4, CYP81F2 and IGMT1 [55, 66, 118] were induced in plants not exposed to cold by *M. brassicae* feeding, but not by *P. brassicae* feeding. In cold-treated plants, expression of these genes was attenuated upon *M. brassicae* feeding, but not altered upon *P. brassicae* feeding. This is consistent with the observation that the performance of *P. brassicae* larvae is identical on cold-treated and control plants, whereas *M. brassicae* larvae perform better on cold-treated plants. Yet, the plant’s defense response invoked by the feeding damage of *P. brassicae* larvae is comparable in untreated and cold-treated *A. thaliana* plants. Since the specialist *P. brassicae* is well adapted to the defense measures [119, 120] it was expected that its performance is not impaired.

Since *M. brassicae* is more sensitive to the defense compounds of *A. thaliana* [114], its performance in untreated plants is negatively affected. In cold-treated plants, though, the *M. brassicae* feeding damage pattern elicited an attenuated defense reaction. These results are in accordance with two other studies that addressed the question of how the experience of prior abiotic stress influences later defense responses against herbivores [42, 43]. Common results of the three studies are: (i) prior exposure of plants to abiotic stress caused a reduced transcriptional induction of tissue damage-inducible defense genes, including attenuated gene expression of e.g. JA- and glucosinolate metabolism-related genes; (ii) the performance of the specialist herbivores *P. rapae* [42] and *P. brassicae* (this study and [43]) was not affected by prior drought or cold treatment of *A. thaliana*; (iii) herbivory led to a shift from the drought- or cold-adapted transcriptome towards herbivore defense, thus accelerating the abiotic stress deacclimation. Yet, the differentially regulated genes in feeding-damaged plants with prior drought or cold experience differed to a great extent. Only two genes, a glutathione S-transferase (GSTU8) and UPF0496 were transcriptionally responding to all tissue damage types when preceded by drought or cold.

**Conclusions**

We show that a prior cold treatment of *A. thaliana* differentially reprogrammed the transcriptional response to leaf tissue damage by artificial wounding and feeding by the specialist herbivore *P. brassicae* or the generalist herbivore *M. brassicae*. The cold-treatment resulted at the transcriptional level in an attenuation of the plant’s damage-induced defense response. We suggest that this attenuation is responsible for the improved larval performance of the generalist *M. brassicae*. In contrast, the specialist *P. brassicae* is unaffected by the damage-induced *A. thaliana* defense measures and accordingly does not benefit from the defense attenuation by a preceding cold treatment of the plants.

**Methods**

**Plant growth**

*Arabidopsis thaliana* Columbia Col-0 seeds (Stock No. N1093) were obtained from the Nottingham Arabidopsis Stock Centre (NASC). Seeds were sown on soil type A (2:2:1, Einheitserde CL P: Einheitserde CL T: Sand) and stratified for 2 days at 4 °C. Thereafter, plants were grown in a growth chamber at short day conditions (8 h/16 h light/dark cycle, 120 μE), 20 °C and 50% relative humidity for 7 weeks. Three-week-old seedlings were transplanted in pots containing soil type B (7:7:3, Einheitserde CL P: Einheitserde CL T: Perlite).

**Insect rearing**

*Pieris brassicae* larvae from in-house captive breeding were reared on savoy cabbage (*Brassica oleracea* var. *sabauda*) as described by [43]. *Mamestra brassicae* were obtained from N. Fatouros (Biosystematics Group, Wageningen University and Research, Wageningen, Netherlands). Larvae were reared on cabbage plants (*Brassica oleracea* var. *sabellica* L.) until pupation. Soil was provided to last instar *M. brassicae* larvae for pupation, while *P. brassicae* pupae were kept on cardboard. Adults of *M. brassicae* were offered water and a sugar-water solution (1:5 w/v). Adult *P. brassicae* butterflies were fed with an aqueous honey solution.

**Plant treatments**

The experimental design is depicted in Fig. 1. Seven-week-old plants were subjected to (i) 5 days cold at 4 °C (P samples), (ii) leaf damage by *P. brassicae* larvae (TP samples), *M. brassicae* larvae (TM samples) or artificial
wounding (T_W samples), (iii) cold followed by leaf damage (P + T_P, P + T_M or P + T_W samples), or (iv) no stimulus (C samples). The stimulus ‘cold’ was applied for 5 days, followed by 1 day under normal growth conditions (20 °C) as memory/deacclimation phase. P1 samples were taken directly after 5 days of cold and P2 samples 3 days after transferring plants back to 20 °C (Fig. 1). The second stress (larval herbivory or artificial wounding) was applied for 2 days following the 1 day memory/deacclimation phase. For treatment with larvae, neonate P. brassicae or M. brassicae larvae were added in a clipcage to leaf number 17. For control, an empty clipcage was placed on leaf number 17 of untreated control (C) and cold-pretreated (P) plants. Artificial wounding was applied by damaging leaf number 17 with forceps for 30 s two times a day for 2 days. The damaged area almost matched the area of damage that larvae feeding inside a clipcage inflicted to a leaf.

Larval performance measurement

Individual seven-week-old plants treated with or without prior cold were subjected to feeding by 15 freshly hatched M. brassicae or P. brassicae larvae on leaf 17. The experiments were repeated 11 times (N = 11 plants) with M. brassicae and 15 times (N = 15 plants) with P. brassicae. Larvae were confined in clipcages with a diameter of 3 cm. Two days later, larval weight and weight gain were determined. Furthermore, the consumed leaf area was assessed by comparing pictures of the leaves taken before and after 2 days feeding using ImageJ [121]. The leaf expansion during the 2 days feeding period was marginal and not taken into account. Subsequently larvae were returned to the plants and allowed to feed upon the whole plant for another 4 days. Two and 4 days later larval weight and weight gain were measured again. Larval performance data were evaluated with “R” [122] and subjected to statistical analysis [123, 124]. Data were tested for normal distribution (Shapiro-Wilk test) and homogenous variances (Levene’s test). If larval weight and weight gain values were not normally distributed and/or did not show variance homogeneity, data were log2 transformed to fulfil the prerequisites for applying unpaired Student’s t-test.

Transcriptome analyses

We analyzed the transcriptome of untreated plants (C), cold-exposed plants (P), damaged plants (T) and cold-exposed and feeding-damaged plants (P + T). We standardized the extent of damage by insects and artificial wounding to be able to ascribe damage-induced transcriptomic changes to the type of damage rather than to the extent of damage. Therefore, plant leaves were exposed to 10 P. brassicae larvae or 20 M. brassicae larvae in a clip cage. After 2 days feeding, the leaf area consumed by the two species was almost identical (Additional file 1: Figure S4). The artificially wounded area was similar as well. For RNA extraction, a 1 cm wide strip from leaf number 17 of C1, C2, P1, P2, T_P, T_M, T_W, P + T_P, P + T_M and P + T_W plants was harvested. The strip was located proximal to the clipcage or wounding site. To minimize effects of circadian clock-dependent transcriptional regulation, all samples were collected from 4 to 6 h after the onset of the daylight phase, i.e. at a time when larvae are actively feeding in nature. After harvesting, the strips were kept frozen in liquid nitrogen. Leaf material of three individual plants was pooled to obtain one biological replicate, and three biological replicates of each sample type were analyzed.

Frozen leaf material was ground in liquid nitrogen, and total RNA was extracted according to Onate-Sánchez [125]. Total RNA was DNase I-digested according to manufacturer’s instructions (Thermo Fisher Scientific). Yield and quality of extracted RNA was determined spectrophotometrically and by denaturing agarose gel electrophoresis.

Genome-wide expression analyses were conducted on ArrayXS Arabidopsis v2 microarrays (series XS-5010; GEO accession GPL19779; Oaklabs GmbH, Henningsdorf, Germany). Microarray data were processed and analyzed with the Bioconductor Linear Models for microarray data (limma) software package [126, 127] as described in Firtzlaff et al. [43]. In short, microarray signals were background-corrected and interarray-normalized. Genes with ≥2-fold expression change and adjusted P-values ≤0.05 (Benjamini and Hochberg false discovery rate procedure) were defined to be differentially expressed genes (DEGs) (Additional file 1: Figure S2). Gene expression data are deposited in the NCBI GEO repository under the accession number GSE114211.

Principle component analysis (PCA) of the transcriptomic data sets was performed using the “ggplot” and “ggbiplot” packages of “R” [122, 128]. Enriched gene ontology (GO) terms were identified using the TAIR GO Term Enrichment for Plants tool (www.arabidopsis.org) provided by PANTHER DB (http://pantherdb.org). If not mentioned otherwise, a Bonferroni correction for multiple testing was applied to reduce false positives.

Additional files

Additional file 1: Figure S1. Relative growth rates of Pieris brassicae and Mamestra brassicae neonate larvae on previously cold-treated or untreated plants. Figure S2. Gene expression changes in plants exposed to larval feeding or artificial wounding compared to untreated control plants. Figure S3. Principle component analysis of transcriptomes of plants exposed to individual treatments. Figure S4. Leaf area consumption by Pieris brassicae and Mamestra brassicae neonate larvae after 2 days feeding upon previously cold-treated or untreated plants. (PDF 348 kb)
Abbreviations
ABA: Abscisic acid; DEG: Differentially expressed gene; ET: Ethylene; GO: Gene ontology; JA: Jasmonic acid; PCA: Principal component analysis; SA: Salicylic acid

Acknowledgments
We thank Nina Fatouros for providing Mamestra brassicae, Charlotte Thomas for general lab support and the members of the Hilker and Kunze labs for valuable discussions.

Authors’ contributions
JO, VL, MH and RK conceived and designed the study. JO performed the experiments and analyzed the data. JO, VL, MH and RK wrote the manuscript. All authors read and approved the final manuscript.

Funding
The research was funded by the Deutsche Forschungsgemeinschaft, Collaborative Research Centre 973, project B4 (www.sfb973.de). The funding body was not involved in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Availability of data and materials
Microarray transcription raw data are deposited in the NCBI Gene Expression Omnibus (GEO) repository under the accession number GSE114211.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests

Author details
1Freie Universität Berlin, Institute of Biology - Applied Genetics, Dahlem Centre of Plant Sciences, Albrecht-Thaer-Weg 6, 14195 Berlin, Germany. 2Freie Universität Berlin, Institute of Biology - Applied Zoology / Animal Ecology, Dahlem Centre of Plant Sciences, Haderslebener Str. 9, 12163 Berlin, Germany.

Received: 16 April 2019 Accepted: 23 July 2019
Published online: 02 August 2019

References
1. Walling LL. The myriad plant responses to herbivores. J Plant Growth Regul. 2000;19(2):195–216.
2. Hirayama T, Shinozaki K. Research on plant abiotic stress responses in the post-genome era: past, present and future. Plant J. 2010;61(6):1041–52.
3. Baxter A, Mittler R, Suzuki N. ROS as key players in plant stress signalling. J Exp Bot. 2014;65(5):1229–40.
4. Zebelo SA, Maffei ME. Role of early signalling events in plant-insect interactions. J Exp Bot. 2015;66(2):435–48.
5. Nabity PD, Zawa JA, DeLucia EH. Indirect suppression of photosynthesis on individual leaves by arthropod herbivory. Ann Bot. 2009;103(4):655–63.
6. Núñez-Farfán J, Fornoni J, Valverde PL. The evolution of resistance and tolerance to herbivores. Annu Rev Ecol Evol S. 2007;38:541–66.
7. Strauss SY, Rudgers JA, Lao JA, Irwin RE. Direct and ecological costs of resistance to herbivory. Trends Ecol Evol. 2002;17(6):278–85.
8. Schaller A. Induced plant resistance to herbivory. Stuttgart: Springer; 2008.
9. Karban R, Baldwin IT. Induced responses to herbivory. Chicago: The University of Chicago Press; 1997. 330 p.
10. Howe GA, Jander G. Plant immunity to insect herbivores. Annu Rev Plant Biol. 2002;59:41–66.
11. Koo AJ, Howe GA. The wound hormone jasmonate. Phytochemistry. 2009;70(13–14):1571–80.
12. Petieter CM, Leon-Reyes A, Van der Ent S, Van Wees SC. Networking by small-molecule hormones in plant immunity. Nat Chem Biol. 2009;5(3):108–16.
13. Verhage A, van Wees SC, Petieter CM. Plant immunity: it's the hormones talking, but what do they say? Plant Physiol. 2010;154(2):536–40.
14. Petieter CM, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SC. Hormonal modulation of plant immunity. Annu Rev Cell Dev Biol. 2012;28:489–521.
15. Wasternack C, Hause B. Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in annals of botany. Ann Bot. 2013;111(6):1021–58.
16. Dezelc C, von Dahl CC, Gaquerel E, Baldwin IT. Different lepidopteran elicitors account for cross-talk in herbivory-induced phytohormone signaling. Plant Physiol. 2009;150(3):1576–86.
17. Mittler R. Abiotic stress, the field environment and stress combination. Trends Plant Sci. 2006;11(1):15–9.
18. Prasch CM, Sonnewald U. Signaling events in plants: stress factors in combination change the picture. Environ Exp Bot. 2015;114–4–14.
19. Stam JM, Knoos A, Li Y, Gols R, van Loon JI, Poelman EH, et al. Plant interactions with multiple insect herbivores: from community to genes. Annu Rev Plant Biol. 2014;65:689–713.
20. Atkinson NJ, Urwin PE. The interaction of plant biotic and abiotic stresses: from genes to the field. J Exp Bot. 2012;63(10):3523–43.
21. Ramesgowda V, Senthil-Kumar M. The interactive effects of simultaneous biotic and abiotic stresses on plants: mechanistic understanding from drought and pathogen combination. J Plant Physiol. 2015;176:47–54.
22. Suzuki N, Rivero RM, Shulaev V, Blumwald E, Mittler R. Abiotic and biotic stress combinations. New Physiol. 2014;203(1):32–43.
23. Voelckel C, Baldwin IT. Herbivore-induced plant vaccination. Part II. Array-studies reveal the transience of herbivore-specific transcriptional imprint and a distinct imprint from stress combinations. Plant J. 2004;38(4):650–63.
24. Hilker M, Schwachtje J, Baier M, Balaladezh S, Bäurle I, Geiselhardt S, et al. Priming and memory of stress responses in organisms lacking a nervous system. Biol Rev Camb Philos Soc. 2016;91(4):1118–33.
25. Conrath U, Beckers GJ, Langenbach CJ, Jaskiewicz MR. Priming for enhanced defense. Annu Rev Phytopathol. 2015;53:97–119.
26. Martinez-Medina A, Fiers V, Heil M, Mauch-Mani B, Petieter CM, Pozo MJ, et al. Recognizing plant defense priming. Trends Plant Sci. 2016;21(10):818–22.
27. Pastor V, Luna E, Mauch-Mani B, Ton J, Fiers V. Primed plants do not forget. Environ Exp Bot. 2013;94:46–56.
28. Meischer MC, De Moraes CM. Plant biology: pass the ammunition. Nature. 2014;510(7504):421–2.
29. Heil M, Ton J. Long-distance signalling in plant defence. Trends Plant Sci. 2008;13(6):264–72.
30. Hilker M, Fatouros NE. Plant responses to insect egg deposition. Annu Rev Entomol. 2015;60:493–515.
31. Hilker M, Fatouros NE. Resisting the onset of herbivore attack: plants perceive and respond to insect eggs. Curr Opin Plant Biol. 2016;32:9–16.
32. Karban R, Yang LH, Edwards RF. Volatile communication between plants that affects herbivory: a meta-analysis. Ecol Lett. 2014;17(1):44–52.
33. Altmann S, Muino JM, Lortzing V, Brandt R, Himmelbach A, Altschmied L, et al. Transcriptomic basis for reinforcement of elm antiherbivore defence mediated by insect egg deposition. Mol Ecol. 2018;27(23):4901–15.
34. Geuss D, Stelzer S, Lortzing T, Steppuhn A. Solanum dulcamara’s response to eggs of an insect herbivore comprises oxidative hydrogen peroxide production. Plant Cell Environ. 2017;40(11):2663–77.
35. Lortzing V, Oberländer J, Lortzing T, Duhrge T, Steppuhn A, Kunze R, et al. Insect egg deposition renders plant defence against hatching larvae more effective in a salicylic acid-dependent manner. Plant Cell Environ. 2019;42:1019–32.
36. Büchel K, McDowell E, Nelson W, Descour A, Gershenzon J, Hilker M, et al. Transcriptome analysis of Z-3-hexenol-treated Zea mays plants reveals distinct transcriptional networks and anti-herbivore defense potential of green leaf volatiles. PLoS One. 2013;8(10):e77465.
37. Ton J, D’Alessandro M, Jourdie V, Jakab G, Kafien D, Held M, et al. Priming by airborne signals boosts direct and indirect resistance in maize. Plant J. 2007;49(1):16–26.
40. Coolen S, Proietti S, Hickman R, Davila Olivas NH, Huang PP, Van Verk MC, et al. Transcriptome dynamics of Arabidopsis during sequential biotic and abiotic stresses. Plant J. 2016;86(3):249–67.
41. Weldegergis BT, Zhu F, Poelman EH, Dicke M. Drought stress affects plant metabolites and herbivore preference but not host location by its parasitoids. Oecologia. 2015;177(3):701–13.
42. Davila Olivas NH, Coolen S, Huang P, Severing E, van Verk MC, Hickman R, et al. Effect of prior drought and pathogen stress on Arabidopsis transcriptome changes to caterpillar herbivory. New Phyol. 2016;210(4):1344–56.
43. Firtzlaff V, Oberländer S, Hinkler M, Kunze R. Pre-exposure of Arabidopsis to the abiotic or biotic environmental stimuli “chilling” or “insect eggs” exhibits different transcriptomic responses to herbivory. Sci Rep. 2016;6:28454.
44. Storz HU, Pitterndrigh BR, Kroymann J, Weniger K, Fritsche J, Bauke A, et al. Stotz HU, Pittendrigh BR, Kroymann J, Weniger K, Fritsche J, Bauke A, et al. Firtzlaff V, Oberländer J, Geiselhardt S, Hilker M, Kunze R. Pre-exposure of Arabidopsis to the abiotic or biotic environmental stimuli “chilling” or “insect eggs” exhibits different transcriptomic responses to herbivory. Sci Rep. 2016;6:28454.
45. Müller R, de Vos M, Sun JY, Sønderby IE, Halkier BA, Wittstock U, et al. Müller R, de Vos M, Sun JY, Sønderby IE, Halkier BA, Wittstock U, et al. Effect of prior drought and pathogen stress on Arabidopsis transcriptome changes to caterpillar herbivory. New Phyol. 2016;210(4):1344–56.
46. Firtzlaff V, Oberländer S, Hinkler M, Kunze R. Pre-exposure of Arabidopsis to the abiotic or biotic environmental stimuli “chilling” or “insect eggs” exhibits different transcriptomic responses to herbivory. Sci Rep. 2016;6:28454.
47. Weldegergis BT, Zhu F, Poelman EH, Dicke M. Drought stress affects plant metabolites and herbivore preference but not host location by its parasitoids. Oecologia. 2015;177(3):701–13.
48. Pittendrigh BR, Kroymann J, Weniger K, Fritsche J, Bauke A, et al. Stotz HU, Pittendrigh BR, Kroymann J, Weniger K, Fritsche J, Bauke A, et al. Firtzlaff V, Oberländer J, Geiselhardt S, Hilker M, Kunze R. Pre-exposure of Arabidopsis to the abiotic or biotic environmental stimuli “chilling” or “insect eggs” exhibits different transcriptomic responses to herbivory. Sci Rep. 2016;6:28454.
49. Kumar R, Bhardwaj U, Kumar P, Mazumdar-Leighton S. Midgut serine proteases and herbivore preference but not host location by its parasitoids. Oecologia. 2015;177(3):701–13.
50. Storz HU, Pitterndrigh BR, Kroymann J, Weniger K, Fritsche J, Bauke A, et al. Stotz HU, Pittendrigh BR, Kroymann J, Weniger K, Fritsche J, Bauke A, et al. Firtzlaff V, Oberländer J, Geiselhardt S, Hilker M, Kunze R. Pre-exposure of Arabidopsis to the abiotic or biotic environmental stimuli “chilling” or “insect eggs” exhibits different transcriptomic responses to herbivory. Sci Rep. 2016;6:28454.
51. Weldegergis BT, Zhu F, Poelman EH, Dicke M. Drought stress affects plant metabolites and herbivore preference but not host location by its parasitoids. Oecologia. 2015;177(3):701–13.
52. Davila Olivas NH, Coolen S, Huang P, Severing E, van Verk MC, Hickman R, et al. Effect of prior drought and pathogen stress on Arabidopsis transcriptome changes to caterpillar herbivory. New Phyol. 2016;210(4):1344–56.
53. Firtzlaff V, Oberländer S, Hinkler M, Kunze R. Pre-exposure of Arabidopsis to the abiotic or biotic environmental stimuli “chilling” or “insect eggs” exhibits different transcriptomic responses to herbivory. Sci Rep. 2016;6:28454.
54. Weldegergis BT, Zhu F, Poelman EH, Dicke M. Drought stress affects plant metabolites and herbivore preference but not host location by its parasitoids. Oecologia. 2015;177(3):701–13.
55. Pittendrigh BR, Kroymann J, Weniger K, Fritsche J, Bauke A, et al. Stotz HU, Pittendrigh BR, Kroymann J, Weniger K, Fritsche J, Bauke A, et al. Firtzlaff V, Oberländer J, Geiselhardt S, Hilker M, Kunze R. Pre-exposure of Arabidopsis to the abiotic or biotic environmental stimuli “chilling” or “insect eggs” exhibits different transcriptomic responses to herbivory. Sci Rep. 2016;6:28454.
56. Weldegergis BT, Zhu F, Poelman EH, Dicke M. Drought stress affects plant metabolites and herbivore preference but not host location by its parasitoids. Oecologia. 2015;177(3):701–13.
85. Bednarek P, Pisiewska-Bednarek M, Svatos A, Schneider B, Doudsik J, Mansurova M, et al. A glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense. Science. 2009;323(5910):101–6.

86. Murum R, Burow M, Bukovinszklierz G, Kazantzidou E, Wittstock U, Dicke M, et al. Formation of simple nitriles upon glucosinolate hydrolysis affects direct and indirect defense against the specialist herbivore, Pieris rapae. J Chem Ecol. 2008;34(10):1311–21.

87. Consales F, Schweizer F, Erb M, Gouhier-Darimont C, Bodenhausen N, Bruessel F, et al. Insect oral secretions suppress wound-induced responses in Arabidopsis. J Exp Bot. 2012;63(2):727–37.

88. Reymond P, Bodenhausen N, van Poecke W, Krishna Murthy V, Dicke M, Farmer EE. A conserved transcript pattern in response to a specialist and a generalist herbivore. Plant Cell. 2004;16(11):3132–47.

89. Shinya T, Hogo Y, Desaki Y, Christaller JT, Okada K, Shihiya N, et al. Modulation of plant defense responses to herbivores by simultaneous recognition of different herbivore-associated elicitors in rice. Sci Rep. 2016;6:2537.

90. Mthofer A, Boland W. Recognition of herbivory-associated molecular patterns. Plant Physiol. 2008;146(3):285–31.

91. Maffei ME, Arimura G, Mithöfer A, Boland W. Recognition of herbivory-associated molecular patterns and effector arsenal of chewing herbivores. Mol Plant-Microbe Interact. 2018;31(11):13–21.

92. Mishina TE, Zeier J. The Arabidopsis flavin-dependent monooxygenase FMO1 is an essential component of biologically induced systemic acquired resistance. Plant Physiol. 2006;141(4):1666–77.

93. König S, Feussner K, Kaever A, Landesfeind M, Thurow C, Karlovsky P, et al. Soluble phenylpropanoids are involved in the defense response of Arabidopsis against Verticillium longisporum. New Phytol. 2014;202(3):823–37.

94. Lanocz A, Hodge D, Jackson RG, George GL, Elias L, Lim EK, et al. The glucosinolasedegradation enzyme GUT72E2 is responsible for monoglucosinol 4-O-glucoside production in Arabidopsis thaliana. Plant J. 2006;48(2):286–95.

95. Mao YC, Liu CJ. ATP-binding cassette-like transporters are involved in the transport of lignin precursors across plasma and vacuolar membranes. Proc Natl Acad Sci U S A. 2010;107(52):22728–33.

96. Zhao Q, Nakashima J, Chen F, Yin Y, Fu C, Yun J, et al. Laccase is necessary for fungal defense in Arabidopsis. Physiol Plant. 2013;152(2):256–67.

97. Schaller F. Enzymes of the biosynthesis of octadecanoid-derived signalling molecules. J Exp Bot. 2001;52(34):11–23.

98. Epple P, Apel K, Bohlmann H. Overexpression of an endogenous thionin enhances resistance of Arabidopsis against Fusiurini oxyospore. Plant Cell. 1997;9(4):509–20.

99. Phlaz M, Vogel H, Kroymann J. The gene controlling the indole glucosinolate modifier quantitave trait locus alters indole glucosinolate structures and aphid resistance in Arabidopsis. Plant Cell. 2009;21(3):985–99.

100. Nalczar J, van Looon JJ, Blatt SE, Harvey JA, Agerbirk N, Dicke M. The impact of the absence of aliphatic glucosinolates on insect herbivory in Arabidopsis. PLoS One. 2008;3(4):e2086.

101. Cascio P, Corgni P, Capuano K, Schilke K, Hogendoorn P, Maffei ME, et al. The impact of the absence of aliphatic glucosinolates on insect herbivory in Arabidopsis. PLoS One. 2017;12(8):e0181999.

102. Mickey M, Mccoy J, Zuber E, Chin DK. Rapid transcriptional and metabolic regulation of the deacclimation process in cold acclimated Arabidopsis thaliana. BMC Genomics. 2017;18(1):731.

103. Zuth F, Bezzak J, Lee YP, Bai M, Hinchka DK. Time-dependent deacclimation after cold acclimation in Arabidopsis thaliana accessions. Sci Rep. 2015;5:12199.

104. Byun YJ, Koo MY, Joo HJ, Ha-Lee YM, Lee DH. Comparative analysis of gene expression under cold acclimation, deacclimation and reacclimation in Arabidopsis. Physiol Plantarum. 2014;152(2):256–74.

105. Beekwilder J, van Leeuwen W, van Dam NM, Bertossi M, Grandi V, Mizzi L, et al. The impact of the absence of aliphatic glucosinolates on insect herbivory in Arabidopsis. Physiol Plantarum. 2014;152(2):256–74.

106. Vicente J, Cascon T, Vicedo B, Garcia-Agustin P, Hamberg M, Castresana C. The Arabidopsis glucosinolate sulfotransferase from Arabidopsis thaliana. J Biol Chem. 2003;278(20):17895–900.

107. Pagter M, Alpers J, Erban A, Kopka J, Zuther E, Hinchka DK. Rapid transcriptional and metabolic regulation of the deacclimation process in cold acclimated Arabidopsis thaliana. BMC Genomics. 2017;18(1):731.

108. Zuth F, Bezzak J, Lee YP, Bai M, Hinchka DK. Time-dependent deacclimation after cold acclimation in Arabidopsis thaliana accessions. Sci Rep. 2015;5:12199.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.