Reciprocal Responses in the Interaction between Arabidopsis and the Cell-Content-Feeding Chelicerate Herbivore Spider Mite[1][W][OPEN]

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Most molecular-genetic studies of plant defense responses to arthropod herbivores have focused on insects. However, plant-feeding mites are also pests of diverse plants, and mites induce different patterns of damage to plant tissues than do well-studied insects (e.g. lepidopteran larvae or aphids). The two-spotted spider mite (*Tetranychus urticae*) is among the most significant mite pests in agriculture, feeding on a staggering number of plant hosts. To understand the interactions between spider mite and a plant at the molecular level, we examined reciprocal genome-wide responses of mites and its host Arabidopsis (*Arabidopsis thaliana*). Despite differences in feeding guilds, we found that transcriptional responses of Arabidopsis to mite herbivory resembled those observed for lepidopteran herbivores. Mutant analysis of induced plant defense pathways showed functionally that only a subset of induced programs, including jasmonic acid signaling and biosynthesis of indole glucosinolates, are central to Arabidopsis's defense to mite herbivory. On the herbivore side, indole glucosinolates dramatically increased mite mortality and development times. We identified an indole glucosinolate dose-dependent increase in the number of differentially expressed mite genes belonging to pathways associated with detoxification of xenobiotics. This demonstrates that spider mite is sensitive to Arabidopsis defenses that have also been associated with the deterrence of insect herbivores that are very distantly related to chelicerates. Our findings provide molecular insights into the nature of, and response to, herbivory for a representative of a major class of arthropod herbivores.

[1] This work was supported by the Government of Canada through Genome Canada and the Ontario Genomics Institute (grant no. OGI–046 to M.G. and V.G.), the Ontario Research Fund-Global Leadership in Genomics and Life Sciences (grant no. GL2–01–035 to M.G. and V.G.), a University of Utah Funding Incentive Seed Grant (to R.M.C.), the Fund for Scientific Research Flanders (postdoctoral fellowship to T.V.L. and grant nos. 3G061011 and 3G009312), the Institute for the Promotion of Innovation by Science and Technology in Flanders (grant no. IWT/SB/101451 to N.W.), the National Institutes of Health (genetics training grant no. T32 GM07464 to E.J.O.), and Ministerio de Economia y Competitividad (Ramón y Cajal contract to V.A.).

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[W] The online version of this article contains Web-only data.

[OPEN] Articles can be viewed online without a subscription.

www.plantphysiol.org/cgi/doi/10.1104/pp.113.231555

Plants have evolved complex systems of defense to deter and/or prevent feeding by two different groups of organisms: pathogens such as bacteria and fungi, and herbivores such as plant-feeding arthropods. These defense mechanisms include preexisting structural barriers and deterrents as well as induced immune defenses that further protect the plant from biotic stressors. Our understanding of plant defenses derives largely from studies of plant-pathogen interactions, where the availability of genetically tractable interacting organisms has been critical in identifying key elements required for immune responses. The induced defense response to pathogens is a multilayered process that is initiated by the recognition of conserved pathogen-associated molecular patterns (PAMPs) by the pattern recognition receptors. Upon recognition of extracellular PAMPs, pattern recognition receptors induce PAMP-triggered plant immunity that restricts the propagation of pathogens.

In an evolutionary arms race, pathogens have evolved effectors that suppress PAMP-triggered plant immunity. However, the specific recognition of pathogen effectors (or their activity) by plant disease resistance proteins leads to effector-triggered immunity and plant resistance (Jones and Dangl, 2006).

In contrast to our elaborate knowledge of plant-pathogen interactions, the understanding of plant-arthropod interactions is still rudimentary. While pathogens that acquire plant nutrients from living cells cause comparatively minor physical damage to the plant, most herbivores disrupt the integrity of plant tissue during feeding. Though incomplete, our current understanding of plant responses to insect herbivores suggests that plants perceive herbivore attack by recognizing general herbivory-associated molecular patterns, which are elicitors originating mainly from the herbivore's oral secretions (Bonaventure, 2012), and by damage-associated molecular patterns (DAMPs) that result from wounding or enzymatic reactions imposed by the herbivore (Pearce et al., 1991; McGurl et al., 1992; Schmelz et al., 2006; Yamaguchi and Huffaker, 2011). The recognition of herbivory, similar to pathogen recognition, triggers a basal defense response in the attacked plant. Jasmonic acid (JA) has been implicated in playing a major role in regulating herbivory-associated molecular pattern/DAMP-induced defense responses against a range of herbivores (Browse, 2009). The conjugated form, JA-Ile, is the major biologically active jasmonate, although the JA precursor 12-oxo-phytodienoic acid (OPDA) is also able to regulate defense responses in some instances (Stintzi et al., 2001). Besides JA, other hormones, in particular salicylic acid (SA) and ethylene (ET), have also been linked to herbivore responses through their ability to either modulate JA metabolism and signaling (Robert-Seilaniantz et al., 2011) or induce JA-independent responses (Li et al., 2006), contributing to the specificity of plant defenses. The functional output of herbivore-induced defense is the synthesis of compounds that affect herbivore performance, such as antidigestive proteins (e.g. protease inhibitors) and volatiles that attract predators and/or parasites (e.g. volatile terpenes; Zhu-Salzman et al., 2008; Wu and Baldwin, 2010; Santamaria et al., 2012).

Among insect herbivores, the type of damage to plant tissue varies. For example, chewing insects such as caterpillars and beetles consume significant portions of the plant tissue. In contrast, others such as thrips combine rasping and sucking to feed, while the phloem-feeding insects like aphids connect their feeding tube to the phloem and thus cause minimal tissue disruption. Another group of arthropods of major ecological and agricultural importance are the chelicerates, which include phytophagous (plant-feeding) mites that pierce plant tissue to feed on cell contents (Tanigoshi and Davis, 1978; Campbell et al., 1990). Of these, the two-spotted spider mite (*Tetranychus urticae*) is the best characterized. This species is a major agricultural pest worldwide and has been documented to feed on over 1,100 different plant species, including more than 150 crop plants. Associated with this extraordinary host range, spider mite rapidly develops pesticide resistance (Van Leeuwen et al., 2010), making it one of the most difficult agricultural pests to control. Recently, a high-quality Sanger-sequenced genome of spider mite has become available (Grbić et al., 2011), as have tools and protocols that allow advanced genomic analyses and genetic mapping studies (Dearden et al., 2002; Grbić et al., 2007; Khila and Grbić, 2007; Van Leeuwen et al., 2012, 2013; Dermauw et al., 2013). Consistent with its agricultural importance, studies of plant responses to spider mite herbivory have focused largely on crop plants such as beans (*Phaseolus vulgaris*) and tomatoes (*Solanum lycopersicum*). JA and SA were implicated in regulating tomato responses to mite feeding (Ament et al., 2004, 2006; Li et al., 2004), and the limited transcriptional data also identified JA-, SA-, and ET-regulated genes to be induced in tomato by spider mite herbivory (Kant et al., 2004). Emission of volatile blends of terpenes and methyl salicylate (MeSA) was shown to be involved in indirect defenses against spider mites in a wide range of plant species (Dicke et al., 1998; Ament et al., 2004).

Among plant species, Arabidopsis (*Arabidopsis thaliana*) affords exceptional opportunities for functional studies of plant-arthropod interactions. Owing in part to its unprecedented genetic and genomic toolkits (Koornneef and Meinke, 2010), the response of Arabidopsis to herbivory by insects in several feeding guilds has already been investigated (Reymond et al., 2004; De Vos et al., 2005; Kempema et al., 2007). Furthermore, in laboratory settings, spider mite is a pest of Arabidopsis and has been documented on a number of related species in the Brassicaceae family to which Arabidopsis belongs (Migeon and Dorkeld, 2013). In this study, we have introduced a novel platform for studies of herbivore-plant interactions that uses genomic and genetic resources developed for Arabidopsis and spider mite, allowing us to examine reciprocal genome-wide responses between a plant and a mite.

RESULTS

Natural Genetic Variation in Arabidopsis to Spider Mite-Induced Damage

To test if Arabidopsis accessions vary in susceptibility to spider mite-induced damage, we placed 10 adult female mites on each of 26 genetically and geographically diverse natural accessions and allowed them to feed for 4 d (Supplemental Table S1). For this and all subsequent experiments, mites were from the reference spider mite London strain adapted to and propagated on bean plants. To quantify the resulting mite-induced damage to Arabidopsis accessions, we assessed the total area of chlorosis, a commonly used diagnostic feature of mite damage on crop plants. Among accessions, chlorosis varied by approximately 20-fold (Fig. 1A), with Blanes (Bla-2) showing the least amount of damage and Kondara (Kon) showing the most damage (2 versus 40 mm^2

of total chlorotic area, respectively). Additional characterization of mite feeding responses in Bla-2 and Kon revealed that Bla-2 had a reduced frequency of dead, trypan blue-positive cell patches relative to Kon. Individual patches were similar in size between Bla-2 and Kon, suggesting that the frequency and extent of feeding in Bla-2 were less (Fig. 1B). Consistent with this finding, adult mites preferred to feed on Kon plants relative to Bla-2 in a choice experiment (Fig. 1C). Associated with feeding preference, larvae took longer to develop on Bla-2 relative to Kon leaves, and larval mortality was higher when mites fed on Bla-2 leaves (Fig. 1D).

Induced Arabidopsis Responses to Spider Mite Feeding

To further understand the response to spider mite herbivory, we assayed transcriptional responses in Bla-2 and Kon, the accessions at the opposing ends of the resistance spectrum. To do so, we challenged plants with mites in both time-course and feeding-site (FS) designs. In field conditions, adult female mites establish new colonies by crawling to new plants or by rafting on wind currents. Thus, for the time-course experiment, each plant was inoculated with 10 mites to mimic a normal (low-density) dispersal scenario. These mites were allowed to feed for 1, 3, 6, 12, and 24 h post infestation, after which shoot tissue was collected and RNA was prepared and hybridized to the ATH1 expression microarray (Supplemental Fig. S1). As compared with noninfested control samples, 841 differentially expressed genes (DEGs) were detected in at least one of the five time points in at least one accession (Fig. 2A; Supplemental Data S1), with absolute fold change (FC) > 2 and Benjamini-Hochberg (BH) false discovery rate adjusted $P < 0.01$ using the Bioconductor package limma (Smyth and Speed, 2003).

Specific responses at feeding sites, especially at early time points, might be missed in the time-course experiment, as only a tiny fraction of plant cells were initially damaged by the 10 mites. Therefore, to robustly capture early/local plant responses at feeding sites, we applied hundreds of mites to completely cover rosette leaves. At 1 h post infestation, when effectively the entire plant was a feeding site, shoot tissue was harvested and RNA was isolated for expression studies as described above. In this FS experiment, we detected 660 genes as differentially expressed relative to the noninfested control in at least one accession. The cumulative number of DEGs in both the time-course and FS data sets was 1,109 (Supplemental Data S1).

Despite the difference in damage caused by mite feeding between Bla-2 and Kon, transcriptional responses to spider mite herbivory in the two accessions were similar (Fig. 2A; Supplemental Data S1; Supplemental Fig. S1). The Bla-2 and Kon accessions

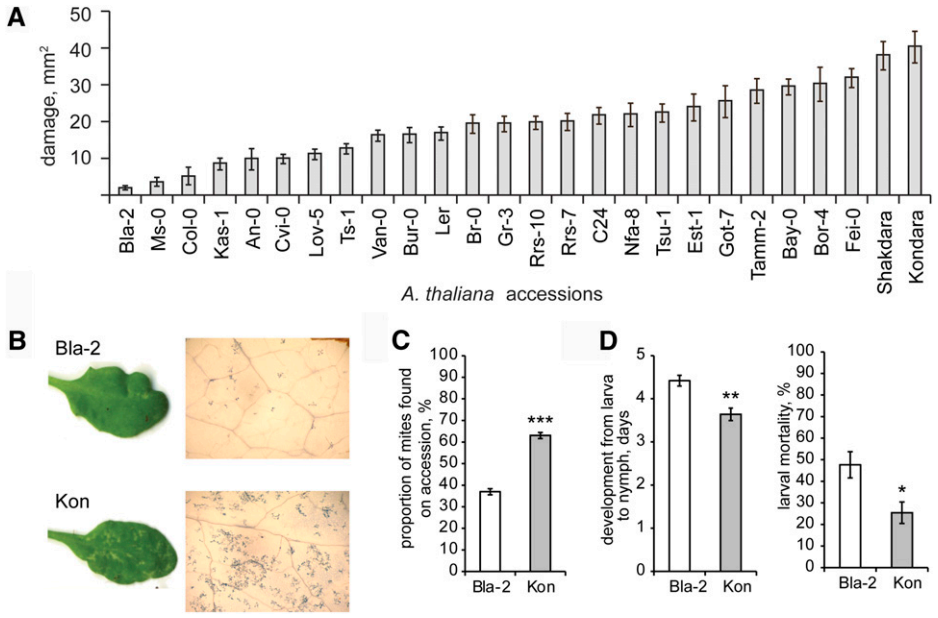

Figure 1. Arabidopsis damage by spider mites. A, Extent of damage, as assessed by the total area of chlorotic spots, of 26 Arabidopsis accessions exposed to 10 mites for 4 d ($n = 6$ plants per accession). Shown are means ± SE. B, Representative damage and trypan blue staining of Bla-2 and Kon leaves after 24 h of spider mite feeding. C, Spider mite accession preference in a choice experiment. Shown are means ± SE percentage of recovered mites ($n = 22$ sets of plants inoculated with 40 mites per set). D, Spider mite performance on Bla-2 and Kon as assessed by mean ± SE days required for larvae to become nymphs and mean ± SE percentage of larval mortality ($n = 5$ sets of 50 larvae). Asterisks represent significantly different comparisons (unpaired Student's t test, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). Replicated experiments of the same comparisons produced similar results. Full names and sources of studied accessions are listed in Supplemental Table S1.

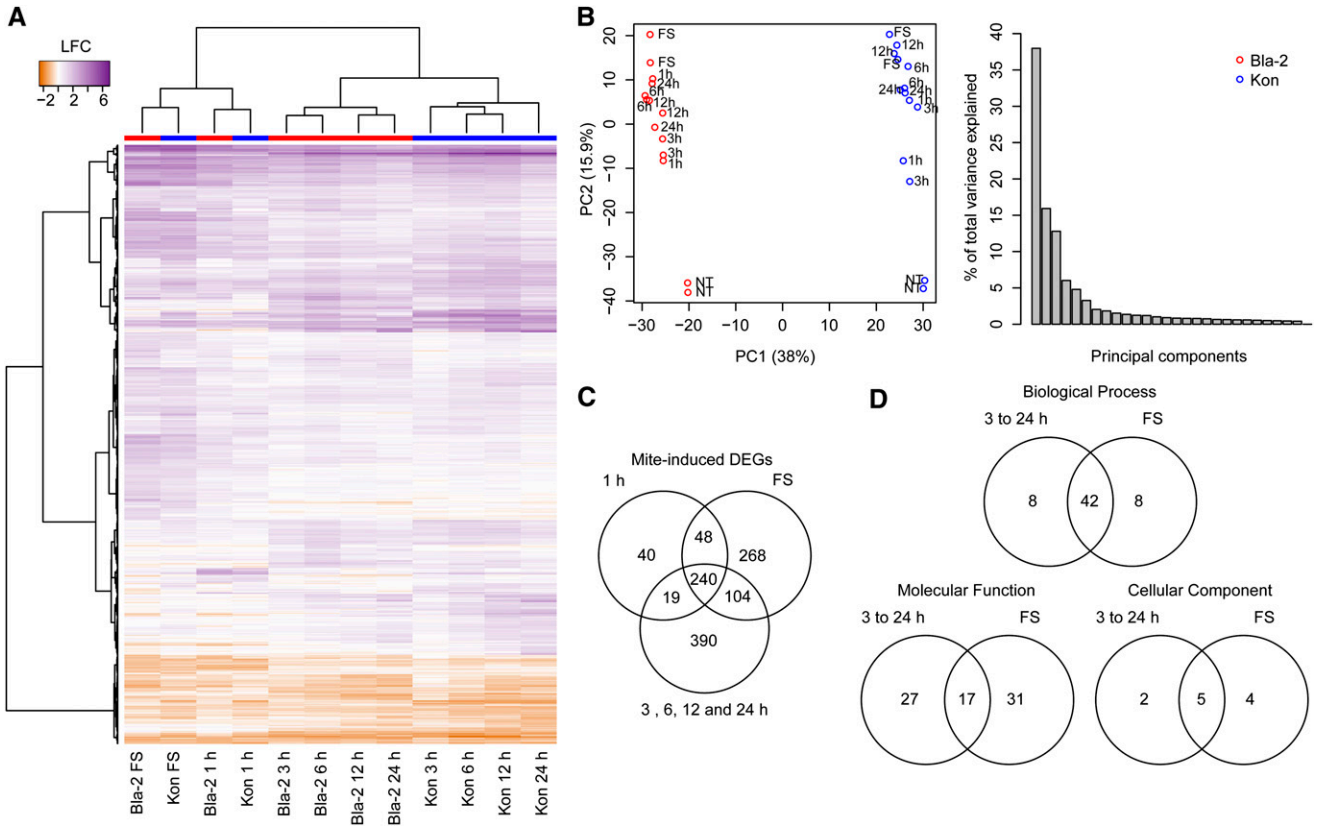

Figure 2. Transcriptional responses to spider mite feeding in Arabidopsis accessions Bla-2 and Kon. A, Hierarchical clustering analysis of $\log_2$ FC (LFC) exhibited by DEGs with absolute FC > 2 and BH-adjusted $P < 0.01$ detected upon spider mite feeding for 1, 3, 6, 12, and 24 h and feeding of hundreds of mites for 1 h (FS sample). The distance metric was Euclidean, and the clustering method was Ward's. B, Principal component analysis of expression measures data for Bla-2 and Kon. C, Analysis of DEGs from 1 h, 3- to 24-h, and FS treatments. D, GO analysis of DEGs detected in FS and 3- to 24-h samples. Up to 50 biological process, molecular function, and cellular component GO categories with weighted Fisher's $P < 0.05$ are compared. Blue samples, Bla-2; red samples, Kon; NT, nontreated samples.

are highly diverse genetically, and in a principal component analysis, the majority of variance in gene expression was due to the factor attributable to accession (principal component 1; Fig. 2B). Nonetheless, the factor attributed to mite treatment, principal component 2, revealed that responses of the two accessions to mite herbivory were similar. Consistent with the expectation that responses should be induced upon exposure of plants to herbivory, the majority of the DEGs were found to be up-regulated (Fig. 2A). Clustering analysis of the 1,109 DEGs revealed that the transcriptional responses for the 1-h and the FS samples were more similar to each other than to those of later time points (3, 6, 12, and 24 h; Fig. 2A). The majority of the 347 DEGs identified in the 1-h samples were present not only in the FS data set but also at later time points (3–24 h; Fig. 2B). An additional 390 induced genes were detected later in the response (Fig. 2B). It is striking that between the 1-h and FS samples, which represent herbivory of 10 versus hundreds of mites for 1 h, only a small number of genes were differentially expressed (55 in Bla-2 and 71 in Kon out of 719; Supplemental Data S2). However, the absolute FC values of genes observed in the FS samples were higher (Fig. 2A; Supplemental Fig. S1); thus, we used the FS data for subsequent analyses of initial plant responses to mite herbivory.

Gene Ontology (GO) enrichment analysis of biological processes revealed that DEGs detected in the FS and 3- to 24-h samples were enriched in genes involved in defense responses common to many biotic stresses (Fig. 2C; Supplemental Data S3). The majority (42) of the top 50 biological process GO terms overlapped between the FS and later time-course samples, indicating that defense programs against mite herbivory are already established within the first 1 h. Among overlapping processes are the biosynthesis of and responses to the JA, SA, abscisic acid (ABA), and ET plant hormones as well as processes associated with early responses to biotic stress, such as responses to fungus/chitin, regulation of hydrogen peroxide metabolism, the respiratory burst involved in defense response, and mitogen-activated protein kinase cascades. However, DEGs annotated to overlapping GO categories can be clearly separated between early and late responses.

For example, response to JA stimulus (GO:0009753) is common to both FS and 3- to 24-h data sets. JA-responsive genes in the FS sample (149) included JA biosynthetic and JA-modifying genes (e.g., *ALLENE OXIDE SYNTHASE* [*AOS*], *ALLENE OXIDE CYCLASE3* [*AOC3*], *LIPOXYGENASE*s [*LOX*], *OXOPHYTODIENOATE-REDUCTASE3*, *OPC-8:0 COA LIGASE1*, *JASMONIC ACID CARBOXYL METHYLTRANSFERASE*, *CYTOCHROMES P450*s [*CYP*] *CYP94C1*, and *CYP94B3*), regulators of hormonal responses (e.g., *JASMONATE-ZIM-DOMAIN PROTEIN*s, *MYC2*, ethylene-responsive transcription factors, *NIM1-INTERACTING1*, and *GLUTAREDOXIN480*), genes involved in secondary messenger response (Ca2+-dependent receptors, e.g. *CALCIUM-DEPENDENT PROTEIN KINASE1* and *CALCIUM-DEPENDENT PROTEIN KINASE32*), DAMP receptors (*PEP1 RECEPTOR1* [*PEPR1*], and *PEPR2*), genes encoding lectin-binding proteins (*LECTIN RECEPTOR KINASE4.1*, and *LECTIN RECEPTOR KINASEI.9*), transcription factors (e.g. *ALTERED TRYPTOPHAN REGULATION1* [*ATR1*], *WRKY DNA-BINDING PROTEIN33*, *BASIC REGION/LEUCINE ZIPPER MOTIF60*, and *SALT TOLERANCE ZINC FINGER*), signaling pathway genes (e.g. *MITOGEN-ACTIVATED PROTEIN KINASE*s *MAPKKK14* and *MPK11*, *CAM-BINDING PROTEIN 60-LIKE G*, *CALMODULIN-LIKE38*, *SYNTAXIN OF PLANTS122*, and *PLANT U-BOX23*), and metabolite biosynthetic genes (e.g. *ANTHRANILATE SYNTHASE ALPHA SUBUNIT1*, *PHYTOALEXIN DEFICIENT3* [*PAD3*], *TERPENE SYNTHASE04* [*TSP04*], and *TSP10*); and the JA-responsive genes exclusively present in 3- to 24-h data set (46 out of 148) included genes directly involved in defense (e.g. *PLANT DEFENSIN1.2*, *PAD4*, *TPS03*, *THIONIN2.1*, and *N-ACETYLTRANSFERASE ACTIVITY1*), SA metabolism (e.g. *BSMT1*, *ISOCHORISMATE SYNTHASE1*, and *SAR DEFICIENT1*), and JA and tryptophan metabolism (e.g. *ACYL-COA OXIDASE1* [*ACX1*], *LOX2*, *ABC TRANSPORTER OF THE MITOCHONDRION3*, *TRYPTOPHAN SYNTHASE ALPHA CHAIN*, and *CYP71A13*). The analysis of top-ranking molecular function and cellular component GO terms also revealed differences in the early and late responses. For instance, GO terms exclusively enriched in the FS sample were associated with perception, signaling, and transcriptional activation, while the GO terms specific to the 3- to 24-h response implicated enzymatic activities involved in the production of defense compounds and metabolic alterations in response to herbivore attack (Supplemental Data S3). Thus, the data sets capture genes involved in both the perception of and signaling in response to mite feeding as well as downstream response genes associated with changes in secondary metabolites and the regulation of biotic stress responses.

Differences in Responses between Bla-2 and Kon

In response to mite herbivory, Bla-2 and Kon rapidly activate strong and broadly overlapping transcriptional responses. Nevertheless, they differ by approximately 20-fold in damage in response to mite feeding. Therefore, we examined preexisting interaccession variation in specific plant defense pathways known to be implicated in responses to biotic stresses. From the ATH1 microarray data, we detected 993 DEGs between Bla-2 and Kon in the absence of spider mite herbivory (Fig. 3A; Supplemental Fig. S2; Supplemental Data S4), and these differences were typically maintained between Bla-2 and Kon plants even after challenge with spider mites (Supplemental Fig. S2). This finding is consistent with recent studies that have documented extensive variation in gene expression among Arabidopsis accessions (Gan et al., 2011). Although polymorphism between accessions can confound the detection of differential gene expression with microarrays at specific loci, we observed interaccession expression variation across many genes associated with responses to biotic stimuli. In particular, genes expressed more highly in Bla-2 were associated with defense-related GO categories (Supplemental Data S5). In stark contrast, genes expressed at higher levels in Kon grouped into various categories (Supplemental Data S5), none of which strongly overlapped with GO terms previously associated with herbivore defense (Fig. 3B).

Among the GO categories for DEGs up-regulated in Bla-2 were biosynthetic processes for SA, JA, and glucosinolates, previously implicated in plant responses to herbivory (Chehab et al., 2008). The JA biosynthetic genes expressed more highly in Bla-2 included *LOX6*, *AT4G39730*, *AT2G22170* (lipoxygenases), *AOC3* (involved in the biosynthesis of OPDA), and *ACX5* and *3-KETO-ACYL-COENZYME A THIOLASE5* (involved in the conversion of OPDA to JA). The JA-responsive genes expressed at higher levels in Bla-2 included *CORONATINE-INDUCED PROTEIN1*, *JASMONATE RESPONSIVE1* and *JASMONATE RESPONSIVE2*. Genes required for the biosynthesis of indole (*CYP79B2*) and aliphatic (*MYB29*, *METHYLTHIOALKYLMALATE SYNTHASE1* and *AT2G25450*) glucosinolates and their activation (*EPITHIOSPECIFIER PROTEIN*) were also expressed at higher levels in Bla-2. While these findings suggest that the differential expression of JA- and glucosinolate-related genes may be responsible for the observed differential susceptibility of Arabidopsis accessions, our subsequent analysis demonstrated that basal levels of metabolites that affect mite herbivory were only modestly different between Bla-2 and Kon. Furthermore, a comparison of the DEGs shared between noninduced Bla-2 and Kon and those induced by spider mites identified 98 genes that were up-regulated in Bla-2 and 26 genes that were up-regulated in Kon that were also further induced by mite feeding (Fig. 3, A and C). While the 98 genes in Bla-2 are associated with GO terms related to plant defense, the 26 genes in Kon have heterogeneous functions, with the majority being related to the general "metabolic process" term (Supplemental Data S5). Collectively, our findings suggest that differences in the constitutive expression of a range of defense-associated genes and their induction upon mite feeding may

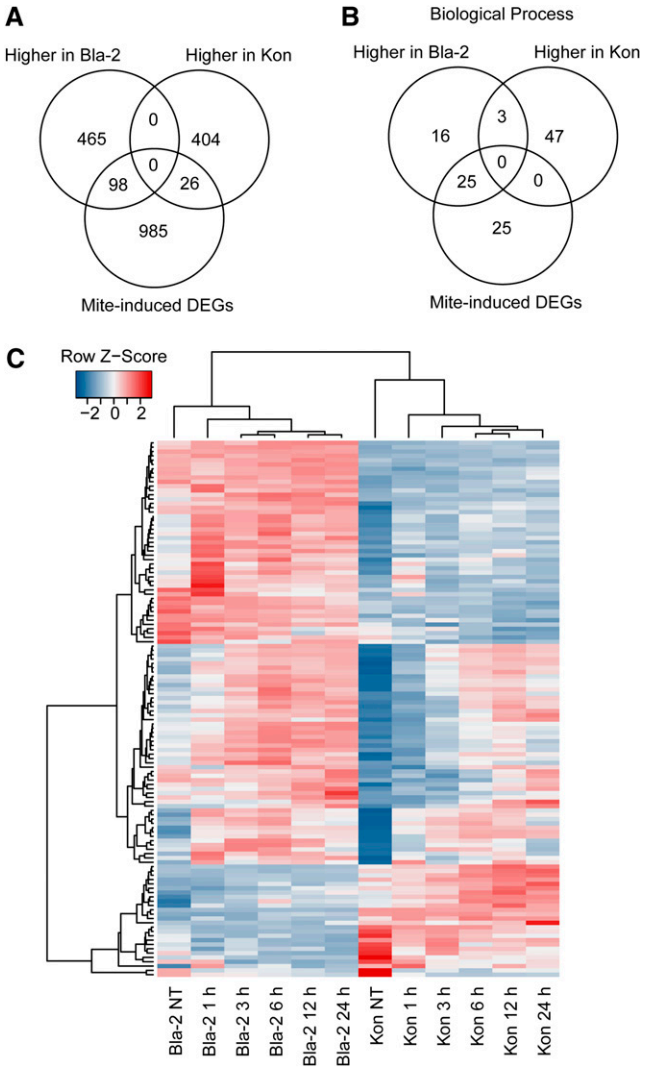

Figure 3. Analysis of a preexisting transcriptional difference between Bla-2 and Kon and its stability upon spider mite feeding. A, Analysis of DEGs detected between nontreated Bla-2 and Kon. B, GO analysis of DEGs detected between nontreated Bla-2 and Kon. Up to 50 biological process GO categories with weighted Fisher's $P < 0.05$ are compared. C, Hierarchical clustering analysis of expression measures of 124 DEGs that were detected as inducible by spider mite feeding and exhibited differences of expression levels in nontreated Bla-2 and Kon. The distance metric was Pearson's correlation coefficient, and the clustering method was average. NT, Nontreated samples.

contribute to the higher level of resistance to mite herbivory observed for Bla-2 relative to Kon.

Meta-Analysis of the Arabidopsis Response to Spider Mite Feeding

To establish the relationship between Arabidopsis responses to spider mite feeding and that of other herbivores, pathogens, and hormones, we performed a meta-analysis using publicly available expression data. To do this, the Genevestigator online tool (Hruz et al., 2008) was used to select and retrieve available experimental data (Supplemental Table S2). To compare between experiments, we used 470 genes that demonstrated a high degree of variability in responses across studies included in this meta-analysis (Supplemental Data S6; Supplemental Fig. S3). Within our data set, we selected the FS, 6-h, and 24-h samples of Bla-2 and Kon, as they were representative of the range of responses observed upon mite herbivory (Fig. 2). Of the 470 genes included in our meta-analysis, 311 (66%) were differentially regulated in our study. The gene list used for the meta-analysis was enriched for genes involved in perception and response to a range of stimuli, including JA, SA, ET, ABA, and auxin (indole-3-acetic acid) pathways (Supplemental Data S7).

Both Bla-2 and Kon responses to spider mite feeding at different times grouped together in a cluster that also included early responses (less than 24 h) to other herbivores as well as wounding (Fig. 4; Supplemental Fig. S2). The early response to spider mite feeding (FS) clustered most closely with the wound response at 1 h, indicating that response to physical damage (tissue and cell piercing) is a major component of the early plant response to spider mite herbivory. Of the eight different hormone treatments for which data were available, only responses to methyl jasmonate/OPDA clustered together with mite-triggered responses. Mite responses further clustered with the response profiles of caterpillar (*Pieris rapae*) and early thrips (*Frankliniella occidentalis*) herbivory, which are known to activate JA signaling (Jander and Howe, 2008). The remaining three clusters (responses to pathogens, abiotic stimuli, and late [more than 24 h] responses to herbivores and wounding; responses to ABA and long-term SA stimulus; and short-term responses to hormones) showed no relatedness with mite-induced responses. Thus, our meta-analysis suggests that JA is the major hormonal signal associated with the Arabidopsis response to spider mite feeding.

Hormone Signaling in Response to Spider Mite Feeding

The similarity of gene expression profiles between spider mite- and methyl jasmonate/OPDA-triggered responses (Fig. 4) suggested that JA-mediated signaling is important in Arabidopsis defense responses to spider mite herbivory. As assessed by mass spectrometry, we found that both JA and the bioactive jasmonate JA-Ile accumulate upon mite herbivory within the 24-h response period (Fig. 5A). To directly test the importance of JA signaling in the response of Arabidopsis to mite herbivory, we used mutants with disrupted JA biosynthesis (*aos*) or signaling (*myc2 myc3 myc4*; Park et al., 2002; Fernández-Calvo et al., 2011). Columbia (Col-0) plants incurred similar levels of damage as Bla-2 in the initial survey of Arabidopsis accessions (Fig. 1) and accumulated JA-Ile in a pattern nearly indistinguishable from that of the resistant Bla-2

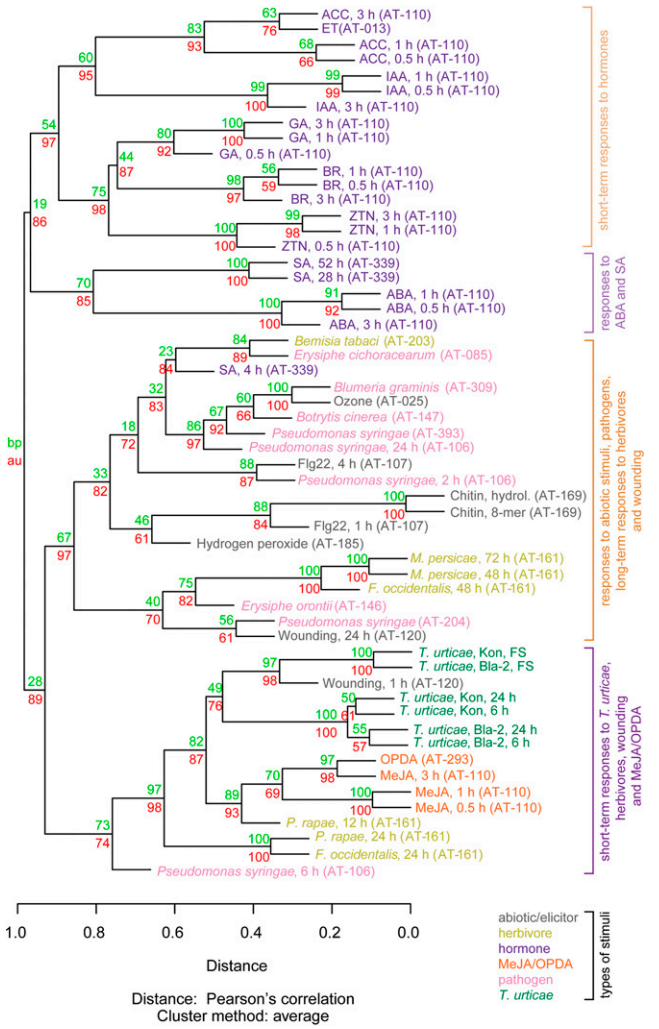

Figure 4. Meta-analysis of Arabidopsis transcriptional responses to a range of biotic, abiotic, and hormonal stimuli. The dendrogram shows the levels of similarity between individual responses. Edge labels are bootstrap *P* values (bp) in green and approximately unbiased *P* values (au) in red. The distance metric was Pearson's correlation coefficient, and the clustering method was average. ACC, 1-Aminocyclopropane-1-carboxylic acid; BR, brassinosteroids; IAA, indole-3-acetic acid; MeJA, methyl jasmonate; ZTN, zeatin.

(Fig. 5A). Both JA-deficient and JA signaling mutants were more severely damaged than wild-type plants upon spider mite feeding. While wild-type plants sustained individual necrotic spots upon mite infestation, leaf damage was significantly expanded in *aos* and *myc2 myc3 myc4* plants, with white and dehydrated leaf patches readily apparent (Fig. 5, B and C). Increased leaf damage in *aos* and *myc2 myc3 myc4* plants was correlated with a dramatic reduction in mite mortality (up to 90% mortality in wild-type plants compared with 5% in mutants; Fig. 5C). In addition, mites progressed significantly faster from the larval to the nymph developmental stage when fed on mutant plants, indicating that JA is key to the establishment of plant defense programs that limit the ability of mites to feed.

In addition to JA, spider mite feeding induced some SA biosynthesis and signaling genes, and SA accumulated in infested plants (Supplemental Fig. S4). However, deficiency in SA and downstream signaling (*enhanced disease susceptibility1-1*, *salicylic acid induction deficient2-2*, *enhanced disease susceptibility5-1*, *nonexpresser of pr genes1-1*, and plants carrying bacterial salicylate hydroxylase nahG) did not significantly affect plant damage or mite performance (Supplemental Fig. S4). These results are consistent with our meta-analysis of transcriptional responses, which suggests that SA is not a major contributor in Arabidopsis defenses against spider mite herbivory.

Indole Glucosinolates Affect Mite Herbivory on Arabidopsis

The prominent effect of JA-related mutants on mite mortality indicates that the synthesis of JA-regulated defense compounds plays a major role in the Arabidopsis defense against spider mites. Mite feeding induced genes associated with the Trp catabolic and indoleacetic acid biosynthetic processes (Supplemental Data S3) that are coupled with the biosynthesis of the plant secondary metabolites indole glucosinolates (IGs). Both basal and induced levels of genes encoding the IG-committing enzymes *CYP79B2* and *CYP79B3* were dependent on JA (Fig. 6B; Supplemental Table S3), indicating that IGs may be one of the functional outputs of the JA-regulated Arabidopsis defense against spider mite herbivory. In addition, levels of indol-3-ylmethyl glucosinolate, 1-methoxy-indol-3-ylmethyl glucosinolates (neoglucobrassicins), and 4-hydroxyindol-3-ylmethyl glucosinolate increased in Bla-2, Kon, and Col-0 plants upon mite feeding (Fig. 6A). To test if IGs affect mite herbivory, we checked if plant damage and mite performance correlate with the levels and the composition of IGs (Fig. 6, C–E). The quadruple knockout mutant *myb28 myb29 cyp79b2 cyp79b3* (qKO) that lacks both IGs and aliphatic glucosinolates (AGs; Sun et al., 2009; Supplemental Fig. S5), the double mutant *cyp79b2 cyp79b3* that lacks IGs (Zhao et al., 2002), and the *cyp81f2* mutant that has reduced levels of a subset of IG metabolites (4-hydroxy-indole-3-ylmethyl and 4-methoxy-indole-3-ylmethyl; Pfalz et al., 2009) all showed increased susceptibility to spider mite herbivory relative to Col-0 plants. Furthermore, we used *atr1D*, a dominant overexpression allele of the *ATR1* transcription factor that activates the expression of the IG biosynthetic genes and overaccumulates IGs (Celenza et al., 2005; Supplemental Fig. S5). The increased plant resistance of *atr1D* plants to mite herbivory was apparent. Likewise, larval mortality was dramatically reduced, and mite development time was faster, in the qKO and *cyp79b2 cyp79b3* mutants. The opposite trend was observed in the *atr1D* genotype, which overproduces IGs that displayed larval mortality of 100% (Fig. 6). These data indicate that IGs profoundly affect mites' ability to feed on Arabidopsis.

In contrast to IGs, mite feeding did not induce the expression of genes involved in the biosynthesis of the

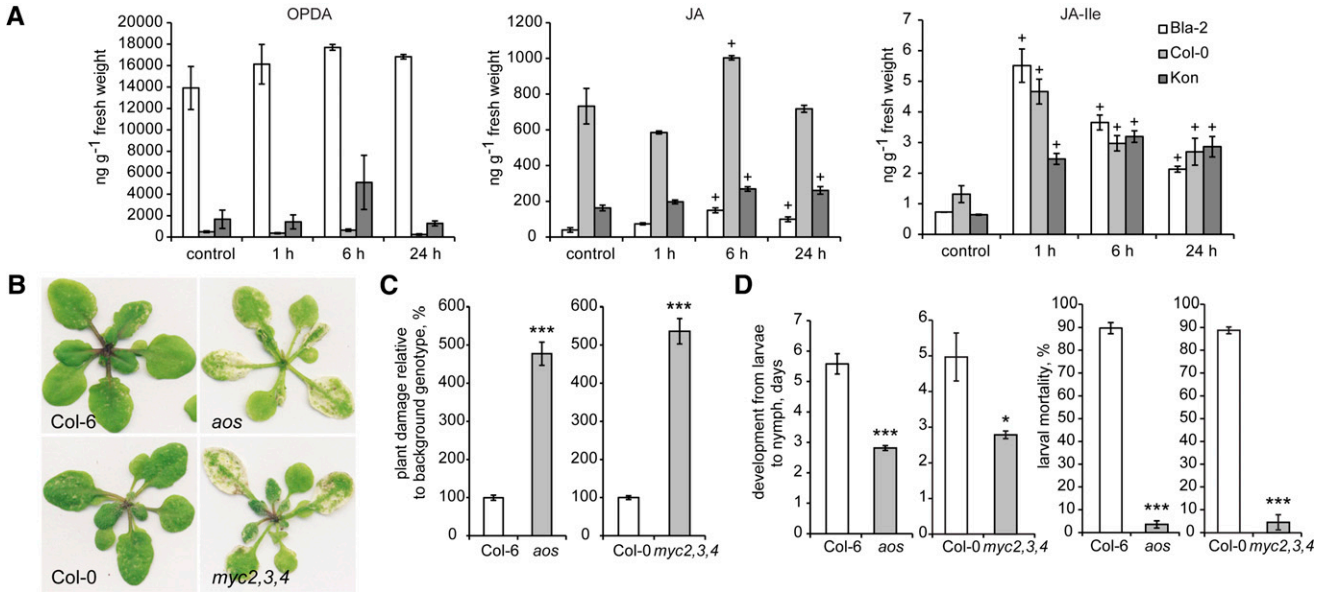

Figure 5. Susceptibility of Arabidopsis JA-related mutants *aos* and *myc2 myc3 myc4* to spider mite herbivory. A, Levels of OPDA, JA, and JA-Ile (ng g^{-1} fresh weight) in 3-week-old Bla-2, Col-0, and Kon plants after spider mite herbivory for 1, 6, and 24 h. Values are means ± SE ($n = 3$). Crosses indicate significant differences for planned comparisons relative to corresponding control plants ($P < 0.05$, pairwise Student's *t* tests with pooled SD and the Holm-Bonferroni adjustment of *P* values). B and C, Susceptibility of Arabidopsis JA-related mutants *aos* (Columbia-6 background) and *myc2 myc3 myc4* (*myc2,3,4*; Col-0 background) to spider mite herbivory. Mean ± SE chlorotic area is expressed relative to the mean chlorotic area of corresponding wild-type plants ($n = 12$). D, Spider mite performance as assessed by mean ± SE days required for larvae to become nymphs and mean ± SE percentage of larval mortality upon feeding on Arabidopsis JA-related mutants ($n = 5$ sets of 50 larvae). Asterisks indicate significant differences from corresponding wild-type plants (unpaired Student's *t* test, $*P < 0.05$, $***P < 0.001$). Individual subgroups of comparisons in C and D are not directly comparable, as they were performed as separate experiments. Replicated experiments of the same comparisons produced similar results.

AGs (Fig. 6B; Supplemental Data S1). Since basal levels of AGs could affect mite herbivory, we checked the effect of mutants with impaired functions of regulators of AG biosynthesis, *myb28*, *myb29*, and *myb28 myb29*, on plant damage and mite performance (Sønderby et al., 2007; Beekwilder et al., 2008). We found that these parameters were similar in mutant plants and the wild type (Supplemental Fig. S6), indicating that AGs do not contribute to defense against spider mites.

To further address the possibility that the observed phenotypes could be due to camalexin, an indole alkaloid phytoalexin whose biosynthesis is also dependent on CYP79B2 and CYP79B3 enzymes, we determined its levels in the plants used in our analysis. Spider mite herbivory induced the accumulation of camalexin, but the camalexin levels in qKO, Col-0, and *atr1D* did not correlate with plant damage or mite performance (Supplemental Figs. S5 and S7). Furthermore, the *pad3-1* mutant that lacks camalexin biosynthesis (Zhou et al., 1999) sustained less damage than the wild type upon mite feeding and did not affect mite performance (Supplemental Fig. S7). Thus, our data suggest that AG and camalexin do not have major effects on spider mite herbivory in Arabidopsis but that both the level and the composition of IGs are important determinants of mites' ability to use Arabidopsis as a host. The similarity in the effects of JA- and IG-deficient mutants on mite performance indicates that IGs are the major defense output of Arabidopsis to mite herbivory.

Spider Mites Respond Transcriptionally to Increasing Levels of IGs

Mite larval mortality was specifically affected by IGs, and we asked whether spider mites are able to mount a transcriptional response after host transfer from bean plants to Arabidopsis lines with varying levels of IGs. Genome-wide expression analysis in mites was carried out using a custom Agilent gene expression microarray (Dermauw et al., 2013). To avoid potential confounding that might arise from differing developmental rates of immature stages by plant genotype, which can affect expression patterns generally, we used adult female mites in these experiments. Reflecting the findings with larvae, adult mortality at 24 h post transfer correlated with IG levels in Arabidopsis lines (qKO, 2.5%; Col-0, 6.1%; and *atr1D*, 9.5%; Fig. 7A).

We detected a total of 613 DEGs (absolute FC > 2 at BH-adjusted $P < 0.05$ in at least one host-transfer comparison) in mites 24 h post transfer from bean to at least one of the Arabidopsis lines (Fig. 7B; Supplemental

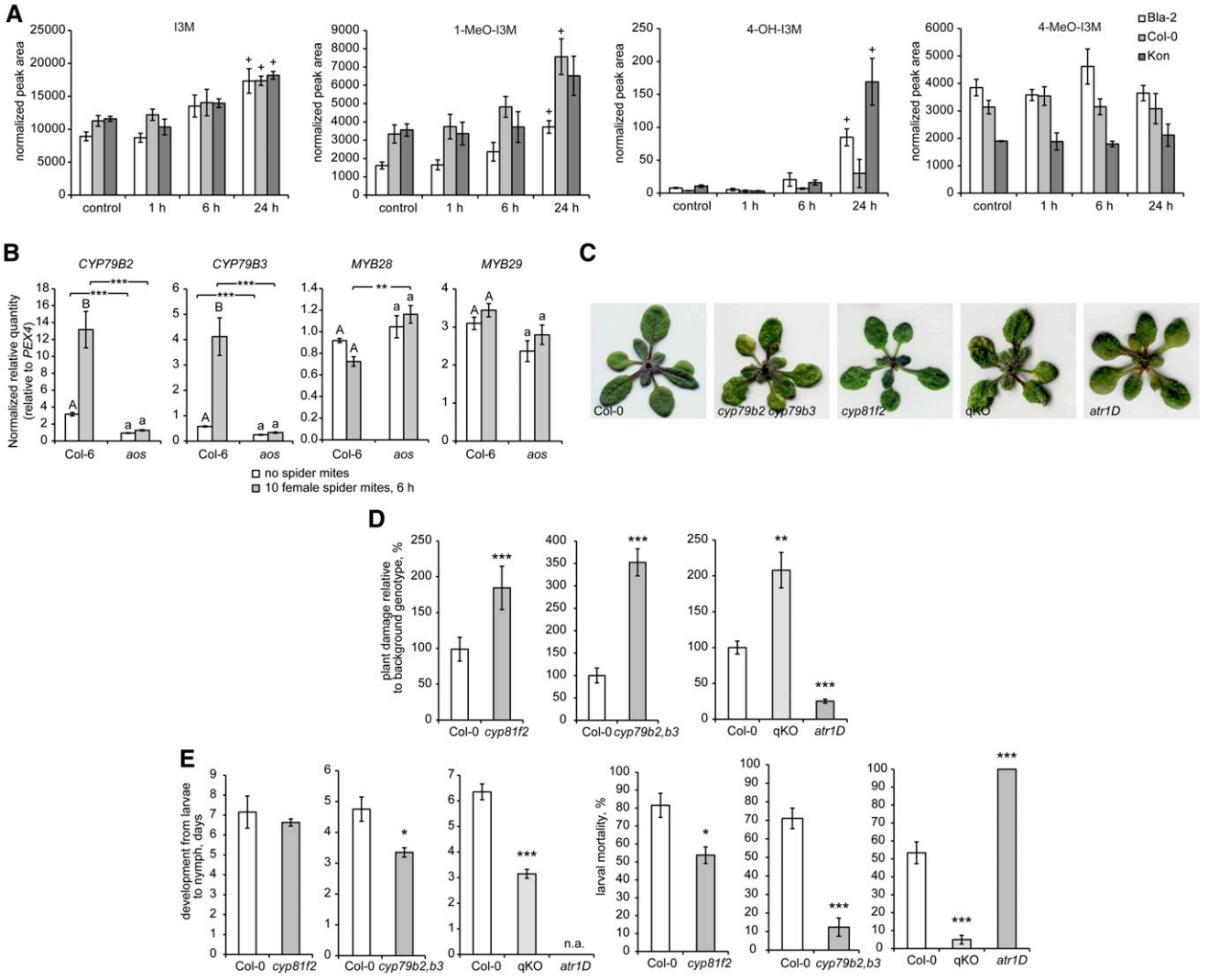

Figure 6. Role of IGs in the Arabidopsis response to spider mite herbivory. A, Relative levels of the IGs indol-3-ylmethyl glucosinolate (I3M), 1-methoxy-indol-3-ylmethyl glucosinolates (neoglucobrassicins; 1-MeO-I3M), 4-hydroxyindol-3-ylmethyl glucosinolate (4-OH-I3M), and 4-methoxyl-3-ylmethyl glucosinolate (4-MeO-I3M; shown as normalized peak areas) in 3-week-old Bla-2, Col-0, and Kon plants after spider mite herbivory for 1, 6, and 24 h. Values are means ± SE ($n = 3$). Crosses indicate significant differences for planned comparisons relative to corresponding control plants ($P < 0.05$, pairwise Student's *t* tests with pooled SD and the Holm-Bonferroni adjustment of *P* values). B, Induction of *CYP79B2*, *CYP79B3*, *MYB28*, and *MYB29* genes in response to spider mite feeding. Shown are means ± SE for FCs of expression levels detected by RT-qPCR in Columbia-6 and *aos* plants ($n = 4$). Letters indicate significant comparisons within a genotype (lowercase, *aos*; uppercase, Columbia-6; Tukey's HSD test, $P < 0.001$). Asterisks indicate significant differences in expression levels within treatment type and between genotypes (Tukey's HSD test, $^{**}P < 0.01$, $^{***}P < 0.001$). C and D, Susceptibility of Arabidopsis mutants with altered levels of glucosinolates to spider mite herbivory. *cyp79b2 cyp79b3* (*cyp79b2,b3*) and *cyp81f2* plants lack all or a subset of IGs, qKO plants lack both indole and aliphatic glucosinolates, and *atr1D* plants have elevated levels of IGs. Mean ± SE chlorotic area is expressed relative to the mean chlorotic area of corresponding wild-type plants ($n = 12$). E, Spider mite performance as assessed by mean ± SE days required for larvae to become nymphs and mean ± SE percentage of larval mortality upon feeding on Arabidopsis mutants with altered levels of glucosinolates ($n = 5$ sets of 50 larvae). Asterisks indicate significant differences from corresponding wild-type plants (unpaired Student's *t* test: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). Individual subgroups of comparisons in D and E are not directly comparable, as they were performed as separate experiments. Replicated experiments of the same comparisons produced similar results. n.a., Developmental time data was not collected due to 100% larval mortality.

Data S8; Supplemental Fig. S8). The number of DEGs was positively related to increasing IG levels and ranged from 313 for qKO, to 391 for Col-0, and to 523 for *atr1D* that overaccumulates IGs, suggesting that spider mites perceive IGs, directly or indirectly, as toxicants or stress factors.

To further understand mite responses to increasing levels of IGs, we focused on genes that showed a consistent increase or decrease in expression levels across the qKO-Col-0-*atr1D* genotype spectrum (absolute FC > 1.25, BH false discovery rate-adjusted $P < 0.05$). We detected 40 genes whose expression levels correlated with increasing levels of IGs (Fig. 7C). These IG dose-dependent responsive genes primarily encoded detoxifying enzymes such as P450 monooxygenases, glycosyltransferases, and lipocalins, all of which have previously been implicated in responses to xenobiotic stress (Li et al., 2007; Dermauw et al., 2013).

To further assess the relevance and specificity of the observed response, we compared our data with a previously published study that described transcriptional responses of spider mites to host transfer from bean to tomato (cv Moneymaker), a species that does not accumulate glucosinolates (Dermauw et al., 2013). Only a limited overlap between the DEGs identified in this study and the DEGs identified after transfer to tomato was apparent (47%, 289 of 613). The overlap with genes that responded to glucosinolates in a dose-dependent manner was similar (50%, 20 of 40) and was even less with genes induced by mite feeding on the *atr1D* mutant that overaccumulates IGs (33%, 56 of 172; Fig. 7D). In addition, the set of P450 monooxygenases that were dose responsive to IG levels in this study exhibited higher induction of gene expression upon transfer of spider mite larvae from bean to Arabidopsis than upon transfer to tomato, as assessed in an earlier study (Grbić et al., 2011). Cumulatively, these findings indicate partial specificity in the response of mites to Arabidopsis and to IGs.

DISCUSSION

The transcriptional responses of Arabidopsis to spider mite feeding during the initial 24-h period are characterized by DEGs that can be partitioned into two broad groups. First, genes induced within the first 1 h were associated with the recognition of herbivory, signaling, and induction of defense programs. They significantly overlapped with DEGs induced upon the application of pathogen elicitors such as chitin (Supplemental Data S3), consistent with the emerging view that plant perception and signaling mechanisms are conserved between pathogen- and herbivore-induced responses (Erb et al., 2012). The second group of DEGs, induced in the 3- to 24-h period, encodes enzymes involved in the biosynthesis of a wide range of secondary metabolites. The expression of early-response genes is maintained throughout mite infestation; thus, later responses reflect the combination of both early- and late-induced DEGs. The spider mite-induced responses were similar to responses induced by caterpillar and thrips, despite differences in tissue damage caused by these herbivores. The conservation among Arabidopsis induced defense responses across a range of biotic stressors suggests that the plant responds with limited specificity to a given attacker. Our data further showed that only a subset of programs induced by mite herbivory were effective against spider mite. In particular, JA biosynthesis and signaling and IGs were central to Arabidopsis's defense, accounting for the profound toxicity of Arabidopsis tissues to both larvae and adult mites.

The other salient feature of the Arabidopsis response to mite herbivory is its limited dependence on the magnitude of the initial signal. The number of mites applied (10 versus hundreds of mites in 1-h versus FS treatments) did not result in proportionally different transcriptional responses, implying the existence of regulatory mechanisms acting to both amplify and restrain the magnitude of the plant responses to mite feeding. Spider mite herbivory induced the expression of *PEPR1* and *PEPR2* DAMP receptors that have been implicated in the amplification of PAMP- and herbivory-induced responses (Huffaker and Ryan, 2007; Huffaker et al., 2013). Likewise, the establishment of systemic defense responses that result in the de novo synthesis of JA throughout the plant is expected to result in the enhancement of defense signaling (Truman et al., 2007; Koo et al., 2009). On the other hand, mite feeding induces the expression of *JASMONATE-ZIM-DOMAIN PROTEIN* repressors that act as negative regulators of JA perception and signaling (Chung et al., 2008) and enzymes (*CYP94B3* and *CYP94C1*) that convert JA-Ile to physiologically inactive forms (Koo et al., 2011; Heitz et al., 2012).

Among Arabidopsis accessions, susceptibility to damage by spider mite varied greatly, mirroring intraspecific variation in the plant response as reported for caterpillar and aphid herbivory (Kliebenstein et al., 2002, 2005; Müller et al., 2010; Züst et al., 2012). The nature of this variation is unclear. On the one hand, common pathways were activated in the resistant Bla-2 and susceptible Kon; however, Bla-2 plants constitutively overexpressed a subset of defense-related genes relative to Kon, and this difference was maintained post mite infestation. Among genes expressed at higher levels in Bla-2 were *LOXs*, *AOS*, *AOC3*, and *ACX5* (involved in JA biosynthesis) and *CYP79B2*, *MYB29*, *METHYLTHIOALKYLMALATE SYNTHASE1*, *GLH-OH*, and *AOP1* (involved in the biosynthesis of glucosinolates; Supplemental Data S6). This correlated with increased levels of OPDA and AGs and altered composition of IGs in Bla-2 relative to Kon (Figs. 5 and 6; Supplemental Fig. S5). We showed that JA and IGs have pivotal roles in establishing effective defense against mite herbivory (Figs. 5 and 6). However, while alterations in JA biosynthesis and IGs profoundly affected mite development and mortality in Col-0, these parameters were only modestly different between Bla-2 and Kon. In addition, levels of bioactive JA-Ile and total levels of IGs were similar between these accessions. Thus, it seems unlikely that changes in the expression of JA and glucosinolate biosynthetic genes can explain the increased susceptibility of Kon relative to Bla-2. Rather, either a

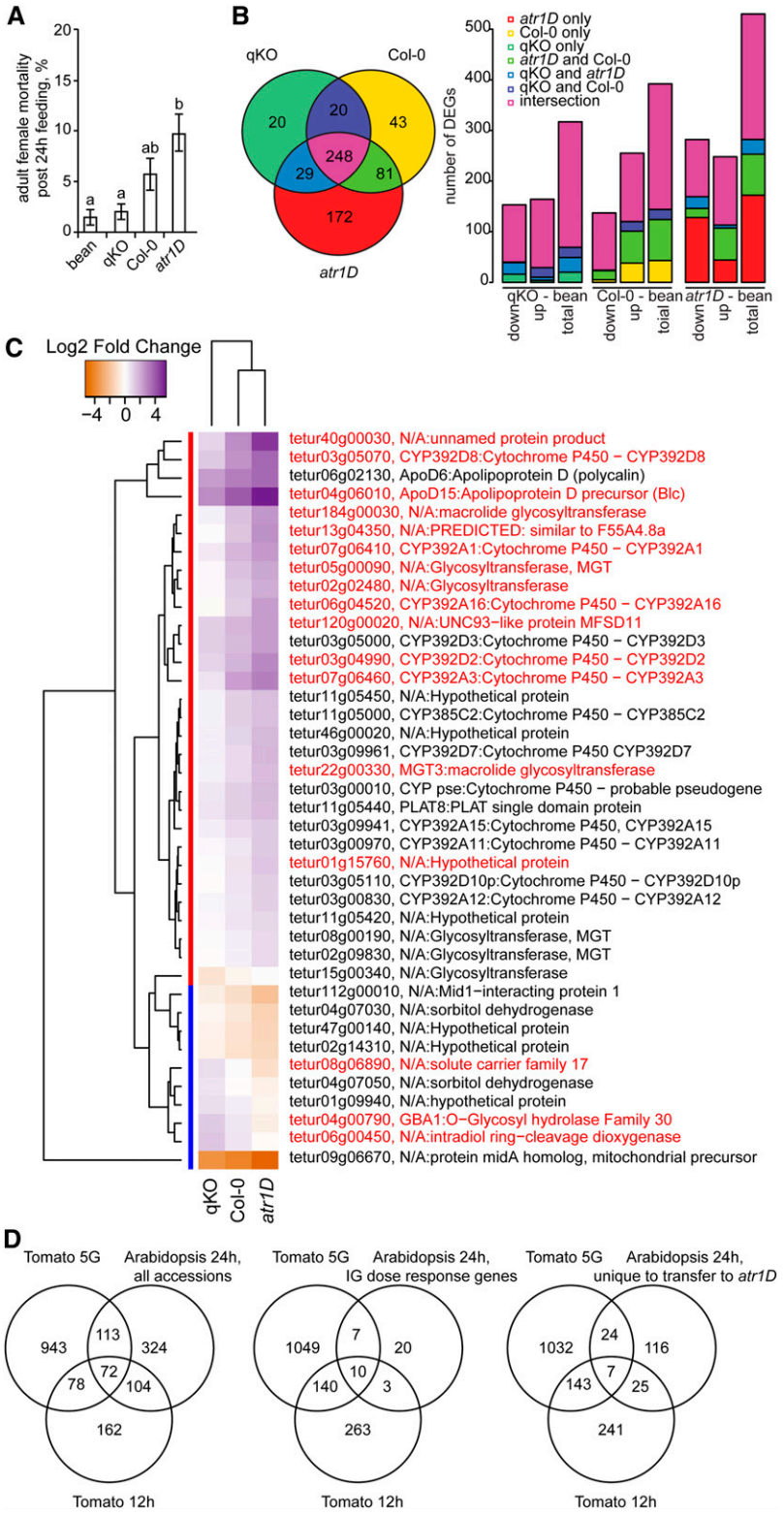

Figure 7. Transcriptional responses of spider mites to feeding on Arabidopsis plants containing different levels of IGs. A, Relative mortality of adult female spider mites 24 h post transfer from bean plants to Arabidopsis lines with varying levels of IGs. Values represent means ± SE percentage of adult female mites that died after 24 h of feeding ($n = 4$ sets of 100 mites; critical $P = 0.05$). B, Number of detected DEGs upon spider mite feeding on qKO, Col-0, and *atr1D* Arabidopsis plants that contain none, normal, and increased levels of IGs, respectively. Overlap categories from the Venn diagram are shown for up- and down-regulated DEGs at right. C, Hierarchical clustering analysis of DEGs with consistent increase or decrease of gene expression levels by at least 25% (FC > 1.25, BH-adjusted $P < 0.05$) within the qKO-Col-0-*atr1D* continuum. Genes with descriptions in red demonstrated FC > 1.5 per step. The distance metric was Euclidean, and the clustering method was average. D, Comparison between DEGs identified in host transfer from bean plants to tomato (Dermauw et al., 2013) and from bean plants to Arabidopsis lines with different IG contents.

specific gene or a group of genes that are constitutively overexpressed in Bla-2 may contribute to the higher level of resistance to mite herbivory observed for Bla-2 relative to Kon.

JA-regulated defenses against spider mites have been described for several plants (Li et al., 2002, 2004; Ament et al., 2004; Schweighofer et al., 2007; Zheng et al., 2007; Zhang et al., 2009), indicating that regulatory

mechanisms leading to mite-induced defense programs are broadly conserved across plant species. In contrast, the conservation of downstream JA-regulated pathways that mediate plant resistance is less clear. For example, tomato emits terpenes (4,8,12-trimethyltrideca-1,3,7,11-tetraene and trans-β-ocimene) and MeSA in a blend of volatile organic compounds in a JA-dependent manner that attracts natural predators of spider mites as an indirect defense against spider mite herbivory (Dicke et al., 1998; Ament et al., 2004). While we did not assay volatiles emitted by Arabidopsis upon mite herbivory, up-regulation of genes required for the biosynthesis of 4,8,12-trimethyltrideca-1,3,7,11-tetraene (*TPS4* and *CYP82G1*), trans-β-ocimene (*TPS2*, *TPS3*, and *TPS10*) and MeSA (*PHENYL ALANINE AMMONIA-LYASE1* and *BSMT1*) was observed, suggesting that these indirect defenses might be conserved between tomato and Arabidopsis. However, while JA-inducible Ser protease inhibitors have been used as reliable markers of JA-induced tomato defenses to spider mite herbivory (Ament et al., 2004; Kant et al., 2004, 2008), only a few out of 50 annotated proteinase inhibitors were weakly induced in Arabidopsis. Instead, spider mite feeding induces the biosynthesis of glucosinolates, metabolites that commonly accumulate in Arabidopsis as a result of herbivory. Spider mite feeding induces the expression of IG biosynthetic genes and the accumulation of IGs (Supplemental Data S1), but feeding of *Spodoptera exigua* (a generalist lepidopteran herbivore) induces the transcription of AG biosynthetic genes, leading to increased accumulation of AGs (Mewis et al., 2005, 2006), despite the common regulation of induced defenses by JA (Fig. 5; Chung et al., 2008). Thus, although there is a conservation of JA-regulated plant defenses against herbivores, there are both plant species- and herbivore-specific responses.

IGs have toxic effect on mites, causing mortality of both larvae and adults (Figs. 6 and 7). In most other cases studied, glucosinolates act as deterrents and antifeedants, causing reduced weight gain and fecundity to feeding herbivores (Kim and Jander, 2007; Kim et al., 2008; Müller et al., 2010). Glucosinolates themselves are not toxic and generally require myrosinase activity for activation. However, IGs affect the aphid's ability to feed on Arabidopsis in a myrosinase-independent manner (Kim and Jander, 2007). The defensive mechanism of IGs to the aphid *Myzus persicae* is based on their postingestive breakdown and conjugation with other herbivory-induced metabolites such as ascorbic acid and Cys within the aphid gut (Kim et al., 2008). The mechanism of IG toxicity is not known in spider mites. Nevertheless, the profound effect of IGs on both mite performance and gene expression suggests that they may be activated by either myrosinases or passage through the mite digestive tract to lead to the formation of canonical IG-derived metabolites, such as nitriles and isothiocyanates, or specific toxic conjugates like indol-3-ylmethyl glucosinolate-Cys (Kim et al., 2008).

Insects that feed regularly on plants in the Brassicaceae family have developed specialized defenses against glucosinolates. Where known, most of these specialist adaptations have been shown to prevent the formation of the most toxic metabolites (as opposed to circumventing their downstream toxic effects; Ratzka et al., 2002; Wittstock et al., 2004; Wheat et al., 2007). For most generalist herbivores, however, both the mode of action and responses to glucosinolates are largely unknown, although for some lepidopteran species, glucosinolates are detoxified by conjugation with glutathione (Schramm et al., 2012), a mechanism also observed in vertebrates (McDanell et al., 1988). General phase I and II detoxifying enzymes have been greatly expanded in the spider mite genome (Grbić et al., 2011; Dermauw et al., 2013). The observed spider mite transcriptional response confirms the importance of such detoxification systems, as some of the major families that respond to IGs are phase I P450s and phase II glycosyltransferase detoxification enzymes. This is in sharp contrast to the response observed in the leaf-mining fly *Scaptomyza flava*, an evolutionarily recent specialist on the Brassicaceae family, in which glucosinolates induce a general stress response (Whiteman et al., 2012).

In conclusion, we have established Arabidopsis and spider mite as a novel platform for studies of plant-herbivore interactions and have used a combination of genomic and genetic resources to assess reciprocal interactions between a plant and an herbivore at both the organismal and molecular levels. On the plant side, spider mites induce defense pathways conserved among many different biotic stressors. Our analysis revealed that only some of the induced programs are effective against spider mites, including JA-regulated accumulation of secondary IG metabolites that affect the ability of spider mite to use Arabidopsis as a host plant. Our analysis of spider mite responses indicates that IGs are perceived by spider mite, but changes in the expression of genes implicated in the detoxification of xenobiotics are insufficient to fully detoxify IGs.

MATERIALS AND METHODS

Material and Growth Conditions

Seeds for Arabidopsis (*Arabidopsis thaliana*) accessions and mutant lines were obtained from the Arabidopsis Biological Resource Center, except for the seed of *myc2 myc3 myc4*, which was acquired from R. Solano (Universidad Autónoma de Madrid), *myb28 myb29* and its corresponding wild-type Columbia from P. Morandini (University of Milan), qKO from B.A. Halkier (University of Copenhagen), and *atr1D* from J. Bender (Brown University). Col-0 was used as the wild type for all experiments, except for the analysis of *aos* in the Columbia-6 background. Plants were grown under 100 to 150 μmol m^{-2} s^{-1} cool-white fluorescent light at 24°C with a 16-h/8-h (light/dark) photoperiod in controlled growth chambers. The London reference spider mite (*Tetranychus urticae*) strain was reared on bean plants (*Phaseolus vulgaris* 'California Red Kidney'; Stokes) in growth chambers at 24°C, 60% relative humidity, and with a 16-h/8-h (light/dark) photoperiod for more than 100 generations.

Plant Infestation with Spider Mites and Plant Damage Assessment

Three-week-old Arabidopsis plants were infested with 10 adult female mites that were allowed to feed for 3 d. Plant damage was assessed as total area of

chlorotic spots based on leaf scans overlaid with a grid of 0.25 mm × 0.25 mm using Adobe Photoshop CS5 software (Adobe Systems). Damage was recorded if a spot covered more than half of the grid unit. The mean chlorotic area of mutant lines was expressed relative to the mean chlorotic area of the corresponding wild-type plants. Experiments were performed on three independent sets of plants.

Choice Experiment

Size-matched 5-week-old Arabidopsis plants were arranged in groups of four plants (two Bla-2 and two Kon plants diagonally) with leaves overlapping and infested with 10 adult female mites per plant that were deposited on leaves adjacent to the overlapping leaves (total of 40 mites per group of plants). After 2 d, mites were recovered from individual plants and counted. The frequency distribution of mites over the different genotypes was calculated and tested for statistical difference from a 50:50 choice proportion using Student's *t* test. Experiments were performed on 23 independent sets of four plants and were repeated three times.

Larvae Isolation and the Analysis of Mite Performance

Infested beans were washed in 0.001% (v/v) Tween 20 solution, and eggs were filtered through a series of fine sieves. To synchronize spider mite development, eggs were deposited on a filter paper and incubated overnight next to a detached leaf collected from the 3-week-old Arabidopsis plants in a petri dish maintained at 24°C, 70% relative humidity, and a long-day photoperiod (16/8 h). The next morning, 50 newly hatched larvae that moved to a detached leaf were retained for analysis. To follow spider mite development, the emerged neonymphs were counted daily and removed from the detached leaf. Mortality was calculated as the ratio of the number of larvae that did not reach the neonymph stage divided by the total number of larvae initially deposited on the leaf.

Mite Damage Visualization

Trypan blue staining (Keogh et al., 1980) was performed on 3-week-old Arabidopsis plants that were infested with 10 adult female mites each for 24 h. Bright-field images were taken using a Zeiss Axiophot microscope (Carl Zeiss).

Hormonal Analyses

Plant hormones were quantified by isotopic dilution mass spectrometry from pooled rosettes of six 3-week-old plants. Isotope-labeled standards were added to plant samples (approximately 0.1 g) before extraction as described previously (Durgbanshi et al., 2005). Ultra-performance liquid chromatography (UPLC)-electrospray ionization-tandem mass spectrometry analyses were carried out on an Acquity SDS system (Waters) coupled to a triple quadrupole mass spectrometer (Micromass). Quantification was accomplished with an external calibration. Planned comparisons between hormone levels determined for nontreated control and each mite treatment time point were performed using pairwise Student's *t* tests with pooled SD and the Holm-Bonferroni adjustment of *P* values (Holm, 1979).

Nontargeted Metabolic Analysis of Semipolar Compounds by UPLC-Quadrupole Time-Of-Flight-Mass Spectrometry

Analysis of glucosinolates and other semipolar compounds was carried out using a UPLC-electrospray ionization-Quadrupole Time-Of-Flight-mass spectrometry system operated in negative or positive electrospray mode as described previously (Malitsky et al., 2008; Böttcher et al., 2009). Briefly, for each sample, rosettes of six 3-week-old plants (approximately 50 mg) were pooled and extracted in 70% (v/v) methanol:water (supplemented with biochanin A) by ultrasonication for 10 min, after which samples were incubated at 80°C for 15 min in a water bath to deter myrosinase activity, centrifuged, and finally filtered through 0.2-mm polytetrafluoroethylene membrane filters (GE Healthcare). Afterward, 10-mL aliquots of samples were directly injected into the UPLC system (Acquity SDS; Waters) interfaced to a Micromass QTOF Premier device. Extraction of mass data was achieved with XCMS software as described previously (Smith et al., 2006; Arbona et al., 2010). Mass chromatographic features were grouped and used for the annotation of glucosinolates and related compounds. This analysis allowed us to detect all common indole glucosinolates and related metabolites but only a subset of the aliphatic glucosinolates (Supplemental Table S4). Relative quantification was achieved by assessing the recovery of internal standard biochanin A and dividing corrected peak areas by actual tissue weight (normalized peak area). Planned comparisons between areas of chromatographic peaks determined for nontreated control and each mite treatment time point were performed using pairwise Student's *t* tests with pooled SD and the Holm-Bonferroni adjustment of *P* values (Holm, 1979).

Real-Time Quantitative Reverse Transcription-PCR and Data Analysis

Total RNA was extracted from Arabidopsis rosettes of 3-week-old plants 6 h post spider mite infestation using the RNeasy Plant Mini Kit, including DNase treatment (Qiagen). Two micrograms of total RNA was reverse transcribed using the Maxima First Strand cDNA Synthesis Kit for reverse transcription (RT)-quantitative PCR (qPCR; Thermo Fisher Scientific). Reactions were performed in triplicate for each biological replicate, using Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific). RT-qPCR was performed on an Agilent Mx3005P qPCR instrument (Agilent Technologies). Primer sequences and amplification efficiencies (*E*) are listed in Supplemental Table S5. *PEROXIN4* (*AT5G25760*) was used as a reference gene (Czechowski et al., 2005). Cycle threshold values of technical replicates were averaged to generate the cycle threshold (Ct) of a biological replicate. For plotting, the expression value for each target gene (*T*) was normalized to the reference gene (*R*), and normalized relative quantity (NRQ) was calculated as follows: $NRQ = (1 + E_R)^{CtR}/(1 + E_T)^{CtT}$. For statistical analysis, normalized relative quantities were $\log_2$ transformed, and factorial ANOVA was used to assess the significance of the main effects (plant genotype and spider mite herbivory) and of the interaction plant genotype × spider mite herbivory for all variables measured throughout the experiments (Rieu and Powers, 2009). The ANOVA results should be interpreted as follows: main significant effects of the plant genotype or spider mite herbivory means that these factors systematically affect a response variable, while a significant plant genotype × spider mite herbivory interaction indicates that genotypes respond in different ways to the spider mite herbivory. ANOVA was followed by Tukey's honestly significant difference (HSD) test.

Microarray Analysis of Arabidopsis Responses to Spider Mite Feeding

We used the Affymetrix GeneChip Arabidopsis ATH1 Genome Array (Affymetrix) to assess gene expression changes in response to spider mite attack, as described previously (Bomblies et al., 2007). Plants were grown under 100 to 150 μmol m^{-2} s^{-1} cool-white fluorescent light at 24°C with a 16-h/8-h (light/dark) photoperiod in controlled growth chambers. In the time-course scenario, 10 adult female spider mites were applied per plant and allowed to feed for 1, 3, 6, 12, or 24 h. In the FS scenario, rosettes were covered with hundreds of mites that were allowed to feed for 1 h. Two biological replicates containing six plants each were generated per treatment. Whole rosettes were used for RNA extraction. Total RNA was prepared using the RNeasy Plant RNA extraction kit (Qiagen). Analysis was performed using the Bioconductor framework. Initial data quality assessment was conducted using arrayQualityMetrics (Kauffmann et al., 2009). Expression measures were computed using robust multiarray average on the complete data set (Irizarry et al., 2003). Detection of differentially expressed genes was performed using limma with BH-adjusted *P* values (Benjamini and Hochberg, 1995; Smyth, 2004).

GO Annotation of Gene Lists

We used topGO with Fisher's test statistic and the "weight01" algorithm (Alexa et al., 2006) to generate a list of top 50 biological process GO annotations and annotate lists of genes that were detected as differentially expressed. The lists were further filtered by applying a cutoff of 0.05 to Fisher's weighted *P* values.

Meta-Analysis

Using the Genevestigator online tool (Hruz et al., 2008), we selected 18 publicly available ATH1 Genome Array platform data sets that captured the Arabidopsis transcriptional response to a range of biotic, abiotic, and hormonal stimuli (for a list of data sets, see Supplemental Table S2). All subsequent data analysis was performed within the Bioconductor framework.

Quality assessment was performed on a per data set basis using arrayQualityMetrics, and arrays of insufficient quality and/or incongruent with the original experimental designs were excluded from subsequent analysis. Expression measures were computed using robust multiarray average on a per data set basis. To select genes informative for the purpose of comparison, we calculated $\log_2$ FC values for all genes and for all relevant contrasts for each experiment. To retain genes that were informative for the inference of relatedness of various experimental treatments, we calculated sample SD of $\log_2$ FC across all experiments and contrasts for every gene. Given that the maximum observed sample SD for the Affymetrix control probe sets across all experiments was 0.61, we selected a sample SD cutoff of $\log_2$ FC across all experiments and contrasts equal to 1 for the selection of informative differentially expressed genes. We retained a data matrix of 470 genes (Supplemental Data S6) and their respective $\log_2$ FC values across experiments for subsequent study comparison. We used Pvclust (Suzuki and Shimodaira, 2006) to assess the relatedness of various experimental treatments. Pearson's correlation coefficient was utilized as a distance metric, and clustering was performed using the average linkage method. A total of 100,000 bootstrap replications were performed.

Microarray Analysis of Spider Mite Responses to Host Transfer to Arabidopsis

Microarray hybridization and scanning procedures for the analysis of spider mite responses to feeding on Arabidopsis plants with varying levels of glucosinolates were done as described previously (Dermauw et al., 2013). Bean is the ancestral host plant for the spider mite strain used in this study. Adult female spider mites were transferred to three Arabidopsis lines (Col-0, qKO, and *atr1D*) or bean, and after 24 h, the gene expression levels were analyzed. Four biological replicates were performed for each of the three comparisons: qKo-Col-0, *atr1D*-Col-0, and Col-0-bean. For each replicate, RNA was extracted from 100 adult female mites using the RNeasy RNA extraction kit (Qiagen). We used limma to preprocess and analyze spider mite microarray two-color data. Background correction was performed using the "normexp" method with offset of 50, global loess normalization was used to normalize data within arrays and quantile normalization with method "Aquantile" was used to normalize data between arrays (Smyth and Speed, 2003; Ritchie et al., 2007).

Transcript profiling data from this article are deposited at Gene Expression Omnibus (GEO) platform GPL16890 as GEO series GSE48771 (for the spider mite data set) and at GEO platform GPL16112 as GEO series GSE49981 (for the Arabidopsis data set).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Experimental setup and expression measures of DEGs detected in Arabidopsis in response to spider mite attack in at least one time point or FS sample.

Supplemental Figure S2. Heat maps of expression measures and FC of DEGs detected between nontreated Bla-2 and Kon plants.

Supplemental Figure S3. Heat map of FC in levels of expression of genes that were used in meta-analysis of the Arabidopsis response to a variety of biotic and abiotic stimuli.

Supplemental Figure S4. Analysis of the involvement of the SA pathway in the Arabidopsis response to spider mite attack.

Supplemental Figure S5. Levels of Trp, camalexin, IGs, and AGs in qKO, Col-0, and *atr1D*.

Supplemental Figure S6. Analysis of the involvement of AGs in the Arabidopsis response to spider mite attack.

Supplemental Figure S7. Analysis of the involvement of camalexin in the Arabidopsis response to spider mite attack.

Supplemental Figure S8. Heat map of FC in levels of expression of DEGs detected in spider mite upon transfer from bean to qKO, Col-0, and *atr1D* Arabidopsis plants.

Supplemental Table S1. Arabidopsis accessions used for the analysis of natural variation of susceptibility to spider mite attack.

Supplemental Table S2. Studies used in meta-analysis of the Arabidopsis response to a variety of biotic and abiotic stimuli.

Supplemental Table S3. Summary tables for ANOVA and relevant Tukey's HSD comparisons for the analysis of qPCR results.

Supplemental Table S4. Summary of analyzed glucosinolates and related compounds.

Supplemental Table S5. Gene-specific primer sequences used for real-time quantitative RT-PCR.

Supplemental Data S1. DEGs and their FC values detected in Arabidopsis in response to spider mite attack in at least one time point or FS sample.

Supplemental Data S2. Subset of DEGs sensitive to the increase of mite number in the FS sample relative to the 1-h sample.

Supplemental Data S3. Comparisons of GO categories of DEGs detected in Arabidopsis in response to spider mite attack in the FS sample and in 3- to 24-h samples.

Supplemental Data S4. DEGs and their FC values detected between nontreated Bla-2 and Kon plants.

Supplemental Data S5. Comparisons of biological process GO categories of DEGs detected between nontreated Bla-2 and Kon plants.

Supplemental Data S6. FC in levels of gene expression of genes that were used in meta-analysis of the Arabidopsis response to a variety of biotic and abiotic stimuli.

Supplemental Data S7. Biological process GO categories of genes used in the meta-analysis.

Supplemental Data S8. DEGs and their FC values detected in spider mite upon transfer from bean to qKO, Col-0, and *atr1D* Arabidopsis plants.

ACKNOWLEDGMENTS

Plants and the spider mite colony were maintained in the Biotron at Western University.

Received October 30, 2013; accepted November 25, 2013; published November 27, 2013.

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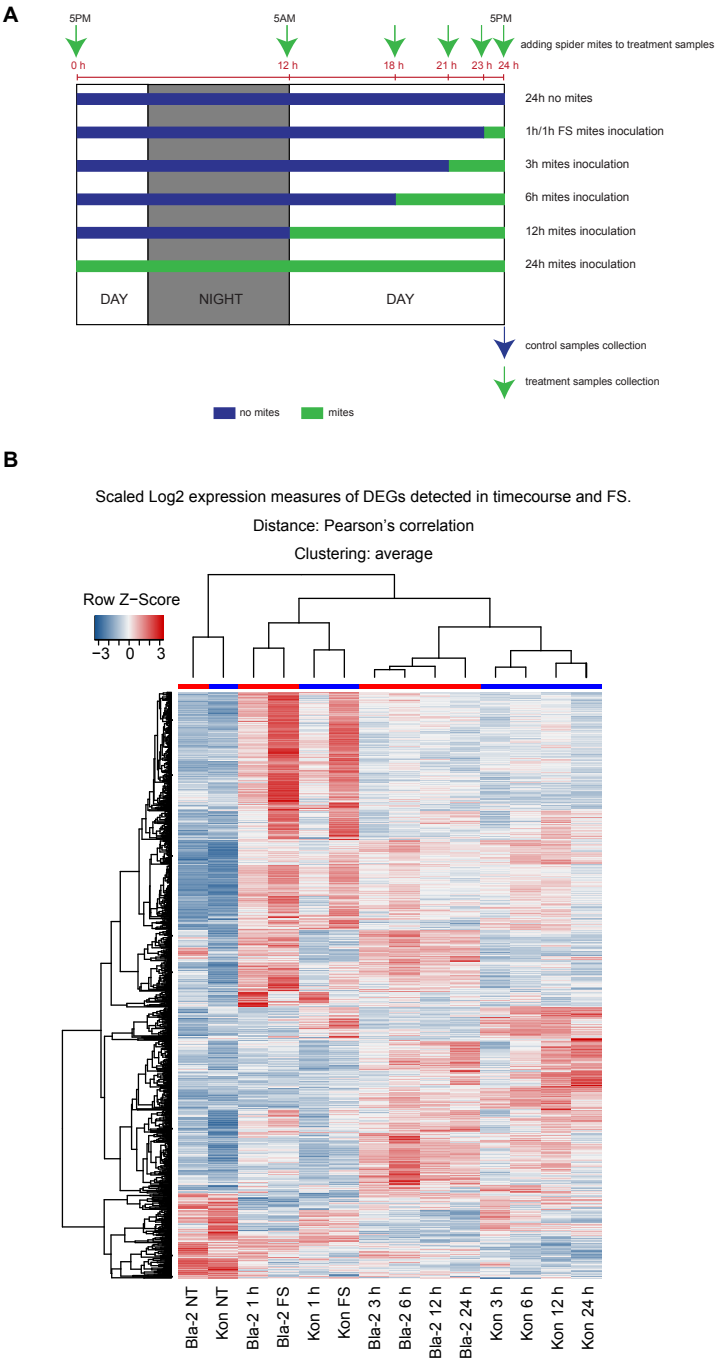

Figure S1. Experimental setup (A) and expression measures of DEGs detected in *A. thaliana* in response to *T. urticae* attack in at least one time-point or FS sample (B).

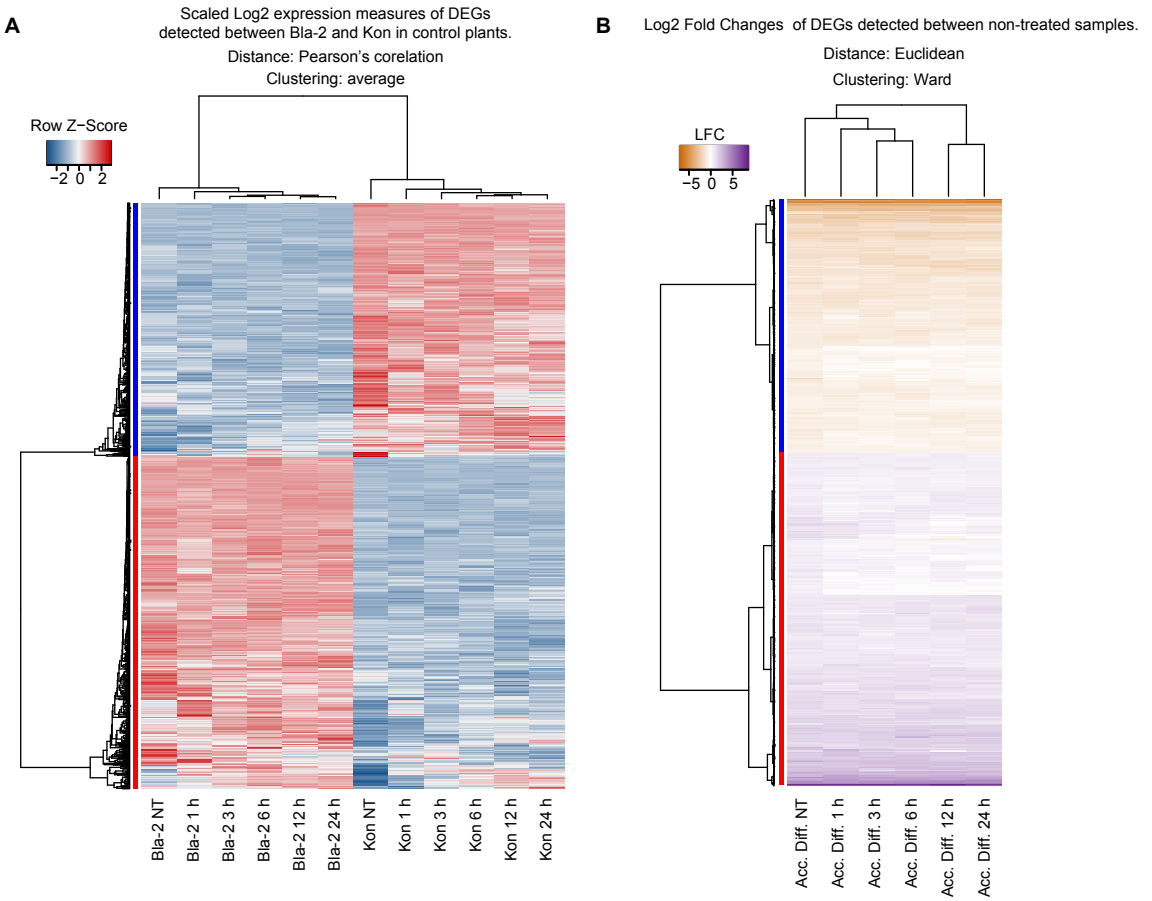

Figure S2. Heatmaps of expression measures (A) and Log2 fold-changes (B) of DEGs detected between non-treated Bla-2 and Kon plants.

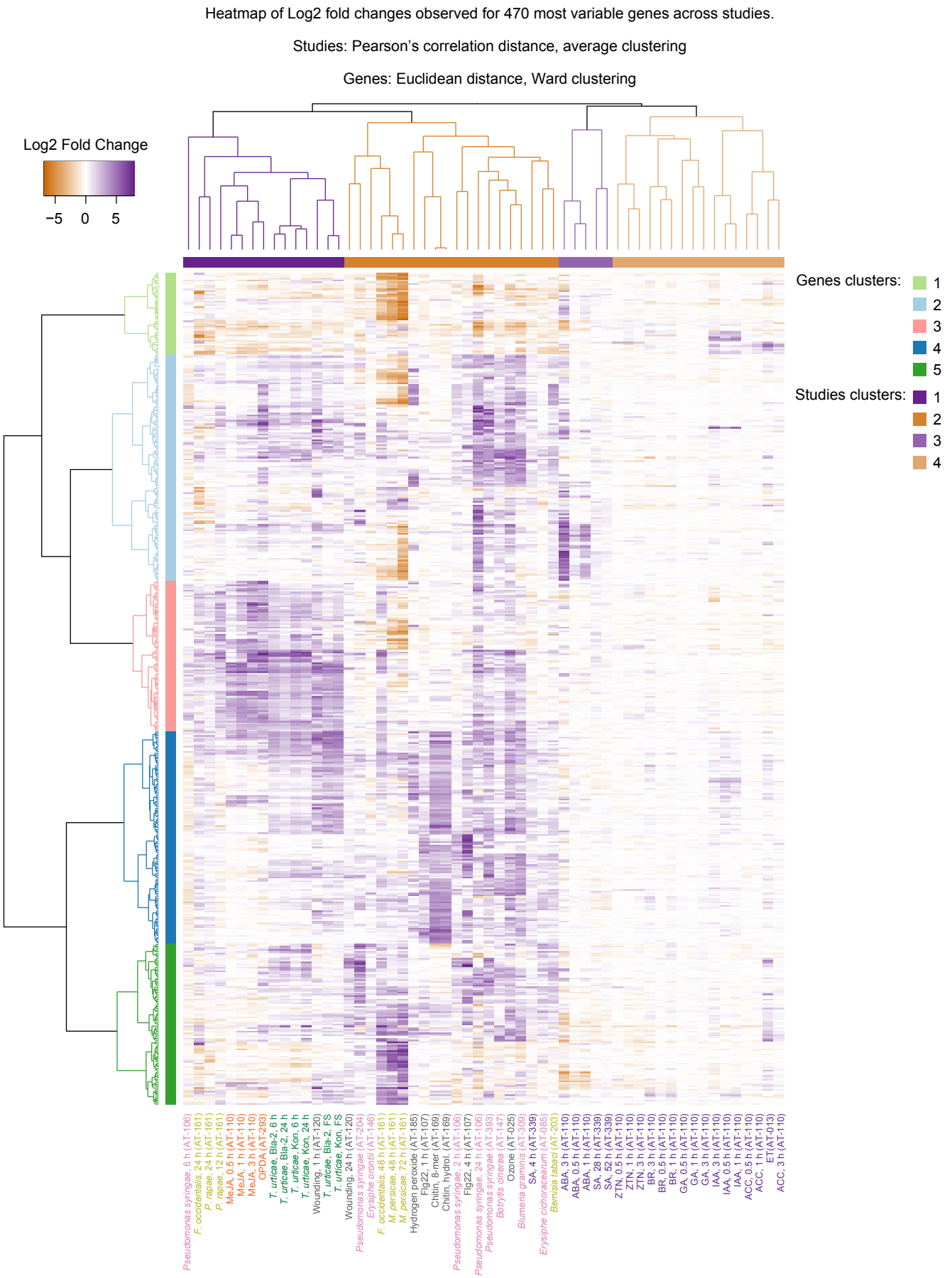

Figure S3. Heatmap of Log2 fold-changes in levels of expression of genes that were used in meta-analysis of *A. thaliana* response to variety of biotic and abiotic stimuli.

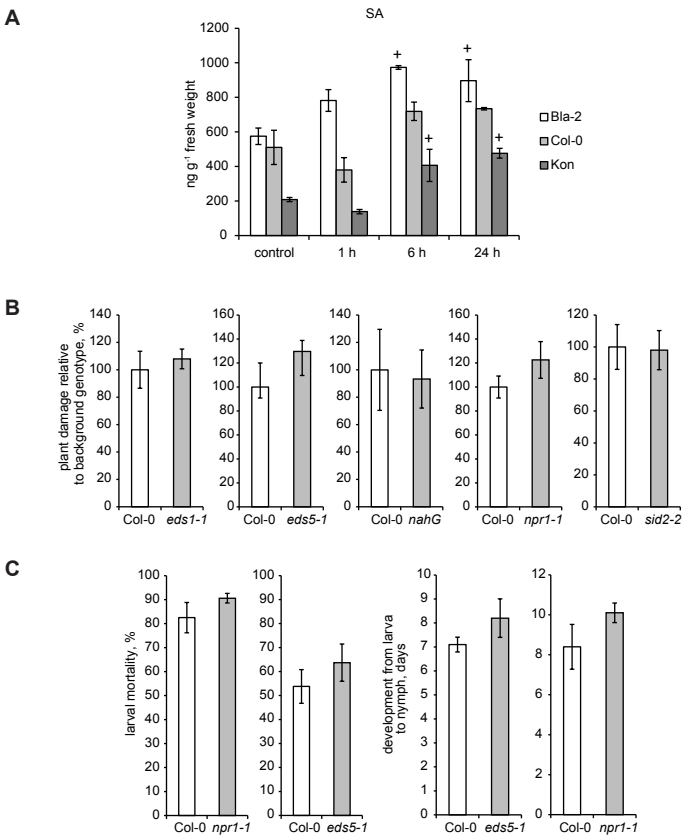

Figure S4. Analysis of involvement of SA pathway in *A. thaliana* response to *T. urticae* attack. A. Levels of SA (ng/g fresh weight [fw]) in 3-week old Bla-2, Col-0 and Kon plants after the spider mite herbivory for 1, 6 and 24 h. Values are means ± SEM (n = 3). Crosses indicate significant difference for planned comparisons relative to corresponding control plants ($P < 0.05$, pairwise t-tests with pooled standard deviations (SD) and Holm-Bonferroni adjustment of P-values). B. Susceptibility of *A. thaliana* SA-related mutants to spider mite herbivory. Mean ± SEM chlorotic area is expressed relative to the mean chlorotic area of corresponding wild type plants (n = 12). C. Spider mite performance as assessed by mean ± SEM days required for larvae to become nymphs and mean ± SEM percentage of larval mortality upon feeding on *A. thaliana* SA-related mutants (n = 5 sets of 50 larvae). Individual sub-groups of comparisons in B and C are not directly comparable as they were performed as separate experiments. Replicated experiments of the same comparisons produced similar results.

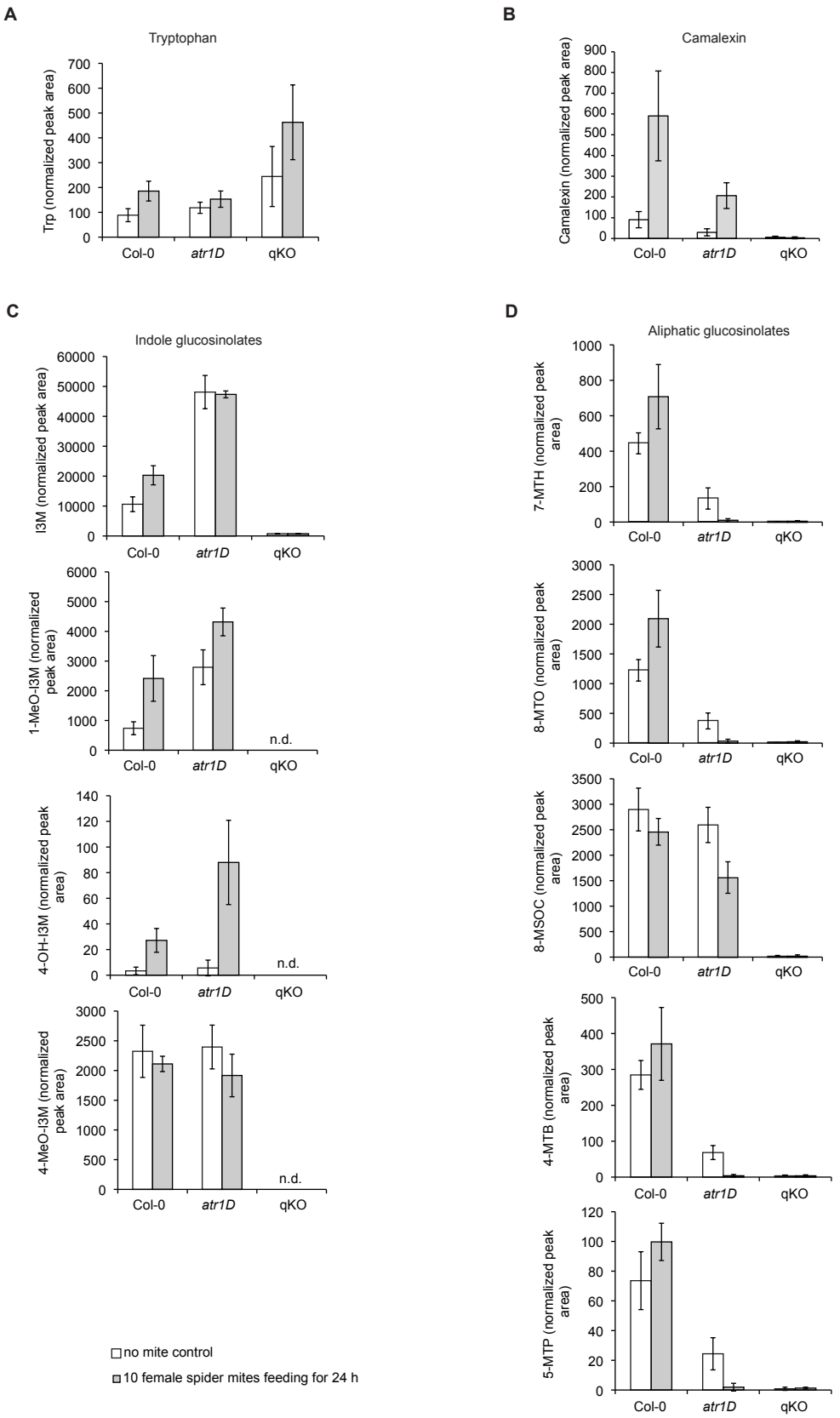

Figure S5. Levels of tryptophan, camalexin, IGs and AGs in qKO, Col-0 and *atr1D*.

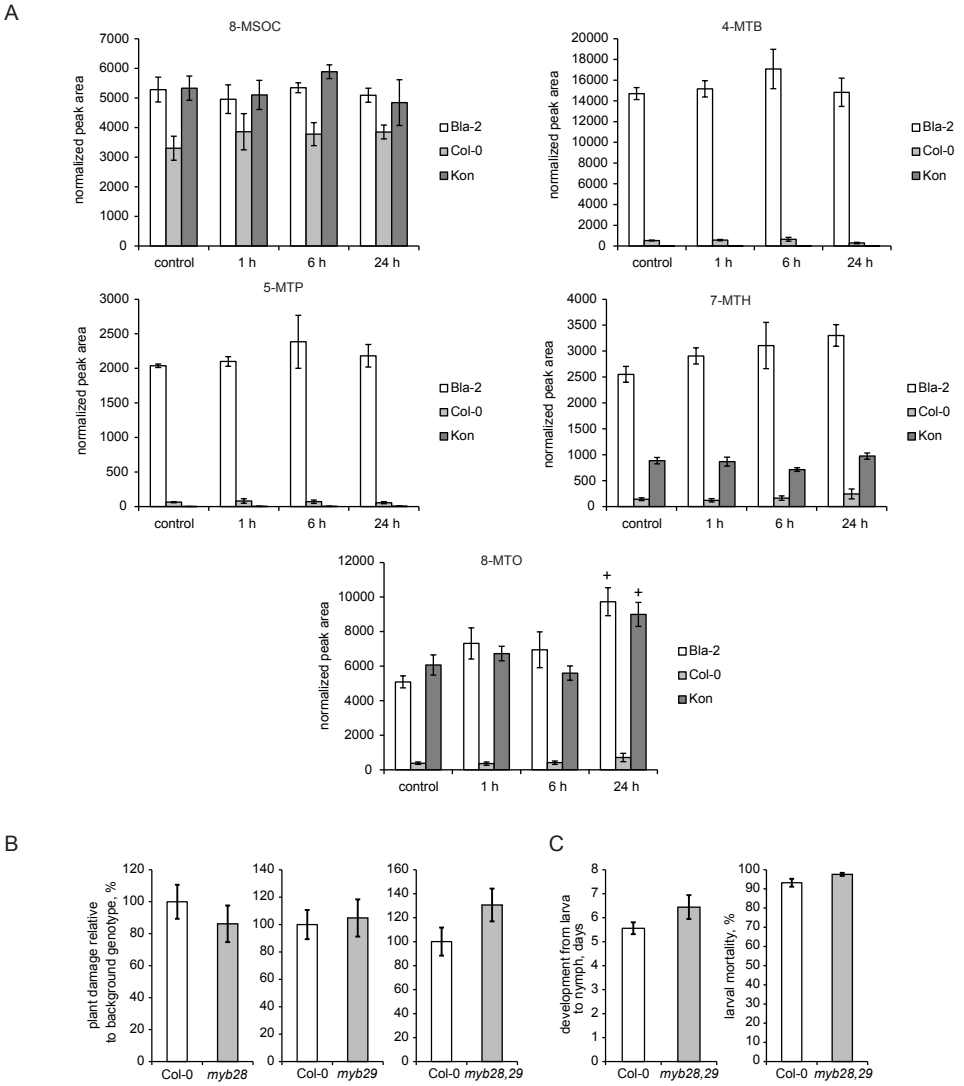

Figure S6. Analysis of involvement of AGs in *A. thaliana* response to *T. urticae* attack. A. Relative levels of subset of AGs (shown as normalized peak area) in 3-week old Bla-2, Col-0 and Kon plants after the spider mite herbivory for 1, 6 and 24 h. Values are means ± SEM (n = 3). Crosses indicate significant difference for planned comparisons relative to corresponding control plants ($P < 0.05$, pairwise t-tests with pooled standard deviations (SD) and Holm-Bonferroni adjustment of P-values). B. Susceptibility of *A. thaliana* AG-related mutants *myb28, myb29* and *myb28 myb29* (labeled as *myb28,29*) to spider mite herbivory. Mean ± SEM chlorotic area is expressed relative to the mean chlorotic area of corresponding wild type plants (n = 12). C. Spider mite performance as assessed by mean ± SEM days required for larvae to become nymphs and mean ± SEM percentage of larval mortality upon feeding on *A. thaliana* AG-related mutants (n = 5 sets of 50 larvae). Individual sub-groups of comparisons in B and C are not directly comparable as they were performed as separate experiments. Replicated experiments of the same comparisons produced similar results.

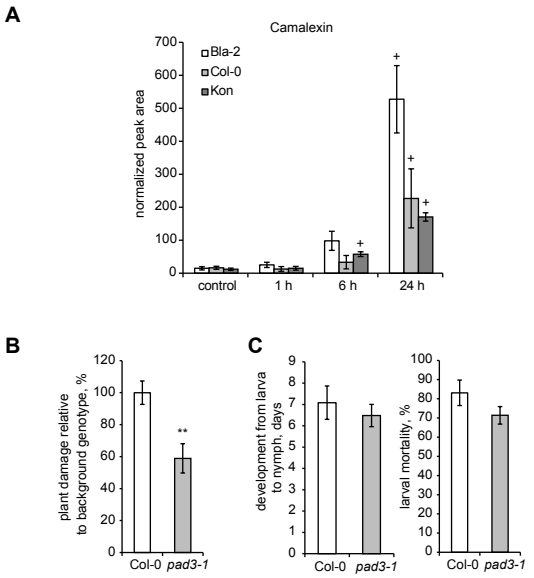

Figure S7. Analysis of involvement of camalexin in *A. thaliana* response to *T. urticae* attack. A. Relative level of camalexin (shown as normalized peak area) in 3-week old Bla-2, Col-0 and Kon plants after the spider mite herbivory for 1, 6 and 24 h. Values are means ± SEM (n = 3). Crosses indicate significant difference for planned comparisons relative to corresponding control plants ($P < 0.05$, pairwise t-tests with pooled standard deviations (SD) and Holm-Bonferroni adjustment of P-values). B. Susceptibility of *A. thaliana* mutant that lacks camalexin to spider mite herbivory. Mean ± SEM chlorotic area is expressed relative to the mean chlorotic area of corresponding wild type plants (n = 12). C. Spider mite performance as assessed by mean ± SEM days required for larvae to become nymphs and mean ± SEM percentage of larval mortality upon feeding on *A. thaliana* lacking camalexin (n = 5 sets of 50 larvae). Asterisks indicate significant difference from corresponding wild type plants (unpaired t-test, ** - $P < 0.01$). Replicated experiments of the same comparison produced similar results.

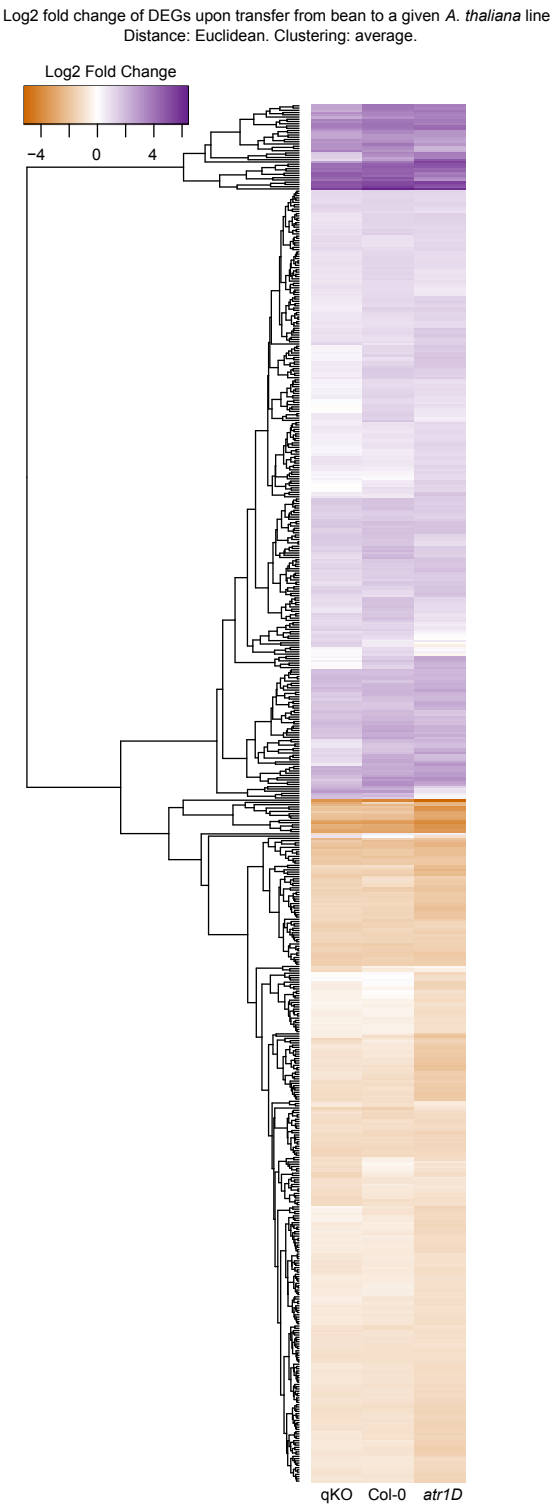

Figure S8. Heatmap of Log2 fold-changes in levels of expression of DEGs detected in *T. urticae* upon transfer from bean to qKO, Col-0 and *atr1D A. thaliana* plants.

Table S1. List of *A. thaliana* accessions used for the initial analysis of natural variation of susceptibility to *T. urticae* attack.

Accession	Full name	NASC stock ID	ABRC stock ID
An-0	Antwerpen	-	CS76091
Bay-0	Bayreuth	-	CS22676
Bla-2	Blanes	N973	-
Bor-4	Borky	-	CS22677
Br-0	Brunn	N995	-
Bur-0	Burren	N1029	-
C24	C24	-	CS22680
Col-0	Columbia	N1093	CS22681
Cvi-0	Cape Verde Islands	-	CS22682
Est-1	Estland	N1151	-
Fei-0	St. Maria d. Feiria	-	CS22684
Got-7	Goettingen	-	CS22685
Gr-3	Graz	N1203	-
Kas-1	Kashmir	-	CS76150
Kondara	Kondara	N6175	-
Ler	Landsberg erecta	N6928	-
Lov-5	Lovvik	-	CS22695
Ms-0	Moscow	N1377	-
Nfa-8	NFA	-	CS22687
Rrs-10	RRS	-	CS22689
Rrs-7	RRS	-	CS22688
Shakdara	Shakdara	N6180	-
Tamm-2	Tammisari		CS22691
Ts-1	Tossa de Mar	N1553	-
Tsu-1	Tsu	-	CS22693
Van-0	Vancouver	N1585	-

ABRC: Arabidopsis Biological Resources Center
NASC: Nottingham Arabidopsis Stock Centre

Table S2. List of studies used in meta-analysis of *A. thaliana* response to variety of biotic and abiotic stimuli.

Genevestigator sample ID	ID prefix in meta-analysis data files
AT-00013	at.013.ethylene
AT-00025	at.025.ozone
AT-00085	at.085.ecic
AT-00106	at.106.psyr
AT-00107	at.107.flg22
AT-00110	at.110.aba/acc/br/ga/iaa/meja/ztn
AT-00120	at.120.wnd
AT-00146	at.146.eoro
AT-00147	at.147.bcin
AT-00161	at.161.focc/mper/prap
AT-00169	at.169.chi
AT-00185	at.185.perox
AT-00203	at.203.btab
AT-00204	at.204.psyr
AT-00293	at.293.opda
AT-00309	at.309.bgra
AT-00339	at.339.sa
AT-00393	at.393.psyr

Table S3. Summary tables for ANOVA and relevant Tukey HSD comparisons for analysis of QPCR results.

ANOVA:

CYP79B2	**Df**	**F**	***P***
Genotype	1	336.744	0
Treatment	1	78.442	0.0000013
Genotype x Treatment	1	31.052	0.0001213

MYB28	**Df**	**F**	***P***
Genotype	1	17.8339	0.001183
Treatment	1	0.8548	0.37341
Genotype x Treatment	1	6.4964	0.025522

CYP79B3	**Df**	**F**	***P***
Genotype	1	246.474	0
Treatment	1	107.442	0.0000002
Genotype x Treatment	1	60.277	0.0000051

MYB29	**Df**	**F**	***P***
Genotype	1	9.3266	0.01001
Treatment	1	2.935	0.11237
Genotype x Treatment	1	0.1722	0.68547

Tukey HSD test results for relevant contrasts of Genotype x Treatment interaction:

CYP79B2	**diff**	**CI, lwr**	**CI, upr**	**adj. *P***
Col-6:control - *aos*:control	1.78	1.1951237	2.3648763	0.0000055
aos:mites - *aos*:control	0.4575	-0.1273763	1.0423763	0.1472303
Col-6:mites - Col-6:control	2.01	1.4251237	2.5948763	0.0000015
Col-6:mites - *aos*:mites	3.3325	2.7476237	3.9173763	0

CYP79B3	**diff**	**CI, lwr**	**CI, upr**	**adj. *P***
Col-6:control - *aos*:control	1.2125	0.5709794	1.8540206	0.0005704
aos:mites - *aos*:control	0.3975	-0.2440206	1.0390206	0.3029606
Col-6:mites - Col-6:control	2.77	2.1284794	3.4115206	0.0000001
Col-6:mites - *aos*:mites	3.585	2.9434794	4.2265206	0

MYB28	**diff**	**CI, lwr**	**CI, upr**	**adj. *P***
Col-6:control - *aos*:control	-0.1675	0.58756279	0.25256279	0.647753
aos:mites - *aos*:control	0.1625	0.25756279	0.58256279	0.6682867
Col-6:mites - Col-6:control	-0.3475	0.76756279	0.07256279	0.1186879
Col-6:mites - *aos*:mites	-0.6775	1.09756279	0.25743721	0.0021559

MYB29	**diff**	**CI, lwr**	**CI, upr**	**adj. *P***
Col-6:control - *aos*:control	0.4075	0.08572159	0.9007216	0.1192966
aos:mites - *aos*:control	0.25	0.24322159	0.7432216	0.4645414
Col-6:mites - Col-6:control	0.1525	0.34072159	0.6457216	0.7959246
Col-6:mites - *aos*:mites	0.31	0.18322159	0.8032216	0.2920559

Table S4. Summary of analyzed glucosinolates and related compounds: quantifier ions used for determinations, retention times, elemental composition and mass deviation from the theoretical values.

Compound name	**Quantifier ion**	**Elemental composition**	**Rt (min)**	**Theoretical *m/z***	**Experimental *m/z***	**Mass deviation (Da)**
Tryptophan	$[M+H]^+$	$C_{11}H_{13}N_2O_2^+$	4.0	205.10	201.09	0.002
4-MTB	$[M-H]^-$	$C_{12}H_{22}NO_9S_3^-$	3.9	420.05	420.046	0.004
I3M	$[M-H]^-$	$C_{16}H_{19}N_2O_9S_2^-$	4.4	447.05	447.054	0.004
8-MSOC	$[M-H]^-$	$C_{16}H_{30}NO_{10}S_3^-$	4.7	492.10	492.102	0.002
4-OH-I3M	$[M-H[^-$	$C_{16}H_{19}N_2O_{10}S_2^-$	4.8	463.05	463.052	0.002
5-MTP	$[M-H]^-$	$C_{13}H_{24}NO_9S_3^-$	5.0	434.06	434.059	0.001
4-methoxy-I3M	$[M-H]^-$	$C_{17}H_{21}N_2O_{10}S_2^-$	5.2	477.06	477.063	0.002
1-methoxy-I3M	$[M-H]^-$	$C_{17}H_{21}N_2O_{10}S_2^-$	5.9	477.06	477.062	0.003
7-MTH	$[M-H]^-$	$C_{15}H_{28}NO_9S_3^-$	6.7	462.09	462.094	0.004
8-MTO	$[M-H]^-$	$C_{16}H_{30}NO_9S_3^-$	7.5	476.11	476.107	0.003
Camalexin	$[M+H]^+$	C11H9N2S+	8.9	201.048	201.052	0.004

Table S5. Gene-specific primer sequences used for real-time quantitative RT-PCR.

Gene ID	**Description**	**Forward primer**	**Reverse primer**	**Amplification efficiency (E)**
AT4G39950	*CYP79B2*	GAAAAGAGGTTGTGCGGCTC	TCTCACTTCACCGTCGGGTA	0.994
AT2G22330	*CYP79B3*	TCTACCGATGCTTACGGGATTG	TACAAGTTCCTTAATGGTTGGTTTG	0.973
AT5G61420	*MYB28*	TGTCTGATTAGGGTTGAAACGGTG	CTATGACCGACCACTTGTTGCCA	1
AT5G07690	*MYB29*	TCCTACAACGGTCGTCTACCA	TTCTTCGGCAGTCCATGCTC	0.932
AT5G25760	*PEX4*	GCTCTTATCAAAGGACCTTCGG	CGAACTTGAGGAGGTTGCAAAG	0.992