GENOTYPIC VARIATION IN BELOW-TO ABOVEGROUND SYSTEMIC INDUCTION OF GLUCOSINOLATES MEDIATES PLANT FITNESS CONSEQUENCES UNDER HERBIVORE ATTACK

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Abstract- Plants defend themselves against herbivore attack by constitutively producing toxic secondary metabolites, as well as by inducing them during herbivore feeding. Induction of secondary metabolites can cross plant tissue boundaries, such as from root to shoot. However, whether the potential for plants to systemically induce secondary metabolites from roots to shoots shows genetic variability, and thus, potentially, is under selection conferring fitness benefits to the plants is an open question. To address this question, we induced 26 maternal plant families of the wild species *Cardamine hirsuta* belowground (BG) using the wound-mimicking phytohormone jasmonic acid (JA). We measured resistance against a generalist (*Spodoptera littoralis*) and a specialist (*Pieris brassicae*) herbivore species, as well as the production of glucosinolates (GSLs) in plants. We showed that BG induction increased AG resistance against the generalist but not against the specialist, and found substantial plant family-level variation for resistance and GSL induction. We further found that the systemic induction of several GSLs tempered the negative effects of herbivory on total seed set production. Using a widespread natural system, we thus confirm that BG to AG induction has a strong genetic component, and it can be under positive selection by increasing plant fitness. We suggest that natural variation in systemic induction is in part dictated by allocation trade-offs between constitutive and inducible GSL production, as well as natural variation in AG and BG herbivore attack in nature.

Key Words - Brassicaceae, Fitness impact of herbivory, Generalist herbivore, Glucosinolates, Induced systemic resistance, Plant-herbivore interaction.

INTRODUCTION

The selective pressure of insect herbivores on plants has led to the evolution of a wide variety of secondary metabolites that can intoxicate or inhibit digestion capacities of the herbivores during feeding (Futuyma and Agrawal 2009; Schoonhoven et al. 2005). While secondary metabolites can be constitutively stored in plant tissues prior to herbivore attack, herbivore feeding on one organ of a plant can induce the *de novo* production, or increase accumulation of the toxins locally, on the same organ, or systemically, on other organs of a plant (Kessler and Baldwin 2002). Within-plant induction of toxic chemicals often reduces the performance of current or subsequent herbivores (Karban and Baldwin 1997; Poelman et al. 2008), and therefore, locally- or systemically-induced chemical defenses should be linked to plant fitness (Agrawal 1998; Agrawal 2000). Moreover, the induction of defenses can cross widely-separated plant organs, such as travelling from roots to shoots (Bezemer et al. 2003). Indeed, a growing body of literature is showing that a range of belowground (BG) organisms can induce defense responses in aboveground (AG) tissues and *vice versa* (Bezemer et al. 2003; Erb et al. 2009; Rasmann and Agrawal 2008; Soler et al. 2005; Staley et al. 2007; van Dam et al. 2005). Reviews on the topic suggest that the magnitude and direction of chemically-mediated AG-BG interactions in plants largely depend on plant genotypic variation as well as the attacking species' identity (Kabouw et al. 2011; van Geem et al. 2013; Vandegehuchte et al. 2011). Therefore, potentially, selection is acting on plants' natural genetic variation to optimize their ability to induce defenses systemically. While significant levels of genetic variation, as well as a heritable genetic basis for both constitutive and inducible defense traits expression has been shown in several systems (Agrawal et al. 2002; Havill and Raffa 1999; Humphrey et al. 2018; Stevens and Lindroth 2005; Underwood et al. 2000; Wagner and Mitchell-Olds 2018), to date, we have practically no information on whether BG-AG defense induction is under positive selection for plants harboring such trait variation in nature. . Measuring BG to AG root induction is also ecologically-relevant since in nature, plants can be potentially in contact with a wide range of root herbivores, that phenologically, can induce the plants before the leaf herbivores arrive (Erb et al. 2008; Huang et al. 2017; Rasmann and Agrawal 2008).

For a trait to be under selection, it needs to display a significant degree of genetically based variation in nature. Whereas most of such variation is generated by random mutation, and evolutionary and genetic mechanisms (Caliskan 2012), the maintenance of genetic variability can also be affected by energetic costs. Optimal defense theory suggests that inducible defenses have evolved as a cost-saving strategy, and the relative allocation of constitutive and inducible defenses in plant organs, individuals or populations depends on predictability of attack from herbivores, the susceptibility of plants to attacks, and the context dependency of the interaction (e.g. environmental variation) (Agrawal et al. 2002; McKey 1974; Zangerl and Bazzaz 1992; Zangerl and Rutledge 1996). In other words, the simultaneous expression of constitutive and induced defense is thought to be costly (Brody and Karban 1992; Rasmann and Agrawal 2009; Strauss et al. 2002; Zangerl and Bazzaz 1992) and should result in negative genetic correlations (trade-offs) between individual traits and between defense deployment strategies (Agrawal et al. 2010). Therefore, high constitutive expression of a defense trait is predicted to be associated with lower induction abilities. While trade-offs between constitutive and induced defenses on the same organs have been shown in several systems (Heil et al. 2004; Rasmann et al. 2015; Rasmann et al. 2011; Thaler et al. 1999; Thaler and Karban 1997), we still lack evidence for whether AG inducibility of defenses after BG induction is trading-off with constitutive defenses.

In brassicaceous plants, glucosinolates (GSLs), Sulphur- and nitrogen-containing plant secondary metabolites, are the main defensive compounds conferring plant resistance against insect herbivores (Howe and Jander 2008). The defensive function of GSLs breakdown products, when either expressed constitutively or when induced, against both specialist and generalist insect herbivores has been amply documented (Agrawal 1998; Agrawal 2000; Agrawal et al. 2002; Baldwin 1998; Karban and Baldwin 1997; van Dam and Oomen 2008; van Dam and Raaijmakers 2005; van Dam et al. 2005). Several individual GSLs show strong inducibility following herbivory and generally, the plant hormone jasmonic acid (JA) is a key player in the regulation of induced plant responses against chewing herbivores such as caterpillars (Farmer et al. 2003; Howe and Jander 2008). Emerging patterns from studies on *Brassica* spp. indicate that BG insect herbivory, or JA application to roots increase total GSLs levels in shoots (Griffiths et al. 1994; Pierre et al. 2012; Soler et al. 2005; van Dam et al. 2004).

For instance, previous work with *Cardamine hirsuta* demonstrated that the overall abundance and identity of GSLs in the leaves is affected by JA induction in the roots (Bakhtiari et al. 2018). Therefore, if genetic variation for root-to-shoot induction exists in nature, it should correlate to plant fitness, particularly, when plants are under herbivore attack.

We here sought for natural genetic variability in BG-to-AG systemic induction in nature and specifically asked the following questions: 1) Does the exogenous application of JA in roots increase resistance against specialist and generalist insect leaf-chewing herbivores? 2) Is there genetically-based variation in resistance against insects and BG-to-AG induction of GSLs? 3) Is there a trade-off between the constitutive and inducible production of shoot GSLs following root induction? and 4) What is the impact of systemic induction of different GSLs on plant fitness? We answered these questions by inducing the roots of 26 maternal half-sib families of *Cardamine hirsuta* (Brassicaceae), measured GSL production in the leaves, and measured the growth of a specialist herbivore, the large cabbage butterfly *Pieris brassicae*, and a generalist noctuid butterfly, *Spodoptera littoralis*, to assess the potential impact of GSLs on adapted and non-adapted herbivores, respectively. Our work builds toward a better understating of the ecological and evolutionary drivers of plant chemical defense variation in nature.

METHODS AND MATERIALS

Plants and Insects - The hairy bittercress, *Cardamine hirsuta* (Brassicaceae) is a common weed growing in a variety of habitats in Europe but mainly at low elevations (Pellissier et al. 2016). Seeds from 26 half-sib families were collected from three different natural populations separated by at least 10 Km (pop A = 9 pop B = 10, and pop C = 7 families) at the foothills of the Swiss Jura mountains. After an overwintering period of four months at 4 °C, seeds were germinated in Petri dishes lined with humid filter paper, and one week after germination, 15 seedlings per family (total of 390 plants) were transplanted independently into plastic potting pots (13 cm width × 10 cm height) filled with 500 ml of sieved soil (1 cm mesh size) mixed with sand in a 3:1 ratio. The soil/sand mixture was sterilized by autoclave at 120 °C for four hrs. Plants were immediately transferred to climate-controlled chamber

and kept at 16h/22°C - 8h/16°C day-night, and 50% relative humidity conditions. Plants were fertilized (universal liquid fertilizer containing N: P: K ratio of 7:3:6% per liter) twice a week until the beginning of experiment. Although our common garden experiment was specifically designed to measure genetic variation across different maternal lines, we would like to acknowledge that the potentially observed genetic differences among families cannot be fully isolated from maternal environmental effects, but because *C. hirsuta* practically completely relies on autogamous selfing for reproduction (Hay et al. 2014), such maternal effects should be minimized in this system. We used the large cabbage butterfly *Pieris brassicae* (Lepidoptera: Pieridae) and the African cotton leaf worm *Spodoptera littoralis* (Lepidoptera: Noctuidae) as specialist and generalist herbivore insects, respectively. *P. brassicae* is a specialist herbivore that feeds exclusively on plants producing GSLs, especially on species of the Brassicaceae (Chew 1988). The caterpillars used in this experiment were obtained from a culture maintained on *Brassica rapae* ssp. *chinensis* (L) plants. *S. littoralis* is a generalist herbivore known to feed on species belonging to more than forty families of plants (Brown and Dewhurst 1975). However, it does not occur in Switzerland, therefore, it functioned as a generalist, non-adapted, herbivore in our study. Eggs were obtained from Syngenta, Stein AG, Switzerland, and newly hatched *S. littoralis* larvae to be used in the bioassays were reared on corn-based artificial diet until the beginning of the experiment.

Experimental Design and Insect Bioassay - After three weeks of growth, we randomly assigned the plants to three treatment groups. Six plants per family were randomly assigned to the JA treatment, another six group plants to the no-induction treatment, and the rest (three plants per family) to the no-herbivory control treatment. Each plant in the JA treatment were inoculated with 20 ml of JA solution in the roots by spiking the solution into the soil, 0.5 cm below the surface. The JA solution consisted of 2.4 μ moles (500 μ g) of JA ($\pm$ - jasmonic acid, Sigma, St Louis, IL, USA) per plant in 10 ml demineralized water and 0.5% EtOH (van Dam and Oomen 2008; van Dam et al. 2004). The no-induction group of plants received 20 ml of 0.5% EtOH in acid water (pH 3.7 with HCl). We chose to induce roots with JA instead of using a root herbivore (e.g. cabbage root maggots), in order to standardize the induction event across all plant families. Moreover, by applying JA, we intentionally

avoided the effect of tissue removal per se on plant fitness. In other words, we were able to measure the fitness impact of defense induction independently from herbivore damage.

To measure the effect of BG induction on leaf chemistry, four days after JA root application, we collected two fully-expanded new leaves per plant in the JA and the no-induction treatments, and immediately froze and stored them at -80°C for further chemical analyses. Since the leaves from both treatments were collected prior to AG herbivory, the plant materials collected from no-induction treatment served for measuring constitutive secondary metabolites expression. Immediately after leaf removal, we infested half of the plants in the herbivory treatments (three plants per family per induction treatment = six plants/family) with two 7-days old *S. littoralis* larvae, and the other half, with one 6-days old *P. brassicae* larvae. We next covered all plants with gauze bags to prevent escape or cross-movement of insects between plants. After one week of herbivory, bags were removed, the insects were retrieved from individual plants, and their combined weight per plant was measured and recorded to obtain the average insect weight per plant. We used the formula $\ln(\text{final weight} - \text{initial weight})$ to determine the insects' weight gain and plant resistance (i.e. lower growth rate indicate that plants are more resistant). After the herbivore bioassay, we allowed the plants to complete their life cycle and produce seeds. To estimate the total seed production on each plant, we first randomly selected one seedpod per plant from 50 plants, measured each pod's length, and counted the number of seeds per pod. Using these data, we fitted a linear regression of the seed number as the function of seedpod length in order to obtain the seed set of each plants based on the seedpod length (equation: $14.92 \times \text{total pods length} + 1.65$). At the end of the experiment, when all seedpods were mature, AG plant parts were separated from roots, oven-dried at 40°C for 48h and weighted to determine their dry biomass, which served as covariate in the statistical analyses (see below).

Glucosinolate Analyses - We assessed the concentration of individual GSLs in leaf tissues in no-induction and root-JA-induction plants prior to the AG herbivore application. This allowed measuring the chemical content of the leaves to which the herbivores were immediately exposed across different treatments, and to measure the direct effect of the root induction treatment on plant chemistry without the confounding effect of additional herbivore feeding. To this end, we ground the fresh leaves to

powder using mortars and pestles in liquid nitrogen. A 100-mg aliquot of fresh leaf powder was then added with 1.0 ml Methanol: H₂O: formic acid (80:19.5:0.5, v/v) and 5 glass beads in Eppendorf tubes, shaken in a tissuelyser (Mixer Mill MM 400, Retsch GmbH, Haan, Germany) for 4 min at 30 Hertz, and centrifuged them at 12800 g for 3 min. The supernatant was then transferred to HPLC vials for liquid chromatography analysis. Glucosinolate identification and quantification was performed using an Acquity UPLC from Waters (Milford, MA, USA) interfaced to a Synapt G2 QTOF from Waters with electrospray ionization, using the separation and identification method as described in Glauser et al. (2012).

Statistical Analyses-All statistical analyses were carried out with R software (R Development Core Team 2017).

1) *Does the exogenous application of JA in roots increase resistance against specialist and generalist insect leaf-chewing herbivores?* To answer this question we performed two ANOVAs on the larval weight gain, for generalist and specialist respectively, with the JA treatment (two levels) as fixed factor.

2) *Is there genetically-based variation in insect resistance and BG-to-AG GSLs induction?* First, we assessed the effect of JA treatment (two levels) and maternal families (26 families) on the abundance and composition of all GSLs simultaneously using a permutational multivariate analysis of variance (PERMANOVA) with the *adonis* function in the package *vegan* (Oksanen et al. 2017). We included plant biomass as covariate to control for potential direct effect of plant size (Züst et al. 2015) on GSL production, and populations as strata in the model. The results were visualized using a non-metric multidimensional scaling (NMDS) ordination. The Bray–Curtis metric was used to calculate a dissimilarity matrix of all compounds among samples for both the PERMANOVA and the NMDS. Second, to address the effect of root JA-addition and family variation on all i) individual GSLs production, ii) the total amount of GSLs, iii) the AG resistance against *P. brassicae* and *S. littoralis* (insect weight gain), iv) and the seed production, we ran linear mixed-effect models with JA treatment as fixed factor, plant families nested within populations as random factor, and plant biomass as

covariate, using the function *lme* in the package *nlme* (Pinheiro et al. 2017). Because families were included as random factor in the initial model, we estimated their effect by running a second model without the nested family factor. Differences between the first and the second models (AIC scores) would inform on potential maternal family variation, which were assessed using log-likelihood ratios and Chi-Square tests (function *Chisquare* in R). In addition, to test for family-level genetic variation in inducibility (G x E) on all individual GSLs and total amount of GSLs per plant, we ran ANCOVAs with JA treatment, plant families nested within populations and their interactions as fixed factors, and plant biomass as covariate, using the function *lm* in R.

3) *Is there a trade-off between the constitutive and inducible production of shoot GSLs following root induction?* To test for trade-offs between the constitutive production and the inducibility of total GSLs among the 26 plant families, we employed a Monte Carlo simulation procedure proposed by Morris et al. (2006) using MATLAB (Version 7.5.0.342 –R2007b, MathWorks Inc., USA). This statistical approach accounts for several issues that have apparently confounded previous attempts to assess a trade-off between constitutive and induced defenses (Morris et al. 2006). Specifically, this approach uses the difference in mean GSL production between JA-treated and control plants for measuring induced production of GSLs, and uses a modified Monte Carlo procedure that takes into account sampling variation due to limited sample size, measurement error from environmental and genetic differences.

4) *What is the impact of systemically inducing different GSLs on plant fitness?* First, we tested for the effect of treatment (3 levels in this case: root JA induction, no-induction, no-herbivory control treatment) on lifetime seed production using mixed effect models with JA treatment as fixed factor and families nested in populations as random factor, including biomass as covariate (*lme* function), followed by pairwise comparisons using *Tukey HSD* post-hoc tests (*lsmeans* function in the package *lsmeans* (Lenth 2016). Second, to estimate the lifetime fitness effect of root JA induction across all different GSLs, we ran mixed effect ANCOVA models with seed production per plant as response variable, individual and total GSLs in interaction with JA induction treatment as continuous and

categorical fixed factors in the model, respectively. Plant families nested within population were included as random factor using the function *lme* in the package *nlme*. The aim here was to detect a significant interaction between JA induction and GSLs on seed set (as a proxy for plant fitness). If this were the case, it would indicate that the effect of JA treatment on a particular GSL compound would affect plant fitness, positively or negatively.

RESULTS

Effect of JA Treatment on Insect Resistance - We found that *S. littoralis* larvae on JA-treated plants grew 47% less compared to control plants (Fig. 1, Table 1), and maternal families responded differently in resistance against this generalist herbivore (Fig. 2a, Table 1). In contrast, *P. brassicae* larval weight gain did not differ between treatments and there was no family effect on larval weight gain (Table 1).

Effect of JA Treatment and Family Level Variations on GSL Production - The GSL profile of the *C. hirsuta* leaves consisted of 28 GSL compounds: 15 aliphatic-GSLs, 8 aromatic-GSLs, 3 indole-GSLs, and 2 unknown GSLs (Supplementary materials Table S1; Fig. 3a). We found that the maternal family background, but not the JA application, affected the multivariate GSL matrix in *C. hirsuta* leaves (Table 2, Fig. 3b). Specifically, maternal families explained 35% of the variance in the PERMANOVA, and such variation was also marginally explained by plant biomass (Table 2). We also found a maternal family effect for 16 out of the 28 GSLs (Table 1), a JA effect for five GSLs (GSL9: glucohirsutin, GSL12: 8-methylthiooctyl gsl, GSL14: hydroxymethylbutyl gsl, GSL17: veratryl gsl, GSL26: neoglucobrassicin; Table 1), and a biomass effect for four GSLs (GSL11: glucoberteroin, GSL24: glucobrassicin, GSL25: methoxyglucobrassicin, GSL26: neoglucobrassicin; Table 1). JA treatment significantly decreased the production of four out of those five compounds, except neoglucobrassicin, which increased its production by 25%. The production of GSL neoglucobrassicin was also significantly affected by plant biomass and the maternal families treatment, which explained 11% of the total variances (Table 1). We found no effect of JA treatment

and maternal family on total levels of GSLs (Table 1). In addition, we found a significant interactive effect of family $\times$ JA (maternal family effect for induced production) for five GSLs (GSL1: glucoraphanin, GSL9: glucohirsutin, GSL10: glucoerucin, GSL13: gluconapoleiferin, GSL20: 5-benzoyloxyphenyl) (Table S2).

Effect of herbivory on Seed Set - Across all families, lifetime seed production in the control (no herbivory) treatment was significantly higher compared to plants in induced and no-induction treatment that experienced herbivory (Fig. 4, $F_{1,144} = 54.70$, $p < .0001$). While *P. brassicae* and *S. littoralis* herbivory generally decreased seed set by 68% and 40%, respectively, we found strong genetic effect on seed set production after *S. littoralis* herbivory (Table 1, Fig. 2b). Finally, we found no significant JA treatment effect on seed set (Table 1, Fig. 4)

Trade-offs Analyses - We detected a significant negative correlation (trade-off), between the constitutive production and the inducibility of total GSLs across all maternal families of *C. hirsuta* ($r = -0.82$, $p = 0.01$, Fig. S1).

Effect of JA Root Induction on Plant Fitness after Herbivore Attack - Mixed effect ANCOVA analyses showed that five GSLs (GSL4: glucoalyssin, GSL8: glucobrassicinapin, GSL10: glucoerucin, GSL11: glucobetteroin, GSL18: glucotropeolin), as well as the total GSLs production interacted with JA treatment for explaining seed production (Fig. 5, Table S3). In other words, JA induction changed the slope of the relationship between the GSLs and seed production from negative to neutral or even positive (Fig. 5). We also found marginally significant effect of JA $\times$ GSL for GSL16: sinalbin and GSL13: gluconapoleiferin.

DISCUSSION

We found that the systemic induction, from below- to aboveground, of *C. hirsuta* plants significantly decreased the weight gain of a generalist leaf chewing herbivore, but such effect was highly variable

across plant maternal families. Analyses of plants' secondary metabolites showed that JA root induction affected the production of several GSLs aboveground, and significantly ameliorated plant fitness after leaf chewing herbivore attack. Below, we discuss the implications of these findings for the ecology and evolution of plant defense against herbivores in natural brassicaceous systems.

Effect of Root JA Treatment on Insect Resistance and Aboveground Glucosinolate Production.

One of the principal results of our study is that the JA root application increased resistance against the generalist herbivore (*S. littoralis*), while JA application had no effect on the specialist herbivore (*P. brassicae*). These results are in line with several previous studies (Bodenhausen and Reymond 2007; Giamoustaris and Mithen 1995; Lankau 2007; Raybould and Moyes 2001). For instance, root JA application to *Brassica oleracea* roots resulted in reduced weight gain of generalist herbivore *Mamestra brassicae*, whereas the specialist *P. rapae* was unaffected (van Dam and Oomen 2008), and root induction resulted in more infestation by AG specialists in field-grown *B. oleracea* plants (Pierre et al. 2013). Indeed, specialist herbivores of the Brassicaceae not only tolerate GSLs, they also utilize these compounds in host recognition (Raybould and Moyes 2001). On the other hand, the negative effect of GSLs on generalist herbivore performance has also been confirmed in previous studies (Rasmann et al. 2015; Schlaeppli et al. 2008; Schweiger et al. 2014; Schweizer et al. 2017), which confirms strong context-dependency in plant-herbivore interaction.

Contrary to general expectations, we did not detect differences in the production of total GSLs between control and JA-treated plants, and found that the GSL production in leaves was related by a large extend to plant biomass, a common phenomenon when studying secondary metabolite production in plants (Glynn et al. 2003; Traw 2002; Züst et al. 2015). Although previous works with Brassicaceae plants showed that BG herbivory, or root induction by JA, increases total levels of GSL in shoots (Griffiths et al. 1994; Pierre et al. 2012; van Dam and Oomen 2008; van Dam et al. 2004), similarly to this one, other studies failed to detect such changes in production of total GSLs (Agrawal 2000; Pierre et al. 2013). These results indicate that the systemic induced responses in plants from BG to AG can be species (as well as genotypic, such as in this study) specific, and that uniquely measuring the total amount can often be misleading in plant-herbivore interaction studies. .

Changes in phytochemical diversity in response to induction is, on the other hand, likely a more important component of plant defense against herbivory (Agrawal 2000; Berenbaum and Zangerl 1996; Lindig-Cisneros et al. 1997). Accordingly, the results of our multivariate analysis showed that among the five families that are distinctive with respect to their GSL profiles (Fig. 1b.), two families exhibited greater resistance against *S. littoralis* (family 5 & 7). In fact, family 7, which showed the most distinctive GSL composition in the NMDS, was the most responsive family to JA treatment in terms of inducibility of overall GSLs and the most-resistant family against herbivory by *S. littoralis*. In addition, in contrast to total GSL levels, we observed that an indolic GSL, neoglucobrassicin, is the only compound that was both significantly induced by JA (Table 1) and also negatively correlated with *S. littoralis* weight gain (results not shown, but results from linear mixed model for testing the interactive effect of the JA treatment and neoglucobrassicin production on *S. littoralis* weight gain: JA effect; $F_{x,y} = 18.34$, $p < 0.001$; neoglucobrassicin effect; $F_{x,y} = 5.34$, $p = 0.02$; and their interaction: $F_{x,y} = 3.46$, $P = 0.07$). Indole GSLs have been shown to be induced by herbivory and to affect the growth and development of insect herbivores in other systems (Irwin et al. 2003; Rostás et al. 2002). Selective induction of indolic GSLs have been reported in *B. napus*, *B. rapae* and *B. juncea* in response to herbivory by flea beetles (Bodnaryk 1992). For instance, the concentration of neoglucobrassicin was increased considerably in leaves of *B. napus* as a result of topical application of methyl JA to aerial part of the plant (Doughty et al. 1995), as well as in *B. rapae* and *B. napus* plants treated with specialist herbivores (Koritsas et al. 1991; Rostás et al. 2002). The same pattern of induction of neoglucobrassicin was observed in the roots of *B. napus* that were damaged by *Delia floralis* root maggots (Hopkins et al. 1998). In another study, the only compound that was shown to affect the performance of *P. rapae* feeding on *B. oleracea* plants was neoglucobrassicin (Harvey et al. 2007).. Together, these results suggest that the total amount of GSLs in Brassicaceous plants can often be misleading when predicting plant resistance, while, on the other hand, individual GSLs bear differential toxicities might be better predictors of plant resistance.

Does Below-to-Aboveground Systemic Induction of GSLs Affect Plant Fitness?

Demonstrating the effect of induced response on plant fitness is crucial for documenting that they truly serve as a defensive response (Erb 2018). We found that herbivory, overall, decreased plant fitness (seed production) by more than 50%, clearly confirming the well-documented negative consequence of herbivory on plant fitness (Agrawal 1998; Agrawal 1999; Kessler and Baldwin 2004; Maron 1998; Mothershead and Marquis 2000). If herbivory decreases plant fitness and plants possess genetic variation for traits affecting herbivory and enhancing fitness, then herbivores may act as strong selective agents for more resistant plants by promoting inducibility of specific toxic molecules. Accordingly, in *C. hirsuta*, we showed that root JA-mediated induced systemic production of seven GSL compounds in shoots increases seed production in plants exposed to shoot herbivory, compared to plants that did not received JA treatment. This fitness impact has important implications. First, inducible systemic resistance may be an example of adaptive plasticity in plants. Adaptive plasticity is defined by the higher fitness of individuals expressing different phenotypes in a particular environment (Vijendravarma et al. 2015). Thus, the induction of GSLs compounds after root damage can be seen as an adaptive plastic response for *C. hirsuta* plants (Agrawal 1999; Agrawal 2000). Nonetheless, to be fully convincing, arguments about adaptive plastic responses should also be linked to ecological setting. In this case, we could speculate that *C. hirsuta* plants are likely damaged in their roots, by e.g. root fly maggots, every spring before pierids or other generalist butterflies start feeding on these plants. To date, due to obvious methodological limitations of measuring rates of root herbivory in the field, we only have anecdotal information on the timing and amount of root damage in natural systems (Johnson and Rasmann 2015). For now we can only speculate that the observed genetic variation in inducibility from below to aboveground is shaped by the natural variation in root and shoot herbivory.

The second implication of our fitness-related results concerns the evolution of the systemic response from root to shoots. In order for such a trait to evolve by natural selection, there must be heritable variation that affects fitness. We detected genetic variation in induced production of five GSL compounds (significant interactive family $\times$ JA effect). Within these five GSLs, two compounds (GSL10 and 13) were among the seven individual compounds found to be positively affecting seed set

when induced by JA. In other words, plant families possessing the ability for increased production of these seven compounds in the induced state could hinder the negative fitness effect of herbivory. Finally, genetic variation in inducibility could also have been maintained by physiological trade-offs. Accordingly, we showed that the inducibility of total GSLs and neoglucobrassicin negatively correlated with constitutive investment in both traits. It is generally assumed that constitutive and induced defenses should trade off, as the anti-herbivore defenses are costly for plants (Karban and Baldwin 1997; Karban and Myers 1989; Zangerl and Bazzaz 1992). Thus, most *C. hirsuta* families employ economy in direct chemical defense production, by favoring either a constitutive or an inducible strategy. Altogether, ecological and physiological trade-offs may contribute in maintaining the necessary genetic variation in inducibility of specific GSLs, ultimately generating the raw material for selection to act upon.

409 **Author contributions**

410 MB and SR conceived and designed the experiments. MB conducted experiments and chemical
411 analyses. MB and SR analysed the data and wrote the manuscript. The authors declare no conflicts of
412 interest.

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REFERENCES

- Agrawal AA (1998) Induced Responses to Herbivory and Increased Plant Performance Science 279:1201-1202 doi:10.1126/science.279.5354.1201
- Agrawal AA (1999) Induced responses to herbivory in wild radish: Effects on several herbivores and plant fitness Ecology 80:1713-1723 doi:doi:10.1890/0012-9658(1999)080[1713:IRTHIW]2.0.CO;2
- Agrawal AA (2000) Benefits and costs of induced plant defense for *Lepidium virginicum* (Brassicaceae) Ecology 81:1804-1813 doi:10.1890/0012-9658(2000)081[1804:BACOIP]2.0.CO;2
- Agrawal AA, Conner JK, Johnson MTJ, Wallsgrove R (2002) Ecological genetics of an induced plant defense against herbivores: Additive genetic variance and costs of phenotypic plasticity Evolution 56:2206-2213 doi:doi:10.1111/j.0014-3820.2002.tb00145.x
- Agrawal AA, Conner JK, Rasmann S (2010) Tradeoffs and negative correlations in evolutionary ecology. In: Evolution since Darwin; the first 150 years, vol 150. 1 edn. Sinauer Associates, Stony Brook, NY, pp 243-268
- Bakhtiari M, Glauser G, Rasmann S (2018) Root JA induction modifies glucosinolate profiles and increases subsequent aboveground resistance to herbivore attack in *Cardamine hirsuta* Frontiers in Plant Science 9 doi:10.3389/fpls.2018.01230
- Baldwin IT (1998) Jasmonate-induced responses are costly but benefit plants under attack in native populations Proceedings of the National Academy of Sciences 95:8113-8118 doi:10.1073/pnas.95.14.8113
- Berenbaum MR, Zangerl AR (1996) Phytochemical diversity. In: Romeo JT, Saunders JA, Barbosa P (eds) Phytochemical Diversity and Redundancy in Ecological Interactions. Springer US, Boston, MA, pp 1-24. doi:10.1007/978-1-4899-1754-6_1
- Bezemer TM, Wagenaar R, Van Dam NM, Wäckers FL (2003) Interactions between above- and belowground insect herbivores as mediated by the plant defense system Oikos 101:555-562 doi:10.1034/j.1600-0706.2003.12424.x
- Bodenhause N, Reymond P (2007) Signaling pathways controlling induced resistance to insect herbivores in *Arabidopsis* Molecular Plant-Microbe Interactions 20:1406-1420 doi:10.1094/MPMI-20-11-1406
- Bodnaryk RP (1992) Effects of wounding on glucosinolates in the cotyledons of oilseed rape and mustard Phytochemistry 31:2671-2677 doi:10.1016/0031-9422(92)83609-3
- Brody AK, Karban R (1992) Lack of a tradeoff between constitutive and induced defenses among varieties of cotton Oikos:301-306
- Brown ES, Dewhurst CF (1975) The genus Spodoptera (Lepidoptera, Noctuidae) in Africa and the Near East Bulletin of Entomological Research 65:221-262 doi:10.1017/S0007485300005939
- Caliskan M (2012) The molecular basis of plant genetic diversity. IntechOpen. doi:10.5772/2639
- Chew FS (1988) Biological effects of glucosinolates. In: Biologically Active Natural Products, vol 380. ACS Symposium Series, vol 380. American Chemical Society, pp 155-181. doi:doi:10.1021/bk-1988-0380.ch01210.1021/bk-1988-0380.ch012
- Doughty KJ, Kiddle GA, Pye BJ, Wallsgrove RM, Pickett JA (1995) Selective induction of glucosinolates in oilseed rape leaves by methyl jasmonate Phytochemistry 38:347-350
- Erb M (2018) Plant defenses against herbivory: Closing the fitness gap Trends in Plant Science 23:187-194 doi:10.1016/j.tplants.2017.11.005
- Erb M et al. (2009) Signal signature of aboveground-induced resistance upon belowground herbivory in maize The Plant Journal 59:292-302 doi:10.1111/j.1365-313X.2009.03868.x
- Erb M, Ton J, Degenhardt J, Turlings TCJ (2008) Interactions between arthropod-induced aboveground and belowground defenses in plants 146:867-874 doi:10.1104/pp.107.112169 %J Plant Physiology

- Farmer EE, Almras E, Krishnamurthy V (2003) Jasmonates and related oxylipins in plant responses to pathogenesis and herbivory Current Opinion in Plant Biology 6:372-378 doi:10.1016/s1369-5266(03)00045-1
- Futuyma DJ, Agrawal AA (2009) Macroevolution and the biological diversity of plants and herbivores 106:18054-18061 doi:10.1073/pnas.0904106106 %J Proceedings of the National Academy of Sciences
- Giamoustaris A, Mithen R (1995) The effect of modifying the glucosinolate content of leaves of oilseed rape (*Brassica napus* ssp. *oleifera*) on its interaction with specialist and generalist pests Annals of Applied Biology 126:347-363 doi:doi:10.1111/j.1744-7348.1995.tb05371.x
- Glauser G, Schweizer F, Turlings TC, Reymond P (2012) Rapid profiling of intact glucosinolates in Arabidopsis leaves by UHPLC-QTOFMS using a charged surface hybrid column Phytochem Anal 23:520-528 doi:10.1002/pca.2350
- Glynn C, Herms DA, Egawa M, Hansen R, Mattson WJ (2003) Effects of nutrient availability on biomass allocation as well as constitutive and rapid induced herbivore resistance in poplar Oikos 101:385-397 doi:10.1034/j.1600-0706.2003.12089.x
- Griffiths DW, Birch ANE, Macfarlane-Smith WH (1994) Induced changes in the indole glucosinolate content of oilseed and forage rape (*Brassica napus*) plants in response to either turnip root fly (*Delia floralis*) larval feeding or artificial root damage Journal of the Science of Food and Agriculture 65:171-178 doi:10.1002/jsfa.2740650208
- Harvey JA, Gols R, Wagenaar R, Bezemer TM (2007) Development of an insect herbivore and its pupal parasitoid reflect differences in direct plant defense Journal of Chemical Ecology 33:1556-1569 doi:10.1007/s10886-007-9323-0
- Havill NP, Raffa KF (1999) Effects of elicitation treatment and genotypic variation on induced resistance in Populus: impacts on gypsy moth (Lepidoptera: Lymantriidae) development and feeding behavior Oecologia 120:295-303
- Hay AS et al. (2014) *Cardamine hirsuta*: a versatile genetic system for comparative studies 78:1-15 doi:10.1111/tpj.12447
- Heil M et al. (2004) Evolutionary change from induced to constitutive expression of an indirect plant resistance Nature 430:205 doi:10.1038/nature02703
- Hopkins RJ, Griffiths DW, Birch ANE, McKinlay RG (1998) Influence of increasing herbivore pressure on modification of glucosinolate content of swedes (*Brassica napus* spp. *rapifera*) Journal of Chemical Ecology 24:2003-2019 doi:10.1023/a:1020729524818
- Howe GA, Jander G (2008) Plant immunity to insect herbivores Annu Rev Plant Biol 59:41-66 doi:10.1146/annurev.arplant.59.032607.092825
- Huang W, Robert CAM, Herv MR, Hu L, Bont Z, Erb M (2017) A mechanism for sequence specificity in plant-mediated interactions between herbivores 214:169-179 doi:10.1111/nph.14328
- Humphrey PT, Gloss AD, Frazier J, Nelson-Dittrich AC, Faries S, Whiteman NK (2018) Heritable plant phenotypes track light and herbivory levels at fine spatial scales Oecologia 187:427-445 doi:10.1007/s00442-018-4116-4
- Irwin RE, Strauss SY, Storz S, Emerson A, Guibert G (2003) The role of herbivores in the maintenance of a flower color polymorphism in wild radish Ecology 84:1733-1743 doi:doi:10.1890/0012-9658(2003)084[1733:TROHIT]2.0.CO;2
- Johnson SN, Rasmann S (2015) Root-feeding insects and their interactions with organisms in the rhizosphere Annual Review of Entomology 60:517-535 doi:10.1146/annurev-ento-010814-020608
- Kabouw P et al. (2011) Effects of soil organisms on aboveground multitrophic interactions are consistent between plant genotypes mediating the interaction Entomologia Experimentalis et Applicata 139:197-206 doi:doi:10.1111/j.1570-7458.2011.01123.x
- Karban R, Baldwin IT (1997) Induced responses to herbivory Chicago : The University of Chicago Press,
- Karban R, Myers JH (1989) Induced plant responses to herbivory Annual Review of Ecology and Systematics 20:331-348 doi:10.1146/annurev.es.20.110189.001555

- Kessler A, Baldwin IT (2002) Plant responses to insect herbivory: The emerging molecular analysis
Annual Review of Plant Biology 53:299-328 doi:10.1146/annurev.arplant.53.100301.135207
- Kessler A, Baldwin IT (2004) Herbivore-induced plant vaccination. Part I. The orchestration of plant
defenses in nature and their fitness consequences in the wild tobacco *Nicotiana attenuata*
The Plant Journal 38:639-649 doi:10.1111/j.1365-3113.2004.02076.x
- Koritsas V, Lewis J, Fenwick G (1991) Glucosinolate responses of oilseed rape, mustard and kale to
mechanical wounding and infestation by cabbage stem flea beetle (*Psylliodes chrysocephala*)
Annals of Applied Biology 118:209-221
- Lankau RA (2007) Specialist and generalist herbivores exert opposing selection on a chemical defense
New Phytologist 175:176-184 doi:10.1111/j.1469-8137.2007.02090.x
- Lenth RV (2016) Least-Squares Means: The {R} Package {lsmeans} Journal of Statistical Software 69:1-
33 doi:10.18637/jss.v069.i01
- Lindig-Cisneros R, Benrey B, Espinosa-García FJ (1997) Phytoalexins, resistance traits, and
domestication status in *Phaseolus coccineus* and *Phaseolus lunatus* Journal of Chemical
Ecology 23:1997-2011 doi:10.1023/B:JOEC.0000006485.38713.8c
- Maron JL (1998) Insect herbivory above- and belowground: Individual and joint effects on plant
fitness Ecology 79:1281-1293 doi:10.1890/0012-9658(1998)079[1281:IHAABI]2.0.CO;2
- McKey D (1974) Adaptive patterns in alkaloid physiology The American Naturalist 108:305-320
- Morris WF, Traw MB, Bergelson J (2006) On testing for a tradeoff between constitutive and induced
resistance Oikos 112:102-110 doi:10.1111/j.0030-1299.2006.14253.x
- Mothershead K, Marquis RJ (2000) Fitness impacts of herbivory through indirect effects on plant-
pollinator interactions in *Oenothera macrocarpa* Ecology 81:30-40 doi:10.1890/0012-
9658(2000)081[0030:FIOHTI]2.0.CO;2
- Oksanen J et al. (2017) Vegan: community ecology package Journal of Statistical Software
- Pellissier L et al. (2016) The simultaneous inducibility of phytochemicals related to plant direct and
indirect defences against herbivores is stronger at low elevation Journal of Ecology 104:1116-
1125 doi:10.1111/1365-2745.12580
- Pierre PS, Dugravot S, Cortesero AM, Poinot D, Raaijmakers CE, Hassan HM, van Dam NM (2012)
Broccoli and turnip plants display contrasting responses to belowground induction by *Delia*
radicum infestation and phytohormone applications Phytochemistry 73:42-50
doi:10.1016/j.phytochem.2011.09.009
- Pierre SP, Dugravot S, Herve MR, Hassan HM, van Dam NM, Cortesero AM (2013) Belowground
induction by *Delia radicum* or phytohormones affect aboveground herbivore communities on
field-grown broccoli Front Plant Sci 4:305 doi:10.3389/fpls.2013.00305
- Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2017) {nlme}: Linear and Nonlinear Mixed
Effects Models Journal of Statistical Software
- Poelman EH, van Loon JJA, Dicke M (2008) Consequences of variation in plant defense for
biodiversity at higher trophic levels Trends in Plant Science 13:534-541
doi:10.1016/j.tplants.2008.08.003
- R Development Core Team (2017) R: A language and environment for statistical computing, 3.4.0
edn. R Foundation for Statistical Computing, Vienna, Austria
- Rasman S, Agrawal AA (2008) In defense of roots: A research agenda for studying plant resistance to
belowground herbivory Plant Physiology 146:875-880 doi:10.1104/pp.107.112045
- Rasman S, Agrawal AA (2009) Plant defense against herbivory: progress in identifying synergism,
redundancy, and antagonism between resistance traits Current opinion in plant biology
12:473-478
- Rasman S, Chassin E, Bilat J, Glauser G, Reymond P (2015) trade-off between constitutive and
inducible resistance against herbivores is only partially explained by gene expression and
glucosinolate production Journal of Experimental Botany 66:2527-2534
doi:10.1093/jxb/erv033
- Rasman S, Erwin AC, Halitschke R, Agrawal AA (2011) Direct and indirect root defences of milkweed
(*Asclepias syriaca*): trophic cascades, trade-offs and novel methods for studying

- subterranean herbivory *Journal of Ecology* 99:16-25 doi:doi:10.1111/j.1365-2745.2010.01713.x
- Raybould A, Moyes C (2001) The ecological genetics of aliphatic glucosinolates *Heredity* 87:383
- Rostás M, Bennett R, Hilker M (2002) Comparative physiological responses in Chinese cabbage induced by herbivory and fungal infection *Journal of Chemical Ecology* 28:2449-2463 doi:10.1023/a:1021427917603
- Schlaeppli K, Bodenhausen N, Buchala A, Mauch F, Reymond P (2008) The glutathione-deficient mutant pad2-1 accumulates lower amounts of glucosinolates and is more susceptible to the insect herbivore *Spodoptera littoralis* *The Plant Journal* 55:774-786 doi:doi:10.1111/j.1365-3113X.2008.03545.x
- Schoonhoven LM, Van Loon B, van Loon JJ, Dicke M (2005) *Insect-plant biology*. Oxford University Press on Demand,
- Schweiger R, Heise AM, Persicke M, Muller C (2014) Interactions between the jasmonic and salicylic acid pathway modulate the plant metabolome and affect herbivores of different feeding types *Plant Cell Environ* 37:1574-1585 doi:10.1111/pce.12257
- Schweizer F, Heidel-Fischer H, Vogel H, Reymond P (2017) Arabidopsis glucosinolates trigger a contrasting transcriptomic response in a generalist and a specialist herbivore *Insect Biochemistry and Molecular Biology* 85:21-31 doi:10.1016/j.ibmb.2017.04.004
- Soler R, Bezemer TM, Van Der Putten WH, Vet LEM, Harvey JA (2005) Root herbivore effects on above-ground herbivore, parasitoid and hyperparasitoid performance via changes in plant quality *Journal of Animal Ecology* 74:1121-1130 doi:10.1111/j.1365-2656.2005.01006.x
- Staley JT, Mortimer SR, Morecroft MD, Brown VK, Masters GJ (2007) Summer drought alters plant-mediated competition between foliar- and root-feeding insects *Global Change Biology* 13:866-877 doi:10.1111/j.1365-2486.2007.01338.x
- Stevens MT, Lindroth RL (2005) Induced resistance in the indeterminate growth of aspen (*Populus tremuloides*) *Oecologia* 145:297-305
- Strauss SY, Rudgers JA, Lau JA, Irwin RE (2002) Direct and ecological costs of resistance to herbivory *Trends in Ecology & Evolution* 17:278-285 doi:10.1016/S0169-5347(02)02483-7
- Thaler JS, Fidantsef AL, Duffey SS, Bostock RM (1999) Trade-offs in plant defense against pathogens and herbivores: A field demonstration of chemical elicitors of induced resistance *Journal of Chemical Ecology* 25:1597-1609 doi:10.1023/a:1020840900595
- Thaler JS, Karban R (1997) A phylogenetic reconstruction of constitutive and induced resistance in *Gossypium* *The American naturalist* 149:1139-1146 doi:10.1086/286042
- Traw MB (2002) Is induction response negatively correlated with constitutive resistance in black mustard? *Evolution* 56:2196-2205
- Underwood N, Morris W, Gross K, Lockwood III JR (2000) Induced resistance to Mexican bean beetles in soybean: variation among genotypes and lack of correlation with constitutive resistance *Oecologia* 122:83-89
- van Dam NM, Oomen M (2008) Root and shoot jasmonic acid applications differentially affect leaf chemistry and herbivore growth *Plant Signaling & Behavior* 3:91-98
- van Dam NM, Raaijmakers CE (2005) Local and systemic induced responses to cabbage root fly larvae (*Delia radicum*) in *Brassica nigra* and *B. oleracea* *Chemoecology* 16:17-24 doi:10.1007/s00049-005-0323-7
- van Dam NM, Raaijmakers CE, Van Der Putten WH (2005) Root herbivory reduces growth and survival of the shoot feeding specialist *Pieris rapae* on *Brassica nigra* *Entomologia Experimentalis et Applicata* 115:161-170 doi:10.1111/j.1570-7458.2005.00241.x
- van Dam NM, Witjes L, Svatoš A (2004) Interactions between aboveground and belowground induction of glucosinolates in two wild Brassica species *New Phytologist* 161:801-810 doi:10.1111/j.1469-8137.2004.00984.x
- van Geem M, Gols R, van Dam N, van der Putten W, Fortuna T, Harvey J (2013) The importance of aboveground–belowground interactions on the evolution and maintenance of variation in plant defense traits *Frontiers in Plant Science* 4 doi:10.3389/fpls.2013.00431

619 Vandegehuchte ML, de la Peña E, Bonte D (2011) Contrasting covariation of above- and belowground
620 invertebrate species across plant genotypes *Journal of Animal Ecology* 80:148-158
621 doi:doi:10.1111/j.1365-2656.2010.01766.x
622 Vijendravarma RK, Narasimha S, Chakrabarti S, Babin A, Kolly S, Lemaitre B, Kawecki TJ (2015) Gut
623 physiology mediates a trade-off between adaptation to malnutrition and susceptibility to
624 food-borne pathogens *Ecology Letters* 18:1078-1086 doi:doi:10.1111/ele.12490
625 Wagner MR, Mitchell-Olds T (2018) Plasticity of plant defense and its evolutionary implications in
626 wild populations of *Boechera stricta* *Evolution* 72:1034-1049 doi:10.1111/evo.13469
627 Zangerl AR, Bazzaz FA (1992) Theory and pattern in plant defense allocation Plant resistance to
628 herbivores and pathogens:363-391
629 Zangerl AR, Rutledge CE (1996) The probability of attack and patterns of constitutive and induced
630 defense: a test of optimal defense theory *The American Naturalist* 147:599-608
631 Züst T, Rasmann S, Agrawal AA (2015) Growth–defense tradeoffs for two major anti-herbivore traits
632 of the common milkweed *Asclepias syriaca* *Oikos* 124:1404-1415 doi:10.1111/oik.02075
633

634 Tables

635 **Table 1.** Mixed effect model table for testing the effect of JA induction treatment in the roots of
636 *Cardamine hirsuta* plants, maternal families, and their biomass on individual and total glucosinolates
637 (GSL*), as well as seed production and *Spodoptera littoralis* larval growth. *C. hirsuta* plant families
638 nested within populations was the random factor. Family effect was calculated from the log-likelihood
639 difference (LLR) between the full model and the model missing the random effect. 16 GSL out of 28
640 showed a significant Family effect. GSL 9, 12, 14, 17, 26 showed significant JA effect. GSL 11, 24,
641 25, 26 showed significant biomass effect
642

GSL †	Factor	Value	Df	t-value/LLR	p-value	
GSL1	JA	0.11	91	1.154	0.252	
	Plant biomass	0	91	-0.896	0.373	
	Family		4	1.735	0.187	
GSL2	JA	-0.062	91	-1.19	0.237	
	Plant biomass	0	91	0.055	0.956	
	Family		4	0	1	
GSL3	JA	0.063	91	0.88	0.381	
	Plant biomass	0	91	0.769	0.444	
	Family		4	12.671	0	***
GSL4	JA	0.047	91	0.433	0.666	
	Plant biomass	0	91	1.362	0.177	
	Family		4	51.395	0	***
GSL5	JA	0.036	91	0.394	0.695	
	Plant biomass	0	91	0.673	0.502	
	Family		4	6.591	0.01	**
GSL6	JA	0.025	91	0.15	0.881	
	Plant biomass	0	91	-0.104	0.918	
	Family		4	0.099	0.753	
GSL7	JA	0	91	-0.005	0.996	
	Plant biomass	0	91	0.118	0.907	
	Family		4	7.301	0.006	**
GSL8	JA	0.047	91	0.284	0.777	
	Plant biomass	0.001	91	1.491	0.139	
	Family		4	18.37	0	***
GSL9	JA	-0.086	91	-2.846	0.006	**
	Plant biomass	0	91	-0.299	0.766	
	Family		4	27.569	0	***
GSL10	JA	-0.02	91	-0.297	0.768	
	Plant biomass	0	91	0.33	0.742	
	Family		4	3.254	0.071	°
GSL11	JA	0.094	91	1.178	0.242	
	Plant biomass	0	91	2.168	0.033	**
	Family		4	19.56	0	***
GSL12	JA	-0.095	91	-1.769	0.08	°
	Plant biomass	0	91	0.808	0.421	
	Family		4	3.021	0.082	°

GSL13	JA	0.036	91	0.605	0.547	
	Plant biomass	0	91	1.476	0.143	
	Family		4	21.435	0	***
GSL14	JA	-0.112	91	-2.165	0.033	**
	Plant biomass	0	91	0.046	0.963	
	Family		4	3.043	0.081	°
GSL15	JA	-0.081	91	-1.019	0.311	
	Plant biomass	0	91	-0.517	0.606	
	Family		4	0.031	0.859	
GSL16	JA	0.062	91	0.884	0.379	
	Plant biomass	0	91	0.172	0.864	
	Family		4	1.783	0.181	
GSL17	JA	-0.094	91	-1.74	0.085	°
	Plant biomass	0	91	0.754	0.453	
	Family		4	2.751	0.097	
GSL18	JA	0.021	91	0.126	0.9	
	Plant biomass	0	91	0.44	0.661	
	Family		4	0	1	
GSL19	JA	-0.092	91	-1.71	0.091	
	Plant biomass	0	91	0.778	0.439	
	Family		4	2.579	0.108	
GSL20	JA	-0.057	91	-1.535	0.128	
	Plant biomass	0	91	-0.393	0.696	
	Family		4	0.857	0.005	**
GSL21	JA	-0.091	91	-1.768	0.081	
	Plant biomass	0	91	-0.052	0.959	
	Family		4	2.735	0.098	°
GSL22	JA	0.113	91	0.804	0.424	
	Plant biomass	0	91	-0.228	0.821	
	Family		4	14.837	0	***
GSL23	JA	-0.091	91	-1.768	0.081	°
	Plant biomass	0	91	-0.052	0.959	
	Family		4	2.735	0.098	°
GSL24	JA	0.069	91	0.634	0.528	
	Plant biomass	-0.001	91	-4.379	0	***
	Family		4	0.812	0.367	
GSL25	JA	-0.009	91	-0.081	0.935	
	Plant biomass	-0.002	91	-5.913	0	***
	Family		4	3.536	0.06	°
GSL26	JA	0.04	91	1.888	0.062	°
	Plant biomass	0	91	-3.367	0.001	**
	Family		4	9.61	0.002	***
GSL27	JA	-0.089	91	-1.649	0.103	
	Plant biomass	0	91	0.199	0.843	
	Family		4	1.311	0.252	

GSL28	JA	-0.109	91	-2.082	0.04	
	Plant biomass	0	91	-0.027	0.978	
	Family		4	1.966	0.161	
GSL Total	JA	0.029	91	0.194	0.847	
	Plant biomass	0	91	0.489	0.626	
	Family		4	0	1	
Seed production	JA	-80.991	116	-1.697	0.092	°
	Plant biomass	0.171	116	1.206	0.23	
	Family		4	19.551	0	***
<i>S. littoralis</i>	JA	-0.835	92	-4.736505	0	***
	Plant biomass	0	92	-0.087	0.93	
	Family		4	13.989	0	***
<i>P. brassicae</i>	JA		92		0.2	
	Plant biomass		92		0.4	
	Family		4		0.32	

Signif. codes: *** < 0.001, ** < 0.01, * < 0.05, ° < 0.1

† GSL1 = Glucoraphanin; GSL2 = Hydroxypropyl gsl; GSL3 = Progoitrin; GSL4 = Glucoalyssin; GSL5 = Glucoputranjivin; GSL6 = Gluconapin; GSL7 = Butyl gsl; GSL8 = Glucobrassicinapin; GSL9 = Glucohirsutin; GSL10 = Glucoerucin; GSL11 = Glucoberteroin; GSL12 = 8-Methylthiooctyl gsl ; GSL13 = Gluconapoleiferin; GSL14 = Hydroxymethylbutyl gsl ; GSL15 = 2-Methylbutyl gsl; GSL16 = Sinalbin; GSL17 = Veratryl gsl; GSL18 = Glucotropeolin; GSL19 = Trimethoxy gsl; GSL20 = 5-Benzoyloxypentyl ; GSL21 = 2-Hydroxy-2-phenylethyl gsl; GSL22 = Gluconasturtiin; GSL23 = Hydroxybenzyl-methylether gsl; GSL24 = Glucobrassicin; GSL25 = Methoxyglucobrassicin; GSL26 = Neoglucobrassicin; GSL27 = Unknown.C16H23NO10S2; GSL28 = Unknown.C19H28N3O12S3.

Table 2. Permutational multivariate analysis of variance (PERMANOVA) table for testing the effect of JA treatment and family on the structure of the glucosinolate (GSLs) matrix.

Factor	Df	MSQ	F value	R ²	P value
JA treatment	1	0.136213	2.04795	0.01494	0.118
Family	25	0.126121	1.89621	0.34592	0.005**
Plant biomass	1	0.192319	2.89149	0.0211	0.054
JA * Family	25	0.063047	0.94791	0.17292	0.571
Residuals	61	0.066512		0.44512	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure legends

Fig. 1. Root to shoot induction of resistance against generalist and specialist herbivores. Boxplots show average weight gain of (a) *Spodoptera littoralis*, and (b) *Pieris brassicae* caterpillars feeding on plants that received jasmonic acid (JA) in the roots 4 days prior herbivory (JA, grey boxes), or received no JA in the roots (Control, open boxes)). Weight gain was calculated as the natural logarithm of the difference between final and initial fresh weight. Asterisks show significant differences across the two treatments ($p < 0.05$).

Fig. 2. Genetic variation in resistance and fitness related traits. Shown are reaction norm plots for the effects of JA treatment in roots on a) the weight gain of *S. littoralis* caterpillars, and b) total seed production across 26 maternal half-sib families of *C. hirsuta* plants subjected to herbivory by *S. littoralis*. Grey lines represent maternal families' averages in the constitutive (Control) state and in the induced state after JA addition the roots (JA). Black lines and dots represent overall averages across families.

Fig. 3. Glucosinolate (GSL) production across *Cardamine hirsuta* half-sib families. **a)** Barplot representation of the concentration of the individual GSLs in leaves of *C. hirsuta* plants that either received JA in the roots 4 days prior to the start of herbivory (JA, grey bars), or did not receive JA treatment in the roots (Control, open bars). Asterisks indicate a significant effect of JA treatment in production of GSLs. GSL1 = Glucoraphanin; GSL2 = Hydroxypropyl gsl; GSL3 = Progoitrin; GSL4 = Glucoalyssin; GSL5 = Glucoputranjivin; GSL6 = Gluconapin; GSL7 = Butyl gsl; GSL8 = Glucobrassicinapin; GSL9 = Glucohirsutin; GSL10 = Glucoerucin; GSL11 = Glucoberteroin; GSL12 = 8-Methylthiooctyl gsl ; GSL13 = Gluconapoleiferin; GSL14 = Hydroxymethylbutyl gsl ; GSL15 = 2-Methylbutyl gsl; GSL16 = Sinabin; GSL17 = Veratryl gsl; GSL18 = Glucotropeolin; GSL19 = Trimethoxy gsl; GSL20 = 5-Benzoyloxypentyl ; GSL21 = 2-Hydroxy-2-phenylethyl gsl; GSL22 = Gluconasturtiin; GSL23 = Hydroxybenzyl-methylether gsl; GSL24 = Glucobrassicin; GSL25 = Methoxyglucobrassicin; GSL26 = Neoglucobrassicin; GSL27 = Unknown.C16H23NO10S2; GSL28 = Unknown.C19H28N3O12S. **b)** Non-multidimensional scaling (nMDS) ordination of the individual glucosinolates found in *C. hirsuta* leaves across 26 plant families at the constitutive state (open dots), or after roots induction with JA (black dots). Numbers besides dots correspond to plant families.

Fig. 4. Herbivore impact on plant fitness. Boxplots show the total seed number produced by 26 maternal half-sib families of *C. hirsuta* plants in plants that did not receive JA in the roots nor treated aboveground herbivory (No herbivory), plants that received jasmonic acid (JA) in the roots 4 days prior to the start of herbivory by *Spodoptera littoralis* or *Pieris brassicae* (*P. brassicae* / JA, *S. littoralis* / JA), and plants subjected to herbivory either by *S. littoralis* or *P. brassicae* but that were not treated by JA in the roots (*P. brassicae* / C, *S. littoralis* / C).

Fig. 5. Fitness benefits of JA root induction during herbivory. Shown are interaction plots indicating the relationship between the total seed production and total glucosinolates, as well as seven individual GSLs that showed interaction with JA across all plants subjected to herbivory by *S. littoralis* in control and JA treatment (see Table S3).

Figures

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5