Diverse allyl glucosinolate catabolites independently influence root growth and development

Ella Katz¹, Rammyani Bagchi², Verena Jeschke³, Alycia R. M. Rasmussen¹, Aleshia Hopper¹, Meike Burow³, Mark Estelle² and Daniel J. Kliebenstein¹,³ x

¹Department of Plant Sciences, University of California, Davis, One Shields Avenue, Davis, CA, 95616, USA
²Section of Cell and Developmental Biology and Howard Hughes Medical Institute, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA
³DynaMo Center of Excellence, University of Copenhagen, Thorvaldsensvej 40, DK-1871, Frederiksberg C, Denmark

xCorresponding Author: Kliebenstein@ucdavis.edu

Short title: Allyl-GSL catabolites and root development

One sentence summary: Allyl-glucosinolate and its catabolites use multiple mechanisms to affect plant growth and development through specific responses that are optimal to any given environment.

Author Contributions: DJK and EK designed the research, analyzed the data and wrote the paper. RB, VJ, ARMR, AH and EK performed the research. All authors contributed to writing and editing the paper.
**ABSTRACT**

Glucosinolates (GSLs) are sulfur-containing defense metabolites produced in the Brassicales, including the model plant Arabidopsis (*Arabidopsis thaliana*). Previous work suggests that specific GSLs may function as signals to provide direct feedback regulation within the plant to calibrate defense and growth, including allyl-GSL, a defense metabolite and one of the most widespread GSLs in Brassicaceae that has also been associated with growth inhibition. Here we show that at least three separate potential catabolic products of allyl-GSL or closely related compounds affect growth and development by altering different mechanisms influencing plant development. Two of the catabolites, raphanusamic acid and 3-butenolic acid, differentially affect processes downstream of the auxin signaling cascade. Another catabolite, acrylic acid, affects meristem development by influencing the progression of the cell cycle. These independent signaling events propagated by the different catabolites enable the plant to execute a specific response that is optimal to any given environment.

**Keywords:** Allyl-GSL, Arabidopsis thaliana, auxin, cell cycle, defense metabolites, plant defense, plant growth.
INTRODUCTION

Pathogens and herbivore attacks are a critical life-long threat to any plant. To survive these attackers, plants have developed a variety of defense mechanisms and resistance strategies, including the production of defensive chemicals (Chen 2008). However, indiscriminate use of these defense chemicals by the plant can have detrimental effects on growth or introduce ecological costs by attracting specialized attackers (Hartmann 2004). Therefore, maximizing the effectiveness while limiting the detriments of these defense chemicals, and the plant immune system in general, requires that they are produced in the proper tissue, cell and developmental stage. This requires a central coordination with the developmental programming of the plant, though the nature of this coordination is yet to be fully understood (Agrawal et al. 1999, Campos et al. 2016, Guo et al. 2018, Kliebenstein 2016, Strauss & Agrawal 1999). While previous models assumed that this coordination was a simple tradeoff between growth and defense, it is now clear that growth, development and defense are in a more complex relationship with the potential for synergism (Coley et al. 1985, Kliebenstein 2016, Wasternack 2017). For example, any cost of defense metabolism is likely at most temporary to allow the plant to deal with the immediate threat and increases the long-term fitness potential.

To fully understand the connection between defense and growth requires understanding how the two sides of this equation mutually interact. Development is well known to influence defense, as there are dramatic changes in a plant’s defense arsenal during its life cycle to adjust to the different biotic attackers prevalent at each stage. For example, plants alter physical defenses like trichomes and spines across their development. Further, plants dramatically shift their chemical defense throughout development (Barton & Koricheva 2010). A well-studied example of how chemical defenses change across the plant life-cycle is displayed by the glucosinolates (GSLs), key insect and pathogen defense metabolites in Cruciferous plants including the model plant Arabidopsis (Arabidopsis thaliana) (Beekwilder et al. 2008). GSL accumulation and composition dramatically changes throughout the life of a plant and can alter the sensitivity to pathogens or insects (Brown et al. 2003, Clay et al. 2009, Hopkins et al. 2009, Korves & Bergelson 2004, Wentzell & Kliebenstein 2008, Wentzell et al. 2008, Wittstock & Burow 2010). In addition to development influencing chemical defenses across time, development also influences defense patterning within a specific tissue. By creating non-uniform defense distributions, it is more difficult for the pathogen to establish an efficient and effective
attack (Kliebenstein 2013, Shelton 2004, 2005; Shroff et al. 2008). Recent work is identifying
the mechanisms by which development influences defense (De Bruyne et al. 2014, Li et al. 2004,
Melotto et al. 2008, Shelton 2004, 2005).

In addition to development modulating defense, there is a developing understanding that
defense outputs, like defense metabolites, can modulate development. For example, GSL studies
in Arabidopsis and other Brassicaceae have started to highlight the potential for defense
metabolites to directly influence growth and development. In the *Raphanus* genus, seedling
phototropism can be regulated by a local gradient of catabolism of a specific GSL, via
influencing the TRANSPORT INHIBITOR RESPONSE (TIR1) auxin receptor (Hasegawa et al.
2000, Yamada et al. 2003). Similarly, the indole GSL catabolism product indole-3-carbinol
(I3C), produced during pathogen attack, inhibits auxin signaling by competing with auxin for
binding the TIR1 auxin receptor, leading to growth arrest (Katz & Chamovitz 2017, Katz et al.
2015b). Further highlighting the potential for defense metabolites to influence growth-related
signaling pathways is 3-hydroxypropylglucosinolate (3OHPGSL), which interacts with the TOR
pathway (Target of Rapamycin, a central developmental regulator), allowing 3OHPGSL to
inhibit growth in both plants and fungi (Malinovsky et al. 2017). While these studies indicate the
potential of defense metabolites to influence growth and development signaling in plants, the
mechanisms behind their coordination are unknown. One question that has not been assessed is if
a single defense metabolite creates a single mechanistic response or signal, or if single defense
metabolites might branch into multiple signals or responses.

To gain a better understanding of how plant defense metabolites may integrate growth
and defense, we studied how allyl-GSL influences growth in Arabidopsis. Allyl-GSL has been
linked to resistance to numerous insects and pathogens and is an attractant to specialist insects
that are adapted to the Brassicaceae (Lankau 2007). Although allyl-GSL is one of the most
widespread aliphatic GSLs within the Brassicaceae, the Brassicaceae also display extensive
variation for the presence or absence of allyl-GSL due to independent losses of the 2-
oxoglutarate-dependent dioxygenases 2 (AOP2) enzyme required for its biosynthesis. This
diversity suggests that the accumulation of allyl-GSL may be detrimental in some environments.
This is further supported by the observation that Brassica genotypes accumulating high allyl-
GSL are less competitive in comparison to low accumulating genotypes (Lankau 2007). In
addition, exogenous or endogenous introduction of allyl-GSL into Arabidopsis accessions
without allyl-GSL leads to a reduction in growth (Burow et al. 2015, Francisco et al. 2016a, Urbancsok et al. 2017, Wentzell & Kliebenstein 2008). Allyl-GSL’s effects on growth are not limited to Brassicaceae but are also linked to allelopathic effects against diverse plants in other plant families (Bialy et al. 1990, Lankau 2007, Uremis et al. 2009, Vaughn & Berhow 1999, Vaughn et al. 2006). However, the mechanism by which allyl-GSL may function or what is/are the most likely bioactive compound(s) are unknown. One possible mechanism of these effects is that allyl-GSL can induce localized stomatal closure in response to a wound and alter the circadian periodicity of Arabidopsis, likely via isothiocyanate (Khokon et al. 2011, Zhao et al. 2008). An alternative possibility is suggested by a genome-wide association study that found an association between natural variation in auxin signaling genes and altered growth in response to allyl-GSL in Arabidopsis (Francisco et al. 2016a,b).

To develop a deeper mechanistic understanding of how allyl-GSL modulates plant growth, we focused on the effect of allyl-GSL and its associated catabolites on Arabidopsis root growth and root morphology. Allyl-GSL effects were dependent on the specific catabolite with three different allyl-GSL-derived catabolites activating at least two distinct signaling processes. One pathway led to altered cell-cycle progression, while the other pathway functioned downstream of auxin perception to modulate the PIN-FORMED(PIN) protein distribution. With each catabolite influencing a different component of root growth and development, this may allow the plant to respond to the specific biotic events that are influencing defense metabolism. Thus, this could allow the plant to create a specific response that is optimal to any given environment. These results extend our understanding of how plants integrate growth and defense and thrive under changing biotic environments.

RESULTS

Allyl-GSL and auxin coordinately influence growth. Arabidopsis accessions present extensive variation in the concentrations of endogenous allyl-GSL, ranging between hundreds to thousands μM in fresh weight, during different growth stages (Chan et al. 2011, Kliebenstein et al. 2001c) (Table 1). About a third of natural Arabidopsis accessions like Col-0 do not produce endogenous allyl-GSL due to a natural knockout in the AOP2 enzyme (Chan et al. 2011, Kliebenstein et al. 2001c), but when the AOP2 enzyme is reintroduced into these genotypes they produce ~150 μM of endogenous allyl-GSL and have reduced growth (Francisco et al. 2016a) (Table 1). In addition
to a response to endogenous allyl-GSL, Col-0 plants growing on exogenous allyl-GSL will accumulate allyl-GSL and show a typical responsiveness to allyl-GSL in comparison to other accessions (Francisco et al. 2016a, Jeschke et al. 2019) (Table 1). In our experiments, we used the Arabidopsis Col-0 accession, as it provides a clean background wherein there is no endogenous compound to confound these experiments. Based on the multiple lines of evidence presented in Table 1, we decided to work with a concentration of 50 μM, which is lower than the endogenous allyl-GSL concentration produced by different accessions and lower than the endogenous concentration produced by AOP2::Col-0 plants, and hence can be considered as physiologically relevant. As a control, we first tested if allyl-GSL was taken up and accumulated in the plants under our experiments (Francisco et al. 2016a). We grew Col-0 seedlings on a medium supplemented with or without allyl-GSL, and after 14 days we measured the GSL content in the seedlings. As expected, accumulation of allyl-GSL was detected only in plants that grew on a medium supplemented with this molecule (Fig. S1A).

Previous genome-wide association mapping of genes that may influence the effect of allyl-GSL on plant growth found an enrichment in putative auxin signaling genes, suggesting a link between allyl-GSL responses and auxin (Francisco et al. 2016b). To investigate this potential connection we analyzed the effect of allyl-GSL on two auxin-mediated responses, inhibition of root elongation and root curliness. To test if allyl-GSL modulates the ability of auxin to inhibit root elongation, Arabidopsis Col-0 seedlings were grown vertically on Murashige and Skoog (MS) medium supplemented with different concentrations of allyl-GSL and auxin (3-indoleacetic acid, IAA).
After 14 days we measured the length of the primary root. IAA and allyl-GSL individually inhibited root growth in dose-dependent manners. Critically, at the higher allyl-GSL concentration allyl-GSL and IAA had a synergistic interaction leading to higher root inhibition than expected when combining the effects of the individual treatments (P-value < 1.55E-08, Fig. 1A,B). Using the same experimental design, we tested for an interaction of auxin and allyl-GSL on root curliness, an auxin-dependent phenotype (Dolan 1998). Arabidopsis Col-0 seedlings were grown vertically on clean MS medium for four days, then transferred to medium supplemented with different concentrations of IAA, allyl-GSL or both. After one additional day the gravity stimuli was applied by tilting the plates to a 45° angle against the gravity vector (Okada & Shimura 1990). After 3 days of growth in tilted position, we counted the number of root curls within 1 cm of the root tip (Mochizuki et al. 2005). Again, the independent addition of allyl-GSL or auxin decreased the number of curls in the roots (Fig. 1C,D. Detailed statistic in Sup.T1). This experiment shows that the addition of allyl-GSL causes the plant to behave as if it was exposed to a higher dose of auxin. The inhibitory effect of auxin on root curls was amplified by the addition of allyl-GSL only in the lower auxin concentrations with no effect at the higher auxin concentrations (Fig. 1C,D). Statistical analysis using an ANOVA showed a highly significant interaction between auxin and allyl-GSL supporting that they have a synergistic relationship (Sup.T1). These results suggest that allyl-GSL inhibits auxin-linked phenotypes and that the two compounds have a synergistic interaction, indicating that they function via a similar pathway.

**Allyl-GSL catabolites inhibit root growth.** While the above activities are linked to the application of allyl-GSL, it is possible that the allyl-GSL is converted into other compound(s) that mediate the identified activities. GSLs are broken down by myrosinase and various modifying enzymes into a diverse set of catabolites that are known to have different activities against biotic attackers (Halkier & Gershenzon 2006, Xue et al. 1992). Thus, we proceeded to test if any common allyl-GSL catabolism products may be responsible for the observed root inhibition.

In roots of Arabidopsis catabolism of allyl-GSL by myrosinase and nitrile specifier proteins results in formation of allyl-nitrile and allyl-isothiocyanate (Burow et al. 2008, 2009; Urbancsok et al. 2017, Wentzell & Kliebenstein 2008) (Fig. 2A). To test if the effect of these catabolites is
similar to allyl-GSL, seedlings were grown on MS medium supplemented with each of these molecules and IAA.

Allyl-isothiocyanate had no detectable effect on root length, with or without IAA (Fig. 2B). One potential explanation for this is that isothiocyanate is considered to be highly reactive and the molecule may have reacted with the media or the external cell walls, decreasing the potential concentration. In contrast, allyl-nitrile affected root length similarly to allyl-GSL (Fig. 2B). Roots of seedlings grown on medium supplemented with allyl-nitrile were shorter than roots of seedlings grown on the control medium. Further, the allyl-nitrile effect was dependent on the level of IAA within the medium (Fig. 2B, detailed statistic in Sup.T1). Higher concentrations of all allyl-GSL catabolites (100 µM) were tested, and showed the same trends (Fig. S2).

Allyl-isothiocyanate can be further converted by separate catabolic pathways that lead to the generation of acrylic acid and raphanusamic acid (Bednarek et al. 2009, Piślewska-Bednarek et al. 2018, Wittstock et al. 2016) (Fig. 2A). Correspondingly, allyl-nitrile can be converted to 3-butenoic acid by nitrilase that has activity against aliphatic nitriles in Arabidopsis (Bartling et al. 1992, 1994; Burow et al. 2008, 2009; Kissen & Bones 2009, Kuchernig et al. 2012, Piotrowski 2008, Piślewska-Bednarek et al. 2018, Urbancsok et al. 2018, Vorwerk et al. 2001, Wajant & Effenberger 2002).

Raphanusamic acid can be synthesized from other GSL-derived isothiocyanate (Bednarek et al. 2009), thus Col-0 plants produce low concentrations of endogenous raphanusamic acid during different growth stages that correlate to total GSL amounts (Bednarek et al. 2009, Jeschke et al. 2019, Sanchez-Vallet et al. 2010) (Table 1). When the plants are grown on exogenous allyl-GSL, higher concentrations of raphanusamic acid accumulate in the plant (Jeschke et al. 2019) (Table 1). The Arabidopsis cell wall irx1-6 mutant shows high resistance to pathogens and presents high endogenous concentrations of raphanusamic acid (Sanchez-Vallet et al. 2010) (Table 1). Different Cruciferous plants produce concentrations of endogenous raphanusamic acid (50-150 µM) before and after pathogen attack (Bednarek et al. 2011, Palliyaguru et al. 2019) (Table 1). These data suggest that working with a concentration of 50 µM (as for allyl-GSL) can be considered as physiologically relevant for raphanusamic acid especially as the Arabidopsis Col-0 accession is amongst the lower GSL-accumulating Arabidopsis accessions. As a control, we tested if raphanusamic acid was taken up and accumulated in the plants under our experiments.
(Francisco et al. 2016a). As expected, accumulation of raphanusamic acid was detected in plants grown on a medium supplemented with this molecule (Fig. S1B).

The separation and quantification of acrylic acid and butenoic acid is difficult, due to the generic structure and low mass of these molecules, and data on the endogenous concentrations of these catabolites do not exist. Given the accepted biosynthetic pathway for raphanusamic acid production from an isothiocyanate, this predicts that that there will be a 1:1 equivalency in the production of raphanusamic acid and carboxy acid precursor from every isothiocyanate (Bednarek et al. 2009, Piślewska-Bednarek et al. 2018, Wittstock & Burow 2010, Wittstock et al. 2016). Therefore, we compromised on a uniform concentration of 50 μM for all of the catabolites.

To test the effect of these catabolites on root length, we grew seedlings on MS media with different concentrations of IAA and each compound. In contrast to allyl-isothiocyanate and allyl-nitrile, all three compounds strongly inhibited root length, and their effect was greater than with either allyl-GSL, nitrile or isothiocyanate (Fig. 2C). Further, there were two distinct inhibition trends. Raphanusamic acid showed an IAA interaction similar to allyl-GSL albeit with stronger effects. In contrast, butenoic acid and acrylic acid had strong effects on root growth across all IAA concentrations. These two molecules have similar structure; butenoic acid has a four-carbon backbone while acrylic acid has a three-carbon backbone (Fig. 2C, additional concentrations in Fig. S2, detailed statistic in Sup.T1). Based on the root growth assays, we conclude that these three catabolites of allyl-GSL are all bioactive, and because of the stronger activity, are probably closer to the actual active compounds responsible for the effects we observed when adding allyl-GSL. The presence of two different activities suggests that these molecules function via at least two mechanisms: butenoic acid and acrylic acid may affect root growth through a similar pathway, while raphanusamic acid works through a different one.

To determine whether allyl-GSL or one of its catabolites produce toxic compounds that lead to root inhibition by causing cell death, seedlings were grown on allyl-GSL or each catabolite for 14 days. All were still green and viable, arguing against toxicity effects. To further check the viability of the seedlings after allyl-GSL treatment we tested if the effect of allyl-GSL on roots growth was reversible. Seedlings were grown vertically on a clean medium, or a medium supplemented with allyl-GSL and IAA. After seven days the seedlings were transferred to the
opposite medium, or to a new medium with the same supplementation. After an additional seven
days the primary roots length of the seedlings was measured. Roots of seedlings grown on a
media with allyl-GSL and IAA and then transferred to a clean media were significantly longer
than the roots of seedlings grown on allyl-GSL and IAA for the entire experiment (Fig. 2D). This
indicates that the seedlings were able to recover from the allyl-GSL treatment. We then
conducted a more direct method to test if allyl-GSL or any of its catabolites cause cell death.
Seedlings were grown on each of the catabolites and stained with trypan blue, which selectively
stains dead cells. Surveying roots from 10 seedlings grown in each of the treatments showed no
evidence of cell death (Fig. 2E). As a positive control for the staining method, seedlings were
treated with 500 mM of NaCl for 24 hours, then imaged. These seedlings showed a strong blue
coloration, indicating massive cell death (Fig. S3). These results show that the application of
allyl-GSL or its catabolites did not lead to cell death.

**Allyl-GSL catabolites affect GSL content.** To further characterize how allyl-GSL and each of
its catabolites affect roots, we checked whether they affect endogenous GSL accumulation in the
plants. Endogenous GSL levels were measured from seedlings grown for 14 days on medium
with each catabolite. We found that allyl-GSL and butenoic acid affected the aliphatic 4-carbon
GSL pathway (Fig. 3). This phenotype was defined by calculating the ratio between 4-
methylsulfinylbutyl (4MSB) to 4MSB and 4-methylthiobutyl (4MTB) (Fig. 3). The amount of
indolic GSL, synthesized from tryptophan, was also impacted, hence presenting an effect on a
parallel GSL pathway. Interestingly, only acrylic acid affected this phenotype, as seedlings that
were grown on a medium with acrylic acid had a higher amount of indolic GSL (Fig. 3, detailed
statistic in Sup.T1).

**Allyl-GSL catabolites have different effects on the root meristem.** To dissect the
developmental process by which the allyl-GSL catabolites influence root growth, we measured
their effects on root morphology. Inhibition of primary root growth can arise from either a
reduction in the number of cells (as a result of an inhibition in cell divisions), or because each
cell is smaller (Beemster & Baskin 1998). As the root growth assays suggest that the catabolites
are closer to the actual active compounds, we focused on testing their effect on roots
morphology. Seedlings were grown on MS medium supplemented with each compound, and the
meristem size and the distance from the tips to the elongation zone of the seedlings were
measured. The distance from the tip to the elongation zone was defined as the distance from the root tip to the first root hair. Seedlings treated with raphanuseramic acid, butenoic acid and acrylic acid had shorter elongation zones in comparison to seedlings grown on control media (Fig. 4A, detailed statistic in Sup.T1). We then measured the meristem size as the number of cells between the quiescent center and the first elongated cell. Only acrylic acid caused a significant reduction in the number of cells in the meristem in comparison to the control roots (Fig. 4B, detailed statistic in Sup.T1). To test how these catabolites may interact with IAA signaling processes, we assayed how these catabolites affect auxin signaling in the meristems. For this purpose, we used plants expressing the interaction domain II of Aux/IAA attached to a VENUS marker (DII-VENUS), which is sensitive to the presence of auxin in a dose-dependent manner (Brunoud et al. 2012). DII-VENUS seedlings were grown on MS medium and treated with one of the allyl-GSL catabolites. The seedlings were imaged using confocal microscopy, and the mean density of the VENUS fluorescence in the root was measured. The root meristem requires a specific concentration of auxin for proper development (Sabatini et al. 1999), hence we quantified the mean fluorescence intensity only in the meristem area, as indicated in Figure 4C. Two hours following treatment with raphanuseramic acid or butenoic acid, the DII-VENUS fluorescence intensity in the meristems was significantly lower in comparison to the control (Fig. 4D, detailed statistic in Sup.T1). In contrast, acrylic acid did not have a significant effect on the DII-VENUS fluorescence intensity. This indicates that the short treatment with raphanuseramic acid or butenoic acid increased auxin-related signaling in the root meristem. In opposition to our hypothesis, these results indicate that even though acrylic acid and butenoic acid have similar root inhibition phenotypes, this likely happens via different mechanisms. This opens the door for a potential third mechanism that affects root length and demonstrates that each allyl-GSL catabolite may have a different mechanism to affect root inhibition. We then continued to dissect the involvement of the catabolites on each one of these processes.

**Acrylic acid affects meristem development.** The presence of fewer cells in the root meristem after acrylic acid catabolite exposure suggested that this compound may affect cell cycle dynamics. To test this hypothesis we used plants expressing cyclin B1-1::GFP (Bush et al. 2015), a cell cycle reporter. Cyclin B1-1 is expressed in G2 and early M phase (Kobayashi et al. 2015), and the expression of this reporter gene indicates cells’ progress through mitosis. Stronger expression in the nucleus than in the cytoplasm is an indication that the cell is at a late G2 phase
(Fig. 4E) (Bush et al. 2015). These seedlings were grown on MS medium, treated with or without acrylic acid, butenoic acid, or raphanusamic acid for two hours and then imaged using confocal microscopy. We counted the number of cells in each root that were in late G2 phase, and calculated their percentage out of all the cells expressing this reporter gene. The number of cells expressing this reporter gene was not significantly different following the treatment with the different catabolites. However, the percentage of cells in late G2 phase was significantly reduced following treatment with acrylic acid compared to the control cells (Fig. 4F). Butenoic acid treatment resulted in a minor decrease in this percentage, while raphanusamic acid treatment resulted in a slight increase in this percentage. However, both were not significant in agreement with their lack of effect on the meristem cell number. This indicates that only acrylic acid, but not butenoic acid or raphanusamic acid, influences cell cycle.

**Raphanusamic acid affects the auxin machinery.** Since raphanusamic acid had a rapid effect on auxin signaling in the meristem and a synergistic interaction with auxin, we tested if this catabolite influences different parts of the auxin signaling machinery. For this we used several auxin marker lines that represent different parts of the auxin machinery and analyzed the effect of raphanusamic acid on those markers at different time points. The different marker lines were grown on MS medium, treated with raphanusamic acid, and imaged using confocal microscopy every 30 minutes. For each time point we quantified the fluorescence signal in the root tips (not only in the meristem area as above) and calculated the relative signal in comparison to the untreated seedlings by dividing the fluorescence of the treated seedlings by the mean fluorescence of the untreated seedlings. Treatment of 60 minutes with raphanusamic acid resulted in a significant reduction in the DII-VENUS intensity in comparison to the untreated seedlings, indicating a quick increase in auxin signaling (Fig. 5A,C). We then tested if raphanusamic acid affects auxin transporters. We first used seedlings expressing the auxin transporter PIN1-GFP marker (Benková et al. 2003) and found a significant reduction in the intensity of this transporter within 60 minutes of exposure to raphanusamic acid (Fig. 5A, S4B). Using seedlings expressing the pPIN7::PIN7-GFP auxin transporter marker, we found that the localization and amount of PIN7 was not affected significantly by raphanusamic acid treatment (Fig. 5A, S4C) (Blilou et al. 2005). We then checked how raphanusamic acid treatment may directly affect the TIR1 auxin receptor using seedlings expressing TIR1-VENUS (Wang et al. 2016). Raphanusamic acid significantly reduced the activity of this reporter 60 minutes
following the treatment (Fig. 5A, S4A). These experiments show that raphanusamic acid affects
auxin signaling and transporters at around 60 minutes following the treatment.

**Butenoic acid affects the auxin machinery.** As butenoic acid also had a rapid effect on auxin
signaling in the meristem, we tested how this molecule affects each of the auxin marker and
receptor lines. Treatment of 30 minutes with butenoic acid treatment resulted in a significant
reduction in the DII-VENUS intensity compared to the untreated seedlings (Fig. 5B,C). We also
found a significant reduction in the intensity of the PIN1 transporter 60 minutes following the
treatment (Fig. 5B, S4B), and a significant reduction in the intensity of the PIN7 transporter in
the root cap 90 minutes following the treatment (Fig. 5B, S4C). Lastly, we found that treatment
with butenoic acid significantly reduced the activity of TIR1-VENUS reporter 90 minutes
following the treatment (Fig. 5B, S4A). Using these marker lines, we conclude that butenoic acid
affects the auxin machinery in a specific order. Butenoic acid first affects auxin signaling, then
auxin transporters PIN1 and PIN7, and finally the auxin receptor TIR1.

Our results clearly show that both raphanusamic acid and butenoic acid affect the auxin
machinery, but the timing of the effects of each one of the catabolites on the different
components of the auxin machinery is different. In combination with their different chemical
structure, this suggests that raphanusamic and butenoic acid may have different molecular
targets. To test whether they have different molecular targets, we analyzed if they interact to
modulate root growth. In pharmacological assays an interaction between two compounds
suggests that they work through the same target (Jia et al. 2009). Seedlings were grown on
medium supplemented with or without butenoic acid, raphanusamic acid, or both, and root
lengths were measured. ANOVA was used to test the effect of each defense catabolite
individually as well as their interaction in respect to root length (Fig. 5E). Within these
conditions, there was no detectable interaction between the two catabolites. In agreement with
the auxin-related marker genes, this suggests that the two catabolites function through different
targets. Hence, we conclude that although butenoic acid and raphanusamic acid both affect the
auxin signaling machinery, this is via independent mechanisms.

**Raphanusamic acid and butenoic acid do not interact with the auxin receptors.** As both
butenoic acid and raphanusamic acid have a synergistic interaction with auxin in respect to root
length, and both have a very quick effect on auxin signaling, an obvious hypothesis was that they
interact with any of the TIR1/AFBs (Auxin signaling F-Box) auxin receptors. This hypothesis was supported by previous work that suggests that a specific GSL catabolite, I3C, interacts specifically with TIR1 in the same binding site as auxin (Katz et al. 2015b). Because the interaction between TIR1/AFBs and the Aux/IAA (IAA) family proteins is facilitated by auxin, we used a yeast two-hybrid (Y2H) system to measure if raphanusamic acid or butenoic acid alter this interaction (Calderón Villalobos et al. 2012). We tested the interaction between the receptors (TIR1 and AFB1-3) expressed in yeast (Saccharomyces cerevisiae, EGY48) and 10 different IAA proteins grown on medium supplemented with different concentrations of auxin, and with or without raphanusamic acid or butenoic acid. Raphanusamic acid or butenoic acid by themselves did not facilitate the TIR1/AFB and Aux/IAA interaction. Furthermore, adding these catabolites with auxin to the growth media did not change the interaction between the receptors in comparison to auxin alone (Fig. 5D, and Fig. S5 for additional concentrations and receptors). We then performed in vitro pull-down assays with Myc:TIR1 and GST:IAA14 protein in the presence of auxin or auxin and raphanusamic acid or butenoic acid (Fig. S5). Again, raphanusamic acid and butenoic acid did not affect the auxin-facilitated interaction between TIR1 and Aux/IAA. These experiments suggest that raphanusamic acid or butenoic acid do not interact directly with the auxin receptors and that the observed effects are via different mechanism(s).

To genetically test the in planta interaction of the catabolites with the auxin machinery and its receptors, we measured their effect on the root growth of the tir1-1 afb2-1 afb3-1 triple mutant. This mutant controls for the partial redundancy between TIR1 and the AFBs (Dharmasiri et al. 2005). We grew Col-0 and the triple mutant seedlings on medium with or without allyl-GSL, raphanusamic acid, and acrylic acid, measured their root length at day seven, then calculated the percentage of elongation compared to the untreated seedlings. The auxin triple mutant affected the sensitivity to allyl-GSL, but in contrast, had no effect on the sensitivity to raphanusamic acid and acrylic acid (Fig. S6). This supports the observation that acrylic acid does not work through the auxin pathway, and it suggests that allyl-GSL might have some additional auxin-related pathways that are yet to be identified.

**Allyl-GSL catabolites affect root growth in diverse species.** The previous suggestions that allyl-GSL may influence other plants led us to test if this extended to the allyl-GSL-related
catabolites. For this, we focused on the effect of allyl-GSL catabolites on the root growth of non-
Brassicaceae plant species that do not produce GSL. We used four different species from
different families [Basil (Ocimum basilicum) - Lamiales, Dill (Anethum graveolens) - Apiales,
Lettuce (Lactuca sativa) - Asterales and Tomato (Solanum lycopersicum) - Solanales] and grew
them on a medium with or without raphanusamic acid, acrylic acid, and butenoic acid, and
measured their root length after five days. All three allyl-GSL catabolites had an effect on the
tested species, but there was specificity to the effects for each catabolite. Acrylic acid inhibited
the root growth of dill, lettuce and basil with no effect on tomato. In contrast, butenoic acid
inhibited the root growth of dill and lettuce while stimulating basil and having no influence on
tomato. Finally raphanusamic acid also inhibited dill and lettuce while dramatically inducing
root growth in tomato (Fig. 6). These results show that the allyl-GSL catabolites can influence
growth across a wide range of species (probably since it works through conserved mechanisms),
and that similar to Arabidopsis, it is likely that there are three different mechanisms being
targeted.

DISCUSSION

Allyl-GSL catabolites work through different mechanisms. In this work we found that the
defense metabolite allyl-GSL affects Arabidopsis root growth and development by three
different catabolic products, with each compound having a unique regulatory effect on root
development (summarized in Fig. 7). Acrylic acid has the most unique mechanism out of the
catabolites that were tested. Acrylic acid was the only molecule that affected meristem
development by influencing the cell cycle in the root tips. In contrast to acrylic acid, both
butenoic acid and raphanusamic acid manipulated several steps of the auxin machinery.
However, each molecule had a different order in which it affected each of the tested parameters,
suggesting that they work through different mechanisms. Supporting the idea that butenoic acid
and raphanusamic acid are working through different pathways was the fact that they showed no
pharmacological interaction. Future work is however required to identify the immediate targets
of these compounds. A few connections between Arabidopsis GSLs and auxin were suggested in
the past. One of them is the antagonistic character of an indolic GSL and auxin (Katz et al.
2015a,b), and another one shows that allyl isothiocyanate treatment induces the expression of 19
auxin-related genes (Kissen et al. 2016). Our results suggest that there are additional connections
between Arabidopsis GSL and auxin. This raises the question of how many other links may exist
given the diversity of GSLs across the Brassicaceae.

**Do different environments favor different molecules?** Following tissue rupture, potentially as
a result of herbivory attack, GSLs will mix with the myrosinase enzyme, and a variety of
breakdown products will be generated. The generation of the different catabolic products is
regulated by specific factors, including the expression of specific enzymes that differ across
tissues and accessions (Brown et al. 2003, Fu et al. 2016, Halkier & Gershenzon 2006, Wentzell
& Kliebenstein 2008). In Arabidopsis Col-0, the nitrile-specifier protein needed for production of
nitriles from exogenously fed allyl-GSL are not expressed in the leaves but are present in the
roots. This leads to allyl-isothiocyanate, the precursor of acrylic acid and raphanusamic acid,
being the prevalent form in Arabidopsis Col-0 leaves when feeding exogenous allyl-GSL. In
contrast, Arabidopsis Col-0 roots expressing the nitrile-specifier protein and hence allyl-nitrile,
the precursor of butenoic acid, are more common in Col-0 roots (Kissen & Bones 2009,
Urbancsok et al. 2017, Wentzell & Kliebenstein 2008, Wentzell et al. 2008). Further, growing
plants in crowded or uncrowded conditions can plastically alter the production of nitriles versus
isothiocyanates in older plants (Wentzell & Kliebenstein 2008). The fact that different GSL
catabolites are favored in specific tissues and in specific conditions implies that the effect of
allyl-GSL on development will be conditioned on the tissue or environment in which the study
occurs. Thus, different environments and situations in the plant’s life will favor the breakdown of
allyl-GSL to different catabolites, creating different signals, and consequently generating
different developmental effects. This creates the opportunity for the plant to potentially use this
to execute a specific response that is optimal to any given environment. As the complexity of the
environment is greater than the number of metabolites in a plant, this suggests that individual
metabolites within a plant should have multiple roles to maximize its efficiency and the fitness of
the plant (Kliebenstein 2018a). Furthermore, presenting complex and less predictable defense
mechanisms by the plant will probably decrease the rate of the counter adaptation of the attacker
(Hopper 1999, Kliebenstein 2018b, Veening et al. 2008). This can be a key aspect of allyl-GSL
potential allelopathic effects, as each of the catabolites also has different effects on other plant
species.
Future perspectives. The role of GSLs as signaling molecules and their effects on developmental processes in the plant are now starting to be revealed, and the accumulation of specific GSLs can provide direct feedback regulation within the plant to calibrate defense and growth. Recent work shows that specific GSLs affect plant growth through different mechanisms, among them an interaction with the auxin machinery and the TOR pathway (Katz et al. 2015b, Malinovsky et al. 2017). Here we expand the list of potential mechanisms influenced by GSL and related catabolites. We show that one GSL catabolite affects cell cycle regulation, and the other two catabolites affect the auxin machinery, probably by different mechanisms than previously shown. Moreover, we showed that even one GSL can have multiple mechanisms to affect plant development. Thus, there is not a single link between defense metabolism and growth but a web of signals from defense metabolism that can influence growth. Future studies are needed to assess how this may be occurring in plants that do not contain GSLs, as other defense metabolites are known to affect plant growth and development (Hartmann 2004).

The fact that the effect of allyl-GSL is dependent upon environmental conditions suggests that future studies aiming to dissect GSL mechanisms will have to consider a precise description of the behavior of each molecule under different environments and different conditions. Adding to this complexity is the fact that some of the catabolites can be synthesized by more than one precursor, rather than only from allyl-GSL. This is the case for butenoic acid, which can also be synthesized from but-3-enyl GSL (which is not produced in Col-0 plants), and raphanusamic acid, which can be synthesized from any GSL-derived isothiocyanate (Bednarek et al. 2009). Furthermore, raphanusamic acid has been linked to influencing other plant processes, including influencing chlorophyll content across a wide range of plant species, including those with and without GSL production (Inamori et al. 1992). This implies that even though GSLs are young compounds, their catabolites can have signaling roles wider than was previously considered. This raises the possibility that there is an interconnected network within plants that may measure multiple components of their metabolism to fine tune plant development and defense.

The complexity of the role of defense metabolites in coordinating defense and growth is known. In this work, we present another layer to this complexity by showing that one metabolite can have multiple mechanisms to affect development under different environments. We show that
allyl-GSL, which can regulate its catabolic products according to the changing biotic conditions, has multiple ways to affect plant growth and development. We propose that this allows the plant to sense the specific processes influencing defense metabolism, and then enable a specific response that is optimal to any given environment. This may apply to other plant species and different defense metabolites also known to have different developmental effects.

MATERIALS AND METHODS

Plant material and growth conditions

Arabidopsis (Arabidopsis thaliana) strains used in this work were all in the Columbia-0 (Col-0) background. Transgenic lines that were used in this work were the following: DII-VENUS (Brunoud et al. 2012), PIN1:GFP (Benková et al. 2003), PIN3:GFP (Xu & Scheres 2005, Zádníková et al. 2010), cyclin B1-1::GFP (Culligan et al. 2006), TIR1-VENUS (Wang et al. 2016), PIN7:GFP (Blilou et al. 2005), tir1-1 afb2-1 afb3-1 (Dharmasiri et al. 2005).

Seeds were surface sterilized with 4.125% (v/v) sodium hypochlorite and 0.01% Tween-20 for 15 minutes, then rinsed five times with distilled water. The seeds were then cultivated on Petri dishes containing Murashige and Skoog (MS) basal salt mixture, 1% sucrose, pH 5.8, with 0.8% agar.

For each experiment, six seeds were sown in a row across a 36 grid square 100 × 100 × 15 mm Petri dish (Fisherbrand) with one seed per grid square, approximately 13 mm apart. Each treatment was replicated across three independent plates within an experiment. Each experiment was conducted at least two independent times and the data combined for analysis (Francisco et al. 2016a,b). After planting on media, plates were stratified for two nights in the dark at 4°C to break dormancy.

Allyl-GSL and its catabolites were purchased from Sigma-Aldrich: allyl-GSL [(-)-Sinigrin hydrate (S1647)], allyl-nitrile (122793), allyl-isothiocyanate (377430), 3-butenolic acid (134716), acrylic acid (147230), raphanusamic acid ([4r]-(-)-2-thioxo-4-thiazolidinecahydroxylic acid (273449)], and indole-3-acetic acid (I5148). To test the effect of the GSL catabolites the different chemicals were dissolved in distilled water and were added to the autoclaved MS to a final concentration of 50 μM (unless mentioned otherwise). All compounds were soluble under these conditions. The plates were placed vertically at 22°C under light/dark conditions of 16 h
white light and 8 h darkness, with light at 100–120 μEi. For short treatments GSL catabolites were added to liquid MS with seven-day old seedlings.

To test root curliness, seedlings were grown vertically on clean MS medium for four days, then transferred to medium supplemented with different concentrations of IAA, allyl-GSL or both. After one additional day the plates were tilted to a 45° angle against the gravity vector, and after 3 days of growth the number of root curls within 1 cm from the tip was counted (Mochizuki et al. 2005, Okada & Shimura 1990).

Root length was measured using ImageJ software (https://imagej.nih.gov/ij/).

**Cell death detection by trypan blue staining**

Seedlings were harvested in acetic acid: ethanol solution (1:3 v/v) for one hour under constant shaking, then acetic acid: ethanol: glycerol (1:5:1 v/v/v) for an additional hour, then stained with trypan blue for one hour (0.05% trypan blue and 0.01% aniline blue in lactic acid: phenol: distilled water: glycerol, 1:1:1:0.75 v/v/v/v) (Chung et al. 2010). The staining solution was removed, and the seedlings were rinsed with distilled water before placing on microscope slides. For positive control: six-day-old seedlings were treated with 500 mM NaCl and stained with trypan blue after 24 hours.

**Confocal microscopy**

Seedlings were submerged in 0.005 mg ml⁻¹ propidium iodide in distilled water, placed on microscope slides, and imaged using a Zeiss LSM700 laser scanning microscope with ×20/NA 0.8. All pictures were taken with the exact settings. Rainbow spectrum was applied for PIN1:GFP pictures. YFP/GFP fluorescence in the root tips was quantified using ImageJ software (https://imagej.nih.gov/ij/). The mean fluorescence was compared to the mean fluorescence of the untreated plants (control). Definition of meristem area (Fig. 4C): the meristematic area was defined with a deltoid shape: 1 row under the quiescent center + 8 rows above the quiescent center, minus 2 rows on each side.

**Measurement of GSL content**

GSLs were measured as previously described (Kliebenstein et al. 2001a–c). Briefly, 4-6 14-day-old seedlings from the same Petri plate were pooled, weighed and harvested in 400 μL of 90%
methanol. Tissues were homogenized for 3 min in a paint shaker, centrifuged, and the 
supernatants were transferred to a 96-well filter plate with DEAE sephadex. The filter plate with 
DEAE sephadex was washed once with water, 90% methanol, and water again. The sephadex-
bound GSLs were eluted after an overnight incubation with 110μL of sulfatase. Individual 
desulfo-GSLs within each sample were separated and detected by HPLC-DAD, identified, 
quantified by comparison to standard curves from purified compounds, and further normalized to 
the fresh weight.

**Uptake of raphanusamic acid**

Col-0 seeds were germinated on medium supplemented with or without 5 μM raphanusamic acid 
(on MS medium contained 3 mM nitrogen and 1.68 mM sulfur). Single seedlings were harvested 
5 days after germination and analyzed for raphanusamic acid as described (Jeschke et al. 2019).

**Yeast-two hybrid assays**

Yeast-two hybrid experiments were performed as described previously (Prigge et al. 2010). 
The TIR1/AFB1-5 bait vector pGILDA and the IAA (IAA1, IAA3, IAA5, IAA6, IAA7, IAA8, 
IAA12, IAA14, IAA18, IAA19, IAA28 and IAA31) prey vector pB42AD (Prigge et al. 2010) 
were co-transformed into yeast (*Saccharomyces cerevisiae*, EGY48). The negative control 
comprised yeast expressing pGILDA or pB42AD empty vectors.

**In vitro pull-down assays**

*In vitro* pull-down assays were performed as described previously (Parry et al. 2009). The 
TIR1:Myc fusion protein was incubated with GST:IAA14 protein in the presence or absence of 
50μm IAA or 50μm IAA plus 150 μm of GSL catabolite. Proteins were purified with 
Glutathione–Agarose beads (Sigma-Aldrich; #G4510), pulled down using monoclonal anti c 
Myc (Sigma-Aldrich; cat# 11814150001), separated by SDS–PAGE and detected using anti- 
GST (Sigma-Aldrich; cat# G7781).
Statistical analyses
Statistical analyses were conducted using R software (https://www.R-project.org/) with the
RStudio interface (http://www.rstudio.com/). Significance was tested via two-way ANOVA
using “stat” package. Specific models are listed in Supplemental Table 1, but they followed the
following general format: in each experiment, Treatment (application of allyl-GSL or associated
catabolites) and Auxin (the auxin concentrations ranging 0-0.5 µM) were considered as fixed
effects. Experiment and Plate were treated as random effects in the linear model.

Accession Numbers
2-oxoglutarate-dependent dioxygenases 2 (AOP2): AT4G03060.

Supplemental Data

Supplemental Figure S1. The uptake of allyl-GSL and raphanusamic acid by Col-0 seedlings.

Supplemental Figure S2. The effect of Allyl-GSL catabolites and auxin on roots length.

Supplemental Figure S3. Trypan Blue staining.

Supplemental Figure S4. The effect of raphanusamic acid and butenoic acid on auxin
responses.

Supplemental Figure S5. The effect of raphanusamic acid and butenoic acid on the interaction
between TIR1/AFBs and Aux/IAA.

Supplemental Figure S6. The effect of allyl-GSL catabolites on roots length of auxin mutant.

Supplemental Table S1. Statistics for figures 1-4.

Acknowledgments
Funding for this work was provided by the NSF award MCB 1906486 and IOS 1665810 to DJK,
the USDA National Institute of Food and Agriculture, Hatch project number CA-D-PLS-7033-H
to DJK, by the Danish National Research Foundation (DNRF99) grant to DJK, and by a
fellowship from the Binational Agricultural Research and Development Fund to DJK and EK
(FI-560-2017). We thank Prof. Rosangela Sozzani (Department of Plant and Microbial Biology,
North Carolina State University), Dr. Tonni Grube Andersen (Max Planck Institute for Plant Breeding Research), and Dr. Michael Prigge (Section of Cell and Developmental Biology and Howard Hughes Medical Institute, University of California, San Diego) for consulting and providing seeds. We thank Prof. Siobhan M Brady (Department of Plant Biology and Genome Center, University of California, Davis) for the use of the Zeiss LSM700 laser scanning microscope.
| Tissue                              | Species/Genotype or ecotype | Conc.    | Ref.                                |
|------------------------------------|-----------------------------|----------|-------------------------------------|
| **Allyl-GSL**                      |                             |          |                                     |
| seeds                              | Arabidopsis/ Radk-1         | 8200 μM  | Katz, unpublished data              |
| seeds                              | Arabidopsis/ Hodja          | 380 μM   | Kliebenstein et al 2001 (Plant Physiology) |
| seedling, 7 DAG                    | Arabidopsis/ CIBC-17        | 108 μM   | Chan et al 2011                     |
| leaves, 3 weeks old                | Arabidopsis/ Kondara        | 519.8 μM | Kliebenstein et al 2001 (Plant Physiology) |
| seedling, 15 DAG                   | Arabidopsis/ AOP2           | 150 μM   | Francisco et al 2016                |
| seedling, 9 DAG, grown on 50 μM allyl-GSL | Arabidopsis/ Col-0    | 60 μM    | Jeschke et al 2019                  |
| **Raphanusamic acid**              |                             |          |                                     |
| seedling, 9 DAG                    | Arabidopsis/ Col-0          | 2 μM     | Jeschke et al 2019                  |
| leaves, 3-4 weeks old              | Arabidopsis/ Col-0          | 4 μM     | Bednarek et al 2009                 |
| leaves, 3 weeks old                | Arabidopsis/ Col-0          | 27 μM    | Sanchez-Vallet et al 2010           |
| seedling, 9 DAG, grown on 50 μM allyl-GSL | Arabidopsis/ Col-0    | 130 μM   | Jeschke et al 2019                  |
| leaves, 3 weeks old                | Arabidopsis/ irx1-6: cell wall mutant with resistance to pathogens | 85 μM | Sanchez-Vallet et al 2010 |
| seedlings, 3 DAG                   | Crucihimalaya lasiocarpa    | 50 μM    | Bednarek et al 2011                 |
| leaves, 4-5 weeks old, inoculated with Botrytis cinerea | Crucihimalaya lasiocarpa | 50 μM    | Bednarek et al 2011                 |
| leaves, 4-5 weeks old, inoculated with Botrytis cinerea | Arabis alpina             | 85 μM    | Bednarek et al 2011                 |

**Table 1:** Allyl-GSL and raphanusamic acid concentrations. Concentrations were determined by grams per fresh weight. DAG: days after germination.

**FIGURE LEGENDS**
Figure 1: The effect of allyl-GSL on root growth and auxin responses. (A) The effect of allyl-GSL and auxin (IAA) on root length of Arabidopsis. Seedlings were grown vertically on MS medium supplemented with IAA (0.1-0.5 µM) or IAA and allyl-GSL (50, 100 µM). Root length was measured at 14 days old. Data are least squared means over two independent experimental replicates with 15-18 seedlings per condition per experiment. Significance was tested via two-way ANOVA (Detailed statistic in Supplemental Table S1). (B) Phenotypes of 14-day-old plants grown with or without IAA and allyl-GSL. Bar=10 mm. (C) The effect of allyl-GSL and IAA on number of root curls within 1 cm of the root tip. Seedlings were grown vertically on MS medium for four days, then transferred to medium with IAA (0.1-0.5 µM) or IAA and allyl-GSL (50 µM). After another day the plates were tilted back 45 degrees and photographed three days later. Results are least squared means over two independent experimental replicates, with 15-18 seedlings per condition per experiment. Significance was tested via two-way ANOVA (**<0.0001, relative to control seedlings. Quadratic curves were fitted to the model. Detailed statistics in Sup.T1). (D) Phenotypes of seedlings grown with or without allyl-GSL. Bar=1 cm.

Figure 2: The effect of allyl-GSL breakdown products and auxin on root length. (A) Allyl-GSL biosynthesis and breakdown pathway (MTB: methylthiobutyl, GSOH: GLUCOSINOLATE HYDROXYLASE, MSB: methylsulfinylbutyl, AOP2: 2-oxoglutarate-dependent dioxygenases 2, NSP: nitrile specifier proteins). (B-C) The effect of allyl-GSL catabolites and IAA on root length. Arabidopsis seedlings were grown vertically on MS medium supplemented with IAA (0.1-0.5 µM) or IAA and the different allyl GSL catabolites. At 14 days old their root lengths were measured. Allyl-GSL catabolites in the media: (B) 50 µM of allyl-GSL, allyl nitrile, or allyl isothiocyanate (allyl ITC). (C) 50 µM allyl-GSL, butenoic acid, acrylic acid, or raphanusamic acid. Results are least squared means over at least two independent experimental replicates, with 15-18 seedlings per condition per experiment. Significance was tested via two-way ANOVA (**<0.0001, relative to control seedlings. Quadratic curved were fitted to the model. Detailed statistic in Supplemental Table S1). (D) Arabidopsis seedlings were grown vertically on clean MS medium or MS media supplemented with 0.5 µM IAA and 50 µM allyl-GSL, as indicated. After seven days the seedlings were transferred to MS medium with the treatments as indicated. At day 14 they were photographed, and the root lengths were measured. Results are least squared means over two independent experimental replicates, with 15-18 seedlings per condition per
experiment. Significance was tested by t-Test, P < 0.0001. Error bars represent standard errors.

(E) Seedlings were grown on MS medium with or without 50 µM allyl-GSL. Seven-day-old seedlings were stained with trypan blue and photographed. Two experiments were conducted, with 5-10 replicates in every experiment per each treatment. Bar = 0.5 mm.

Figure 3: The effect of allyl-GSL catabolites on GSL accumulation. Arabidopsis seedlings were grown on MS medium supplemented with 50 µM of allyl-GSL breakdown products. At 14 days old GSL content was measured using HPLC. Results are least squared means over at least two independent experimental replicates, with 2-3 replicates per condition per experiment. Significance was tested via two-way ANOVA, followed by Dunnett’s test (* < 0.05, ** < 0.01, ***<0.0001, relative to control untreated seedlings. Detailed statistics in Sup.T1). Error bars represent standard errors.

Figure 4: The effect of allyl-GSL breakdown products on meristem development. (A) Arabidopsis seedlings were grown on MS medium with allyl-GSL breakdown products. At five days old the seedlings were photographed, and the length from the root tips to the first root hair was measured. (B) At seven days old cell walls were stained using propidium iodide, imaged using confocal microscopy, and the number of cells from the quiescent center to the first elongated cell were counted. (C) Confocal images of seven-day-old DII-VENUS seedlings stained with propidium iodide. The meristematic area as measured is defined with a deltoid shape (1 row under the quiescent center + 8 rows above the quiescent center, minus 2 rows in each side). Bar = 50 µm. (D) Seven-day-old DII-VENUS seedlings grown on MS medium and treated for two hours with allyl-GSL breakdown products in liquid MS. The seedlings were imaged using confocal microscopy. The relative integrated density of the VENUS fluorescence in the meristem area was quantified. (E) A cell expressing stronger cyclin B1-1::GFP expression in the nucleus then in the cytoplasm. Bar = 5 µm. (F) Percentage of G2 cells. Seven-day-old seedlings expressing cyclin B1-1::GFP were grown on MS medium, treated for two hours with allyl-GSL catabolites in liquid MS, and imaged using confocal microscopy. The number of cells expressing stronger nuclear GFP intensity compared with the cytoplasm was counted, and their percentage in respect to the total number of cells expressing GFP was calculated. Results are least squared means over two independent experimental replicates, with 5-10 seedlings per condition per experiment. Error bars represent standard errors. For all panels significance was
tested via two-way ANOVA (* < 0.05, ***<0.0001, relative to control untreated seedlings. Detailed statistic in Supplemental Table S1).

Figure 5: **The effect of raphanusamic acid and butenoic acid on auxin responses.** (A-B) Arabidopsis seedlings expressing the different auxin markers were grown on MS medium. At seven days old they were treated with or without 50 µM raphanusamic acid (A) or butenoic acid (B) and imaged using confocal microscopy. The integrated density of the fluorescence markers in the root tips was measured for each treatment in every time point, and the relative intensity was calculated in comparison to the control seedlings (untreated). Results are least squared means over two independent experimental replicates, with 5-10 seedlings per condition per experiment. Error bars represent standard errors. Significance was tested via two-way ANOVA (* < 0.05, ** < 0.01, ***<0.0001, relative to control untreated seedlings). The auxin markers lines are DII-VENUS, TIR1-VENUS, PIN1:GFP, PIN7:GFP. (C) Images of seven-day-old DII-VENUS seedlings treated with 50µM of raphanusamic acid for 60 minutes or 50 µM of butenoic acid for 30 minutes and the control. Bar is 50 µm. (D) Yeasts expressing TIR1/AFBs and IAA protein were grown on a medium containing auxin (20 µM), or auxin and raphanusamic acid or butenoic acid (150 µM). The interaction between the proteins was analyzed after an overnight incubation at 30°C. (E) Arabidopsis seedlings were grown vertically on MS medium supplemented with or without 50 µM of raphanusamic acid and butenoic acid. At 14 days old the seedlings were photographed, and their roots were measured. Results are least squared means over two independent experimental replicates, with 15-18 seedlings per condition per experiment. Error bars represent standard errors. Significance was tested via two-way ANOVA (* < 0.05, ***<0.0001, relative to control untreated seedlings).

Figure 6: **The effect of allyl-GSL catabolites on root length of different species:** Basil, dill, lettuce and tomato seedlings were grown vertically on MS medium supplemented with or without 50 µM of the different allyl-GSL catabolites. At five days old their root lengths were measured. Results are least squared means over two independent experimental replicates, with 12-24 seedlings per species per treatment. Error bars represent standard errors. Bars with different letters represent statistically different values within species according to ANOVA followed by Tukey’s HSD post hoc test (P < 0.05).
Figure 7: **Allyl-GSL catabolites inhibit root growth through different pathways.**

Raphanusamic acid and butanoic acid manipulate several steps of the auxin machinery, while acrylic acid influences the cell cycle in the root tips. PIN: PIN-FORMED, TIR1: TRANSPORT INHIBITOR RESPONSE.

**REFERENCES**

Agrawal AA, Strauss SY, Stout MJ. 1999. Costs of induced responses and tolerance to herbivory in male and female fitness components of wild radish. *Evolution*. 53(4):1093–1104

Bartling D, Seedorf M, Mithöfer A, Weiler EW. 1992. Cloning and expression of an Arabidopsis nitrilase which can convert indole-3-acetonitrile to the plant hormone, indole-3-acetic acid. *Eur. J. Biochem.* 205(1):417–424

Bartling D, Seedorf M, Schmidt RC, Weiler EW. 1994. Molecular characterization of two cloned nitrilases from Arabidopsis thaliana: key enzymes in biosynthesis of the plant hormone indole-3-acetic acid. *Proc. Natl. Acad. Sci. USA*. 91(13):6021–6025

Barton KE, Koricheva J. 2010. The ontogeny of plant defense and herbivory: characterizing general patterns using meta-analysis. *Am. Nat.* 175(4):481–493

Bednarek P, Piślewska-Bednarek M, Svatos A, Schneider B, Doubsky J, et al. 2009. A glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense. *Science*. 323(5910):101–106

Bednarek P, Pišlewska-Bednarek M, Ver Loren van Themaat E, Maddula RK, Svatoš A, Schulze-Lefert P. 2011. Conservation and clade-specific diversification of pathogen-inducible tryptophan and indole glucosinolate metabolism in Arabidopsis thaliana relatives. *New Phytol.* 192(3):713–726

Beekwilder J, van Leeuwen W, van Dam NM, Bertossi M, Grandi V, et al. 2008. The impact of the absence of aliphatic glucosinolates on insect herbivory in Arabidopsis. *PLoS One*. 3(4):e2068

Beemster GT, Baskin TI. 1998. Analysis of cell division and elongation underlying the developmental acceleration of root growth in Arabidopsis thaliana. *Plant Physiol.* 116(4):1515–1526

Benková E, Michniewicz M, Sauer M, Teichmann T, Seifertová D, et al. 2003. Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell*. 115(5):591–602
Bialy Z, Oleszek W, Lewis J, Fenwick GR. 1990. Allelopathic potential of glucosinolates (mustard oil glycosides) and their degradation products against wheat. *Plant Soil*. 129(2):277–281

Blilou I, Xu J, Wildwater M, Willemsen V, Paponov I, et al. 2005. The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots. *Nature*. 433(7021):39–44

Brown PD, Tokuhisa JG, Reichelt M, Gershenzon J. 2003. Variation of glucosinolate accumulation among different organs and developmental stages of Arabidopsis thaliana. *Phytochemistry*. 62(3):471–481

Brunoud G, Wells DM, Oliva M, Larrieu A, Mirabet V, et al. 2012. A novel sensor to map auxin response and distribution at high spatio-temporal resolution. *Nature*. 482(7383):103–106

Burow M, Atwell S, Francisco M, Kerwin RE, Halkier BA, Kliebenstein DJ. 2015. The Glucosinolate Biosynthetic Gene AOP2 Mediates Feed-back Regulation of Jasmonic Acid Signaling in Arabidopsis. *Mol. Plant*. 8(8):1201–1212

Burow M, Losansky A, Müller R, Plock A, Kliebenstein DJ, Wittstock U. 2009. The genetic basis of constitutive and herbivore-induced ESP-independent nitrile formation in Arabidopsis. *Plant Physiol*. 149(1):561–574

Burow M, Zhang Z-Y, Ober JA, Lambrix VM, Wittstock U, et al. 2008. ESP and ESM1 mediate indol-3-acetonitrile production from indol-3-ylmethyl glucosinolate in Arabidopsis. *Phytochemistry*. 69(3):663–671

Bush MS, Crowe N, Zheng T, Doonan JH. 2015. The RNA helicase, eIF4A-1, is required for ovule development and cell size homeostasis in Arabidopsis. *Plant J*. 84(5):989–1004

Calderón Villalobos LIA, Lee S, De Oliveira C, Ivetac A, Brandt W, et al. 2012. A combinatorial TIR1/AFB-Aux/IAA co-receptor system for differential sensing of auxin. *Nat. Chem. Biol*. 8(5):477–485

Campos ML, Yoshida Y, Major IT, de Oliveira Ferreira D, Weraduwage SM, et al. 2016. Rewiring of jasmonate and phytochrome B signalling uncouples plant growth-defense tradeoffs. *Nat. Commun*. 7:12570

Chan EKF, Rowe HC, Corwin JA, Joseph B, Kliebenstein DJ. 2011. Combining genome-wide association mapping and transcriptional networks to identify novel genes controlling glucosinolates in Arabidopsis thaliana. *PLoS Biol*. 9(8):e1001125

Chen M-S. 2008. Inducible direct plant defense against insect herbivores: A review. *Insect Science*. 15(2):101–114
Chung C-L, Longfellow JM, Walsh EK, Kerdieh Z, Van Esbroeck G, et al. 2010. Resistance loci affecting distinct stages of fungal pathogenesis: use of introgression lines for QTL mapping and characterization in the maize--Setosphaeria turcica pathosystem. *BMC Plant Biol.* 10:103

Clay NK, Adio AM, Denoux C, Jander G, Ausubel FM. 2009. Glucosinolate metabolites required for an Arabidopsis innate immune response. *Science.* 323(5910):95–101

Coley PD, Bryant JP, Chapin FS. 1985. Resource availability and plant antiherbivore defense. *Science.* 230(4728):895–899

Culligan KM, Robertson CE, Foreman J, Doerner P, Britt AB. 2006. ATR and ATM play both distinct and additive roles in response to ionizing radiation. *Plant J.* 48(6):947–961

De Bruyne L, Höfte M, De Vleesschauwer D. 2014. Connecting growth and defense: the emerging roles of brassinosteroids and gibberellins in plant innate immunity. *Mol. Plant.* 7(6):943–959

Dharmasiri N, Dharmasiri S, Weijers D, Lechner E, Yamada M, et al. 2005. Plant development is regulated by a family of auxin receptor F box proteins. *Dev. Cell.* 9(1):109–119

Dolan L. 1998. Pointing roots in the right direction: the role of auxin transport in response to gravity. *Genes Dev.* 12(14):2091–2095

Francisco M, Joseph B, Caligagan H, Li B, Corwin JA, et al. 2016a. The Defense Metabolite, Allyl Glucosinolate, Modulates Arabidopsis thaliana Biomass Dependent upon the Endogenous Glucosinolate Pathway. *Front. Plant Sci.* 7:774

Francisco M, Joseph B, Caligagan H, Li B, Corwin JA, et al. 2016b. Genome Wide Association Mapping in Arabidopsis thaliana Identifies Novel Genes Involved in Linking Allyl Glucosinolate to Altered Biomass and Defense. *Front. Plant Sci.* 7:1010

Fu L, Wang M, Han B, Tan D, Sun X, Zhang J. 2016. Arabidopsis Myrosinase Genes AtTGG4 and AtTGG5 Are Root-Tip Specific and Contribute to Auxin Biosynthesis and Root-Growth Regulation. *Int. J. Mol. Sci.* 17(6):

Guo Q, Yoshida Y, Major IT, Wang K, Sugimoto K, et al. 2018. JAZ repressors of metabolic defense promote growth and reproductive fitness in Arabidopsis. *Proc. Natl. Acad. Sci. USA.* 115(45):E10768–E10777

Halkier BA, Gershenzon J. 2006. Biology and biochemistry of glucosinolates. *Annu. Rev. Plant Biol.* 57:303–333

Hartmann T. 2004. Plant-derived secondary metabolites as defensive chemicals in herbivorous insects: a case study in chemical ecology. *Planta.* 219(1):1–4
Hasegawa T, Yamada K, Kosemura S, Yamamura S, Hasegawa K. 2000. Phototropic stimulation induces the conversion of glucosinolate to phototropism-regulating substances of radish hypocotyls. *Phytochemistry*. 54(3):275–279

Hopkins RJ, van Dam NM, van Loon JJA. 2009. Role of glucosinolates in insect-plant relationships and multitrophic interactions. *Annu Rev Entomol*. 54:57–83

Hopper KR. 1999. Risk-spreading and bet-hedging in insect population biology. *Annu Rev Entomol*. 44:535–560

Inamori Y, Muro C, Tanaka R, Adachi A, Miyamoto K, Tsujibo H. 1992. Phytogrowth-Inhibitory Activity of Sulphur-Containing Compounds. I. Inhibitory Activities of Thiazolidine Derivatives on Plant Growth. *Chem. Pharm. Bull.* 40(10):2854–2856

Jeschke V, Weber K, Moore SS, Burow M. 2019. Coordination of glucosinolate biosynthesis and turnover under different nutrient conditions. *Front. Plant Sci*. 10:1560

Jia J, Zhu F, Ma X, Cao Z, CaoZW, et al. 2009. Mechanisms of drug combinations: interaction and network perspectives. *Nat. Rev. Drug Discov.* 8(2):111–128

Katz E, Chamovitz DA. 2017. Wounding of Arabidopsis leaves induces indole-3-carbinol-dependent autophagy in roots of Arabidopsis thaliana. *Plant J*. 91(5):779–787

Katz E, Nisani S, Sela M, Behar H, Chamovitz DA. 2015a. The effect of indole-3-carbinol on PIN1 and PIN2 in Arabidopsis roots. *Plant Signal. Behav*. 10(9):e1062200

Katz E, Nisani S, Yadav BS, Woldemariam MG, Shai B, et al. 2015b. The glucosinolate breakdown product indole-3-carbinol acts as an auxin antagonist in roots of Arabidopsis thaliana. *Plant J*. 82(4):547–555

Khokon MAR, Jahan MS, Rahman T, Hossain MA, Muroyama D, et al. 2011. Allyl isothiocyanate (AITC) induces stomatal closure in Arabidopsis. *Plant Cell Environ.* 34(11):1900–1906

Kissen R, Bones AM. 2009. Nitrile-specifier proteins involved in glucosinolate hydrolysis in Arabidopsis thaliana. *J. Biol. Chem*. 284(18):12057–12070

Kissen R, Øverby A, Winge P, Bones AM. 2016. Allyl-isothiocyanate treatment induces a complex transcriptional reprogramming including heat stress, oxidative stress and plant defence responses in Arabidopsis thaliana. *BMC Genomics*. 17(1):740

Kliebenstein DJ. 2013. Making new molecules--evolution of structures for novel metabolites in plants. *Curr. Opin. Plant Biol*. 16(1):112–117

Kliebenstein DJ. 2016. False idolatry of the mythical growth versus immunity tradeoff in molecular systems plant pathology. *Physiol. Mol. Plant Pathol*. 95:55–59
Kliebenstein DJ. 2018a. Plant nutrient acquisition entices herbivore. *Science*. 361(6403):642–643.

Kliebenstein DJ. 2018b. Quantitative genetics and genomics of plant resistance to insects. In *Annual Plant Reviews Online*, ed. JA Roberts, pp. 235–262. Chichester, UK: John Wiley & Sons, Ltd.

Kliebenstein DJ, Gershenzon J, Mitchell-Olds T. 2001a. Comparative quantitative trait loci mapping of aliphatic, indolic and benzylic glucosinolate production in Arabidopsis thaliana leaves and seeds. *Genetics*. 159(1):359–370.

Kliebenstein DJ, Kroymann J, Brown P, Figuth A, Pedersen D, et al. 2001b. Genetic control of natural variation in Arabidopsis glucosinolate accumulation. *Plant Physiol.* 126(2):811–825.

Kliebenstein DJ, Lambrix VM, Reichelt M, Gershenzon J, Mitchell-Olds T. 2001c. Gene duplication in the diversification of secondary metabolism: tandem 2-oxoglutarate-dependent dioxygenases control glucosinolate biosynthesis in Arabidopsis. *Plant Cell*. 13(3):681–693.

Kobayashi K, Suzuki T, Iwata E, Nakamichi N, Suzuki T, et al. 2015. Transcriptional repression by MYB3R proteins regulates plant organ growth. *EMBO J.* 34(15):1992–2007.

Korves T, Bergelson J. 2004. A novel cost of R gene resistance in the presence of disease. *Am. Nat.* 163(4):489–504.

Kuchernig JC, Burow M, Wittstock U. 2012. Evolution of specifier proteins in glucosinolate-containing plants. *BMC Evol. Biol.* 12:127.

Lankau RA. 2007. Specialist and generalist herbivores exert opposing selection on a chemical defense. *New Phytol.* 175(1):176–184.

Li L, Zhao Y, McCaig BC, Wingerd BA, Wang J, et al. 2004. The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. *Plant Cell*. 16(1):126–143.

Malinovsky FG, Thomsen M-LF, Nintemann SJ, Jagd LM, Bourgine B, et al. 2017. An evolutionarily young defense metabolite influences the root growth of plants via the ancient TOR signaling pathway. *Elife*. 6:

Melotto M, Underwood W, He SY. 2008. Role of stomata in plant innate immunity and foliar bacterial diseases. *Annu. Rev. Phytopathol.* 46:101–122.
Mochizuki S, Harada A, Inada S, Sugimoto-Shirasu K, Stacey N, et al. 2005. The Arabidopsis WAVY GROWTH 2 protein modulates root bending in response to environmental stimuli. *Plant Cell.* 17(2):537–547

Okada K, Shimura Y. 1990. Reversible root tip rotation in Arabidopsis seedlings induced by obstacle-touching stimulus. *Science.* 250(4978):274–276

Palliyaguru DL, Salvatore SR, Schopfer FJ, Cheng X, Zhou J, et al. 2019. Evaluation of 2-Thiothiazolidine-4-Carboxylic Acid, a Common Metabolite of Isothiocyanates, as a Potential Biomarker of Cruciferous Vegetable Intake. *Mol. Nutr. Food Res.* 63(3):e1801029

Parry G, Calderon-Villalobos LI, Prigge M, Peret B, Dharmasiri S, et al. 2009. Complex regulation of the TIR1/AFB family of auxin receptors. *Proc. Natl. Acad. Sci. USA.* 106(52):22540–22545

Piotrowski M. 2008. Primary or secondary? Versatile nitrilases in plant metabolism. *Phytochemistry.* 69(15):2655–2667

Piślewska-Bednarek M, Nakano RT, Hiruma K, Pastorczyk M, Sanchez-Vallet A, et al. 2018. Glutathione Transferase U13 Functions in Pathogen-Triggered Glucosinolate Metabolism. *Plant Physiol.* 176(1):538–551

Prigge MJ, Lavy M, Ashton NW, Estelle M. 2010. Physcomitrella patens auxin-resistant mutants affect conserved elements of an auxin-signaling pathway. *Curr. Biol.* 20(21):1907–1912

Sabatini S, Beis D, Wolkenfelt H, Murfett J, Guilfoyle T, et al. 1999. An auxin-dependent distal organizer of pattern and polarity in the Arabidopsis root. *Cell.* 99(5):463–472

Sanchez-Vallet A, Ramos B, Bednarek P, López G, Piślewska-Bednarek M, et al. 2010. Tryptophan-derived secondary metabolites in Arabidopsis thaliana confer non-host resistance to necrotrophic Plectosphaerella cucumerina fungi. *Plant J.* 63(1):115–127

Shelton AL. 2004. Variation in chemical defences of plants may improve the effectiveness of defence. *Evolutionary Ecology Research.* 6(5):709–726

Shelton AL. 2005. Within-plant variation in glucosinolate concentrations of Raphanus sativus across multiple scales. *J. Chem. Ecol.* 31(8):1711–1732

Shroff R, Vergara F, Muck A, Svatos A, Gershenzon J. 2008. Nonuniform distribution of glucosinolates in Arabidopsis thaliana leaves has important consequences for plant defense. *Proc. Natl. Acad. Sci. USA.* 105(16):6196–6201

Strauss SY, Agrawal AA. 1999. The ecology and evolution of plant tolerance to herbivory. *Trends Ecol. Evol. (Amst.).* 14(5):179–185
Urbancsok J, Bones AM, Kissen R. 2017. Glucosinolate-Derived Isothiocyanates Inhibit Arabidopsis Growth and the Potency Depends on Their Side Chain Structure. *Int. J. Mol. Sci.* 18(11):

Urbancsok J, Bones AM, Kissen R. 2018. Benzyl Cyanide Leads to Auxin-Like Effects Through the Action of Nitrilases in Arabidopsis thaliana. *Front. Plant Sci.* 9:1240

Uremıs I, Arslan M, Sangun MK, Uygur V, Isler N. 2009. Allelopathic potential of rapeseed cultivars on germination and seedling growth of weeds. *Asian Journal of Chemistry*. 21(3):2170–2184

Vaughn SF, Palmquist DE, Duval SM, Berhow MA. 2006. Herbicidal activity of glucosinolate-containing seedmeals. *Weed Science.* 54:743–74

Vaughn SFV, Berhow MA. 1999. Allelochemicals Isolated from Tissues of the Invasive Weed Garlic Mustard (Alliaria petiolata). *J. Chem. Ecol.* 25(11):2495–2504

Veening J-W, Smits WK, Kuipers OP. 2008. Bistability, epigenetics, and bet-hedging in bacteria. *Annu. Rev. Microbiol.* 62:193–210

Vorwerk S, Biernacki S, Hillebrand H, Janzik I, Müller A, et al. 2001. Enzymatic characterization of the recombinant Arabidopsis thaliana nitrilase subfamily encoded by the NIT2/NIT1/NIT3-gene cluster. *Planta.* 212(4):508–516

Wajant H, Effenberger F. 2002. Characterization and synthetic applications of recombinant AtNIT1 from Arabidopsis thaliana. *Eur. J. Biochem.* 269(2):680–687

Wang R, Zhang Y, Kieffer M, Yu H, Kepinski S, Estelle M. 2016. HSP90 regulates temperature-dependent seedling growth in Arabidopsis by stabilizing the auxin co-receptor F-box protein TIR1. *Nat. Commun.* 7:10269

Wasternack C. 2017. A plant’s balance of growth and defense - revisited. *New Phytol.* 215(4):1291–1294

Wentzell AM, Boeye I, Zhang Z, Kliebenstein DJ. 2008. Genetic networks controlling structural outcome of glucosinolate activation across development. *PLoS Genet.* 4(10):e1000234

Wentzell AM, Kliebenstein DJ. 2008. Genotype, age, tissue, and environment regulate the structural outcome of glucosinolate activation. *Plant Physiol.* 147(1):415–428

Wittstock U, Burow M. 2010. Glucosinolate breakdown in Arabidopsis: mechanism, regulation and biological significance. *Arabidopsis Book.* 8:e0134

Wittstock U, Kurzbach E, Herfurth AM, Stauber EJ. 2016. Glucosinolate Breakdown. In *Glucosinolates*, Vol. 80, pp. 125–169. Elsevier
Xu J, Scheres B. 2005. Dissection of Arabidopsis ADP-RIBOSYLATION FACTOR 1 function in epidermal cell polarity. *Plant Cell*. 17(2):525–536

Xue JP, Lenman M, Falk A, Rask L. 1992. The glucosinolate-degrading enzyme myrosinase in Brassicaceae is encoded by a gene family. *Plant Mol. Biol.* 18(2):387–398

Yamada K, Hasegawa T, Minami E, Shibuya N, Kosemura S, et al. 2003. Induction of myrosinase gene expression and myrosinase activity in radish hypocotyls by phototropic stimulation. *J. Plant Physiol.* 160(3):255–259

Zádníková P, Petrásek J, Marhavy P, Raz V, Vandenbussche F, et al. 2010. Role of PIN-mediated auxin efflux in apical hook development of Arabidopsis thaliana. *Development*. 137(4):607–617

Zhao Z, Zhang W, Stanley BA, Assmann SM. 2008. Functional proteomics of Arabidopsis thaliana guard cells uncovers new stomatal signaling pathways. *Plant Cell*. 20(12):3210–3226
Figure 1: The effect of allyl-GSL on root growth and auxin responses. (A) The effect of allyl-GSL and auxin (IAA) on root length of Arabidopsis. Seedlings were grown vertically on MS medium supplemented with IAA (0.1-0.5 μM) or IAA and allyl-GSL (50, 100 μM). Root length was measured at 14 days old. Data are least squared means over two independent experimental replicates with 15-18 seedlings per condition per experiment. Significance was tested via two-way ANOVA (Detailed statistic in Supplemental Table S1). (B) Phenotypes of 14-day-old plants grown with or without IAA and allyl-GSL. Bar=10 mm. (C) The effect of allyl-GSL and IAA on number of root curls within 1 cm of the root tip. Seedlings were grown vertically on MS medium for four days, then transferred to medium with IAA (0.1-0.5 μM) or IAA and allyl-GSL (50 μM). After another day the plates were tilted back 45 degrees and photographed three days later. Results are least squared means over two independent experimental replicates, with 15-18 seedlings per condition per experiment. Significance was tested via two-way ANOVA (***<0.0001, relative to control seedlings. Quadratic curves were fitted to the model. Detailed statistics in Sup.T1). (D) Phenotypes of seedlings grown with or without allyl-GSL. Bar=1 cm.
Figure 2: The effect of allyl-GSL breakdown products and auxin on root length. (A) Allyl-GSL biosynthesis and breakdown pathway (MTB: methylthiobutyl, GSOH: GLUCOSINOLATE HYDROXYLASE, MSB: methylsulfinylbutyl, AOP2: 2-oxoglutarate-dependent dioxygenases 2, NSP: nitrile specifier proteins). (B-C) The effect of allyl-GSL catabolites and IAA on root length. Arabidopsis seedlings were grown vertically on MS medium supplemented with IAA (0.1-0.5 μM) or IAA and the different allyl GSL catabolites. At 14 days old their root lengths were measured. Allyl-GSL catabolites in the media: (B) 50 μM of allyl-GSL, allyl nitrile, or allyl isothiocyanate (allyl ITC). (C) 50 μM allyl-GSL, butenoic acid, acrylic acid, or raphanusamic acid. Results are least squared means over at least two independent experimental replicates, with 15-18 seedlings per condition per experiment. Significance was tested via two-way ANOVA (***<0.0001, relative to control seedlings. Quadratic curved were fitted to the model. Detailed statistic in Supplemental Table S1). (D) Arabidopsis seedlings were grown vertically on clean MS medium or MS media supplemented with 0.5 μM IAA and 50 μM allyl-GSL, as indicated. After seven days the seedlings were transferred to MS medium with the treatments as indicated. At day 14 they were photographed, and the root lengths were measured. Results are least squared means over two independent experimental replicates, with 15-18 seedlings per condition per experiment. Significance was tested by t-Test, P < 0.0001. Error bars represent standard errors. (E) Seedlings were grown on MS medium with or without 50 μM allyl-GSL. Seven-day-old seedlings were stained with trypan blue and photographed. Two experiments were conducted, with 5-10 replicates in every experiment per each treatment. Bar = 0.5 mm.
Figure 3: The effect of allyl-GSL catabolites on GSL accumulation. Arabidopsis seedlings were grown on MS medium supplemented with 50 µM of allyl-GSL breakdown products. At 14 days old GSL content was measured using HPLC. Results are least squared means over at least two independent experimental replicates, with 2-3 replicates per condition per experiment. Significance was tested via two-way ANOVA, followed by Dunnett’s test (* < 0.05, ** < 0.01, *** < 0.0001, relative to control untreated seedlings. Detailed statistics in Sup.T1). Error bars represent standard errors.
Figure 4: The effect of allyl-GSL breakdown products on meristem development. (A) Arabidopsis seedlings were grown on MS medium with allyl-GSL breakdown products. At five days old the seedlings were photographed, and the length from the root tips to the first root hair was measured. (B) At seven days old cell walls were stained using propidium iodide, imaged using confocal microscopy, and the number of cells from the quiescent center to the first elongated cell were counted. (C) Confocal images of seven-day-old DII-VENUS seedlings stained with propidium iodide. The meristematic area as measured is defined with a deltoid shape (1 row under the quiescent center + 8 rows above the quiescent center, minus 2 rows in each side). Bar = 50 μm. (D) Seven-day-old DII-VENUS seedlings grown on MS medium and treated for two hours with allyl-GSL breakdown products in liquid MS. The seedlings were imaged using confocal microscopy. The relative integrated density of the VENUS fluorescence in the meristem area was quantified. (E) A cell expressing stronger cyclin B1-1::GFP expression in the nucleus then in the cytoplasm. Bar = 5 μm. (F) Percentage of G2 cells. Seven-day-old seedlings expressing cyclin B1-1::GFP were grown on MS medium, treated for two hours with allyl-GSL catabolites in liquid MS, and imaged using confocal microscopy. The number of cells expressing stronger nuclear GFP intensity compared with the cytoplasm was counted, and their percentage in respect to the total number of cells expressing GFP was calculated. Results are least squared means over two independent experimental replicates, with 5-10 seedlings per condition per experiment. Error bars represent standard errors. For all panels significance was tested via two-way ANOVA (* < 0.05, ** < 0.001, relative to control untreated seedlings. Detailed statistic in Supplemental Table S1).
Figure 5: The effect of raphanusamic acid and butenoic acid on auxin responses. (A-B) Arabidopsis seedlings expressing the different auxin markers were grown on MS medium. At seven days old they were treated with or without 50 μM raphanusamic acid (A) or butenoic acid (B) and imaged using confocal microscopy. The integrated density of the fluorescence markers in the root tips was measured for each treatment in every time point, and the relative intensity was calculated in comparison to the control seedlings (untreated). Results are least squared means over two independent experimental replicates, with 5-10 seedlings per condition per experiment. Error bars represent standard errors. Significance was tested via two-way ANOVA (* < 0.05, ** < 0.01, ***<0.0001, relative to control untreated seedlings). The auxin markers lines are DII-VENUS, TIR1-VENUS, PIN1:GFP, PIN7:GFP. (C) Images of seven-day-old DII-VENUS seedlings treated with 50 μM of raphanusamic acid for 60 minutes or 50 μM of butenoic acid for 30 minutes and the control. Bar is 50 μm. (D) Yeasts expressing TIR1/AFBs and IAA protein were grown on a medium containing auxin (20 μM), or auxin and raphanusamic acid or butenoic acid (150 μM). The interaction between the proteins was analyzed after an overnight incubation at 30°C. (E) Arabidopsis seedlings were grown vertically on MS medium supplemented with or without 50 μM of raphanusamic acid and butenoic acid. At 14 days old the seedlings were photographed, and their roots were measured. Results are least squared means over two independent experimental replicates, with 15-18 seedlings per condition per experiment. Error bars represent standard errors. Significance was tested via two-way ANOVA (* < 0.05, ***<0.0001, relative to control untreated seedlings).
Figure 6: The effect of allyl-GSL catabolites on root length of different species: Basil, dill, lettuce and tomato seedlings were grown vertically on MS medium supplemented with or without 50 μM of the different allyl-GSL catabolites. At five days old their root lengths were measured. Results are least squared means over two independent experimental replicates, with 12-24 seedlings per species per treatment. Error bars represent standard errors. Bars with different letters represent statistically different values within species according to ANOVA followed by Tukey's HSD post-hoc test (P < 0.05).
Figure 7: **Allyl-GSL catabolites inhibit root growth through different pathways.** Raphanusamic acid and butanoic acid manipulate several steps of the auxin machinery, while acrylic acid influences the cell cycle in the root tips. PIN: PIN-FORMED, TIR1: TRANSPORT INHIBITOR RESPONSE.
Agrawal AA, Strauss SY, Stout MJ. 1999. Costs of induced responses and tolerance to herbivory in male and female fitness components of wild radish. Evolution. 53(4):1093–1104

Bartling D, Seedorf M, Mithöfer A, Weiler EW. 1992. Cloning and expression of an Arabidopsis nitrilase which can convert indole-3-acetonitrile to the plant hormone, indole-3-acetic acid. Eur. J. Biochem. 205(1):417–424

Bartling D, Seedorf M, Schmidt RC, Weiler EW. 1994. Molecular characterization of two cloned nitrilases from Arabidopsis thaliana: key enzymes in biosynthesis of the plant hormone indole-3-acetic acid. Proc. Natl. Acad. Sci. USA. 91(3):6021–6025

Barton KE, Koricheva J. 2010. The ontogeny of plant defense and herbivory: characterizing general patterns using meta-analysis. Am. Nat. 175(4):481–493

Bednarek P, Pislewska-Bednarek M, Svatos A, Schneider B, Doubsky J, et al. 2009. A glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense. Science. 323(5910):101–106

Bednarek P, Pislewska-Bednarek M, Ver Loren van Themaat E, Maddula RK, Svatůš A, Schulze-Lefert P. 2011. Conservation and clade-specific diversification of pathogen-inducible tryptophan and indole glucosinolate metabolism in Arabidopsis thaliana relatives. New Phytol. 192(3):713–726

Beemster GT, Baskin TI. 1998. Analysis of cell division and elongation underlying the developmental acceleration of root growth in Arabidopsis thaliana. Plant Physiol. 116(4):1515–1526

Benková E, Michniewicz M, Sauer M, Teichmann T, Seifertová D, et al. 2003. Local, efflux-dependent auxin gradients as a common module for plant organ formation. Cell. 115(5):591–602

Bialy Z, Oleszek W, Lewis J, Fenwick GR. 1990. Allelopathic potential of glucosinolates (mustard oil glycosides) and their degradation products against wheat. Plant Soil. 129(2):277–281

Blilou I, Xu J, Wildwater M, Willemsen V, Paponov I, et al. 2005. The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots. Nature. 433(7021):39–44

Brown PD, Tokuhisa JG, Reichelt M, Gershenzon J. 2003. Variation of glucosinolate accumulation among different organs and developmental stages of Arabidopsis thaliana. Phytochemistry. 62(3):471–481

Brunoud G, Wells DM, Oliva M, Larrieu A, Mirabet V, et al. 2012. A novel sensor to map auxin response and distribution at high spatio-temporal resolution. Nature. 482(7383):103–106

Burow M, Atwell S, Francisco M, Kerwin RE, Halkier BA, Kliebenstein DJ. 2015. The Glucosinolate Biosynthetic Gene AOP2 Mediates Feed-back Regulation of Jasmonic Acid Signaling in Arabidopsis. Mol. Plant. 8(8):1201–1212

Burow M, Losansky A, Müller R, Ploegh A, Kliebenstein DJ, Wittstock U. 2009. The genetic basis of constitutive and herbivore-induced
ESP-independent nitrile formation in Arabidopsis. Plant Physiol. 149(1):561–574
Burrow M, Zhang Z-Y, Ober JA, Lambrich VM, Wittstock U, et al. 2008. ESP and ESM1 mediate indol-3-acetonitrile production from indol-3-ylmethyl glucosinolate in Arabidopsis. Phytochemistry. 69(3):663–671

Bush MS, Crowe N, Zheng T, Doonan JH. 2015. The RNA helicase, eIF4A-1, is required for ovule development and cell size homeostasis in Arabidopsis. Plant J. 84(5):989–1004

Calderón Villalobos LIA, Lee S, De Oliveira C, Ivetac A, Brandt W, et al. 2012. A combinatorial TIR1/AFB-Aux/IAA co-receptor system for differential sensing of auxin. Nat. Chem. Biol. 8(5):477–485

Campos ML, Yoshida Y, Major IT, de Oliveira Ferreira D, Weraduwage SM, et al. 2016. Rewiring of jasmonate and phytochrome B signalling uncouples plant growth-defense tradeoffs. Nat. Commun. 7:12570

Chen M-S. 2008. Inducible direct plant defense against insect herbivores: A review. Insect Science. 15(2):101–114

Chung C-L, Longfellow JM, Walsh EK, Kerdieh Z, Van Esbroeck G, et al. 2010. Resistance loci affecting distinct stages of fungal pathogenesis: use of introgression lines for QTL mapping and characterization in the maize–Setosphaeria turcica pathosystem. BMC Plant Biol. 10:103

Clay NK, Adio AM, Denoux C, Jander G, Ausubel FM. 2009. Glucosinolate metabolites required for an Arabidopsis innate immune response. Science. 323(5910):95–101

Coley PD, Bryant JP, Chapin FS. 1985. Resource availability and plant antiherbivore defense. Science. 230(4728):895–899

Culligan KM, Robertson CE, Foreman J, Doerner PB. 2006. ATR and ATM play both distinct and additive roles in response to ionizing radiation. Plant J. 48(6):947–961

De Bruyne L, Höfte M, De Veesschauwer D. 2014. Connecting growth and defense: the emerging roles of brassinosteroids and gibberellins in plant innate immunity. Mol. Plant. 7(6):943–959

Dharmasiri N, Dharmasiri S, Weijers D, Lechner E, Yamada M, et al. 2005. Plant development is regulated by a family of auxin receptor F box proteins. Dev. Cell. 9(1):109–119

Dolan L. 1998. Pointing roots in the right direction: the role of auxin transport in response to gravity. Genes Dev. 12(14):2091–2095

Francisco M, Joseph B, Caligagan H, Li B, Corwin JA, et al. 2016a. The Defense Metabolite, Allyl Glucosinolate, Modulates Arabidopsis thaliana Biomass Dependent upon the Endogenous Glucosinolate Pathway. Front. Plant Sci. 7:774

Francisco M, Joseph B, Caligagan H, Li B, Corwin JA, et al. 2016b. Genome Wide Association Mapping in Arabidopsis thaliana Identifies Novel Genes Involved in Linking Allyl Glucosinolate to Altered Biomass and Defense. Front. Plant Sci. 7:1010
Fu L, Wang M, Han B, Tan D, Sun X, Zhang J. 2016. Arabidopsis Myrosinase Genes ATGG4 and ATGG5 Are Root-Tip Specific and Contribute to Auxin Biosynthesis and Root-Growth Regulation. Int. J. Mol. Sci. 17(6):

Guo Q, Yoshida Y, Major IT, Wang K, Sugimoto K, et al. 2018. JAZ repressors of metabolic defense promote growth and reproductive fitness in Arabidopsis. Proc. Natl. Acad. Sci. USA 115(45):E10768–E10777

Halkier BA, Gershenzon J. 2006. Biology and biochemistry of glucosinolates. Annu. Rev. Plant Biol. 57:303–333

Hartmann T. 2004. Plant-derived secondary metabolites as defensive chemicals in herbivorous insects: a case study in chemical ecology. Planta. 219(1):1–4

Hasegawa T, Yamada K, Kosemura S, Yamamura S, Hasegawa K. 2000. Phototropic stimulation induces the conversion of glucosinolate to phototropism-regulating substances of radish hypocotyls. Phytochemistry. 54(3):275–279

Hopkins RJ, van Dam NM, van Loon JJA. 2009. Role of glucosinolates in insect-plant relationships and multitrophic interactions. Annu Rev Entomol. 54:57–83

Hopper KR. 1999. Risk-spreading and bet-hedging in insect population biology. Annu Rev Entomol. 44:535–560

Inamori Y, Muro C, Tanaka R, Adachi A, Miyamoto K, Tsujibo H. 1992. Phytogrowth-Inhibitory Activity of Sulphur-Containing Compounds. I. Inhibitory Activities of Thiazolidine Derivatives on Plant Growth. Chem. Pharm. Bull. 40(10):2854–2856

Jeschke V, Weber K, Moore SS, Burow M. 2019. Coordination of glucosinolate biosynthesis and turnover under different nutrient conditions. Front. Plant Sci. 10:1560

Jia J, Zhu F, Ma X, Cao Z, Cao ZW, et al. 2009. Mechanisms of drug combinations: interaction and network perspectives. Nat. Rev. Drug Discov. 8(2):111–128

Katz E, Chamovitz DA. 2017. Wounding of Arabidopsis leaves induces indole-3-carbinol-dependent autophagy in roots of Arabidopsis thaliana. Plant J. 91(5):779–787

Katz E, Nisani S, Sela M, Behar H, Chamovitz DA. 2015a. The effect of indole-3-carbinol on PIN1 and PIN2 in Arabidopsis roots. Plant Signal. Behav. 10(9):e1062200

Katz E, Nisani S, Yadav BS, Woldemariam MG, Shai B, et al. 2015b. The glucosinolate breakdown product indole-3-carbinol acts as an auxin antagonist in roots of Arabidopsis thaliana. Plant J. 82(4):547–555

Khokon MAR, Jahan MS, Rahaman T, Hossain MA, Muroyama D, et al. 2011. Allyl isothiocyanate (AITC) induces stomatal closure in Arabidopsis. Plant Cell Environ. 34(11):1900–1906

Kissen R, Bones AM. 2009. Nitrile-specifier proteins involved in glucosinolate hydrolysis in Arabidopsis thaliana. J. Biol. Chem. 284(18):12057–12070
