Specificity of herbivore-induced secondary metabolite responses in tall goldenrod, *Solidago altissima*

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Abstract: Plants respond to herbivore damage with a bewildering array of metabolic responses. To what degree such responses are specific to particular herbivore species and how plants respond to multiple attackers are questions of extensive scientific debate because their answers are fundamental to the understanding of the ecological consequences of plant induced responses to herbivory. In a field experiment, I investigate the nature of secondary metabolite and defensive protein production in *Solidago altissima* when elicited by two different herbivore species, the larvae of the galling fly *Eurosta solidaginis* and the chrysomelid beetle *Trirhabda virgata*, individually or at the same time. The chemical analysis of leaf tissue reveals differential responses in both trypsin proteinase inhibitor production and volatile organic compound emission when goldenrod plants are damaged by either herbivore individually or the two in combination. Our findings suggest damage-specific elicitation and signal transduction for each herbivore individually *and* for when both attack simultaneously.

Introduction

Everyday plants face a unique challenge in their lives: they must obtain nutrients and reproduce, all without moving an inch, making them a seemingly easy target for herbivores and parasites. However, plants have evolved a number of ways of coping with their enemies. Three categories of plant defenses are currently differentiated by scientists studying plant-insect herbivore interactions (Karban and Baldwin, 1997). First, many plants have evolved to tolerate a certain degree of herbivore damage without a significant reduction in fitness (Stowe et al. 2000). Second, plants can have direct defenses such as

physical structures (e.g. thorns, spines and prickles) or chemicals that are toxic, anti-digestive, or anti-nutritive to reduce their palatability to herbivores (Duffey and Karban, 1996). A third category, indirect defenses, facilitates the top-down control of herbivore populations by providing food, shelter (e.g. ant plants) or prey-searching signals (volatile organic compounds (VOCs)) for their natural enemies (Karban and Baldwin, 1997). In this way, the plant becomes indirectly defended by organisms of the third trophic level. Any or all of these mechanisms might be utilized by a specific plant, and they may function synergistically or antagonistically (Bostock et al. 2001, Kessler and Baldwin 2004).

Direct and indirect chemical defenses are assumed to be costly for a plant to produce (Baldwin, 2001). Therefore, the production of these compounds is often dependent on induction by environmental stresses such as herbivore attack. By this process, the plant is able to recommit resources from growth and reproduction to defending itself only when necessary (Herms and Mattson, 1992). The ecological effects of herbivore-induced chemical responses have been extensively studied in the past few decades. It has been found that these induced chemical responses do indeed provide plants with resistance against herbivores while maintaining fitness (Kessler and Baldwin, 2004). Such induced resistance has been seen both directly through compounds that are toxic or inhibit protein digestion, such as nicotine (Baldwin and Zhang, 1997) and proteinase inhibitors (Glawe and Zavala, 2003), respectively, and indirectly by attraction of predators via VOCs (De Moraes, 2001; Dicke, 2000; Kessler and Baldwin, 2001), or the provision of food such as extrafloral nectar in ant plants (Heil et al. 2001).

Novel research has examined the impact of these induced chemical responses on arthropod communities. However, few studies have investigated the effect of multiple herbivores feeding on the same plant at the same time (Rodriguez-Saona et al. 2005). Plant-mediated interactions of different herbivore species might be affected either by cross-resistance or facilitation, depending on the specificity of the herbivore elicitation and the functional specificity of plant defensive responses (Bostock et al 2001, Kessler and Baldwin, 2004). The metabolic specificities of such plant responses to multiple herbivores as well as their ecological consequences have rarely been studied yet are critical to understanding the role of plant traits in shaping arthropod community structure.

Current research suggests that induced chemical responses involve a rearrangement of the plant's metabolism, away from primary metabolism (e.g. growth and reproduction) and toward secondary metabolism (e.g. defensive compounds) as a mechanism to alleviate fitness costs of herbivory (Baldwin, 2001). Induced endogenous responses result from both mechanical damage and chemical elicitation from the saliva of herbivores, resulting in the differential expression of one or more chemical pathways (e.g. jasmonic acid, salicyclic acid, or ethylene). These metabolic pathways are often involved in "chemical cross-talk" which can be envisioned as a tunable dial of responses (Reymond and Farmer, 1998). In this model, specific mechanical and chemical elicitation from different herbivores acts on each of these chemical pathways to a different extent. For instance, in *Nicotiana attenuata* (tobacco) the mechanical damage plus chemical elicitation of *Manduca sexta* caterpillars induces a jasmonic acid (JA) (Halitschke et al 2001) and ethylene burst, leading to the release of specific VOCs (Halitschke et al 2000, Kessler and Baldwin 2001), while at the same time attenuating nicotine production (Winz

et al 2001). However, mechanical damage without chemical elicitation causes an increased production of nicotine. While a direct nicotine defense might work against generalist herbivores, specialized attackers are likely resistant to such alkaloids. Therefore, in the case of chemical elicitation from these herbivores it was proposed that the plant responds by induced VOC production leading to an indirect defense rather than relying on direct defenses (Kahl et al. 2000).

While pathway crosstalk may prove effective for plants in distinguishing and responding to generalist versus specialized herbivores, when two herbivores are attacking at the same time, the differing plant responses may have different ecological consequences. In some instances, it has been shown that a plant can respond independently and effectively to multiple simultaneous elicitations, for instance one that induces the JA and another which induces the salicylic acid (SA) pathway (Thaler et al. 2001, Thaler et al. 2004). On the other hand, the response to one attacking herbivore may dominate the metabolic reconfiguration of the whole plant independently of the elicitation of the attacker. As a consequence, the plant response to one attacking pathogen or herbivore might compromise the responses to another herbivore or pathogen, leaving the plant more open to attack by secondary attackers (Voelckel and Baldwin, 2004, Kessler and Baldwin 2004, Rodriguez-Saona et al, 2005).

In order to decipher the effects of cross-talking metabolic responses when two herbivores are actively feeding on a plant, we set up a series of field experiments in which goldenrod plants were attacked by both gall fly and beetle herbivores. *Solidago altissima* (Tall Goldenrod) is a target for many herbivore species (Root and Cappuccino, 1992). Perhaps one of the most noticeable of these herbivores is gall flies, *Eurosta*

solidaginis, which are specialist herbivores that oviposit their eggs in the apical meristems of *S. altissima*. *E. solidaginis* larvae release chemicals which lead to the trapping of carbon based nutrients and the growth of large spherical 'ball galls' on the *S. altissima* stem around them (Anderson et al. 1989). These galls nourish the larvae and protect them throughout the winter months until they are fully grown and ready to eclose the following spring (Irwin and Lee, 2000). While these galls clearly procure nutrients from the plant, many questions can be asked about how they affect *S. altissima* secondary metabolism and thus its ability to defend against other herbivores. Preliminary data showed a strong induced resistance of goldenrod to *T. virgata* beetle larvae, another specialist herbivore, in response to their feeding activity (Kessler and Hogan, unpublished data). Although the exact biochemical processes have not yet been determined, changes in secondary metabolite production are likely to play a major role in this resistance.

Scientists are begining to understand the biochemical signaling that mediates plant induced resistance to herbivores and pathogens. Different signaling pathways are induced by differential elicitation (Kessler and Baldwin, 2002). The methyl jasmonate signaling pathway is often upregulated in response to herbivory and has been shown to be crucial in plant wound responses in many species (Creelman and Mullet, 1997, Kessler and Baldwin 2002). Among other effects, the activation of this signaling pathway can lead to a reduction in the protein concentration of the leaves being attacked and also to an increase in the activity of proteinase inhibitors in the plant (Koiwa et al. 1997). Reducing protein content lowers the nutritive value of the leaf material that the herbivore consumes, while ingested proteinase inhibitors prevent the herbivore from being able to

break down the proteins it does obtain into usable peptides. This set of responses essentially leads to the starvation of herbivores feeding on the plant. In addition, jasmonic acid signaling in response to wounding increases the production of potentially toxic or antidigestive secondary metabolites which may contribute to the plants increased resistance (Duffey and Stout, 1996). The responses described above are typical and widespread in response to many chewing insect herbvivores. However, responses to specialist and piercing-sucking insect herbivores have been shown to frequently alter these typical responses through the crosstalk with other signaling pathways (McCloud and Baldwin, 1997, Thaler et al. 2004). The specific chemical responses facilitated by this pathway crosstalk are what I hope to decipher in this series of experiments.

I hypothesize that the hormonal signaling elicited by ball gall forming *E. solidaginis* alters *S. altissima*'s secondary metabolite production in a different way than does the chewing damage of leaf beetle *T. virgata*. Three scenarios seem possible. If *E. solidaginis* larvae dominate in altering the effect of plant wound signaling, then the host plant's chemical defenses meant to ward off other herbivores, such as *T. virgata*, may be compromised, making the plant more vulnerable to attack by the beetle larvae. A second possibility would be that beetle damage acts to override the responsive signaling caused by *E. solidaginis* herbivory, which may alter the plant's interaction with *E. solidaginis*. It may also be the case that the two elicitations cause an integrated response in the plant which is effective against both herbivores or that the two responses remain independent.

To determine the effects of *E. solidaginis* attack on *S. altissima*'s metabolic ability to respond to a second herbivore, we set up field experiments in Ithaca, NY from June-August 2006, involving attack by *T. virgata* beetles secondary to ball gall

formation. Leaf samples and headspace volatile emissions were collected from these plants, and used to test for differences in proteinase inhibitor activity, phenolic contents, and VOC production.

Materials and Methods

Field Experiments

Three field experiments were conducted (July 24, July 31, and August 7, 2006) at Cornell University's Wipple Farm in Ithaca, NY. The field site was formerly a farm and is now mowed every few years, allowing goldenrod to remain the dominant plant species. On each of these days, sixteen *S. altissima* plants, which showed minimal damage to upper leaves, were selected within a ten square meter area, eight that had a naturally initiated ball gall and eight that did not. Whether all or any of these plants are clonal is unknown. Five *T. vigrata* beetles were placed on the young upper leaves of half of the plants in each group and kept in this area with mesh bags ("BREATHER", Palm Tree Packaging Inc. Apopka, FL). The beetles were left to feed on the plants for four days and then removed. To the other eight plants, similar mesh bags were applied, but with no beetles. Therefore, each of these three trials, there were four treatments, e.g. control, beetles only, gall only, beetle and gall. (n = 4 for each treatment)

After four days, volatile organic compound emissions were collected using the protocol described below. Following volatile emission collection, four young leaves from near the top of each plant, 4-6 inches above gall level, where the beetles and/or mesh bags had been placed, were collected and immediately placed in liquid nitrogen for transportation and preservation. These leaf tissue samples were stored at -80 degrees Celsius until they were extracted for compound analysis.

Protein Extraction Assays

While still frozen in liquid nitrogen, approximately 150µg of leaf material was ground manually in a 2mL Eppendorf tube. To these crushed samples, 300µl of extraction buffer (1L 0.1M Tris-Cl ph 7.6, 50g PVPP, 2g phenylthio urea, 5g diethyl dithiocarbamate, 18.6g Na_2EDTA) was added. The samples were vortexed and then centrifuged for 20 minutes at 4 degrees Celsius at 13000rpm. Proteins remain in the supernatant, which was transferred into a new vial. The protein concentration of the supernatant was assayed utilizing Bradford reagent and a BioTek 96-well photospectrometer as explained below.

Protein Assay

10µl of supernatant from protein extraction was diluted in 90µl of ddH_20 to which 200µl of Bradford reagent (SIGMA) was added. Samples were tested in triplicate in 96 well plates along with IgG standards (from bovin serum, SIGMA) of known concentration. Concentrations were measured in a BioTek 96-well photospectrometer at 595nm after 10 minutes of incubation and 30 seconds of shaking. Assays of each sample and standard were replicated three times and the measurements were averaged to determine concentration using a linear standard curve.

Proteinase Inhibitor Assays

Radial diffusion assays (Jongsma et al. 1994) were utilized to determine the concentration of proteinase inhibitors (PI) in the protein extracts. Agarose gels (1.8% in

0.1M Tris-Cl buffer) were made containing 0.002g/ml of trypsinase (added at 50°C) and poured into three inch square plates. Nine 4mm evenly spaced holes were bored into the gels after they solidified. On each gel, five of these holes were used to measure the diffusion of standards (Soybean Trypsin PI, SIGMA) with known concentrations; the other four were used for leaf extracts. 35µl of standard or protein extract was added to each of these holes and allowed to diffuse for 14-16 hours at 4°C. 25ml of a staining solution (48mg Fast Blue B salt, 0.5ml water, 0.1M Tris-Cl to final volume 90ml added to 24mg APNE dissolved in 10ml N,N-dimethylformamide) (Jongsma et al. 1994) was then poured over the gels and the plates incubated for 55 minutes with the solution at 37°C. Everywhere on the gel where the trypsinase is active and APNE is broken down, a dark violet staining can be observed. In areas that contained diffused trypsin PI's, this reaction is inhibited and the gel remains clear. Thus, the diameter of the inhibition zone is directly correlated with the activity of trypsin PIs in the plant sample. After incubation, the staining solution is washed off with water and the diameters of the inhibition zones are measured and related (log function) to those of original standards (soybean trypsin PI (SIGMA)) that were applied simultaneously on the same agar reaction plate.

Phenolic Extraction Assays

While still frozen in liquid nitrogen, approximately 50µg of leaf material was separated and ground manually in 2mL Eppendorf tubes. To these crushed samples, 750µl of 80% methanol:water extraction buffer was added. Samples were incubated at 4°C with constant shaking for two hours. Samples were then centrifuged for 20 minutes at 4°C at 13000rpm and the supernatant taken off for HPLC analysis. These samples were

run on a Hewlett Packard 1100 series HPLC to determine identity and quantity of phenolic compounds present. The HPLC was equipped with a Gemini C18 reverse phase column (Phenomenex, 150 x 4.60mm, 3 micron). Acidified water (0.25% H3PO4) and acetonitril were used in a gradient as mobile phase as described elsewhere (Keinanen et al 2001). Caffeic acid derivatives (including chlorogenic acid) and flavonoids (including rutin) were identified and quantified by comparing retention times and UV spectra of the samples with those of authentic standards for chlorogenic acid and rutin, using Chemstation software. Unidentified caffeic acid derivatives and flavonoid compounds were treated as chlorogenic acid and rutin equivalents, respectively, in the quantitative analysis.

VOC Emission Assays

Plastic chambers (400mL, PE) were affixed to the top of each plant and attached via tubing to 12V air pumps (GAST® MOA-P101-VN) using an open-flow trapping design (Kessler and Baldwin, 2001). Headspace emissions from these plants were pumped from the chambers through ORBO-32 trapping vials filled with activated charcoal (SIGMA).

Volatile organic compounds were eluted from the charcoal traps using 300µl dichloromethane after spiking with tetraline solution (85.9ng/10ul toluene) that functions as an internal standard. The samples were stored in 1.5ml GC glass vials and run on a Varian 2200GC/MS/MS equipped with an autosampler and an AT-5ms capillary column (Alltech, 30m length, 0.25mm diameter). Compounds were quantified by relating their peak area to that of the internal standard tetraline. Compound identity was determined by

comparing the retention times and mass spectra of the compounds in the samples with those of authentic standards.

Genotypic Differences in Phenolic Compound Production

To assess genotypic variation in phenolic production, clones from 12 different field populations were maintained under lab conditions for 2 months in single ramets in separate ½ gallon pots with metro-mix soil. The original clones were collected from populations around Ithaca, NY. Each clone was grown for two months and the resulting new ramets were cut off and planted into separate pots. 2-4 replicate plants of each clone population were used for analysis. 100mg of leaf tissue from ech plant was harvested, extracted, and analyzed for phenolic compound content as described above.

Statistical Analysis

Standard ANOVA analysis was used with treatments as independent factors and the compound concentrations as dependent variables. For proteinase inhibitor analysis, the proteinase inhibitor/protein (μg/g) concentration was the dependent variable and data were square root transformed to meet ANOVA requirements for equality of variance. For the phenolic assay, concentrations are provided in μg/g fresh mass and data were also square root transformed. For the volatile emissions, the dependent variable was relative signal intensity, using gas chromatography, compared to an internal tetraline standard. Data transformation was performed by calculation of the natural log to meet ANOVA requirements.

Means of treatments were compared using a Student-Newman-Keuls *post hoc* test of an ANOVA. If means of treatments were only compared to the mean of the control treatment (pre-planned contrast) Fisher's protected least significant difference (PLSD) of an ANOVA was used for statistical evaluation.

Results

Trypsin proteinase inhibitor (TPI) assays revealed variant concentrations of PIs in leaf samples that were subjected to different herbivore treatments (ANOVA, F=7.113, P=0.0063, Figure 1). Plants naturally attacked by galling flies showed a marginally significant increase in TPI activity compared to undamaged control plants (Fisher's PLSD of an ANOVA, P = 0.052), while there is no elevated TPI production in plants attacked by beetles (Fisher's PLSD Post hoc of an ANOVA, P = 0.78). Plants attacked by both galls and beetles show a significant decrease in TPI production relative to the control (Fisher's PLSD Post hoc of an ANOVA, P = 0.025).

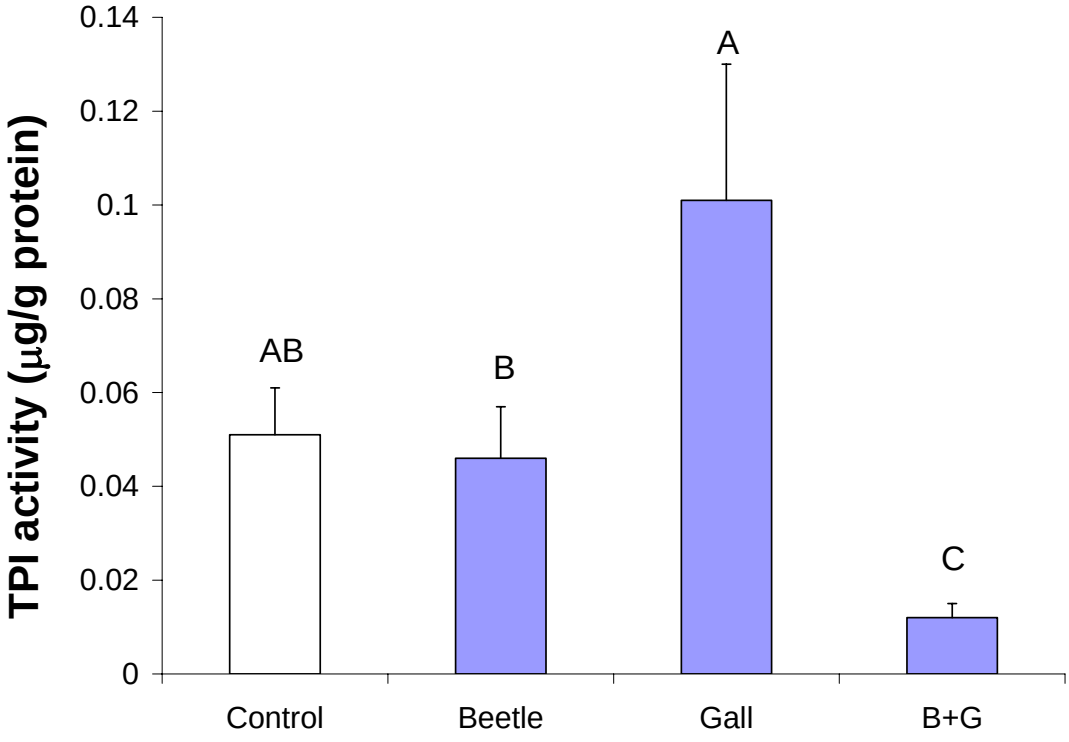

Figure 1. Mean Trypsin Protein Inhibitor activity (+SEM) in tissue from undamaged control plants, plants with beetle damage, plants with ball galls, and plants attacked by both galling flies and beetles (B+G). Different letters designate significantly different means [$P<0.05$] as informed by a Student-Newman-Keuls *post hoc* test of an ANOVA.

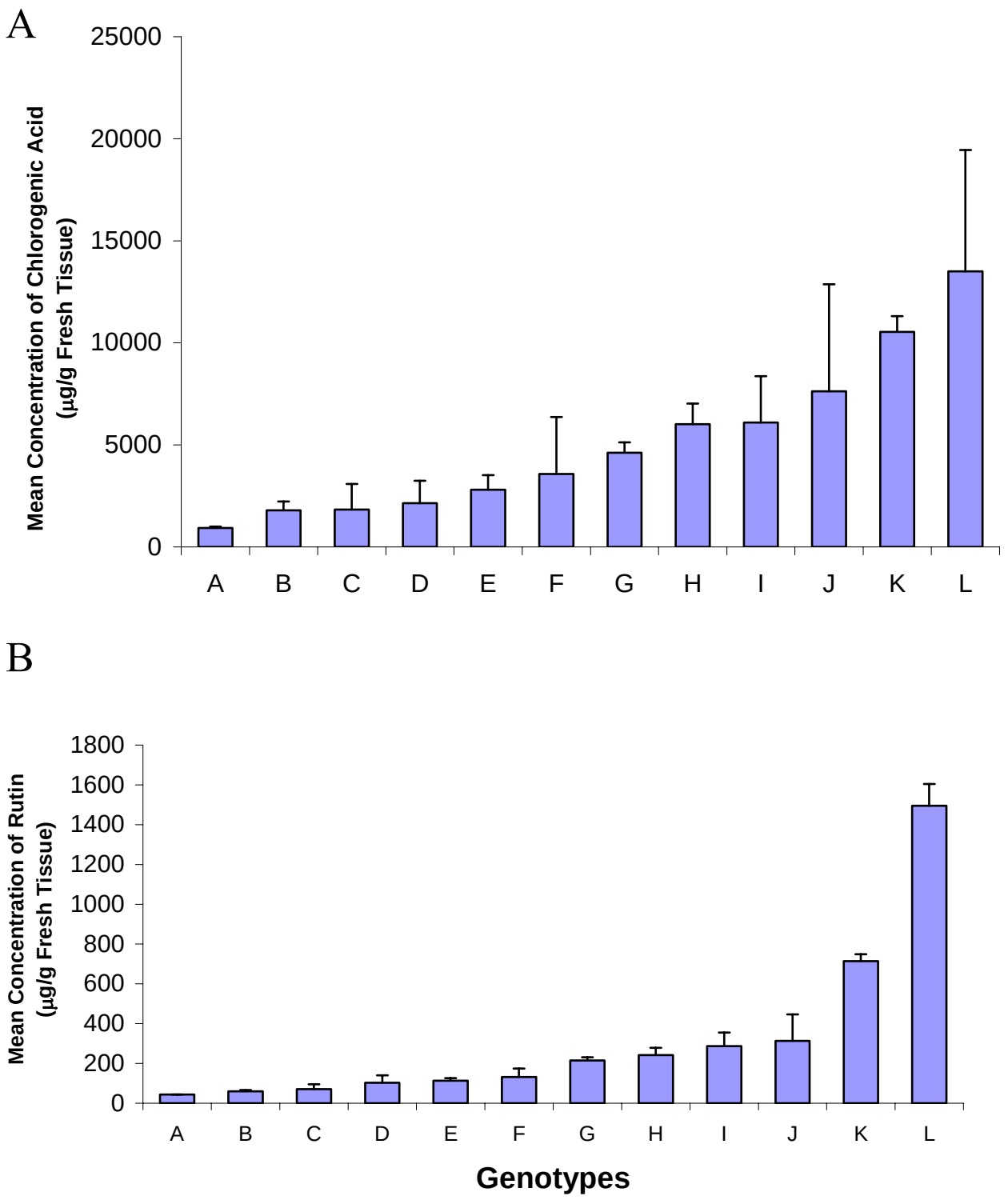

Figure 2. Mean concentration (+SEM) of A) chlorogenic acid and B) rutin from 12 different clonal populations (A-L) that were undamaged.

In contrast to the TPI activity, the production of none of the analyzed phenolic compounds was altered in response to the various herbivore attack treatments suggesting that there is no herbivore-induced alteration of phenolic production in *S. altissima*. However, significant genotypic variation was observed for a number of phenolic compounds including up to a 14-fold difference in chlorogenic acid [ANOVA, $F = 2.299$, $P = .0597$] and as much as a 35-fold difference in rutin [ANOVA, $F = 2.432$, $P = .0485$] content when the constitutive phenolic content of twelve *S. altissima* clones was compared. (Figure 2)

Volatile organic compound assays reveal a number of different categories of plant response. The emission of certain compounds, such as *E*-2-hexenal, was upregulated in plants with galls independent of the additional beetle attack, but were not induced by beetle feeding [ANOVA, $F = 4.280$, $P = 0.0313$] (Figure 3A). Other compounds such as an Unknown Compound at retention time (RT) 8.844 min showed similar upregulation in plants only attacked by beetles [ANOVA, $F = 5.126$, $P = 0.0185$] (Figure 3B).

Some volatile compounds show a trend toward upregulation in beetle + gall treatments, such as α-Pinene [ANOVA, $F = 2.302$, $P = 0.1337$] (Figure 3C). Other compounds, such as a not yet identified compound at RT 8.709 min, are specifically inhibited by galls, even when beetles are present, but are unaffected when beetles alone are attacking the plant [ANOVA, $F = 2.279$, $P = 0.1363$] (Figure 3D).

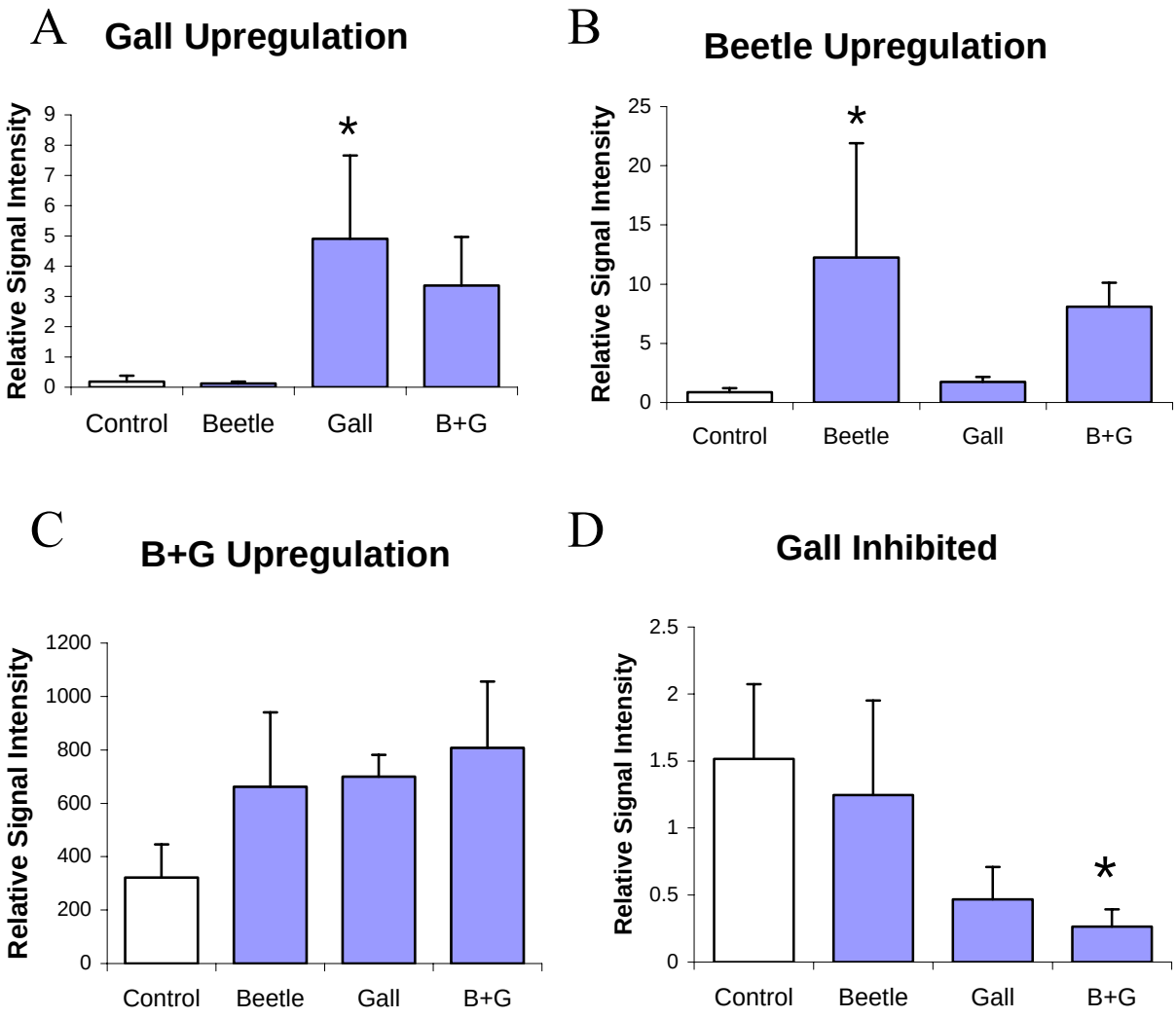

Figure 3. Mean relative signal intensity +SEM) of A) *E*-2-Hexenal, B) Unknown compound at RT 8.8, C) α-pinene, and D) Unknown Compound at RT 8.7 emitted from the headspace of undamaged control plants and plants damaged by *T. virgata* beetles, *E. solidaginis* gall fly larvae, and by both herbivores (B+G). Stars designate VOC emissions significantly different [$P<0.05$] from the control as informed by a Fisher's PLSD of ANOVA. The compounds shown in this figure represent different categories of responses to each type of herbivore damage.

The production of a number of compounds was either upregulated or inhibited significantly by specific treatments. Table 1 shows all the compounds whose production was significantly altered after herbivore attack. More compounds are expected to be differentially regulated in response to herbivore damage but were not statistically apparent due to the low sample sizes.

Table 1. Effect of Herbivore Attack on Volatile Compound Emissions. Compounds whose production was significantly altered, as informed by ANOVA, in response to herbivore attack are organized in four response categories.

Herbivory and Effect	RT	Compound
Gall Upregulated	7.87	*E*-2-hexenal
	18.891	Camphor
	17.996	Unknown Sesquiterpene
Beetle Upregulated	8.844	Unknown Compound
	12.143	*Trans*-ocimene
	15.227	Unknown Green Leaf Volatile
	16.391	Unknown Green Leaf Volatile
	18.891	Farnesene
B+G Upregulated	9.878	*α*-Pinene
	15.015	Unknown Compound
	17.605	*cis*-jasmone
	18.78	Unknown sesquiterpene
Gall Inhibited	8.709	Unknown compound

Discussion

Our results demonstrate specific responses to damage by different herbivore species both in terms of trypsin proteinase inhibitor (TPI) production and VOC emissions. This suggests that the plants are able to detect and specifically respond to unique elicitors from different herbivores, in this case gall flies and beetles.

Upregulation of TPI activity in plants with galls is very distinctive. This suggests that the presence of galls elicits signaling pathways in a way that directly leads to production of TPIs. In other plant species a strong involvement of jasmonate signaling in the elicitation of PI production has been demonstrated (Thaler et al. 2004; Halitschke et al. 2003), which suggests an involvement of this wound signaling pathway in *S. altissima*'s response to *E. solidaginis* as well. However, in plants attacked by *T. virgata*, no such upregulation is seen. Therefore, it appears that plants are able to distinguish between and specifically respond to beetle versus gall fly damage. This ability to distinguish between different herbivores, for instance lepidopteran larvae, has been shown in past studies (Voelckel and Baldwin, 2004). It would follow then, that beetle attack likely induces a different series of chemical pathways, leading to a response distinct from that to gall flies. Here it is important to note that *Trirhabda* beetles are unaffected by TPIs, a type of serine protease inhibitor, because they primarily rely on cysteine proteases for protein digestion (Gruden and Strukelj. 1998). A very specific response, e.g. producing only cysteine protease inhibitors in response to beetle damage, can be hypothesized. However, further studies remain to be done to study the changes in cysteine proteinase inhibitor activity in goldenrod being attacked by *Trirhabda* beetles.

One of the most interesting results of our PI analysis was the discovery that, though significantly upregulated in plants with galls, and unaffected in plants with beetle damage, TPIs were in fact significantly less active in plants with both beetle and gall fly damage. This suggests that the goldenrod is responding in an entirely different way when being fed upon by both beetles and gall fly larva as opposed to one or the other. To my knowledge this is the first time such a specific alteration of secondary metabolite

production has been observed in response to two herbivore species. Previous studies suggested either an integration of responses through pathway crosstalk (Thaler et al. 2004), an imprint of the response to one herbivore over that of another herbivore species (Voelckel and Baldwin 2004), or that the PI response to one herbivore was unaffected by attack of a second (Rodriguez-Saona et al, 2005). It is likely the independent elicitations are inducing independent chemical pathways, which through the process of pathway crosstalk are causing unique responses. Whether this response is specifically adaptive, or simply the result of mixed signals is unknown, however it does have interesting implications for the interactive chemical signaling pathways in plants.

Similar results were discovered in volatile organic compound emission data. Once again, specific compounds seemed to be specifically reduced based on different treatments. Indeed, the goldenrod were not just emitting certain compounds in response to herbivore attack, but rather responding individually to gall flies and beetles. Multiple compounds, such as *trans*-ocimeme, farnesene, and a few other unknown green leaf volatiles were distinctly upregulated only in plants attacked by beetles and not in those with ball galls. Likewise, other compounds including *E*-2-hexenal, camphor, and an unknown sesquiterpene were upregulated only in plants with gall damage, but not those that had beetle elicitation as well. Still other compounds, such as α-pinene, were upregulated most significantly in the plants that had both beetle and gall damage.

These findings once again suggest specific responses to specific elicitations. It is clear that goldenrod can distinguish between beetle and gall fly elicitors, and that these elicitations result in specific signal transduction. This results in differential pathway crosstalk, likely inducing different promoters and leading to specific metabolic responses

to each treatment. Such differences in transcriptional response to differing herbivores has been observed in *Arabidopsis* with phloem feeding aphids and a pathogen (De Vos and Van Oosten, 2005).

It should be noted that, since galling occurred naturally, the treatment cannot be assumed to be randomly assigned. Rather, it is plausible that gall flies selected plants with specific genotypes or gene expression to oviposit their eggs. This is one minor limitation of our method and leaves open the possibility that gall flies can tell which plants are most able to defend again their larvae and choose not to ovitposit in these specific genotypes.

To what extent this specificity in the plant's response has an ecological function remains unknown. However, one could hypothesize significant consequences of specific VOC signals for the plant's indirect defenses. Past research has demonstrated a plant's ability to use VOCs to attract the predators of its herbivores (Takabayashi and Sabelis, 2006). *T. vigrata* beetles and *E. solidaginis* gall flies are both specialists on goldenrod, but have very different predators. Gall larvae are most susceptible to parasitoids (Thompson, 1994), whereas beetles are more likely to be fed upon by predatory bugs of the family pentatomidae (Evans, 1982). Given this, it could be beneficial to the goldenrod's indirect defenses to respond specifically to each attacking herbivore in terms of volatile emissions to attract different predators and parasitoids.

Given the induced difference in TPIs and VOCs we found, it was surprising not to also find significant differences in induced phenolic activity amongst the different treatments. Five caffeic acid derivatives, including chlorogenic acid, as well as five flavonoids, including rutin, were analyzed for treatment specific induction. However,

none of the compounds were induced by herbivore damage. We have reason to believe, however, that an inducible response may have occurred but was masked by natural genetic variation in phenolic production.

To examine this possibility, we analyzed the variation in production of both chlorogenic acid and rutin in 12 different clonal popultions of goldenrod, harvested from the field and maintained in the lab. Significant variation in both chlorogenic acid and rutin production was found amongst these lines (Figure 2), suggesting that the difference was genetic. We suspect that this natural variance in phenolic production masked induced effects in our other experiments. This can be remedied in the future by performing our experiments with clonal plants.

In summary, we observe the ability of *Solidago altissima* to recognize and distinguish between individual chemical and physical elicitations from two specialist herbivores, *Trirhabda vigrata* and *Eurosta solidaginis*. These herbivores differentially induce the plant leading to specific chemical pathway cross-talk, specific signal transduction and induction of secondary metabolite production. Although the underlying transcriptional and biochemical mechanisms are far from being understood we hypothesize significant effects of the specific plant responses for the interaction of the plant with its arthropod community on multiple trophic levels.

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