

Scaptomyza Nigrita Herbivory and Inducible Glucosinolates in *Cardamine Cordifolia*

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ABSTRACT

Brassicaceae plants, such as bittercress (*Cardamine cordifolia*) produce characteristic glucosinolates, which are secondary metabolites used as defense compounds to deter pathogens and insect herbivores, such as the leaf mining fly (*Scaptomyza nigrita*). These compounds are produced constitutively and are also inducible. Although induced defense is well studied in other mustards, little work has been done on *C. cordifolia* defense. In this study, I quantified induced glucosinolate production caused by *S. nigrita* attack using HPLC. I expected that attack by *S. nigrita* would induce *C. cordifolia* to produce glucosinolates at higher concentrations. In addition, I expected that the local response would be more robust than the systemic response because glucosinolates are costly for the plant to produce (van Dam and Raaijmakers 2005). Also, given the geographic mosaic model of co-evolution, I expected variation in the response between plants from different geographic locations (Thompson 1988). Also, percent leaf area mined was found to be a proxy for larval weight gain.

KEYWORDS

Induced defense, Plant-insect interactions, Leaf-mining fly, Bittercress, Local vs. systemic response, Plant defense, Local adaptation, Geographic variation

INTRODUCTION

Brassicaceae plants, such as bittercress (*Cardamine cordifolia*) produce characteristic glucosinolates, or mustard oils, which are secondary metabolites used primarily as defense compounds to deter pathogens and insect herbivores, such as the leaf mining fly (*Scaptomyza nigrita*). Glucosinolates are broken down by myrosinases, yielding toxic molecules such as nitriles and isothiocyanates (Mewis *et al.* 2006). Glucosinolates are amino-acid derived molecules comprised of aromatic, indolic and

aliphatic types and are present constitutively or are inducible. Constitutive glucosinolate compounds are always present in leaves at a basal concentration; they serve as a deterrent against generalist insect herbivores. These chemicals deter generalists, but actually attract Crucifer specialists and stimulate oviposition by females (Mewis *et al.* 2006; Poelman *et al.* 2010). Induced glucosinolates are defense compounds produced at higher concentrations to deter herbivorous insects and pathogens after damage has occurred (Poelman *et al.* 2010).

Glucosinolate induction is well studied in other mustard plants. Previous studies have shown that herbivory increases certain glucosinolate compound concentrations (Mewis *et al.* 2006). *Arabidopsis thaliana*, the most closely related genomic model plant to *C. cordifolia*, exhibits increased aliphatic glucosinolate concentrations when attacked by various herbivore guilds, as well as increased indole glucosinolate gene expression and accumulation for certain insect guilds (Mikkelsen 2003; Mewis *et al.* 2006). Chewing insects induce the jasmonic acid signaling cascade, which affects both local and systemic responses (Mewis *et al.* 2006). Local responses are often robust, but there is species-specific variation in the weaker systemic induced responses (van Dam and Raaijmakers 2005). In *Brassica* spp., root damage causes a systemic response, while above ground damage yields stronger local responses (Van Dam *et al.* 2003). In addition to herbivory, stress induces increased glucosinolate production (Louda and Rodman 1983a; Collinge and Louda 1988; Louda and Collinge 1992). *C. cordifolia* also produces glucosinolates as a response to herbivory, but little is known about the concentrations associated with the response or the effect on herbivores (Louda and Collinge 1992).

C. cordifolia is known to produce about a dozen isothiocyanate-yielding glucosinolates (IYG) and oxazolidinethione-yielding glucosinolates (OYG) in amounts that vary between geographic locations (Rodman and Louda 1984). Variation in glucosinolate compositions and concentrations may mediate *C. cordifolia* susceptibility to herbivory and local adaptation by herbivores (Louda and Rodman 1996; Mewis *et al.* 2006). This follows the geographic mosaic model of co-evolution, in which plants and herbivores become locally adapted to one another via a local evolutionary arms race (Thompson 1988). *Scaptomyza flava*, a congener of *S. nigrita*, is a mustard specialist but is sensitive to glucosinolates in *Arabidopsis* (Whiteman *et al.*, 2010). Glucosinolate composition and concentration also varies between *C. cordifolia* organs, genotype and concentrations fluctuate seasonally (Rodman and Louda 1965; Louda and Rodman 1983a; Poelman *et al.* 2009).

Although Rodman and Louda have previously examined the constitutive glucosinolate profiles, there has been little research on the induced glucosinolates (Rodman and Louda 1965; 1984). The induced glucosinolates are important for deterring herbivores and quantifying these compounds may be helpful for determining local insect resistance and metabolic costs to the plant. We now possess the capability to measure all glucosinolates present using High Pressure Liquid Chromatography (HPLC), which was not the case when Rodman and Louda performed these analyses and allows for identification and quantification of all glucosinolates. In this study, I quantified induced glucosinolate production caused by *S. nigrita* attack using HPLC. I expected that attack by *S. nigrita* would induce *C. cordifolia* to induce glucosinolates at higher concentrations. In addition, I expected that the local (within the leaf attacked by the

larva) response would be more robust than the systemic (in the same plant, but in a leaf not directly attacked) response because glucosinolates are costly for the plant to produce (van Dam and Raaijmakers 2005). Also, given the geographic mosaic model of co-evolution, I expected variation in the response between plants from different geographic locations (Thompson 1988).

METHODS

Study System

Cardamine cordifolia A. Gray (Bittercress, Cruciferae) is a native, rhizomatous mustard plant that grows between 2150m and 3550m elevation throughout the southern Rocky Mountains, Sierra Nevada Mountains, and Cascade Mountains (Harrington 1954). Because bittercress grows from rhizomes, each cluster of plants can be assumed to be one distinct genotype, making it amenable to experimental manipulations. This herbaceous perennial grows 30-75cm tall in moist ground along streams or in meadows, and they are often found in spruce (*Picea engelmanni* Parry) or willow (*Salix* spp.) shade (Barrell 1969). Plants can also grow in the sun but often are shorter and have smaller leaves than plants in the shade (Collinge and Louda 1988). Vegetative growth begins immediately after snowmelt (Louda and Collinge 1992). Bittercress flowers from late June through July and fruits mature in August and early September (Louda and Rodman 1983b).

Scaptomyza nigrita Wheeler (Diptera: Drosophilidae), a leaf-mining fly, utilizes bittercress for oviposition sites, adult food and larval food. It is one of over 28 species of herbivorous insects to utilize bittercress in the study region (Louda and Collinge 1992), but it is likely the most important herbivore in terms of plant fitness. The fly's range

overlaps with that of *C. cordifolia*; it lives from California to Wyoming and up to Alaska. Adult flies are most active in mid-June. Females oviposit on lower or mid-stem leaves immediately after snowmelt, early in the plant's growth season (Collinge and Louda 1989a; 1989b). The females' preferred oviposition sites are the lowest leaves of the plant, which are developmentally the oldest and therefore available when females are ovipositing. Also, these leaves have the lowest glucosinolate concentrations in the plant. These variables are confounded and not mutually exclusive. Notably, adult females create feeding punctures with their ovipositors and are therefore herbivores as well. The flies select plants growing in full sun rather than in shaded locations for oviposition (Collinge and Louda 1988; 1989a; 1989b). When the larvae hatch in early to mid-June, they form characteristic foliar mines for several weeks and then pupate.

Location

The research sites selected are along the 401 Trail and Emerald Lake by the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Gunnison County, Colorado (38°57'56N, 106°59'12W). Having two research sites will allow me to analyze for a geographic difference between constitutive and induced glucosinolate concentrations and composition. The growing season in Gothic is from late May to late August. The site elevations are 10037.1m and 10440.5m for the 401 Trail and Emerald Lake, respectively.

Study Design and Methodology

Undamaged, intact plants were collected from each research site. The plants were selected from the same cluster (genotype) at the two sites. Plants were all selected from shade habitat and visually inspected for damage. Fifteen undamaged plants in the field were used to find the natural constitutive glucosinolate profiles. Fifteen undamaged

plants in the lab were used to determine the constitutive profile in the lab setting because the plants were stressed from being transplanted. The other fifteen undamaged plants in the lab were used to find the induced glucosinolate levels by introducing larvae to the plants. Larvae were selected from the same field site that the plant was from by bringing nurse plants into the lab and transferring larvae to the experimental plant.

Plants left in the field were netted using bridal veil to prevent female flies from stippling or ovipositing on the plants. Plants taken to the lab were planted together in a flat rather than potted individually. They were watered daily and fertilized weekly using MiracleGro (Scotts Corporations, Marysville, OH) as and placed under fluorescent, cool, white lights with a 16:8 hour light: dark photoperiod and some ambient light from a window. Plants were rotated among shelves to minimize effects of position on growth.

I began by manipulating the undamaged plants in the lab. I introduced field-collected larvae into the lowest undamaged leaf of a treatment plant using a larval transfer procedure using methods described in Whiteman *et al.* (2010). Larvae were weighed on a microgram balance before being transferred. The larvae were allowed to mine the leaves for one to seven days, which is sufficient time for glucosinolate induction (Whiteman *et al.* 2010). After this time, the mined leaves were harvested from the plant, photographed and two leaf discs per leaf were taken using a standard sized hole punch and immediately preserved with liquid nitrogen vapor until glucosinolate analysis. The larva from each leaf was removed and weighed again. The photographs were analyzed using ImageJ64 (National Institute of Mental Health, Bethesda, MD) to determine the area mined per leaf and the damage on un-manipulated plants. In addition, a leaf above the mined leaf was harvested for glucosinolate analysis to determine if the defense

response is throughout the plant (systemic) or localized. Leaves were collected and photographed for local and systemic leaves in three groups: undamaged field plants, intact lab plants and experimental lab plants from both the 401 Trail and Emerald Lake.

Leaf discs were pooled together and sent to Dr. Georg Jander at Cornell for glucosinolate analysis (who is a collaborator of Dr. Noah Whiteman's and a specialist on glucosinolates). Using multiple leaf discs, rather than only a couple whole leaves per treatment, allowed me to perform HPLC on more individual plants and therefore obtain an average that was representative of more individual plants. The leaf discs were shipped on dry ice to Cornell on July 22 and 27. We subjected each leaf disc to HPLC analysis to determine the concentration of all glucosinolate constituents present.

Data Analysis

T-tests and ANOVA were run in Excel to compare the leaf area mined throughout the controls and to compare the controls to the experimental group. The initial damage levels and larval weights (initial, final and gain) were compared between sites using t-tests in Excel. The LWG was compared to the percent LAM using a general linear regression in JMP.

RESULTS

The leaves in the control groups, both systemic and local, had an average 2.256 ± 5.134 percent damage and the mined leaves had an average $20.789 \pm 12.015\%$ leaf area mined (LAM). Although there was high variation within the LAM, there are nonetheless significant differences between the damage level on control and experimental leaves ($t = 7.73$, $n = 186$, $p = 1.29E-8$). There was no significant difference in damage level between systemic and local leaves among the control plants ($F = 1.27$, $n = 116$, $p =$

0.286). Comparing the percent LAM between the two sites also yields non-significant differences ($t = 6.80$, $n = 26$, $p = 0.504$). The initial larvae weight ($t = 0.41$, $n = 25$, $p = 0.683$), final larvae weight ($t = -0.328$, $n = 25$, $p = 0.746$) and larval weight gain (LWG) ($t = -0.272$, $n = 25$, $p = 0.788$) were statistically similar comparing the two test sites.

The LWG was compared to the percent LAM using a general linear regression ($R^2 = 0.7012$) and ANOVA ($F = 30.5116$, $N = 15$, $p < 0.001$) (Fig. 1).

DISCUSSION

All control plants had statistically similar initial damage levels; therefore the glucosinolate profile comparisons were not comparing variation between different damage levels but rather variation between the mined leaves and control leaves, which was significant. Also, the larval weight gain did not vary between geographic locations, so the comparison between locations was not comparing different treatment intensities.

There was strong correlation between LAM and LWG, indicating that LAM may be used as a proxy for LWG in the future. This is possible because *C. cordifolia* leaves are very two-dimensional. Also, in this study, LAM was actually more accurate than LWG because some larvae lost weight by excreting gut content, but nonetheless induced defense in the plant. Using LAM as a proxy for LWG is possible and should be used in the future to decrease larval mortality during transfers and increase data accuracy.

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LITERATURE CITED

- Barrell, J. 1969. Flora of the Gunnison Basin: Gunnison, Saguache and Hinsdale counties, Colorado. Natural Lands Institute, Rockford, Illinois.
- Chew, F.S. 1975. Coevolution of pierid butterflies and their cruciferous foodplants. I. The relative quality of available resources. *Oecologia (Berlin)*. 20: 117-127.
- Collinge, S.K. and S.M. Louda. 1988. Herbivory by leaf miners in response to experimental shading of a native crucifer. *Oecologia (Berlin)*. 75: 559-566.
- Collinge, S.K. and S.M. Louda. 1989a. Influence of plant phenology on the insect herbivore/bittercress interaction. *Oecologia*. 79: 111-116.
- Collinge, S.K. and S.M. Louda. 1989b. *Scaptomyza nigrita* Wheeler (Diptera: Drosophilidae), a Leaf Miner of the Native Crucifer, *Cardamine cordifolia* A. Gray (Bittercress). *Journal of the Kansas Entomological Society*. 62 (1): 1-10.
- Harrington, H.D. 1954. Manual of the plants of Colorado. Sage Books, Denver, Colorado, USA.
- Louda, S.M. and S.K. Collinge. 1992. Plant Resistance to Insect Herbivores: A Field Test of the Environmental Stress Hypothesis. *Ecology*. 73 (1): 153-169.

- Louda, S.M. and J.E. Rodman. 1983a. Concentration of Glucosinolates in Relation to Habitat and Insect Herbivory for the Native Crucifer *Cardamine Cordifolia*. *Biochemical Systematics and Ecology*. 11 (3): 199-207.
- Louda, S.M. and J.E. Rodman. 1983b. Ecological Patterns in the Glucosinolate Content of a Native Mustard, *Cardamine cordifolia*, in the Rocky Mountains. *Journal of Chemical Ecology*. 9 (3): 387-422.
- Mewis, I., J.G. Tokuhisa, J.C. Schultz, H.M. Appel, C. Ulrichs and J. Gershenzon. 2006. Gene expression and glucosinolate accumulation in *Arabidopsis thaliana* in response to generalist and specialist herbivores of different feeding guilds and the role of defense signaling pathways. *Phytochemistry*. 67: 2450-2462.
- Mikkelsen, M.D., B.L. Petersen, E. Glawisching, A.B. Jensen, E. Andreasson, B.A. Halkier. 2003. Modulation of CYP79 genes and glucosinolate profiles in *Arabidopsis* by defense signaling pathways. *Plant Physiology*. 131(1): 298-308.
- Poelman, E.H., J.J.A. Van Loon, N.M. Van Dam, L.E.M. Vet and M. Dicke. 2009. Chemical diversity in *Brassica oleracea* affects biodiversity of insect herbivores. *Ecology*. 90 (7): 1863-1877.
- Poelman, E.H., J.J.A. Van Loon, N.M. Van Dam, L.E.M. Vet and M. Dicke. 2010. Herbivore-induced plant responses in *Brassica oleracea* prevail over effects of constitutive resistance and result in enhanced herbivore attack. *Ecological Entomology*. 35: 240-247.
- Rodman, J.E. and S.M. Louda. 1965. Seasonal Flux of Isothiocyanate-yielding Glucosinolates in Roots, Stems and Leaves of *Cardamine cordifolia*. *Biochemical Systematics and Ecology*. 13 (4): 406-412.

- Thomson, J.N. 1988. Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomology Experimental Applications*. 47: 3-14.
- Van Dam, N.M., L. Witjes and A. Svatos. 2003. Interactions between aboveground and belowground induction of glucosinolates in two wild *Brassica* species. *New Phytologist*. 161(3): 801-810.
- Van Dam, N.M and C.E. Raaijmakers. 2005. Local and systemic induced responses to cabbage root fly larvae (*Delia radicum*) in *Brassica nigra* and *B. oleracea*. *Chemoecology*. 16 (1): 17-24.
- Whiteman, N.K., Groen, S.C., Chevasco, D., Beckwith, N., Gregory, T.R., Denoux, C. Mammarella, N., Ausubel, F.M. and N.E. Pierce. Mining the plant-insect interface with a leafmining *Drosophila* of *Arabidopsis*. *Molecular Ecology* (In review).

TABLES

FIGURES

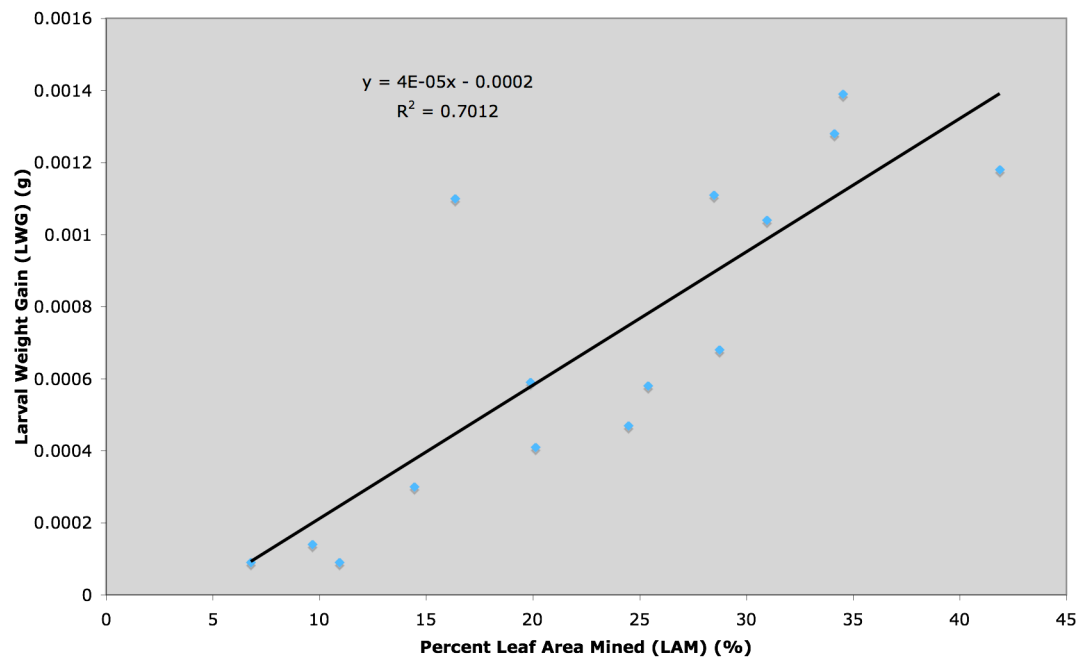


Fig. 1. Can percent leaf area mined (LAM) be used as a proxy for larval weight gain (LWG)? (F= 30.5116, N= 15, p= <0.001)