

**Jasmonate-induced plant defenses hinder growth in *Cardamine cordifolia*  
(Brassicaceae)**

Student: Hajin Kim  
Mentor: Noah Whiteman

Independent Research  
Summer 2010

Abstract: Plants face trade-offs when allocating resources to defense versus growth. To test the hypothesis that induced defenses are costly, I treated *Cardamine cordifolia* with a 1 mM solution of the canonical wounding pathway hormone jasmonic acid (JA) or a control solution. Comparisons across treated and control plants demonstrated a significant cost of JA-induced defenses in terms of leaf and petiole growth rates. Treated plant leaf and petiole lengths decreased following treatment, while untreated control plants showed no significant change. Effect sizes (Cohen's d) for differences between the two treatment groups were stronger than those from longer-term studies of herbivory, suggesting that the 1 mM JA application, commonly used in related literature, may be greater than concentrations naturally elicited in response to herbivory in this species. Thus, this study supports the hypothesis that JA-induced defenses are costly, but further investigation is needed to evaluate ecologically relevant costs of JA-induced defenses in this system.

Keywords: *Cardamine cordifolia*, induced defense, jasmonic acid

## Introduction

Far from passive victims of herbivory as presented in popular imagination, plants have evolved both constitutive and inducible defenses in the face of attack by natural enemies such as herbivores and microbial pathogens. Constitutive defenses are those that are constantly present: Spines on a cactus, for example, prevent grazing by large vertebrates. In contrast, inducible defenses are those that rise in concentration in response to damage or elicitors from natural enemies; these defenses typically reduce herbivore fitness or hinder future herbivory.

Theoretically, induced defenses evolved in the context of life history tradeoffs and resource constraints. There may be significant costs to deploying these weapons, so ecological theory predicts that plants use these tools when benefits outweigh costs, conserving resources when defense is unnecessary (Karban 1993).

In the mustard *Cardamine cordifolia* (Brassicaceae), commonly known as heartleaf bittercress, herbivory has been shown to dramatically inhibit plant growth: Plants sustaining herbivore attack grew significantly less in total leaf area, number of new leaves, plant stature, and fruit production than did those protected by insecticide (Louda 1984). These differences were significantly greater than the simple area of the leaf mined, suggesting herbivory exacted a larger growth cost than just leaf area loss due to foraging. Here, I tested the hypothesis that a component of this growth cost is due to the plant's induced defenses.

The hypothesis that *C. cordifolia*'s defenses can be induced is supported by herbivory data from the specialist leaf-mining fly *Scaptomyza nigrita* (Insecta: Drosophilidae), one of the most important herbivores of this plant (Collinge and Louda 1989). Mustards such as *C. cordifolia* are characterized by their production of glucosinolates, amino acid-derived secondary metabolites that are toxic to herbivores and give mustards their characteristic sharp flavor (Fahey et. al 2001). Specialists such as *S. nigrita* likely have developed some tolerance to these defenses, thereby necessitating increased levels of defensive chemicals to impact herbivore feeding; that *S. nigrita* is likely not entirely indifferent to glucosinolates is bolstered by evidence that a congener is sensitive to varying levels of these compounds, despite also being a specialist (Whiteman et al. 2010).

Inducibility of *C. cordifolia*'s defenses is further supported by evidence that these plants actively alter glucosinolate levels when stressed. In an experiment manipulating levels of light intensity, Louda and Rodman (1983) removed surrounding willows to artificially impose additional light on the naturally shade-loving plants. In response to these changes, *C. cordifolia* ramets increased levels of glucosinolates for the duration of the flowering season. These levels dropped the following year, suggesting it was stress and not environmental differences that prompted the change and thus that these changes are inducible.

If these defenses are induced, reallocation of resources towards defense may contribute to part of the growth costs exacted by herbivory. To test this hypothesis, I employed a jasmonic acid (JA) treatment to evaluate the impact of JA-induced defenses on *C. cordifolia* in a controlled, laboratory setting. JA is a canonical plant hormone and signal transducer of wounding-mediated defenses that strongly induces downstream plant defenses against herbivores and necrotrophic pathogens (Karban and Baldwin 1997). JA's application in the absence of herbivory allowed me to disentangle the cost of JA-induced defenses from the impact of herbivory itself. Previous studies on other systems have been mixed on the impact of JA on plant growth, with evidence of growth costs in some systems while other studies demonstrate no significant impact on plant development (Karban 1993; Baldwin 1998; Thaler 1999; Agrawal 1999; Agrawal 2000;). Traits assessed in this study included leaf length, petiole length, number of leaves, leaf death, plant death, and total aboveground biomass, providing a comprehensive appraisal of JA-induced defense growth costs in the absence of herbivory.

## Methods

### *Study species and area*

*Cardamine cordifolia* A. Gray (bittercress, Brassicaceae) is perennial mustard typically found in wet, shady areas, often in the shade of willows, although sufficient soil moisture allows the plant to also grow in direct sun. Flowering occurs in late June, with seed set in August or early September (Louda and Rodman 1983).

Plants were taken from near the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, and the study was conducted in late June-July 2010. Specifically, I collected plants from near the 401 Trail (38°N 106°W), where *C. cordifolia* grows in dense stands.

### *Experimental set-up*

I reared 64 *C. cordifolia* plants from rhizomes collected from the same patch in the field so as to isolate a single genotype and thereby eliminate genotypic variation; bittercress grows in clonal patches connected via rhizomes. Plants were pulled from the field, cut down to rhizomes, then replanted and grown in potting soil in the lab, so that resulting growth would be unaffected by uncontrolled herbivory. Because these rhizomes were not over-wintered, no flowering stalks formed and plants instead grew only basal rosettes.

To enhance plant growth, I supplemented plants weekly with common garden nitrogen-based fertilizer (MiracleGro: Scotts Miracle-Gro Company, Ohio) and employed 16 hr light / 8 hour dark periods, similar to summer conditions in the Rockies. Due to the many 40-watt florescent lights lining the laboratory for plant growth, temperatures ranged from 70 to 90 degrees Fahrenheit, with temperatures regularly above 85 degrees during the day. Of the 64 original rhizome pieces planted, 38 had begun to display plant growth once treatment commenced and were included in the study; the remaining 26 with no visible growth were discarded.

### *Treatments*

The 38 plants of varying sizes were randomly assigned to one of two treatment groups of 19 plants each (JA-treatment and control); plants were first grouped by size and then randomly assigned treatment groups, to ensure random assignment without skewing group size.

Experimental and control treatments commenced two and a half weeks after growth began, once plant leaf development was well underway. Treatment plants were sprayed with a 1 mM concentration<sup>1</sup> of JA, diluted in water and ethyl alcohol. Control plants were treated only with a water and ethyl alcohol control solution. Both treatments were applied evenly to plants via identical spray bottles, with 120 sprays per flat (treatment), ensuring equal and thorough

---

<sup>1</sup> Previous experiments demonstrate use of 0.5 mM to 1.5 mM concentrations (Thaler 1999)

application. All plants were treated outside, with separation between JA and control plants, to ensure that JA mists would not accidentally reach control plants. After treatment, plants were left to dry 10-15 minutes in the sun before being returned to the lab. JA and control sprays were applied three times. The first treatment was applied on July 15, 2010; the second, on July 21, 2010; the third, on July 23, 2010.

Each group of plants was housed on separate but identical trays in Johnson Lab at RMBL, and tray position was regularly rotated to eliminate any positioning impact within the laboratory. Because trays were identical and position was rotated, tray-specific effects were minimized. Tray separation among treatments helped isolate plant response to JA, so that control plants were less impacted by proximity to JA treated plants.

#### *Data collection*

Data on leaf length, petiole length, and number of leaves per plant were collected prior to and after the first two treatments; data were collected six days after the first treatment and two days after the second. After the first treatment, leaves and sometime even entire plants began to die; number of dead leaves and plants were also assessed. Dead leaves were not included in growth measurements. Three days following the third treatment, biomass was measured by harvesting aboveground biomass and oven-drying it at 105° C for 72 hours.

#### *Statistics*

I used unpaired, two-tailed Student's t-tests to test for differences in leaf length, petiole length, and number of leaves before commencing treatment to validate that that these groups were unbiased. One-tailed Student's T-tests were used to determine differences between groups after treatment began; tests were one-tailed because JA treatment was expected to curtail growth. Paired T-tests were used to compare before-and-after effects within each treatment group, using initial measurements taken before treatment commenced as "before" values. Cohen's d effect sizes were calculated to compare effect sizes across variables and also to compare effect sizes in this study with those of a previous herbivory study by Louda (1984). Cohen's d is a statistic often used in meta-analyses to compare effect sizes. Chi-squared tests were used to determine if there were differences in leaf mortality or plant death between the two treatment groups.

Pearson's correlation analysis was used to test whether other variables (e.g., original plant height) could explain differences across treatments. All tests were conducted in Microsoft Excel 2003, with a significance threshold of 0.05.

## Results

Plants initially demonstrated no differences in any plant measures (Table 1). After treatment, plants treated with JA demonstrated significantly less growth than control plants, as measured by leaf length growth and petiole length growth. Results intensified after the first treatment, and effect sizes were large. However, no difference in final aboveground biomass between treatment groups was found.

### *Leaf length*

Relative to control plants, JA-treated plants had significantly smaller average leaf lengths and lower leaf length growth rates, with differences exacerbating over treatments. Before treatment, there was no difference between control and JA plant leaf lengths ( $t = 0.28$ ,  $p = 0.78$ ). After the initial treatment, control plant leaves were on average 0.31 cm longer than JA plant leaves ( $t = 1.42$ ,  $p = 0.08$ ). Length difference intensified after the second treatment, with an average difference of 0.43 cm ( $t = 1.87$ ,  $p = 0.04$ ; Figure 1).

Leaf length absolute growth and percentage growth showed similar differences between JA treatment and control plants (Figure 2). After the first treatment, JA plant leaves contracted on average 0.22 cm (-17% growth), while control plants grew on average 0.02 cm (1% growth) (Absolute growth:  $t = 1.81$ ,  $p = 0.04$ ; Percentage growth:  $t = 1.89$ ,  $p = 0.03$ ). The second treatment exacerbated these differences; control plants showed slightly positive growth and JA plants further declined ( $t = 2.32$ ,  $t = 0.01$ ).

While control plants grew on average, this growth was not significant (After first treatment:  $t = 0.29$ ,  $p = 0.39$ ; After second treatment:  $t = 1.21$ ,  $p = 0.12$ ). JA-treated plants demonstrated reduction in leaf length (After first treatment:  $t = -2.00$ ,  $p = 0.03$ ; After second treatment:  $t = -2.01$ ,  $p = 0.03$ ).

### *Petiole length*

No differences in petiole length were observed between the two groups, but I found differences in petiole length growth rates. After the second treatment, JA plant petiole lengths had declined on average 0.55 cm since initial measurements, while control plant petiole lengths grew on average 0.40 cm ( $t = 2.16$ ,  $p = 0.02$ ; Figure 3); that translated into a 22% decline for JA plants and a 7% increase for control plants ( $t = 2.23$ ,  $p = 0.02$ ; Figure 3).

Control plants demonstrated only a slight trend in growth after the second treatment ( $t = 1.52$ ,  $p = 0.08$ ). JA plants contracted after both the first and second treatments (After first treatment:  $t = -1.83$ ,  $p = 0.04$ ; After second treatment:  $t = -1.56$ ,  $p = 0.07$ ).

### *Number of leaves*

After the first and second treatments, there was a trend for control plants to have more leaves than did JA-treated plants (After first treatment:  $t = 1.47$ ,  $p = 0.08$ ; After second treatment:  $t = 1.55$ ,  $p = 0.07$ ). The two treatments did not result in differences in the growth of number of leaves.

Number of leaves in control plants did not change after either treatment. JA-treated plants lost leaves after each treatment (After first treatment:  $t = -1.93$ ,  $p = 0.04$ ; After second treatment:  $t = -1.73$ ,  $p = 0.05$ ).

### *Leaf and plant survivorship*

JA treatment did not impact the probability that a plant would develop dead leaves, and no difference in number of dead leaves across treatment groups was found after the first treatment. After the second treatment, JA-treated plants demonstrated lower leaf survivorship ( $\chi^2 = 2.81$ ,  $0.05 < p < 0.10$ ; Figure 4). I found no difference in plant survivorship between treatment groups.

### *Correlations between initial size and growth variables*

No strong correlations were found between initial measurements and growth variables. However, final aboveground biomass was strongly correlated with initial measurements (Table 2).

### *Effect sizes (Cohen's d)*

After the second treatment, effect size for leaf length growth rates were strong, ranging from 0.88 to 1.00 (Table 3). Effect size for petiole length growth rates were similarly strong, though slightly weaker, ranging from 0.84 to 0.92. Effect sizes for leaf count differences after the second treatment were lower, at 0.60 (“medium”).

### Discussion

This study tested the hypothesis that bittercress incurs growth costs due to JA-induced defenses; results supported this hypothesis, as JA-treated plants had significantly lower leaf growth rates, petiole growth rates, and leaf counts than untreated control plants. Because JA-induced defenses are most commonly induced in response to herbivory, the patterns I found are likely a proxy for part of the signal induced by herbivores.

### *Interpretation of results*

The strong differences found in growth rates (both in leaf and petiole lengths) between the JA-treated plants and the control group are notable because JA-treated plants demonstrated significant decline: over 20% decrease in leaf length after the second treatment. In contrast, control plants displayed little positive growth, but differences between the two groups were significant, indicating that JA-treated plants shrunk due to the JA treatment.

JA-treated plants appeared to contract in two ways: Leaf shrinkage itself, wherein the tips of the leaves browned, curled under and began to die off (Figure 5), and leaf abscission and death; larger leaves may have been dying in the JA plant as a result of treatment, resulting in smaller average leaf and petiole size. Both phenomena indicate that JA-induced defense costs may not result in slower growth rates evenly spread throughout the plant – and thus simply be a reallocation of resources from growth to defense – but may in fact divert resources from whole leaves or part of leaves altogether. This is further supported by the lack of difference in final aboveground biomass between treatment groups; aboveground biomass includes dead leaf material, so the lack of difference, as well as the strong correlations found between aboveground

biomass and initial plant measurements, indicated control plants did not proactively grow more than JA-treated plants. Instead, JA-treated plants contracted and killed off leaves, reducing leaf measurements and measured growth rates, but the total amount of dry weight did not differ from that of control plants. Further study on the impact of JA on individual leaves, rather than plant averages, would help evaluate this hypothesis.

The results also suggested a potential “catch-up” effect; measurements taken six days after the first treatment found fewer significant differences between the two groups, while measurements taken two days after the second treatment found much stronger differences. Part of this might be due to the additive effect of the second treatment, but part may also be due to the discrepancies in time lag before measurement; perhaps after the first treatment, JA-treated plants, with six days to grow, had more time to “catch up” to the control plants after the impact of treatment. Daily measurements in repeated studies would help assess the temporal variation in impact.

Time lags may also have been influential in the limited significance found in differences in leaf count between the two groups. In Louda’s 1984 study, *C. cordifolia* were treated in the field with insecticide, limiting herbivory, and were compared to untreated plants to assess the growth costs of herbivory. Louda found significant differences in basal leaf<sup>2</sup> initiation between treated and untreated plants, with herbivore-free plants growing up six times as many new basal leaves as exposed plants after two months of growth. In this shorter study allowing for only over a week of plant growth after initial treatment, leaf count differences were not significant. However, strong trends indicate that more time or a larger sample size may provide results closer to Louda’s.

### *Effect size and significance*

Cohen’s d effect size calculations found that leaf length growth rates were proportionally impacted the most, followed by petiole height growth rate and leaf count (Table 3). This contributes to the hypothesis that JA treatment resulted in plants diverting resources from individual plant parts, as opposed to growth more generally, as leaves and petioles incurred proportionally different costs.

---

<sup>2</sup> Basal leaves are the appropriate comparison to this study, as the rhizomes I grew were not over-wintered and thus did not send up flowering stalks. Instead, they grew only basal rosettes.

Interestingly, these results are at odds with the direction of effect sizes calculated from Louda's 1984 study. In her results, effect size was much larger for leaf count of basal leaves (Cohen's  $d = 1.81$ ), than for leaf area (Cohen's  $d = 0.44$ ), which she found correlated to leaf length ( $R^2 = 0.895$ ); in this study, effect size for leaf length (Cohen's  $d = 0.63$ ) was similar to that for number of leaves (Cohen's  $d = 0.60$ ). If JA-induced defenses explain part of the growth costs of herbivory, then these results suggest that they act through a mechanism that more heavily impacts leaf length, whereas other components of herbivory more strongly influence leaf count. Moreover, JA treatment is not entirely equivalent to herbivory; for example, a study comparing predator attraction to spider mite-infested Lima bean plants versus JA-treated plants found that the carnivorous mite was less attracted to the JA-treated group, demonstrating differing impacts of JA application and herbivory (Dicke et. al 1999). A longer study including an herbivory treatment might shed more light on these differences between studies.

Comparing effect sizes across these two studies – and not just directionally within each study – is also illuminating. Effect sizes in this much shorter study were stronger for leaf length (Cohen's  $d = 0.63^3$ ) than Louda's effect sizes for leaf area (Cohen's  $d = 0.44$ ). Two potential explanations come to mind: First, application of 1 mM concentration of JA may be stronger than the levels of JA bittercress typically experience as a consequence of herbivory; Second, there may be another component of herbivory that actually tempers the influence of JA-induced defenses on leaf length growth. For example, herbivores could be suppressing plant defenses using herbivore associated molecular patterns (HAMPs). Further study on the degree of exposure to JA following herbivory would be instructive.

### *Significance*

While the costs of induced defenses are theoretically elegant, studies of these costs are inconsistent in their results. Agrawal (2000) found that in another Brassicaceae, *Lepidium virginicum*, induced plant changes in biomass varied based on plant density, and Baldwin (1998) demonstrated reductions in viable seed production in *Nicotiana attenuate* due to methyl JA treatment. Karban, in contrast, observed no cost to the desert shrub *Gossypium thurberi* for

---

<sup>3</sup> Effect sizes above 0.9 mentioned earlier refer to differences in leaf length growth rates, not differences in leaf lengths themselves.

induced resistance (1993) and Thaler (1999) similarly observed no measurable cost to tomato plants in terms of seedling survivorship or plant biomass after JA treatment. These mixed responses were recapitulated in a survey of studies assessing the costs of plant traits that confer resistance to herbicides, pathogens, and herbivores: Bergelson and Purrington (1996) found that only 44 of the 88 experiments demonstrated costs. This study thus adds to this body of literature with evidence that JA-induced defenses do incur growth costs for *C. cordifolia*. Researchers studying herbivory in this system should distinguish between the visible costs of damage and additional costs due to slower growth rates.

These results have additional implications for agriculture. While the use of JA as a pesticide on grown plants has widely been eschewed due to growth costs similar to those evidenced by this paper, researchers have begun developing techniques to treat seeds with JA, believing that such treatment will confer only the benefits and not costs of induced defense (von Borcke, unpublished). As comparisons of these results to other studies have shown, users of JA as pesticide should be cautious of varying responses across species.

#### *Suggestions for further study*

Further study into the relative importance of JA-induced defense costs, other pathways activated by herbivory, and the benefits of these defenses is warranted.

This study demonstrated that JA-induced defenses are costly to plants, but the 1 mM JA concentration selection was based on common application in related literature as opposed to the idiosyncrasies of bittercress reactions to herbivory. A study documenting more typical concentrations of JA induced by herbivory would lay the foundation for an investigation of more ecologically relevant costs of JA-induced defenses; how strong is this effect?

With relative strength of JA-induced defense costs established, these costs can then be better compared to overall costs of herbivory, and could logically lead to investigation of other pathways activated by herbivory that similarly incur sacrifices to *C. cordifolia*.

These sacrifices often occur for a reason; plants would not funnel resources into these defenses with no benefit. Examination of the benefits of JA-induced defenses would also logically follow this study: Do *S. nigrita* larvae perform differentially on induced plants versus controls? Do ovipositing females prefer non-induced plants? Costs alone fail to fully explain the significance of JA-induced defenses.

### Acknowledgments

I am grateful to my mentor and Principal Investigator, N Whiteman, for incredible guidance and support, my fellow lab members A Gloss, H Affeldt, and M Fremgen for assistance and feedback, E Mooney for critical suggestions, D Campbell for advice on lab equipment and generosity in sharing her scale, and C Boggs for good counsel on plant growth methodology. I thank also RMBL for providing me this opportunity for scientific pursuit.

### Literature cited

- Agrawal, Anawag. 1999. Induced responses to herbivory in wild radish: effects of several herbivores and plant fitness. *Ecology* 80 (5): 1713-1723.
- Agrawal, Anawag. 2000. Benefits and costs of induced plant defense for *Lepidium virginicum* (Brassicaceae). *Ecology* 80 (7): 1804-1813.
- Baldwin, Ian. 1998. Jasmonate-induced responses are costly but benefit plants under attack in native populations. *Proceedings of the National Academy of Sciences* 95 (14): 8113-8118.
- Bergelson, Joy, and Colin Purrington. 1996. Surveying patterns in the cost of resistance in plants. *The American Naturalist* 148 (3): 536-558.
- Cipollini, Donald and Micelle Sipe. 2001. Jasmonic acid treatment and mammalian herbivory differentially affect chemical defenses and growth of wild mustard (*Brassica kaber*). *Chemoecology* 11 (3): 137-143.

- Collinge, Sharon and Svata Louda. 1988. Patterns of resource use by a drosophilid (dipteran) leaf miner on a native crucifer. *Annals of the Entomological Society of America* 81(5): 733-41.
- Collinge, Sharon and Svata Louda. 1989. *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.
- Dicke, Marcel, Rieta Gols, Daniel Ludeking, and Maarten Posthumus. 1999. Jasmonic acid and herbivory differentially induce carnivore-attracting plant volatiles in lima bean plants. *Journal of Chemical Ecology* 25(8): 1907-1922.
- Fahey, Jed, Amy Zalcmann, and Paul Talalay. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* 56(1): 5-51.
- Karban, Rick. 1993. Costs and benefits induced resistance and plant density for a native shrub, *Gossypium thurberi*. *Ecology* 74 (1): 9-19.
- Karban, Rick and Ian Thomas Baldwin. 1997. *Induced Responses to Herbivory*. Chicago: University of Chicago Press.
- Louda, Svata. and James E. Rodman. 1983. 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, Svata. 1984. Herbivore effect on stature, fruiting, and leaf dynamics of a native crucifer. *Ecology*: 65 (5): 1379-1386.
- Thaler, Jennifer. 1999. Induced resistance in agricultural crops: Effects of Jasmonic acid on herbivory and yield in tomato plants. *Environmental Entomology* 28 (1): 30-37.

Von Borcke, Lars. Insect pest protection through jasmonic acid seed treatment. 2008. Lancaster University. Unpublished.

<<http://www.pbltechnology.com/documents/Techs/07.430%20Seed%20Treatment%20Tech%20Sheet.pdf>>.

Whiteman, Noah, Simon Groen, Daniela Chevasco, Ashley Bear, Noor Beckwith, T. Ryan Gregory, Carine Denoux, Nicole Mamarella, Frederick Ausubel, Naomi Pierce. 2010. University of Arizona, Tuscon. Forthcoming.

## Tables

**TABLE 1:** Initial mean plant measurements prior to treatment for Control and JA treatment groups of *Cardamine cordifolia*. Measurements include average leaf length, average petiole length, and average count of leaves. Student's T-tests assessing differences across the two groups are two-tailed.

|                                  | N  | Mean leaf length (cm) ( $\pm$ SE) | Mean petiole length (cm) ( $\pm$ SE) | Mean leaf count ( $\pm$ SE) |
|----------------------------------|----|-----------------------------------|--------------------------------------|-----------------------------|
| <b>Control</b>                   | 19 | 1.18 (0.19)                       | 2.96 (0.46)                          | 3.95 (0.37)                 |
| <b>JA</b>                        | 19 | 1.13 (0.17)                       | 2.91 (0.48)                          | 3.58 (0.35)                 |
| <b>Student's T-test p-values</b> |    | 0.78                              | 0.93                                 | 0.48                        |

**TABLE 2:** Correlations (Pearson's r [p-value]) between final aboveground biomass measures (taken July 26, 2010), as compared to initial plant measurement values (taken July 15, 2010) for *Cardamine cordifolia*. \* indicates significance at a critical p-value of 0.05 for t-tests establishing correlation coefficient difference from 0.

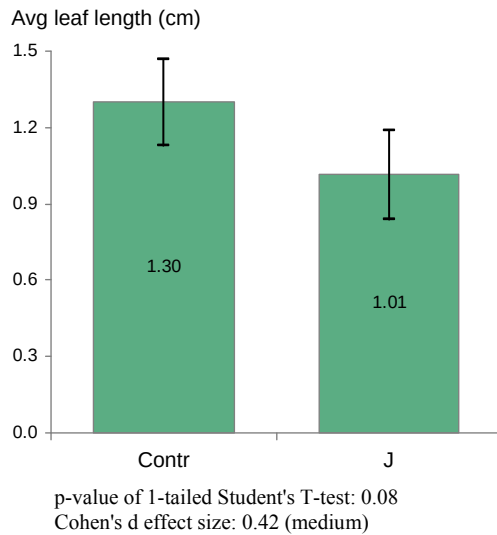
|                                | Initial leaf length (cm) | Initial petiole length (cm) | Initial leaf count (#) |
|--------------------------------|--------------------------|-----------------------------|------------------------|
| <b>Aboveground biomass (g)</b> | 0.83 (0.00)*             | 0.77 (0.00)*                | 0.56 (0.00)*           |

**TABLE 3:** Cohen's d effect sizes for Student's T-tests assessing differences in growth rates between Control and JA treatment groups of *Cardamine cordifolia*. \* indicates significant at a critical p-value of 0.05.

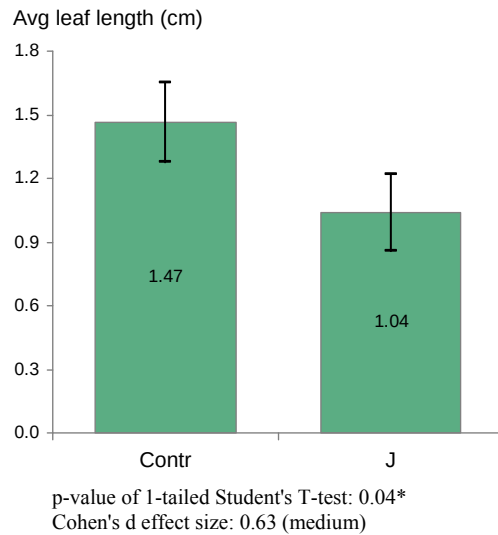
|                                                                             | T-test p-value (Control vs. JA) | Cohen's d effect size | Effect size translation |
|-----------------------------------------------------------------------------|---------------------------------|-----------------------|-------------------------|
| <b>Leaf length differences</b>                                              | 0.04*                           | 0.63                  | Medium                  |
| <b>Leaf length growth: Impact of two treatments, July 15 - 23</b>           | 0.01*                           | 0.90                  | Large                   |
| <b>Leaf length growth %: Impact of two treatments, July 15 - 23</b>         | 0.01*                           | 0.97                  | Large                   |
| <b>Leaf length growth: Impact of second treatment only, July 21-23</b>      | 0.01*                           | 1.00                  | Large                   |
| <b>Leaf length growth %: Impact of second treatment only, July 21-23</b>    | 0.02*                           | 0.88                  | Large                   |
| <b>Petiole length growth: Impact of two treatments, July 15 - 23</b>        | 0.02*                           | 0.84                  | Large                   |
| <b>Petiole length growth %: Impact of two treatments, July 15 - 23</b>      | 0.02*                           | 0.86                  | Large                   |
| <b>Petiole length growth: Impact of second treatment only, July 21-23</b>   | 0.01*                           | 0.92                  | Large                   |
| <b>Petiole length growth %: Impact of second treatment only, July 21-23</b> | 0.32                            | 0.18                  | Small                   |
| <b>Leaf count after two treatments, July 23</b>                             | 0.07                            | 0.60                  | Medium                  |

## Figures

**1A**

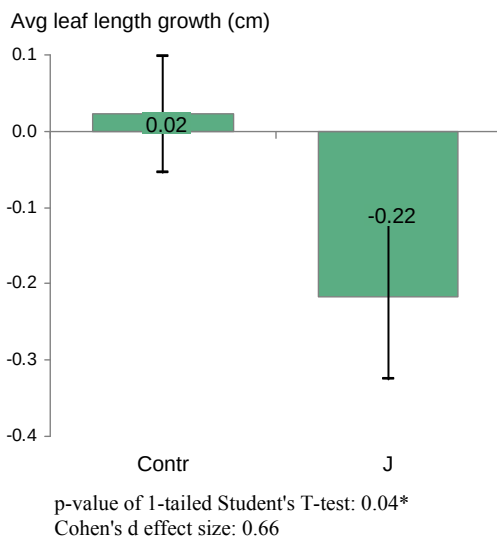


**1B**

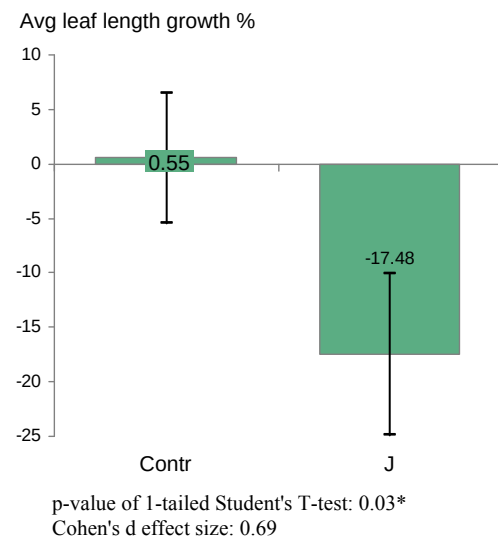


**Figure 1 A & B** Measurements of leaf length differences in plants treated with solvent (Control) or 1 mM jasmonic acid (JA). **A** Mean ( $\pm$ SE) leaf length after initial treatment (Treatment: July 15, 2010; Measurement: July 21, 2010). **B** Mean ( $\pm$ SE) leaf length after second treatment (Treatment: July 21, 2010; Measurement: July 23, 2010). \* indicates significance at a critical p-value of 0.05.

**2A**



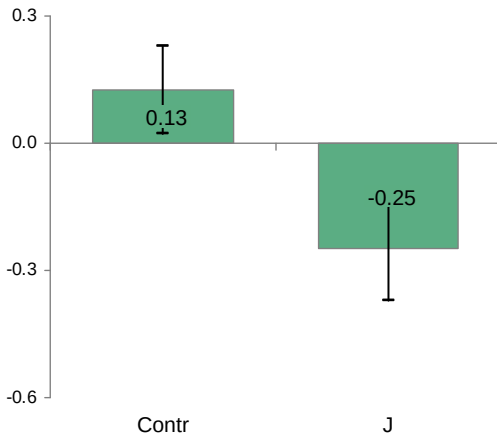
**2B**



**Figure 2 A & B** Measurements of leaf length growth rate differences in plants treated with solvent (Control) or 1 mM jasmonic acid (JA). **A** Mean ( $\pm$ SE) leaf length growth following initial treatment (Treatment: July 15, 2010; Measurement: July 21, 2010). **B** Mean ( $\pm$ SE) leaf length percentage growth following initial treatment (Treatment: July 15, 2010; Measurement: July 21, 2010). \* indicates significance at a critical p-value of 0.05.

**2C**

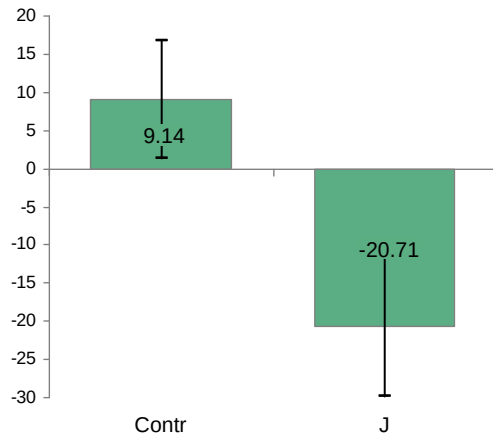
Avg leaf length growth (cm)



p-value of 1-tailed Student's T-test: 0.01\*  
Cohen's d effect size: 0.97

**2D**

Avg leaf length growth %



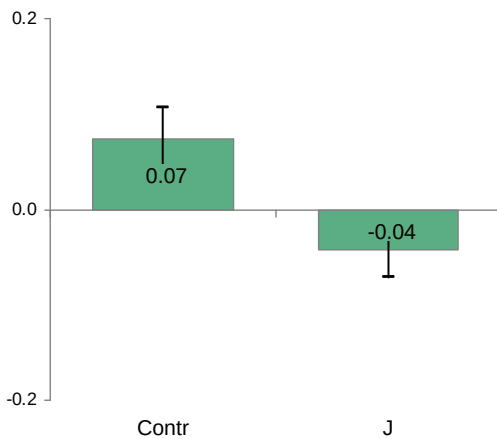
p-value of 1-tailed Student's T-test: 0.01\*  
Cohen's d effect size: 1.00

**Figure 2 C & D** Measurements of leaf length growth rate differences in plants treated with solvent (Control) or 1 mM jasmonic acid (JA). **C** Mean ( $\pm$ SE) leaf length growth following the second treatment, as compared to initial values (Growth from July 15 to July 23, 2010). **D** Mean ( $\pm$ SE) leaf length percentage growth following the second treatment, as compared to initial values (Growth from July 15 to July 23, 2010).

\* indicates significance at a critical p-value of 0.05.

**2E**

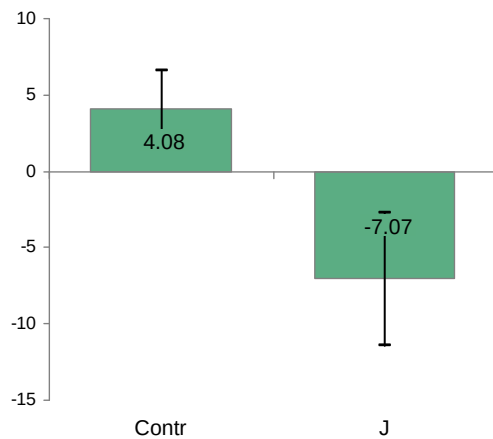
Avg leaf length growth (cm)



p-value of 1-tailed Student's T-test: 0.01\*  
Cohen's d effect size: 1.00

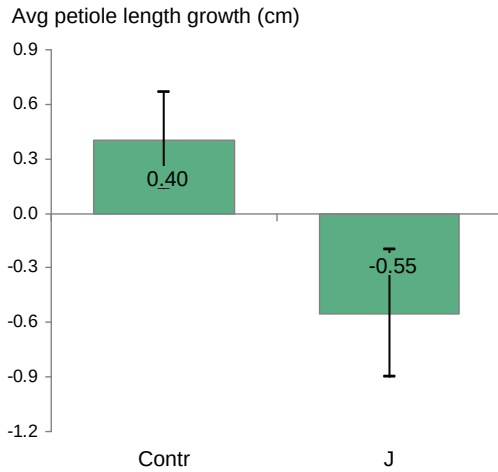
**2F**

Avg leaf length growth %

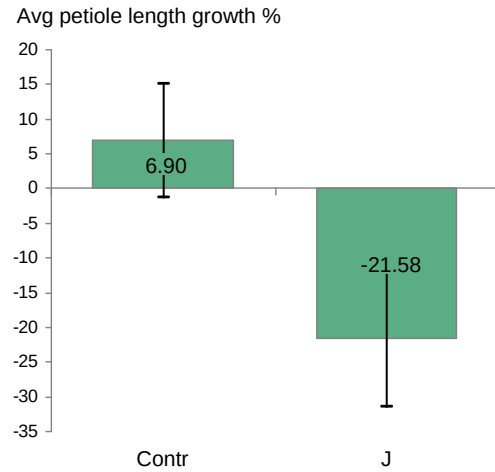


p-value of 1-tailed Student's T-test: 0.02\*  
Cohen's d effect size: 0.88

**Figure 2 E & F** Measurements of leaf length growth rate differences in plants treated with solvent (Control) or 1 mM jasmonic acid (JA). **E** Mean ( $\pm$ SE) leaf length growth following the second treatment, as compared to measurements taken immediately prior to the first treatment (Growth from July 21 to July 23, 2010). **F** Mean ( $\pm$ SE) leaf length percentage growth following the second treatment, as compared to initial values (Growth from July 21 to July 23, 2010). \* indicates significance at a critical p-value of 0.05.

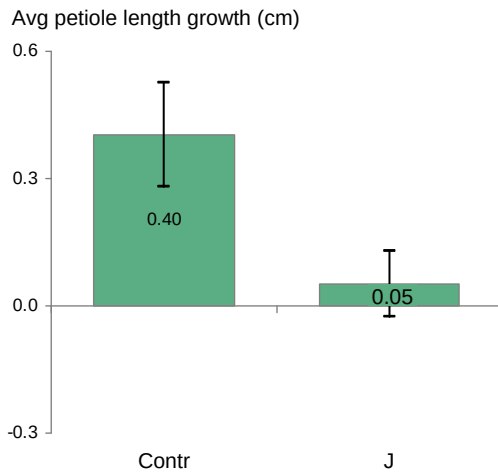
**3A**

p-value of 1-tailed Student's T-test: 0.02\*  
Cohen's d effect size: 0.84

**3B**

p-value of 1-tailed Student's T-test: 0.02\*  
Cohen's d effect size: 0.86

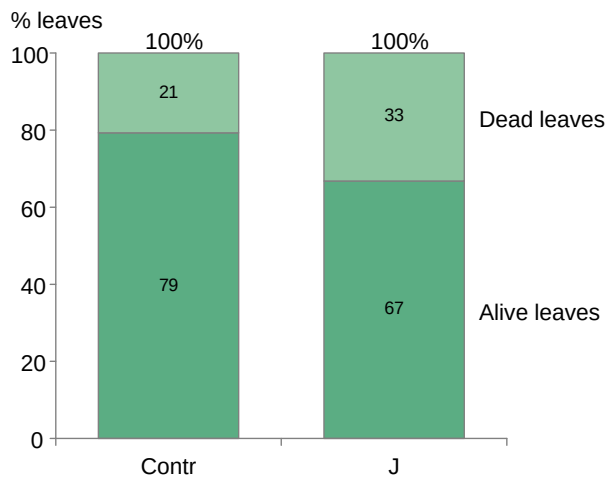
**Figure 3 A & B** Measurements of petiole length growth rate differences in plants treated with solvent (Control) or 1 mM jasmonic acid (JA). **A** Mean ( $\pm$ SE) petiole length growth following the second treatment, as compared to initial values (Growth from July 15 to July 23, 2010). **B** Mean ( $\pm$ SE) petiole length percentage growth following the second treatment, as compared to initial values (Growth from July 15 to July 23, 2010). \* indicates significance at a critical p-value of 0.05.

**3C**

p-value of 1-tailed Student's T-test: 0.01\*  
Cohen's d effect size: 0.92

**Figure 3 C** Measurement of petiole length growth rate differences in plants treated with solvent (Control) or 1 mM jasmonic acid (JA). Mean ( $\pm$ SE) petiole length growth following the second treatment, as compared to measurements taken immediately prior to the first treatment (Growth from July 21 to July 23, 2010).

4



**Figure 4** Comparison of leaf survivorship percentages in plants treated with solvent (Control) versus 1 mM jasmonic acid (JA), following two treatments (Treatments on July 15 and July 21; Measurement on July 23, 2010).

5A



5B



**Figure 5** Photo of *Cardamine cordifolia* leaf decaying at edges. **A** Top of leaf. **B** Bottom of leaf.