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The response of *Heracleum sphondylium* (Apiaceae) and its herbivores to simulated herbivory and soil nutrient augmentation

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Abstract– Plants may face trade-offs in growth or fitness when cued by herbivores to induce a physical and/or chemical response. For an induced response to be considered a defense, the response should benefit the plant in terms of its growth, fitness, or survival. Here, we examined the response of *Heracleum sphondylium* (cow parsnip) to simulated herbivory and/or nutrient augmentation within a natural population in Colorado. We measured length of new leaves, trichome density on leaves, and the abundance of leaf holes and Hemiptera on plants two weeks following the treatments. Simulated herbivory significantly decreased the number of Hemiptera on the leaves of the plants, and we found a trend of fewer leaf holes on clipped plants. However, simulated herbivory and nutrient augmentation did not significantly affect the trichome density and the length of new leaves. These results are the first to suggest that *Heracleum sphondylium* may induce a chemical response rather than a physical response as measured by leaf trichome density when the plant experiences foliage herbivory. Additionally, we conclude that *Heracleum sphondylium* may have a mechanism by which it can compensate for the loss of photosynthetic tissue when soil nutrients are not limiting. These results are consistent with past studies that have shown a closely related species, *Pastinaca sativa* (wild parsnip), induces a chemical response as a result of herbivory.

Key words: *herbivory;* Heracleum sphondylium; *cow parsnip; constitutive response; induced response; induced defense; trichomes*

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Herbivory presents a challenge to plants: to allocate their resources efficiently so as to optimize their defense towards herbivores as well as their growth and fitness (Zangerl and Rutledge 1996, Zangerl et al. 1997). Although constitutive defenses provide plants with consistent protection against herbivores, inducible defenses can be considered a plastic trait that, hypothetically, allows a more efficient allocation of resources that may improve a plant's fitness in an unpredictable environment (Agrawal *et al*. 1999). According to the optimal defense theory, in the absence of

herbivores, plants should allocate a greater portion of their acquired resources to growth and reproduction than towards unnecessary anti-herbivore defenses (Zangerl and Rutledge 1996, Agrawal *et al*. 1999). In contrast, plants under greater herbivory pressure would benefit from defenses if the costs of herbivory are greater than the cost to induce a defense (Zangerl and Rutledge 1996, Agrawal *et al*. 1999).

Physical and chemical expressions of inducible response systems have been divided into three categories: response, resistance, and defense (Agrawal 1999 and references therein). Induced responses to herbivory can be phenotypically expressed either through physical or chemical changes within the plant (Harvell 1990). Past studies have documented a range of responses to herbivory that include changes in leaf toughness, trichome density, nitrogen uptake, and secondary chemistry (Agrawal 1999). Induced resistance includes a change in the plant by way of a physical or biochemical mechanism that decreases herbivores' preferences for an individual (Agrawal 1999). For an induced response to be considered a defense, the response should benefit the plant in terms of the plant's growth, fitness, or survival (Agrawal 1999).

While some have hypothesized that plants that induce a physical or chemical response to herbivory may compromise subsequent growth and reproduction, such trade-offs are not supported by consistent data (Harvell 1990, Karban 1993, Zangerl and Rutledge 1996, Zangerl *et al*. 1997, Agrawal 1999, Agrawal *et al*. 1999). Karban (1993) found that chemically induced *Gossypium thurberi* did not differ in growth or female fitness as compared to control plants. In contrast, Agrawal (1999) found that annual wild radish plants (*Raphanus raphanistrum* and *R. sativus*) both increased and decreased in fitness depending on the variables measured. When measuring female fitness, induced wild radish plants produced an increased number of fruits and seeds. In a separate study that used a multivariate analysis to incorporate alternative measures of fitness, such as time to first flower and aspects of male fitness, Agrawal *et al*. (1999) reported an overall decrease in fitness of the wild radish plants. Accordingly, cost-benefit analyses of trade-offs involved for induced responses to herbivory remain unclear and most likely depend on various biotic and abiotic factors such as herbivore pressure and limiting resources (Karban 1993).

If costs and/or benefits do exist for various plants that induce a response to herbivory, then how are the trade-offs expressed, and what are the potential abiotic factors that could affect the magnitude of costs and benefits of resistance? For our experiment, we chose to study *Heracleum sphondylium* (cow parsnip) located in Gunnison County, Colorado. Several parsnips in the parsley family (Apiaceae) are known to produce xanthotoxin, a toxic furanocoumarin inducible to various degrees in the reproductive parts, leaves, and roots of the plants (Zangerl and Rutledge 1996). Within the parsley family, inducible chemical responses to herbivory have been most widely studied in *Pastinaca sativa* (wild parsnip) (Zangerl *et al*. 1997). An inducible physical or chemical response against herbivores has never been studied, however, for the closely related species, *Heracleum sphondylium*. The aim of our study was to assess how *Heracleum sphondylium* responds to herbivory and the possible trade-offs the plant may experience when responding to herbivore pressure. To determine whether *Heracleum sphondylium* induces a physical and/or chemical response to herbivory, we simulated leaf herbivory, then measured leaf trichome density, number of leaf holes and herbivore abundance on plants in a natural population of *Heracleum sphondylium*. Additionally, we assessed whether soil nutrients might be a limiting factor for the growth and potential response of *Heracleum sphondylium* to herbivory.

If *Heracleum sphondylium* responds to herbivory in a similar manner as *Pastinaca sativa*, then we would expect that *Heracleum sphondylium* induces a toxic furanocoumarin as a resistance mechanism to deter generalist herbivores. Accordingly, we would not expect the plants to respond to simulated herbivory with a physical mechanism to deter herbivores, such as increased trichome densities. Nonetheless, we predict that herbivore preference for the plants that experience simulated herbivory should decrease. If soil

nutrients limit the plants' abilities to induce a response, we would expect to see a greater response in plants with experimentally augmented nutrients. Finally, if there is an immediate resource trade-off so that an induced response comes at the expense of a temporarily reduced growth rate, we would expect to see less new growth in the plants with simulated herbivory.



Fig. 1. *Heracleum sphondylium* (Apiaceae). Photograph© used with permission from Al and Betty Schneider.

Materials and Methods

Heracleum sphondylium (Fig. 1) is a caulescent, polycarpic perennial found in moist and often shaded patches throughout North America and Asia. The plant has been documented in the western two-thirds of Colorado approximately between 1,500-3500 meters elevation. Stalks are 80-200 cm. tall terminating at the primary umbel and leaflets that are 10-40 cm. long. Secondary umbels develop on lateral shoots and produce 25-50 umbellets each with 400-1,600 small flowers. Primary umbels produce 35-60 umbellets with 800-2,000 flowers each (Hickey and King 1981). Most herbivory damage on *Heracleum sphondylium* within our study area appears to be caused by insects, although the plants may occasionally be grazed by ungulates. The most abundant herbivores found on the plants in our study were several insects in the order Hemiptera, and to a lesser extent Homoptera.

The experiment was conducted near the Rocky Mountain Biological Laboratory (RMBL) in Gunnison County, Colorado (elevation 2900 meters). The study site was approximately 2 km east of the RMBL, along the Copper Creek Trail within the Maroon Bells - Snowmass Wilderness area. The study area was approximately 20 meters by 20 meters. The site was a southwest-facing slope dominated by *Populus tremuloides* and *Heracleum sphondylium.* A few shrubs and herbaceous plants, notably *Hydrophyllum fendleri*, were interspersed among *Heracleum sphondylium* plants. At the base of the slope and within 10 meters of the edge of the study area was a seasonal creek, and beyond the creek was a grassy meadow.

In order to test whether nutrient augmentation and/or clipping affect plant growth, trichome production, or herbivory on *Heracleum sphondylium*, we set up a 2 X 2 factorial experiment. We had 4 treatment combinations: 2 clipping treatments (clipping or not) X 2 fertilizer treatments (fertilizer addition or water addition). Before applying treatments, we identified 12 blocks of 4 plants of similar size, for a total sample size of 48 plants. Within each block of four plants, we randomly assigned the 4 treatments. Plants in each block were approximately 0.5 meters apart.

Treatments were applied June 10-11, 2002. Using scissors, we clipped approximately 50% of the leaf area of plants assigned to the 'clipping' treatment. This was done by clipping half of every leaf by cutting straight across the leaves so that the distal portions of the leaves were removed in one piece. The unclipped plants were untouched.

Fertilized plants received 250 ml of a Miracle Gro® solution, which contained nitrogen, phosphorous, potassium, and small amounts of other nutrients and minerals. Watered plants received 250 ml of water. Plants were fertilized and watered a second time on June 18, 2002. To minimize run-off from either the addition of fertilizer or water, the liquids were slowly and systematically administered at the base of each plant and there was visual confirmation of absorption into the top layer of the soil.

Growth, trichome production, and herbivory were measured June 24-25, 2002.

Trichome production– To determine whether simulated herbivory and/or the application of fertilizer affected trichome growth, we measured the density of trichomes on the new leaf growth two weeks after the application of the treatments. We defined new leaf growth of the clipped plants as the leaves that came from leaf buds that had opened after the treatments. We sampled the largest and smallest new leaf from each clipped plant, which were representative of early and late-leaf growth. If the clipped plants did not have new leaves, we did not include these plants in our sample. For the measure of trichome density, we omitted 10 plants because they grew no new leaves after the treatments were applied. For the non-clipped plants, we sampled leaves that were of comparable sizes to the new leaves that were sampled from the clipped plants. These leaves, however, were not restricted to new leaf growth because, like the clipped plants, some of the unclipped plants did not have newly opened leaves.

We stored all leaf samples for less that 24 hours in zip-lock bags until the trichome densities were measured at the Rocky Mountain Biological Laboratory. A hole-punch was used to remove a consistent area from the same place on each leaf. Using dissecting microscopes set at 100x, we counted the number of trichomes in a 1 mm^2 area on the hole-punched portions of the leaves. To guarantee that the size of the sampled areas was consistent on each leaf, wire grids that designated 1 mm^2 areas were placed onto the sampled portions of the leaves that were under the microscope.

Physical growth– To assess whether herbivory or fertilizer affected the growth of *Heracleum sphondylium*, we measured the length of new leaves. All measurements were made in the field. Each plant had several stalks with new leaf growth, and new leaves grew from the topmost node of each stalk. The length of the longest leaf growing from each node was recorded for all stalks with new leaf growth. The average length of the longest leaf per node was calculated on a per plant basis.

Measures of herbivory: herbivore abundance and damage– At the study site, the number of leaf holes was counted on all the leaves growing from the tallest stalk of each plant. We also included bite marks on the edge of the sampled leaves as holes. We defined one continuous bite mark as one hole. Additionally, we counted both juvenile and adult Hemipterans on all of the stalks, leaves, and buds of each plant. Statistical analysis was performed using Stat View©. We performed two-way ANOVA's to test whether variance in trichome production, physical growth, or subsequent herbivory could be explained by simulated herbivory and/or the application of fertilizer treatments.

Results

Trichome production– Independently, clipping and fertilizer did not significantly affect the density of trichomes on early-leaf growth two weeks following the treatments (two-way ANOVA: F=1.194, P=0.28 and F=0.024, P=0.88 respectively), nor was there a significant interaction between clipping and fertilizer on the density of trichomes for early-leaf growth (F=2.421, P=0.130, Fig. 2a). Additionally, we found no significant main effects of clipping and fertilizer on the average number of trichomes per leaf area on late-growth (two-way ANOVA: F=0.042, P=0.839 and F=0.302, P=0.586 respectively). There was no significant clipping x fertilizer interaction for the average trichome density on late-leaf growth (two-way ANOVA: F=0.192, P=0.664, Fig. 2b). Hence, trichome densities on early and late-leaf growth were not significantly affected by simulated herbivory and/or the application of fertilizer two weeks following the treatments.

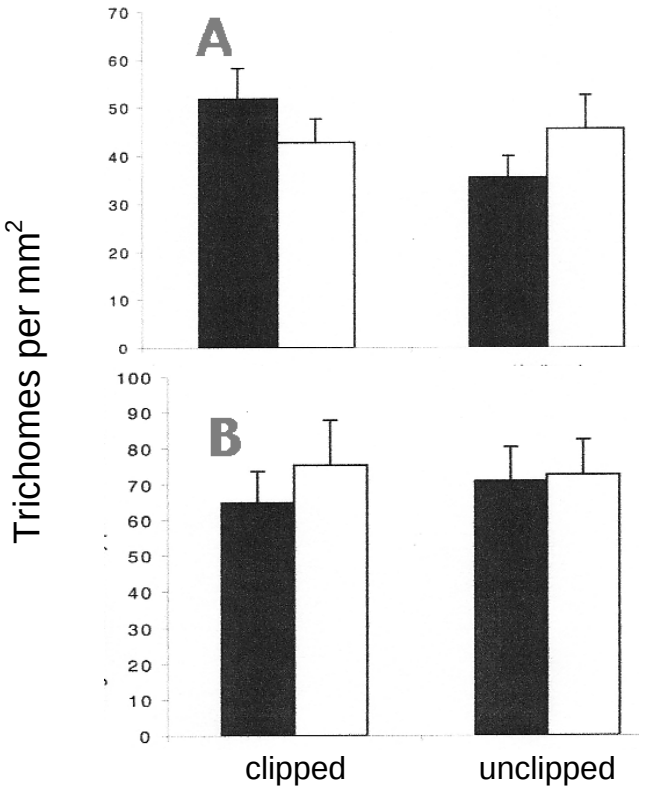


Fig. 2. The effect of clipping and fertilizer on the average trichome density per mm^2 of leaf area on early-leaf growth (A) and late-leaf growth (B). Bars represent means ± SE, black are fertilized, white unfertilized.

We did find a significant size-dependent effect of leaf size on trichome density (paired $t = 6.28$, $df = 33$, $P < 0.0001$).

Physical growth responses– The average length of new leaves was not significantly affected by clipping, fertilizer, or a clipping x fertilizer interaction (Table 1). The average length of new leaves from clipped and unclipped plants that had not received the nutrient treatment were 4.03 cm ± 0.81 S.E and 2.65 cm ± 0.70 S.E. respectively. From clipped and unclipped plants that had received additional nutrients, the average length of new leaves were 4.20 cm ± 0.67 S.E. and 4.34 cm ± 0.41 S.E respectively.

Herbivore Abundance– Clipping significantly affected the average number of Hemiptera on *Heracleum sphondylium* (Table 1). On average, there were fewer Hemiptera on clipped plants than unclipped plants (X=6.08 ±1.14 SE and X=9.25 ± 1.06 SE respectively). There was no significant effect of fertilizer or clipping x fertilizer on the average number of Hemiptera found on *Heracleum sphondylium* (Table 1).

Damage by herbivores– Although we found no significant difference in the number of leaf holes per tallest stalk between clipped and unclipped plants (Table 1, Fig. 3), the data show a trend of fewer holes on clipped plants. On average, the clipped plants had 48.59 ± 7.1 S.E and the unclipped plants had 66.67 ± 7.77 S.E holes. Additionally, fertilizer had no significant effect (Table 1, Fig. 3). The plants augmented with nutrients had 62.33 ± 8.32 S.E. holes and plants without additional nutrients had 52.92 ± 6.74 S.E. holes. There was also no significant clipping x fertilizer interaction on the number of leaf holes per tallest stalk (Table 1, Fig. 3).

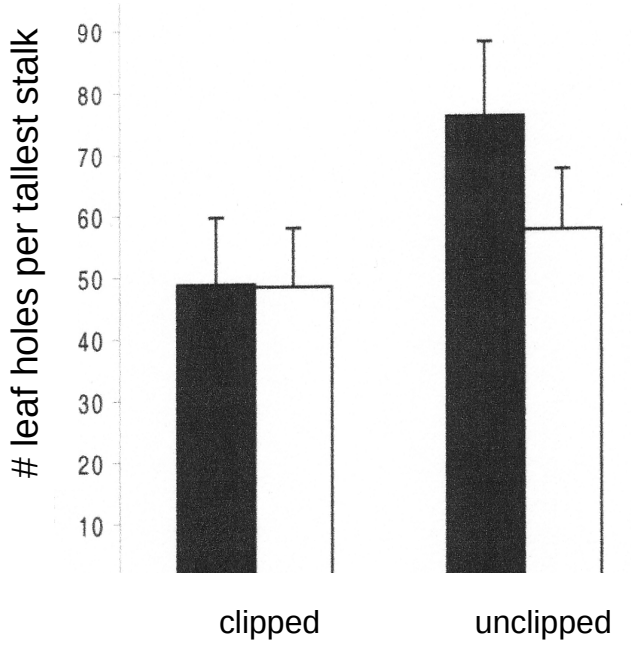


Fig. 3. The effect of clipping and fertilizer on the average number of leaf holes per tallest stalk. Means also include bite

marks (defined as one hole) on the sides of leaves. Bars represent means ± SE, black are fertilized, white unfertilized.

Table 1. Results of a two-way analysis of variance testing for the effects of clipping and fertilizer treatments on growth (length of new leaves) and herbivory (number of Hemiptera found on the plants, and number of leaf holes). * indicates $p < 0.05$.

		Length of new leaves			# Hemiptera			# Leaf holes		
Source	df	MS	F	p	MS	F	p	MS	F	p
Clipping	1	1.69	0.02	0.89	120	4.23	0.046*	3924	2.96	0.093
Fertilizer	1	42.2	0.46	0.50	14.1	0.50	0.491	1064	0.81	0.375
Clipping x fertilizer	1	42.2	0.46	0.50	70.1	2.46	0.124	990.1	0.75	0.392
Residual	44	91.8			28.5			1327		

Discussion

Although *Heracleum sphondylium* did not induce a physical response to herbivory as measured by trichome density and length of new leaves, we did find significantly fewer herbivores (Hemiptera) and a trend of fewer leaf holes per tallest stalk on clipped plants. These results suggest that *Heracleum sphondylium* responds to herbivory in a similar manner as other plants in its family, specifically, *Pastinaca sativa* (wild parsnip), and may induce a chemical defense rather than a physical defense as a response to herbivory. Moreover, because our physical measure of growth did not significantly differ between clipped and unclipped plants, any response by *Heracleum sphondylium* did not appear to have an immediate growth cost. The data also indicated that available nutrients did not limit the response or growth of the plants in this study because no signicant differences were found between the responses of the fertilized and unfertilized plants.

If inducible defenses are considered a plastic trait expressed in a manner that minimizes the plant's cost of defense, then theoretically, expressing the defense phenotype in an environment with high levels of herbivory will benefit the plant in terms of its growth and fitness (Zangerl and Rutledge 1996, Agrawal *et al*. 1999). We found, however, that the length of new leaves on plants that experienced simulated herbivory were not significantly different from the unclipped plants. This result is consistent with several past studies (Karban 1993 and references therein). However, other studies have detected some growth benefits and costs related to inducible responses (Harvell 1990 and references therein). One explanation for not detecting any growth costs or benefits in this study is our use of a clipping treatment to simulate herbivory. The clipping treatment minimized the damage per leaf area removed. Approximately 50% of the total leaf area was removed while a relatively small area of the leaves were actually damaged. We did attempt to minimize the possible lack of a clipping effect by clipping all leaves on the plants that were assigned to the herbivory treatment. Even so, if the magnitude of inducing a chemical response to herbivory is a function of leaf area damaged, our results may underestimate the true costs or benefits of *Heracleum sphondylium* inducing a response to herbivory. If we simulated the behavior of natural herbivores by increasing the area of damaged tissue per area of tissue removed, we would have possibly detected a greater response.

Consequently, from our finding that *Heracleum sphondylium* did not experience a growth cost (or benefit) measured by the length of new leaves after 50% of the total leaf tissue was removed, we suggest that the plants in this study may have had the ability to physically compensate for the reduction of photosynthetic tissue, at least in the short term. Further, it seems possible that *Heracleum sphondylium* may have some mechanism whereby it does not suffer from inducing a chemical defense. Despite the

possibility that the loss of photosynthetic tissue is an independent effect from a chemical induction, the two effects can be additive (Agrawal *et al*. 1999). A past study exhibited that wild parsnip experienced a decrease in the photosynthetic capacity of damaged and undamaged tissues when fed on by herbivores (Zangerl *et al*. 2002). One mechanism, however, that can possibly explain our results is that *Heracleum sphondylium* may have allocated energy stored in below ground reserves to its shoots in order to maintain a similar rate of growth before and after herbivory (Ta *et al*. 1990). Alternatively, it has been suggested that as a part of a whole-plant response to foliage herbivory, some plants can decrease their root growth (Calion *et al*. 1990) or increase the rate at which their roots acquire resources (Ruess 1988). In addition, a well-known response of damaged plants is an increased photosynthetic rate of the remaining undamaged leaves (Kolodny-Hirsch *et al*. 1986, Welter 1989). It is possible, however, that increased photosynthetic activity of undamaged tissue is not applicable to the plants manipulated in this experiment because every leaf of the experimental plants was subjected to the clipping treatment. In addition, the data suggest that soil nutrients were not a limiting resource for the growth of the plants at our particular study site in the summer of 2002. While it is possible that the augmented nutrients were not perfectly contained to the assigned plants, it seems that *Heracleum sphondylium* may have the ability to withstand an intermediate level of tissue loss for our measure of growth when soil resources are not limiting. Further experimentation is necessary to determine (1) the mechanisms by which physical compensation may occur, and (2) whether a reduction in photosynthetic tissue results in any detectable costs when *Heracleum sphondylium* is limited by resources.

Past studies have shown inconsistent results regarding whether an induced response to herbivory affects a plant's fitness (Agrawal 1999, Agrawal *et al*. 1999). Some have shown that seed production and mass as well as number of fruits and fruit mass increased as a result of inducing a chemical defense (Agrawal 1999, Lohman *et al*. 1996). In contrast, other studies have suggested that an induced response decreases a plant's fitness (Lohman *et al*. 1996, Agrawal *et al*. 1999). It has been reported that induced wild parsnips were visited less by pollinators (Lohman *et al*. 1996), and induced wild radish plants experienced an increased time to first flower and produced less seeds as well as pollen grains (Agrawal *et al*. 1999). Our study did not evaluate whether fitness consequences resulted from an induced response to herbivory. However, given the preliminary evidence that *Heracleum sphondylium* induces a chemical response to herbivory, we suggest that further studies assess the following in relation to fitness: (1) whether both male and female fitness consequences occur as a result of inducing a response to herbivory, (2) whether induced compared to constitutive defenses are expressed consistently or optimally throughout different parts of a plant in order to maximize the plant's responses to an attack with minimal cost, and (3) whether greater herbivore pressure will affect additional measures of growth and survival such as decreased photosynthetic productivity.

An additional explanation for our finding of fewer herbivores on clipped plants is that there was less leaf tissue available on those plants. However, insect herbivores generally tend to feed on younger, more tender growth, whereas the clipped leaves were at least two weeks old at the time of our herbivore counts. Therefore we do not think that the lesser availability of older leaf tissue on clipped plants entirely explains the difference we observed in herbivore numbers between the clipping treatments.

In summary, this study presents preliminary evidence that *Heracleum sphondylium* induces a chemical defense, as has been documented for *Pastinaca sativa*, when fed on by herbivores. In addition, the study raises further questions regarding the costs and benefits *Heracleum sphondylium* may experience with the loss of photosynthetic tissue and the induction of a chemical defense as a consequence of foliage herbivory. Further testing is necessary to detect whether *Heracleum sphondylium* actually produces secondary metabolites as a response to herbivory. Moreover, chemical analysis is required to determine the specific chemical that *Heracleum sphondylium* may induce when fed on by herbivores. Nevertheless, this is the first study to

suggest that *Heracleum sphondylium* produces a secondary metabolite as a response to herbivory.

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