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ORIGINAL PAPER

Costs of induced defenses for the invasive plant houndstongue (*Cynoglossum officinale* L.) and the potential importance for weed biocontrol

Justin B. Runyon[1] · **Jennifer L. Birdsall**[1]

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Abstract Inducible plant defenses—those produced in response to herbivore feeding—are thought to have evolved as a cost-saving tactic that allows plants to enact defenses only when needed. The costs of defense can be significant, and loss of plant fitness due to commitment of resources to induced defenses could affect plant populations and play a role in determining the success or failure of weed biocontrol. We used methyl jasmonate (MeJA) to experimentally induce defenses without herbivores in invasive houndstongue plants (*Cynoglossum officinale* L.) in the field and measured resulting growth and fitness (plant size, seed number, and seed weight). MeJA-treated plants emitted large amounts of plant volatiles and produced leaves with twice as many trichomes as untreated plants. Plants with activated defenses had fewer leaves, were smaller, and produced nutlets that weighed less than plants not investing in defenses. These data indicate that herbivore-induced defenses are costly for houndstongue plants in their invaded range and represent significant indirect costs of herbivory beyond direct feeding damage (e.g., loss of photosynthetic tissue). Notably, the magnitude of defenses elicited upon feeding varies greatly by herbivore species and a better understanding of the costs of defense could help us predict which potential biocontrol herbivores are most likely to be effective.

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✉ Justin B. Runyon
jrunyon@fs.fed.us

[1] Rocky Mountain Research Station, Forestry Sciences Laboratory, USDA Forest Service, Bozeman, MT, USA

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Introduction

To ward off herbivore attack, plants perceive herbivore feeding and respond by producing induced defenses including toxic compounds in leaves, trichomes, and plant volatiles that attract herbivore natural enemies (Howe and Jander 2008; Karban and Baldwin 1997). Induced defenses likely evolved because they are costly for plants to produce, directly in terms of resource allocation (e.g., diverting limited resources from growth toward defense) and indirectly via various ecological costs (e.g., reducing pollinator attraction) (Gershenzon 1994; Heil 2002; Hoballah et al. 2004; Steppuhn and Baldwin 2008). Accompanying induced defenses are often fundamental changes in plant primary metabolism, including down-regulation of photosynthesis (Nabity et al. 2009; Schwachtje and Baldwin 2008; Zangerl et al. 2002). Such changes are thought to prioritize defense over growth (Schwachtje and Baldwin 2008), but also increase the costs of defense. The discovery in the early 1990s that the phytohormone jasmonic acid (JA) mediates many anti-herbivore responses (e.g., Farmer and Ryan 1990), allowed the costs of induced defenses to be assessed by applying JA or its methyl ester (MeJA) to elicit plant defenses without herbivore feeding. For example, in the absence of herbivores, treatment with JA or MeJA significantly reduced seed production in wild tobacco (*Nicotiana attenuata*) (Baldwin 1998), tomato (Redman et al. 2001), *Arabidopsis thaliana* (Cipollini 2002), and soybean plants (Accamando and Cronin 2012). Development and use of molecular tools like mutants and transformed plants have provided further documentation and insights into the costs of induced defenses and the consequences for plant fitness (Cipollini 2007; Robert et al. 2013; Zavala et al. 2004; Zuest et al. 2011).

Classical biological control (hereafter "biocontrol"), the deliberate introduction of herbivores from the native range of a weed to suppress weed abundance in the invaded range, is a crucial management tool because it is one of few that can provide long-term control of widespread invasive plants. Major biocontrol successes include control of prickly pear (*Opuntia* spp.) using the cactus moth, *Cactoblastis cactorum*, in Australia (Dodd 1940) and St. John's wort (*Hypericum perforatum*) using the *Chrysolina* flea beetle in western North America (Holloway and Huffaker 1951; Huffaker and Holloway 1949). Such spectacular successes bolster biocontrol and show the great potential this tool holds for controlling invasive plants at a landscape scale. However, biocontrol agents often fail to impact weed populations once established, sometimes despite becoming extremely abundant. The worldwide success rate of biocontrol attempts against invasive plants has been estimated at only 20–30 % (Crawley 1989; Julien 1989; van Klinken and Raghu 2006). This is troubling because testing and approval of each new biocontrol agent take many years and hundreds of thousands of dollars to complete (McFadyen 1998), and ineffective agents can cause unwanted ecological effects even if they are host-specific (Carvalheiro et al. 2008; Pearson and Callaway 2005). A better understanding of the factors affecting biocontrol success and failure is needed to better predict, prior to release, which agents are most likely to be effective (Carson et al. 2008). Choosing effective agents, which has been called the "holy grail" of weed biocontrol (McFadyen 1998), will simultaneously improve our ability to manage invasive plants and minimize unwanted ecological side effects. An overlooked factor that could affect biocontrol outcomes and agent efficacy is the cost to weeds of producing defenses induced by biocontrol herbivores because such costs can be an important component of the overall impact of herbivory on plant fitness (Karban and Baldwin 1997), and the magnitude of defenses induced upon feeding varies dramatically with herbivore species (Howe and Jander 2008). This suggests that introducing biocontrol herbivore species that induce large defenses could reduce invasive plant fitness more than those herbivore species that induce little or no defenses, due to the additional defense costs incurred.

In this study, we used MeJA to examine the costs of induced defenses for houndstongue (*Cynoglossum officinale*), an invasive plant in North America. Houndstongue is a monocarpic biennial or short-lived perennial that is native to Eurasia and was introduced to North America in the mid-nineteenth century (Upadhyaya et al. 1988). Houndstongue plants typically spend year one as a rosette of large leaves (each leaf 10–30 cm long and 2–6 cm wide), bolts and flowers in year two, then dies, but plants can remain a rosette for more than 1 year (Upadhyaya et al. 1988). Houndstongue is a troublesome noxious weed in most western US states and Canadian provinces, and is toxic to livestock due to production of pyrrolizidine alkaloids (Baker et al. 1989; Knight et al. 1984). Each flower produces up to four nutlets covered in barbed prickles, each containing a single seed; plants reproduce by seed only (Upadhyaya et al. 1988). At an invaded field site in Montana, we compared growth (number of leaves, plant height, width, and volume) and lifetime fitness (number and weight of nutlets, germination success) of houndstongue plants treated with a low MeJA dose, high MeJA dose, and untreated control plants. Our goals were to assess whether or not induced defenses are costly for houndstongue and if so discuss the implications for biocontrol. We also lay out a framework for testing if induced defenses hold value for understanding and predicting successful biocontrol of plants.

Methods

Study system

This research was conducted in 2010 and 2011 in meadows at approximately 2000 m elevation in the Northern Rocky Mountains of southwest Montana, USA. The study area covered about 40 ha in the Gallatin Mountains near Lick Creek (N45°30.5′, W110°58.5′) and consisted of a mosaic of meadows and forests. Meadows were predominately grasses (ca. 55 % cover) and forbs (ca. 30 % cover) with scattered sagebrush, *Artemisia tridentata* ssp. *vaseyana* (Rydb.); forests were dominated by lodgepole pine (*Pinus contorta* Douglas). The study area was grazed by cattle every 3 years, but no grazing occurred during the two-year study period. Meadows were invaded (ca. 10 % cover) by houndstongue, *Cynoglossum offinale* L. (Boraginaceae). We chose houndstongue for several reasons: It is a common weed where we work in the Northern Rocky Mountains; it is largely a biennial so lifetime fitness can more easily be estimated; there are currently no biocontrol herbivores or pathogens of houndstongue available in the USA, and feeding by native herbivores is rare; thus, we do not need to use insecticides or cages to exclude herbivory; and the search for, and testing of, potential biocontrol agents to suppress houndstongue abundance is ongoing.

Plant selection and application of MeJA

We selected 40 triplicates of small rosettes of similar size (5.8 ± 0.6 leaves, 21.1 ± 0.7 cm tall, $n = 120$ plants) that were at least 0.5 m apart (average minimum distance between plants in a triplicate was 1.7 m); triplicates were spread out over approximately 40 ha. Plants were randomly assigned to a treatment, and each triplicate marked with wire stake flagging and by engraving plant number and treatment into the plastic cap of 7.6-cm (3-inch)-long steel roofing nails that were placed about 5 cm from crown on north side of each plant. Triplets were marked using survey whiskers.

To activate induced defenses, we applied methyl jasmonate (MeJA; 95 %) in lanolin paste (both from Sigma-Aldrich, St. Louis, MO, USA) to plants following Baldwin et al. (1996). Jasmonic acid mediates many induced plant defenses against herbivores, and numerous studies have applied jasmonic acid or its methyl ester to plants to experimentally elicit defenses, e.g., (Accamando and Cronin 2012; Baldwin 1998; Cipollini 2002; Redman et al. 2001). Doses of lanolin paste, containing quantified amounts of MeJA, were prepared on the same day as their application. Doses were prepared by weighing MeJA in a glass vial and then pipetting liquefied lanolin (heated in a 50 °C water bath) into the vial to obtain the desired concentration of MeJA; contents were mixed well and individual doses pipetted onto wax paper and frozen until use. Three treatments were applied: control (lanolin only; 30 µl per plant), low MeJA (150 µg; 30 µl per plant of 5 mg MeJA/mL lanolin), and high MeJA (300 µg; 30 µl per plant of 10 mg MeJA/mL lanolin). The amounts of MeJA applied have been shown to induce defenses similar in degree to insect feeding in other plant species (Baldwin 1998; Thaler et al. 2001). Wax paper with doses was transported to the field and kept on ice in coolers until applied. Each dose was divided in half and applied to the two newest (youngest) fully expanded leaves of a plant (two leaves per plant were treated). Lanolin paste was applied in a thin layer to the adaxial (upper) leaf surface with a wooden popsicle stick by carefully smearing the paste over an approximately 1-cm-wide band over the width in the middle of the leaf. Treatments were applied to the same plants twice per year about 3 weeks apart in June and July (24 June and 16 July in 2010; 01 July and 22 July in 2011). Background herbivory was very minor because few native herbivores feed on houndstongue and there are no biocontrol agents at the site. Leaves in early spring sometimes received “exploratory” feeding (a few tiny holes) from adults of a native flea beetle (*Longitarsus* sp.) that persists on other Boraginaceae species at the site, and also occasionally some feeding by grasshoppers in late summer, but feeding damage was minor and evenly distributed among the treatments.

Measuring induced defenses: volatiles and trichomes

We measured changes in an indirect defense, plant volatiles, and in a direct defense, trichomes, in the field following a single application of MeJA as measures and indicators of the activation of induced defenses. Volatiles were collected 1–5, 10, 20, and 25 days after MeJA treatment from four randomly chosen plants from each treatment using portable volatile collection systems with controlled push–pull airflow and hydrocarbon filters enclosed in a waterproof case (Volatile Assay Systems, Rensselaer, New York). Plants were enclosed in clear Teflon bags (50 cm wide × 75 cm deep; American Durafilm Co., Holliston, MA, USA), and clean air was pushed into the bag through a side port via Teflon tubing (0.75 L/min); air was pulled out through another side port (0.5 L/min) through volatile traps containing 30 mg of the adsorbent HayeSep-Q (Restek, Bellefonte, Pennsylvania). Volatile emissions were collected for 5 h each day between 10:00 and 15:00. Volatiles were eluted from traps with 200 µL of dichloromethane, and 500 ng of *n*-nonyl-acetate was added as an internal standard. Samples were analyzed using an Agilent 7890A gas chromatograph (GC) coupled with a 5975C mass spectrometer and separated on a HP-1ms (30 m × 0.25 mm inside diameter, 0.25 µm film thickness) column; helium was used as the carrier gas. The GC oven was maintained at 35 °C for 3 min and then increased by 5 °C per min to 125 °C, then 25 °C per min to 250 °C. Quantifications were made relative to the internal standard using ChemStation software (Agilent Technologies, Wilmington, DE, USA), and identifications of compounds were confirmed by comparing retention times and mass spectra with commercial standards. Comparisons were made among treatments for each sampling period using one-way analysis of variance (ANOVA), and individual means were compared with Tukey’s honestly significantly different (HSD) means separation test. Volatile data were square-root transformed to meet variance assumptions. All statistics were performed using SAS (version 8.2; SAS Institute, Cary, NC, USA).

We assessed trichome density on old leaves (fully expanded at time of treatment) and new leaves (fully expanded since treatment) on plants 30 days after application of MeJA in the field. Two 0.4-cm-diameter (12.57 mm^2) leaf disks were punched midway between the leaf tip and leaf base, one on either side of the midrib, and trichomes on the adaxial (upper) leaf surface were counted using a dissecting microscope. To calculate trichome density per leaf, the number of trichomes on each side of the midrib was averaged. Data for trichome density and nutlet number and weight were analyzed using one-way ANOVA.

Measuring plant size and reproduction

We assessed the performance of plants in late June, late July, and early September each year. The number of leaves that were at least half expanded was counted for rosettes; flowering stems produce dozens to hundreds of small stem leaves and bracts which were not counted in July and September of year two (when plants flowered). The height of the tallest stem or leaf was measured from soil surface using a meter stick. Two diameters were recorded: the first diameter at the greatest plant width and a second diameter perpendicular to greatest width. Plant volume was estimated using calculations for a cylinder, which is a reasonable estimate of plant size due to growth form of this species: $V = H \times \pi \times ((D1 + D2)/4)^2$ where $D1$ and $D2$ are the two diameters of the plant and H is plant height. In mid-September of year two, once plants had senesced (most leaves and stems were brown), nutlets were collected and stored in brown paper bags at room temperature for about 2 months. Mature nutlets were then counted and 20 nutlets from 20 plants of each treatment (400 total per treatment) were randomly selected and weighed to nearest 0.1 mg. Seed viability was tested by determining mean percent germination of three reps of 20 randomly selected seeds (60 seeds per treatment) following methods of Moyer et al. (2007). Nutlets were soaked in water for 1 h, and seeds were separated from the pericarp and seed coat using a scalpel. Seeds were placed in petri dishes on moist filter paper and held at 25 °C in darkness. Seeds with 5 mm or greater length radicals after 72 h were considered germinated. Data on plant size were analyzed with repeated measures ANOVA with treatment (control, high JA, low JA) as a fixed effect; individual means were compared with Tukey's honestly significant different (HSD) means separation test. Data on reproduction were analyzed using one-way ANOVA.

Results

MeJA application induced plant volatiles and trichomes

Total amount of volatiles emitted by houndstongue plants significantly increased ($P < 0.05$) within 2 days of MeJA application, seemingly peaked on day five, and remained significantly elevated for 20 days (Fig. 1). Total volatiles emitted by control plants remained relatively constant at about 0.1 µg/h (mean for all days: 0.096 ± 0.032 µg/h), whereas those treated with MeJA emitted more than 12 µg/h at peak emission (mean for all days: 3.93 ± 0.89 µg/h). Twelve total volatile compounds were induced: (*Z*)-β-ocimene, (*E*)-β-ocimene, linalool, α-farnesene, methyl salicylate, 1 unidentified monoterpene, and six unidentified sesquiterpenes. Three of these compounds (*E*)-β-ocimene, linalool, and α-farnesene accounted for most of the increase in volatiles (about 80 % of total). Total mean amounts of volatiles emitted by plants treated with low MeJA tended to be less than plants treated with high MeJA (Fig. 1), but this was not significant on any day.

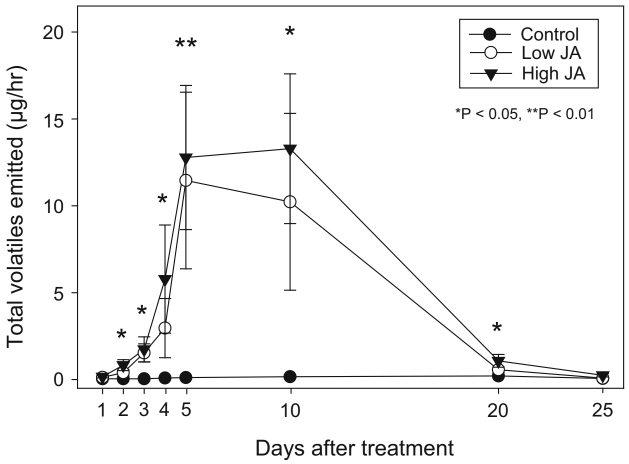

Fig. 1 A single application of methyl jasmonate (MeJA) induced houndstongue plants (*Cynoglossum officinale* L.) in the field to emit large amounts of volatile compounds (mean ± SE, $n = 4$). Significant difference between treatments: $*P < 0.05$, $**P < 0.001$

Application of MeJA also induced plants to produce leaves with more trichomes (Fig. 2). Mean trichome density on new leaves, measured 30 days after one MeJA treatment, was significantly greater than corresponding new leaves on control plants (ANOVA: $F_{2,15} = 50.37$, $P < 0.001$). Trichome density approximately doubled from 12.5 ± 0.6 trichomes per mm^2 in control plants to 22.5 ± 0.4 in low MeJA and 24.3 ± 1.4 trichomes per mm^2 in high MeJA plants (Fig. 2). Density of trichomes did not differ between low and high MeJA treatments (Fig. 2c, $P > 0.05$). MeJA treatment did not alter trichome density on old leaves that were fully expanded at time of treatment (ANOVA: $F_{2,15} = 0.23$, $P = 0.7949$).

MeJA-treated plants were smaller and produced fewer leaves

MeJA treatment had a significant effect on the number of leaves (Fig. 3a; ANOVA: $F_{2,116} = 8.96$, $P = 0.0002$). In September of year 1, about 3 months after first MeJA application, the number of leaves on plants treated with high MeJA (3.9 ± 0.2 leaves, $n = 40$) was significantly fewer than control (4.7 ± 0.2 leaves, $n = 40$) but not low MeJA-treated plants (4.3 ± 0.2 leaves, $n = 40$). In June of year 2, just prior to flowering, rosettes treated with low MeJA (10.8 ± 1.0 leaves, $n = 37$) and high MeJA (9.0 ± 0.8 leaves, $n = 39$) had significantly fewer leaves than control plants (13.5 ± 1.2 leaves, $n = 40$) (Fig. 3a).

Application of MeJA significantly altered other measures of plant size including plant height (Fig. 3b;

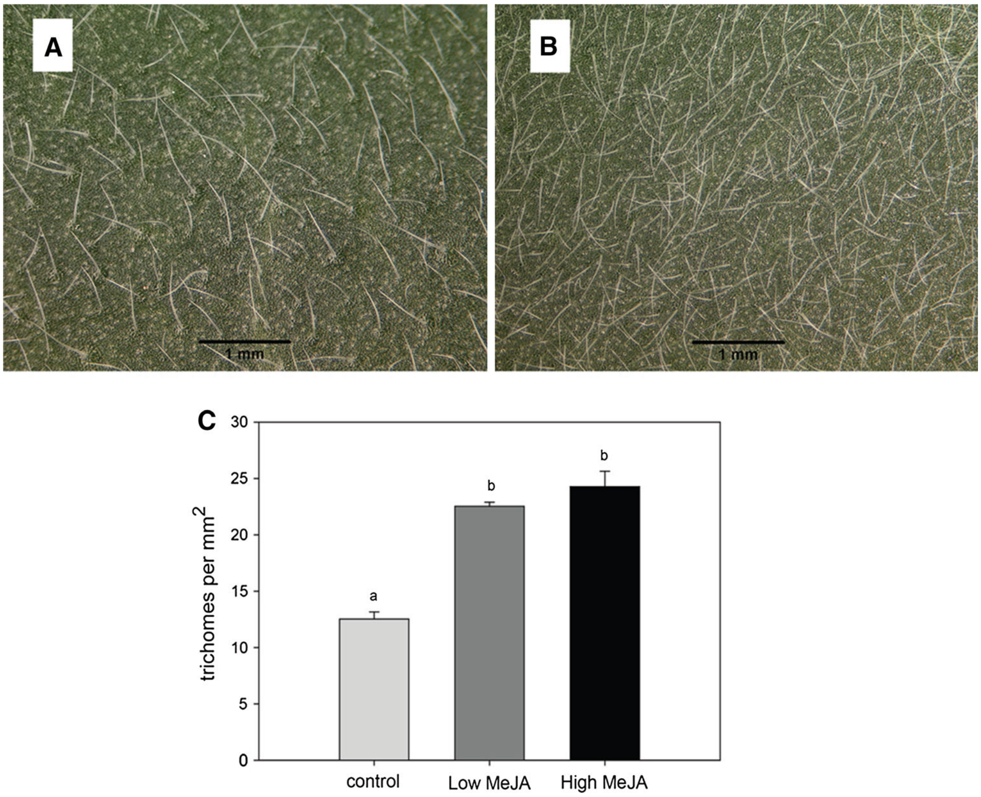

Fig. 2 A single application of methyl jasmonate (MeJA) induced houndstongue plants (*Cynoglossum officinale* L.) in the field to produce leaves with more trichomes. Photographs show newest fully expanded leaf 30 days after treatment with **a** lanolin only (control) or **b** lanolin with MeJA; **c** trichomes per mm^2 leaf area on newest fully expanded leaf 30 days after plant was treated with lanolin (control), low dose of MeJA, and high dose of MeJA (mean ± SE, $n = 6$). *Different letters* indicate significant differences between treatments ($P < 0.05$)

ANOVA: $F_{2,116} = 18.7$, $P < 0.0001$), maximum plant width (Fig. 3c; ANOVA: $F_{2,116} = 12.4$, $P < 0.0001$), and estimated plant volume (Fig. 3d; ANOVA: $F_{2,116} = 20.1$, $P < 0.0001$). Plant height was not affected by MeJA treatment in year 1, but in year 2 plants treated with high MeJA were significantly shorter than control plants on all sampling dates (Fig. 3b). The final, end of life, plant height was less for high MeJA plants (60.4 ± 2.9 cm, $n = 20$) than control plants (69.3 ± 1.6 cm, $n = 22$); the height of low MeJA plants was intermediate (64.8 ± 2.2 cm, $n = 21$). The final maximum plant width and plant volume followed a similar trend except that high MeJA-treated plants showed a significant reduction at the end of year 1 (Fig. 3c, d). All measures of size displayed a similar pattern: Plant size was significantly reduced by treatment with high MeJA, whereas low MeJA-treated plants were intermediate in size to control and high MeJA-treated plants (Fig. 3). A small number of plants died during the study (approximately 2 per treatment), but nearly half of the plants did not bolt and flower in year 2 which reduced sample sizes from 40 to near 20 for measurements of reproduction.

MeJA treatment did not significantly affect the number of nutlets produced per plant, which was highly variable, but high MeJA treatment did reduce the weight of nutlets (Fig. 4a, b). The mean number of nutlets produced by low MeJA-treated (341 ± 145 nutlets, $n = 21$) and high MeJA-treated (345 ± 130 nutlets, $n = 20$) plants tended to be less, but not significantly different from control plants (363 ± 126 nutlets, $n = 22$; Fig. 4a). However, the mean weight of nutlets produced by high MeJA-treated (19.1 ± 0.51 mg, $n = 400$) plants was significantly less than those produced by low MeJA-treated (21.2 ± 0.36 mg, $n = 400$) or control plants (22.3 ± 0.59 mg, $n = 400$) (Fig. 4b). MeJA treatment had no effect on germination success of seeds (ANOVA: $F_{2,21} = 0.14$, $P = 0.87$). Mean percent germination rate for seeds from control plants was 85.9 ± 3.6, for low MeJA-treated plants was 83.9 ± 5.0, and high MeJA-treated plants was 82.6 ± 4.4 ($n = 24$).

Discussion

We have provided evidence that induced defenses are costly for invasive houndstongue plants in a real-world setting: Plants in the field experimentally tricked into producing anti-herbivore defenses had fewer leaves, were smaller, and produced lighter seeds than plants not enacting defenses (Figs. 3, 4). Application of MeJA induced houndstongue plants to emit large amounts of plant volatiles (Fig. 1) and nearly doubled the number of trichomes on leaves (Fig. 2). Trichome production has been shown to be costly in terms of growth and reproduction for some plant species (Hare et al. 2003; Mauricio 1998; Sletvold

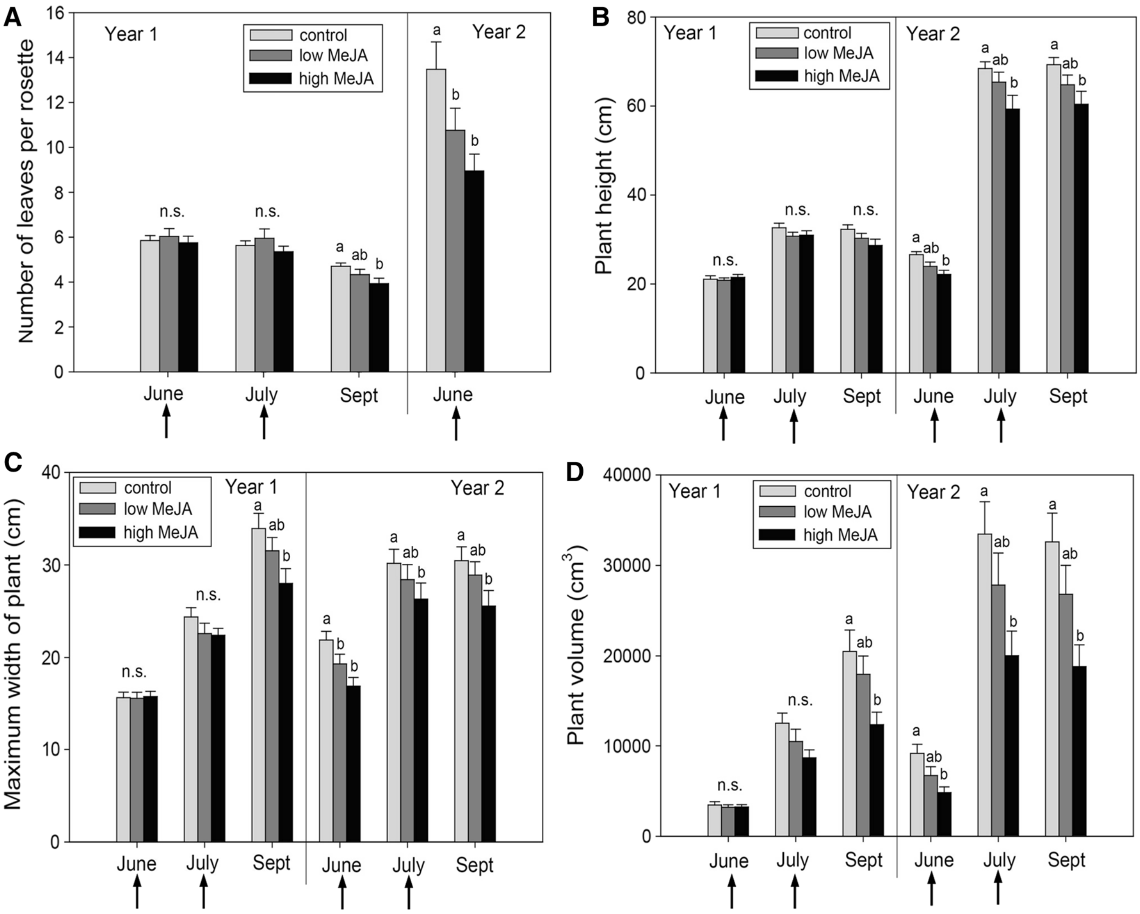

Fig. 3 Treatment with methyl jasmonate (MeJA) reduced the size of houndstongue plants (*Cynoglossum officinale* L.) in the field: mean ± SE of the number of leaves (**a**), plant height (**b**), maximum plant width (**c**), and estimated plant volume (**d**). MeJA or lanolin only was applied in late June and late July in both years (indicated by *arrows*). Plants bolted and flowered in year two; rosette leaves were not counted once plants bolted in July and September. *Different letters* indicate significant differences between treatments ($P < 0.05$, $n \geq 20$)

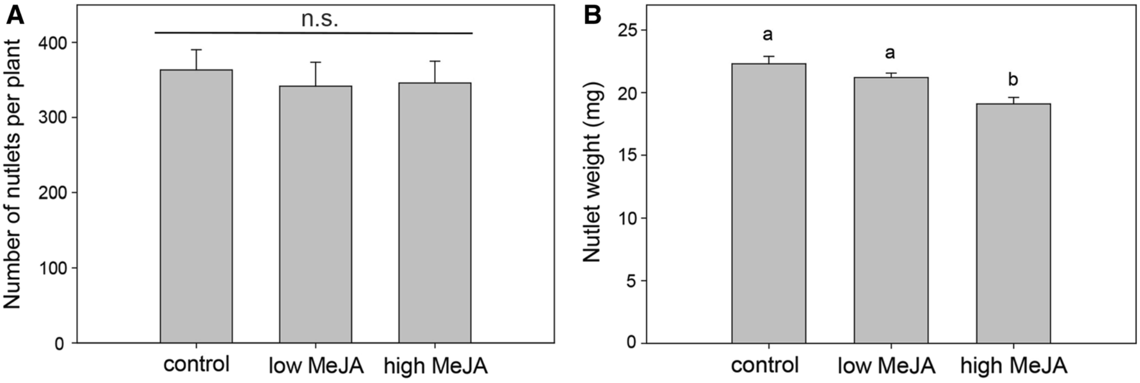

Fig. 4 Effect of treatment with methyl jasmonate (MeJA) on **a** number of nutlets and **b** weight of nutlets of houndstongue plants (*Cynoglossum officinale* L.) in the field. MeJA or lanolin only was applied in late June and late July the year prior and year of flowering. *Different letters* indicate significant differences between treatments ($P < 0.05$); *n.s.* no significant differences between treatments ($P > 0.05$)

et al. 2010; Zuest et al. 2011), but little or no costs have been detected for other species (Agren and Schemske 1993; Kaplan et al. 2009). The costs to plants of producing volatiles are less studied, but did significantly reduce growth, reproduction, and seed germination in maize (*Zea mays* L.) (Hoballah et al. 2004; Robert et al. 2013) and delayed growth of *Arabidopsis thaliana* (Aharoni et al. 2003). The metabolic costs of producing volatiles are predicted to be relatively low due to the small quantities emitted (Halitschke et al. 2000), but ecological costs could be substantial, e.g., by increasing a plant's apparency to enemies (Halitschke et al. 2008; Runyon et al. 2006) or by interfering with pollinator attraction (Kessler et al. 2011; Burkle and Runyon 2016). Our MeJA treatments almost certainly induced other traits not measured including within-plant alkaloids, which are produced by and inducible in houndstongue (pyrrolizidine alkaloids) (Eigenbrode et al. 2008), and production of which is known to incur large fitness costs in other plants species (Baldwin 2001). Not surprisingly, it seems traits other than trichomes and volatiles contributed to suppression of growth and reproduction in houndstongue since the magnitude of trichomes and volatiles induced by low and high MeJA treatments generally did not differ (Figs. 1, 2), but some measures of fitness did (e.g., nutlet weight; Fig. 4b).

Although we did not directly measure how MeJA induction affected houndstongue at the population level—the ultimate measure of success for biocontrol—the observed deleterious effects are important for individual plant fitness and could, in turn, affect populations. For example, plant size appears to be universally positively correlated with fecundity, and larger plants often outcompete and suppress the growth of smaller neighbors (Schwinning and Weiner 1998). In fact, competition among neighboring individuals is a fundamental process affecting populations and shaping plant communities (Berger et al. 2008), and costs of defense are sometimes only seen or magnified when plants are grown in competition with other plants (Van Dam and Baldwin 2001; Zavala et al. 2004). Furthermore, the production of lighter seeds by MeJA-treated houndstongue plants (Fig. 4b) could negatively affect fitness because seed mass is positively correlated with seedling growth, survival, and competitive ability (Moles and Westoby 2004). Collectively, these findings suggest that, for houndstongue, the costs entailed by inducible defenses are an important aspect of herbivory and could affect plant abundance. However, the conditions under which reductions in growth and reproduction translate into meaningful impacts on plant populations are poorly understood. Regardless, at present, induced defenses are probably rarely activated in houndstongue in the invaded range due to low levels of natural herbivory. These findings suggest that introducing herbivore species that induce large defenses in houndstongue (vs. those that induce little or no defenses) should reduce plant size and seed weight due to costs of defense, even if these defenses effectively reduce herbivore damage because the plants still must incur the costs of producing defenses.

We likewise lack an understanding of the factors leading to success or failure in weed biocontrol: Under what circumstances does herbivore feeding reduce populations of invasive plants? Understanding costs of induced defenses could help answer this question for several reasons. First, costs of inducible defenses can be an important component of the overall impact of herbivory on plant fitness (Fig. 5; Karban and Baldwin 1997). The costs of inducible defenses are likely the minimum impact that herbivory will have on a plant, assuming successful feeding and development of the herbivore, as predicted for specialist biocontrol herbivores. Furthermore, the costs of inducible defenses should be quantifiable, for example by experimentally inducing anti-herbivore defenses to plants in the invaded range. Second, the magnitude of plant defenses induced upon feeding varies dramatically with herbivore species. Some herbivores, like chewing caterpillars, tend to induce large chemical and metabolic changes upon feeding (Howe and Jander 2008; Zangerl et al. 2002). In contrast, some herbivores evade or suppress plant defenses, such as in galling herbivores that generally induce little or no plant defenses (Tooker and De Moraes 2009, 2011; Tooker et al. 2008) possibly by actively suppressing the JA defense pathway (Tooker and Helms 2014). Our results for houndstongue suggest that magnitude of costs (Figs. 3, 4) is correlated with magnitude of defenses induced (i.e., amount of MeJA applied). Interestingly, galling herbivores are favored and disproportionately used by biocontrol programs because

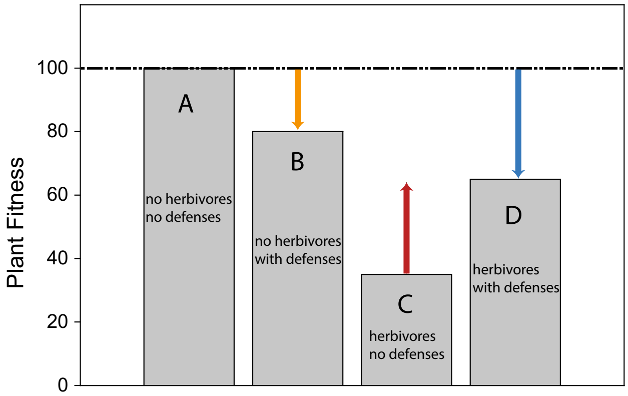

Fig. 5 Schematic diagram illustrating the relative impacts of herbivory and the costs of defense on plant fitness. Fitness is shown for hypothetical plants *A* at optimal conditions and free of herbivores, *B* not experiencing herbivory but with induced defenses activated, *C* attacked by herbivores but without induced defenses, and *D* attacked by herbivores with induced defenses. Plants *A* and *D* frequently occur in nature; plants *B* and *C* do not but can be experimentally created to examine costs of defense. In this study, we applied MeJA to create houndstongue plants in condition *B*; plants in condition *C* can be created using mutant or transgenic plants. The *orange arrow* is the cost of producing induced defenses, the *blue arrow* is the overall impact of herbivory, and the *red arrow* (difference between *C* and *D*) is the benefit to the plant of producing induced defenses

they are highly host-specific (Harris and Shorthouse 1996), a strict requirement for regulatory approval. Whether galling biocontrol agents are less effective on average than other types of agents is not known, due to lack of rigorous, quantitative assessment of biocontrol outcomes (Carson et al. 2008; Morin et al. 2009).

Further study is needed to determine whether inducible defenses hold any predictive value for biocontrol of invasive plants. Do problematic invasive plants express induced defenses when reunited with specialist herbivores? If so, are these defenses costly in terms of competitiveness? One other study has examined costs of defenses in an invasive plant and found that induction was substantially costly to growth of garlic mustard, *Alliaria petiolata* (Cipollini and Lieurance 2012). This suggests that such costs exist for at least one other invasive plant species. Big gaps remain in our knowledge about the types and magnitude of defenses induced by different herbivore taxa. Understanding how induced plant defenses differ with herbivore identity (e.g., insect order, family), feeding type (e.g., chewing, piercing/sucking, galling), and feeding site (leaves, roots, flowers) could identify generalities and allow development of a framework to make predictions about which potential herbivores will most negatively affect the target weed. We are currently surveying the magnitude of defenses induced by past biocontrol successes and failures to determine whether trends exist, e.g., do effective agents tend to induce more defensive changes upon feeding than ineffective ones?

There are growing pressure and need to identify, prior to release, which agents are most likely to be effective. This is due to the ever-expanding need to manage invasive plants and to minimize unwanted nontarget effects caused by ineffective biocontrol herbivores. In this study, we found that eliciting induced defenses in invasive houndstongue plants in the field had deleterious effects on plant size and reproduction. This suggests that defense costs alone can represent an important part of the damage caused by herbivores (Fig. 5) and this tradeoff between growth and defense could be exploited in biocontrol.

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