Constraints on the evolution of resistance to gall flies in Solidago altissima: resistance sometimes costs more than it is worth

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Summary

- Plant populations frequently maintain submaximal levels of resistance to natural enemies, even in the presence of substantial genetic variation for resistance. Identifying constraints on the evolution of increased resistance has been a major goal of researchers of plant–herbivore interactions.
- In a glasshouse study, we measured relative costs and benefits of resistance of tall goldenrod (Solidago altissima) to the gall-inducing tephritid Eurosta solidaginis. We exposed multiple ramets of 26 goldenrod genets to nutrient or shade stress and to oviposition by E. solidaginis.
- The presence of a gall cost a ramet an average of 1743 seeds, but the cost differed 10-fold across environments. Plant genets varied widely for an induced ‘hypersensitive’ response in which meristem cells become necrotic and kill E. solidaginis hatchlings before gall induction. There was no evidence that this highly effective resistance trait carried an allocation cost. However, the response carried a risk of autotoxicity, as necrosis killed the apex of 37% of the unga led ramets. On average, a damaged apex cost each ramet 5015 seeds.
- Autotoxicity may constrain the resistance of S. altissima to an intermediate level, and variation in environmental conditions may alter the relative costs and benefits of resistance and tolerance, thus maintaining genetic variation within goldenrod populations.

Introduction

The recognition that plant populations often maintain intermediate levels of resistance to their natural enemies, despite possessing genetic variation for resistance, has spurred much investigation into the factors that might constrain the evolution of greater resistance (Parker, 1992; Bergelson & Purrington, 1996; Rausher, 1996; Agrawal et al., 1999; Pilson, 2000; Hakes & Cronin, 2011b; Kliebenstein, 2014). In short, resistance is expected to evolve only to the point at which the benefits of increasing resistance are balanced by the costs (Coley et al., 1985; Fagerström et al., 1987; Simms & Rausher, 1987; Cipollini et al., 2014; Heath et al., 2014). The benefit to a plant is obvious: reducing damage that can negatively affect fitness. The potential costs are more complicated and can include costs that are both internal and external to a plant (Koricheva, 2002; Strauss et al., 2002; Puustinen et al., 2004; Agrawal, 2011; Gols, 2014).

Internal costs generally involve pleiotropy, wherein alleles that enhance resistance also have negative effects on other traits that contribute to plant performance (Rausher, 1996; Vila-Aiub et al., 2011). Most commonly, these are viewed as ‘allocation costs’ that result from the diversion of energy and resources toward defense and away from growth and reproduction (Bergelson & Purrington, 1996; Stowe, 1998). Internal costs can also occur when a trait that confers resistance against herbivores has toxic side-effects on the plant itself (i.e. ‘autotoxicity costs’) (Lieberei et al., 1989; Simms & Fritz, 1990; Simms, 1992). External (or ecological) costs involve interactions with other organisms, such as pollinators, other herbivores or natural enemies of herbivores (Agrawal et al., 1999; Strauss et al., 2002; Ness, 2006; Wise, 2009). Defense may also be constrained by epistatic selection between resistance (traits that reduce the amount of damage) and tolerance (traits that reduce the fitness impact of damage) (Fineblum & Rausher, 1995; Stowe, 1998; Leimu & Koricheva, 2006). Specifically, the greater the tolerance of herbivory, the less fitness impact the damage has, and the weaker the selection for increased resistance will be (Simms & Triplett, 1994; Abrahamson & Weis, 1997; Mauricio et al., 1997; Pilson, 2000; Fornoni et al., 2003; Carmona & Fornoni, 2013). In other words, greater tolerance translates to lower benefits of resistance.

While costs may constrain resistance to an intermediate level, spatial or temporal variation in the relative balance of costs and benefits may further maintain variation in resistance levels (Carmona & Fornoni, 2013; Turley et al., 2013). It is well known that environmental conditions (e.g. light or nutrient availability) can affect the expression of resistance (Coley et al., 1985; Maddox & Cappuccino, 1986; Fritz, 1990; Stowe et al., 1994; Koricheva et al., 1998; Ballhorn et al., 2011) and tolerance (Whitham et al., 1991; Hawkes & Sullivan, 2001; Wise & Abrahamson, 2005, 2007). Environmental stress may also affect the
relative costs and benefits of plant defenses (Rhoades, 1979; Fagerström et al., 1987; Bergelson, 1994; Fornoni et al., 2004b; Sampedro et al., 2011; Hakes & Cronin, 2012; Cipollini et al., 2014). Therefore, spatial or temporal variation in the environmental conditions experienced by a plant population could vary the selective optimum for resistance and thus maintain genetic variation for resistance in the population and its descendants (Johnson & Stinchcombe, 2007; Núñez-Farfán et al., 2007; Hakes & Cronin, 2011a,b; Carmona & Fornoni, 2013; Puente & Agren, 2014).

The main goal of this study was to investigate costs and benefits of resistance in Solidago altissima (‘tall goldenrod’, Asteraceae) to one of its major herbivores, the gall-inducing fly Eurosta solidaginis (Tephritidae), in order to provide insight on constraints to the evolution of resistance. Several studies have documented negative effects of E. solidaginis galls on goldenrod growth and reproduction (Hartnett & Abrahamson, 1979; Stinner & Abrahamson, 1979; McCrea & Abrahamson, 1985; McCrea et al., 1985; Abrahamson & Weis, 1997). Therefore, one might expect natural selection to favor an increase in resistance. One of goldenrod’s most effective resistance mechanisms against E. solidaginis is a hypersensitive response to larval feeding, wherein meristem cells surrounding a newly hatched larva become necrotic, thereby either poisoning or starving the larva (Abrahamson et al., 1991; Hess et al., 1996; Abrahamson & Weis, 1997). The result for the plant is that no gall is formed, and the plant is spared the concomitant loss of fitness.

Several studies have shown that goldenrod populations contain substantial genetic variation for resistance to E. solidaginis (Maddox & Root, 1987; McCrea & Abrahamson, 1987; Craig et al., 2000; Halverson et al., 2008a), and for the hypersensitive necrotic response in particular (Anderson et al., 1989; Abrahamson et al., 1991; Abrahamson & Weis, 1997). Given the effectiveness of the necrotic response in resisting a harmful herbivore, there must be some factor that constrains the evolution of this trait and maintains populations at resistance levels below their potential.

In this study, we tested the hypothesis that the benefits of this necrotic response may be balanced by internal costs of resistance. Specifically, we exposed clonal replicates of 26 goldenrod genets to oviposition by E. solidaginis and grew the plants under different environmental treatments for the following purposes: to document the range of variation among genets for resistance to gall formation; to measure potential allocation and autotoxicity costs of the necrotic response; and to examine whether environmental conditions (namely nutrient or light availability) can alter the relative costs and benefits of resistance and thus help maintain variation in resistance.

Materials and Methods

Study system

Solidago altissima L. is a rhizomatous, perennial herb native to eastern North America (Abrahamson & Weis, 1997). This species, noted for its large displays of insect-pollinated, golden-yellow flowers in late summer, has an inflorescence composed of up to several thousand small capitula (heads), each of which is composed of c. 2–5 perfect disk florets surrounded by c. 9–19 pistillate ray florets (Strausbaugh & Core, 1978; Wise et al., 2008). Each floret matures a single-seeded, wind-dispersed fruit (achene).

The goldenrod ball gall inducer, Eurosta solidaginis (Fitch), is a common, specialist herbivore of S. altissima (Uhler, 1951; Abrahamson & Weis, 1997). The short-lived, non-feeding adults emerge from galls in late spring. Females oviposit on apical-leaf buds of young goldenrod ramets. A female generally makes a linear series of ovipunctures into a bud before laying an egg, but once she has decided to puncture a bud, she will usually lay an egg in it (Anderson et al., 1989). Larvae hatch within 1 wk (How et al., 1993), and the presence of a larva in an apical meristem stimulates the production of a roughly spherical gall, which becomes apparent near the top of the stem within c. 3 wk (Weis & Abrahamson, 1985; Craig et al., 2000). There is one larva per gall, and usually just one gall per ramet, although doubly galled ramets are not uncommon, and triply galled ramets occur occasionally (Hess et al., 1996). Larvae feed until host-stem senescence and undergo diapause in galls until the following spring.

By the time a gall is visible, the stem has usually elongated such that the apex is several centimeters above the gall. The stems of galled ramets grow more slowly, but other than the gall itself, there is generally little effect of galling on the morphology of the stems (Hartnett & Abrahamson, 1979; McCrea et al., 1985). However, the majority of ovipunctured stems do not end up forming galls, and the proportion of stems that form galls is highly variable among plant genets (Anderson et al., 1989; Abrahamson & Weis, 1997; Wise & Abrahamson, 2008b). Natural enemies, such as parasitic wasps, inquilinous beetles and birds, kill many E. solidaginis larvae, but not until after gall formation (Weis et al., 1992; Abrahamson & Weis, 1997). Therefore, host-plant resistance is the probable cause of the great majority of cases in which a gall does not form.

Source of plants

In early April 2003, we excavated rhizomes from 26 different genets of S. altissima in a large field population in Union County, PA, USA (40°57.8’N, 76°57.4’W). Because S. altissima grows as discrete patches of genetically identical ramets, ramets in different clumps can generally be assumed to belong to separate genets. To ensure that the rhizomes we collected were from different genets, we collected from clumps of ramets that were separated from each other by >30 m. A sample of tissue from each genet was analyzed by flow cytometry to determine ploidy level. The 26 genets had identical cytotypes, indicating they were of the same ploidy level – presumably hexaploid, since this is the only ploidy level found in the northeastern USA in previous surveys of S. altissima (Semple et al., 1984, 2015; Halverson et al., 2008b).

Clonal progeny from these 26 genets were propagated under controlled conditions each year from 2003 to 2007 for two purposes: to generate multiple ramets per genet for a series of experiments, and to purge the genets of potential maternal influences
that may have carried over from microhabitat differences in their source environment (Roach & Wulff, 1987). Each spring, ramets were propagated in a glasshouse at Bucknell University from 2-cm³ segments of the previous year’s rhizomes for all 26 genets. These segments were measured by the displacement of 2 ml of water as a length of rhizome was dipped into a 100-ml graduated cylinder. The rhizome segments were planted in plastic flats in ProMix™ BX medium (Premier Horticulture Ltd, Dorval, QC, Canada). Emerging ramets were later transplanted from the flats into 16.5-cm-diameter plastic azalea pots. After a few weeks, at least five ramets per genet were transplanted from these pots into 27-cm-diameter plastic pots to grow rhizome material for the next year’s studies. Except in 2003, these large pots were moved outside to randomized locations on a row of wooden pallets located adjacent to the glasshouse for the remainder of the growing season. These plants were in open sunlight, but were isolated from sources of herbivorous insects. Fresh growing medium was used each year, and the plants were fertilized regularly with Peters Professional® 15-16-17 N-P-K fertilizer (J.R. Peters Inc., Allen-town, PA, USA). Rhizomes were removed from the pots in the winter and stored with growing medium in plastic zip-seal bags in a dark cold room until propagation the following spring.

In early April 2007, we made cuttings of rhizomes that were grown in summer 2006 and stored under refrigeration over the winter. From each of the 26 genets, we cut numerous rhizome segments of equal size (2 cm³) and planted the rhizomes in ProMix™ BX in flats in the glasshouse. From 15 to 20 May, we transplanted ramets from the flats into 16.5-cm-diameter plastic azalea pots and randomly assigned experimental treatments. By early June, most ramets had formed an apical leaf bud and were an appropriate size for oviposition by E. solidaginis.

Overview

This study was originally designed to measure goldenrod’s tolerance of galling under environmental stresses. Once we realized that some genets were completely resistant to gall formation, we shifted the main focus to investigate the hypersensitive necrotic response that appeared to be responsible for this resistance. For simplicity, we describe the experimental design with the revised focus in mind, cautioning that some imbalances in the design are a side-effect of this change in focus after the experiment was begun. Other imbalances in sample size per genet resulted from a set of ramets that were sacrificed for chemical analyses before flowering. These ramets were not available for the part of the study involving calculations of fitness costs and benefits. The results of the chemical analyses are not reported in this paper, but are mentioned here only to explain some of the imbalance in sample size among genets.

Oviposition and galling outcome

A total of 461 ramets (between 12 and 24 ramets per each of 26 genets) were exposed to Eurosta solidaginis flies in a mesh-screen cage built on top of a glasshouse bench. Flies were reared in growth chambers from galls collected in local fields the previous winter, using procedures described elsewhere (Cronin & Abrahamson, 1999). On the morning of 5 June 2003, we placed c. 100 S. altissima potted ramets (from the set transplanted into 16.5-cm azalea pots in May) into the screened cage and released c. 100 flies (a mix of males and females) into the cage. We checked the ramets at least three times per day and removed a ramet once it had received a linear series of ovipunctures, which indicated a high likelihood of oviposition. We replaced the ramets until the last of the 461 gall-treatment ramets were successfully ovipunctured, which occurred on 12 June. Eight additional ramets for each of the 26 genets were allocated to a control group (i.e. there were 208 control ramets). These control ramets were treated identically to the gall-treatment ramets, except that they were never exposed to gall flies.

Roughly half of the ramets (ovipunctured and control) for each genet were allocated to a nutrient-stress experiment and half to a shade-stress experiment. These two experiments were conducted concurrently on eight adjacent benches in a glasshouse, as detailed in the following sections (see also Supporting Information Fig. S1). The fate of each ovipunctured ramet was recorded as one of three outcomes: a gall formed; no gall formed but the apex was damaged; or there was no gall or visible damage to the apex.

The galling-outcome experiment included a replicate set of ramets (for 10 of 26 genets) that were sacrificed for chemical analyses before flowering. These ramets were not available for the part of the study involving calculations of fitness costs and benefits. The results of the chemical analyses are not reported in this paper, but are mentioned here only to explain some of the imbalance in sample size among genets.

Nutrient-stress experiment

The original design of the nutrient-stress experiment involved a factorial cross of fertilizer treatment (high vs low), galling (galled vs not) and genet (n = 26), repeated in two completely randomized blocks on adjacent glasshouse benches. Within a block, ramets were placed in 104 evenly spaced positions in six rows per bench (one or two ramets allocated for chemical analysis were placed on the end of each row on each bench). The spacing among positions was maximized such that ramets would not compete for light when fully grown.

For each position assigned to the galled treatment, we generally had two ovipunctured ramets available so that one would serve as an alternate ramet as insurance in case the other ramet did not form a gall. The alternate ramet was placed right next to the original and the two temporarily shared a single numbered position on the benches. After 3 wk (when galls would be visible), the plan was to remove ramets as necessary so that there would be just one ramet per position. If neither of a pair of oviposited ramets sharing a position formed a gall, then the plan was to remove both ramets and replace them with the alternate ramet from the same genet in another position in which both ramets formed a gall. The high-nutrient ramets received Peters Professional® 15-16-17 N-P-K at a rate of 59 ml of a mixture of 3.9 cm³ fertilizer

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per liter of water (i.e. one-quarter cup of fertilizer solution at a concentration of one tablespoon of solid fertilizer per gallon of water) each week from 3 June until the middle of September, when ramets began senescing. This rate represents a realistic value for a benign natural environment. The low-nutrient ramets were fertilized only once – in mid-June – when all ramets in both the nutrient-stress and the shade-stress experiments were fertilized to ensure that they would receive enough nutrients to enable flowering.

Three weeks after the oviposition period, a minority of ovipunctured ramets were forming galls (in total, only 29% formed galls), and the galling success rate was quite variable among genets. Moreover, in many of the ovipunctured ramets that did not form galls, the shoot apex was dying as an apparent side-effect of a hypersensitive necrotic response to oviposition, and the severity of this autotoxicity also was variable among genets (Fig. 1). The low and variable galling rate made it impossible to follow through with the original intent of the experiment. However, we realized that it opened up the possibility to address other questions regarding relative costs and benefits of resistance to galling and tolerance of galling, such as how they might vary among environmental conditions. We adjusted the design of the experiment accordingly.

We saved all of the oviposited ramets that formed galls and all that did not form a gall but exhibited apex damage caused by oviposition. All of the control (not-ovipunctured) ramets were retained so that we could calculate fitness costs associated with resisting galls (through the hypersensitive response) and with tolerating galls (i.e. forming galls). There were six instances in which the ovipunctured ramet (and its alternate ramet) assigned to a position neither formed a gall nor had a damaged apex. In these cases, we left one of the undamaged ramets in that position to minimize imbalance in the overall sample sizes across genets. These six undamaged ramets were pooled into the control group. The revised design still included two fertilizer levels, 26 genets and two blocks, but the galling treatment was considered to have three levels: galled, damaged apex and control. We repositioned the pots on the benches to maximize space between ramets within rows, and again once the ramets at the ends of the rows were removed for chemical analysis (Fig. S1). Not counting the ramets sacrificed for chemical analysis, 206 ramets were included in the nutrient-stress experiment, allocated across galling and nutrient treatments as summarized in Table 1.

### Shade-stress experiment

The original shade-stress experiment consisted of a light treatment (ambient vs shaded), galling treatment (galled vs ungalled) and genet \( (n = 26) \), repeated in two blocks, randomized with respect to galling treatment and genet. As with the nutrient-stress experiment, this experiment also included extra ramets from 10 genets for chemical analyses and alternate ramets for the galled treatment. Unlike the nutrient-stress experiment, it was not possible to randomize the positions of the ramets with respect to the environmental stress. Instead, each block contained an ambient-light bench and a shaded bench. Shading was accomplished by attaching a custom-made 80% shade cloth (PAK Unlimited Inc., Cornelia, GA, USA) to a PVC-pipe frame constructed over one of two benches per block. As with the nutrient-stress experiment,

### Table 1

<table>
<thead>
<tr>
<th>Galling treatment</th>
<th>Nutrient treatment</th>
<th>Shade treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Galled</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>Apex damaged</td>
<td>17</td>
<td>34</td>
</tr>
<tr>
<td>Control (undamaged)</td>
<td>57</td>
<td>53</td>
</tr>
</tbody>
</table>

Fig. 1 Ramets of tall goldenrod (Solidago altissima) displaying a range of hypersensitive necrotic responses to Eurosta solidaginis oviposition. (a) The necrotic response was too small to prevent the formation of a gall. The arrow marks a small scar of necrotic tissue around the area of oviposition. (b) The necrotic response was sufficient to prevent the formation of a gall. The arrow marks a moderate-sized scar of necrotic tissue around the area of oviposition. (c) The necrotic response was severe enough to kill the apex (marked by arrow). This ramet was in the high-nutrient, high-light treatment, so it had plenty of resources to activate axillary meristems to help compensate for the damaged apex (cf. Wise & Abrahamson, 2008a). The photos were taken on 31 July (7–8 wk after oviposition).
we redesigned this experiment after 3 wk, retaining all of the ovipunctured ramets that either formed a gall or exhibited apex damage and the control ramets. There were seven instances in which the ovipunctured ramet (and its alternate ramet) in an assigned position did not form a gall or have a damaged apex. In these cases, we left one of the undamaged ramets in that position and pooled the ramet into the control group. We shifted the remaining ramets on the benches as necessary to maximize spacing between pots (Fig. S1). Across galling and light treatments, a total of 235 ramets were available at the end of this experiment (Table 1).

### Plant performance measurements

We used seed production to quantify fitness impacts of gall flies and environmental stresses. To ensure cross-pollination, we placed a Natupol® ‘Class C’ bumblebee (Bombus impatiens) hive (Koppert Biological Systems, Romulus, MI, USA) in the glasshouse once the first ramets began to flower. The bumblebees proved extremely effective, visiting every ramet repeatedly until flowering ceased.

To estimate seed production for each ramet, we counted all of a ramet’s capitula once that ramet finished flowering, but before achenes started dispersing. We collected five capitula from scattered locations on the ramet’s inflorescence. These capitula were dissected to count the seeds and obtain a mean seed/capitula value for the ramet. That mean was then multiplied by the total number of capitula on that ramet to estimate the total number of seeds produced by the ramet.

### Data analysis

**Oviposition and galling outcome** We fitted a nominal logistic model to assess the influence of environmental stress on galling outcome (namely gall formation, no gall but a damaged apex, or no gall and an undamaged apex) for the 461 ramets that received ovipunctures. For that model, we included ramets from both the nutrient-stress and light-stress experiments and considered environmental stress to be a single treatment factor with three levels: nutrient stress, shade stress and a low-stress control (i.e. fertilized ramets in ambient light). The nutrient-stress group included the ambient-light ramets from the shade-stress experiment and the unfertilized ramets from the nutrient-stress experiment because these groups were treated identically in the two experiments. In total, the nutrient-stress, shade-stress and control group included 236, 113 and 112 ramets, respectively. Genet was also included in the logistic model to investigate genetic variation for galling outcome, and thus for resistance to galling. Likelihood-ratio tests and Wald tests were used to assess the significance of the two explanatory variables (environmental stress and genotype). All statistical analyses were performed using JMP-IN 4.0.4 (SAS Institute, Cary, NC, USA).

**Nutrient-stress experiment** A factorial ANOVA was used to investigate the effects of gall flies and nutrient levels on goldenrod performance. The explanatory factors included block, plant genet, fertilizer treatment and gall treatment (control, galled or damaged apex). The model also included the two-way interactions of fertilizer-by-genet and fertilizer-by-gall treatment. A genotype-by-gall treatment interaction could not be included because, for several genets, there were either no ramets that formed galls or no ramets with a damaged apex. Genet, block and the fertilizer-by-genet interaction were treated as random-effects factors. The response variable for the ANOVA was the square-root of seed number, which was the transformation that produced the best-behaved residuals (better homoscedasticity among environmental treatments). All ANOVAs were performed using the ‘traditional’ (expected mean square) technique in JMP-IN 4.0.4.

**Shade-stress experiment** Because of the split-plot design of the shade-stress experiment, the ANOVA model was slightly more complicated than for the nutrient-stress experiment. Specifically, the shade treatment was considered the main plot, and block was the experimental unit for the main plots. Thus, the ANOVA model nested block within the shade treatment. The split plots included the two other main factors (gall treatment and genet) within the shade-treatment main plot. The model also included the two-way interactions of shade-by-genet and shade-by-gall treatment. Block, genet and shade-by-genet were treated as random-effects factors. The response variable was square-root transformed seed number per ramet.

**Allocation costs of resistance** To test for the presence of allocation costs of resistance, we performed a regression of the mean fitness (seed production) for each genet in the absence of ovipunctures (i.e. for ramets not exposed to flies) against the resistance of each genet, which was quantified as the proportion of ovipunctured ramets that did not form galls. In the absence of gall-fly oviposition, the benefits of resisting gall formation will be absent, but the allocation costs of resistance will still be present. A negative regression coefficient would suggest the presence of an allocation cost of resistance (Simms & Rausher, 1987; Mauricio, 1998; Rausher, 2001). We performed a separate regression for the three environmental treatments in these experiments (nutrient stress, shade stress, control) to see whether the expression of these costs depended on the plants’ environment. As in the galling outcome component described above, the nutrient-stress group comprised ramets from both the low-nutrient treatment from the nutrient-stress experiment and the ambient-light treatment from the shade-stress experiment. The ramets in these two groups were treated identically, and they were statistically indistinguishable in seed production.

**Cost–benefit analysis of resisting vs tolerating galls** We performed calculations to predict the number of seeds that would be expected to be lost to a ramet that formed a gall and to a ramet that did not form a gall after oviposition. Those that formed galls can be considered ‘tolerators’, and the cost of that tolerance was quantified as the mean number of seeds that a gall cost a ramet. Those ramets that did not form galls can be considered ‘resistors’, and the cost of that resistance was quantified...
as the probability of resistance causing apical damage (i.e. autotoxicity), multiplied by the mean number of seeds lost due to having a damaged apex.

To quantify mean seed losses, we ran an ANOVA on seed production using four predictor variables: block, genet, gall treatment and environmental-stress treatment. As with the nominal logistic model for galling outcome and the allocation cost analysis described above, environmental stress had three levels: nutrient stress, shade stress and a low-stress control (i.e. fertilized ramets in ambient light). Block and genet were treated as random-effects factors. As above, the factorial design of the nutrient-stress experiment and the split-plot design of the shade-stress experiment require different types of ANOVAs to determine statistical significances of the treatments. However, for the current analysis, we were interested in calculating least-squares means and standard errors of seed production for the control, galled and damaged-apex ramets in the three different environmental treatments, rather than in calculating $P$-values for any particular comparisons.

**Results**

**Oviposition and galling outcome**

Of the 461 ramets ovipunctured in this experiment, 133 (29%) formed a gall, 122 (26%) had a damaged apex but no gall and 206 (45%) had no signs of damage other than scarring on the stem in the area of the ovipunctures (Fig. 1). These outcomes varied significantly among plant genets ($\chi^2 = 90.503$, df = 50, $P = 0.0004$), with the most susceptible genet forming galls on 79% of its ovipunctured ramets, and with four genets forming no galls on any ramet (Fig. 2). Among-genet variation for stem-apex damage by necrosis was nearly as great, ranging from 4% to 81% of the ovipunctured ramets across the 26 genets.

Galling outcomes also varied significantly among environmental treatments ($\chi^2 = 26.49$, df = 4, $P < 0.0001$; Fig. 3). Nutrient-stressed ramets were only 78% as likely as control ramets to form galls, while shade-stressed ramets were 125% as likely as control ramets to form galls. Of ramets that did not form galls, nutrient-stressed ramets were twice as likely and shade-stressed ramets were nearly three times as likely to display apex damage from autotoxicity as were the control ramets (Fig. 3).

**Nutrient-stress and shade-stress experiments**

The environmental stresses imposed in these experiments significantly reduced goldenrod’s seed production (Table 2). On average, low-nutrient ramets produced only 14% as many seeds as high-nutrient ramets (least squares (LS) means ± SE: 11,107 ± 2,481 vs 79,481 ± 2,666 seeds per ramet, respectively). Shaded ramets produced c. 53% as many seeds as those in ambient light (6,949 ± 811 vs 13,225 ± 831 seeds per ramet, respectively). Damage by *Eurosta* also significantly depressed goldenrod reproduction (Table 2). Averaged across the environmental treatments, the presence of a gall reduced seed production by c. 7%, and a damaged apex reduced seed production an average of 19%.

The ramets also displayed significant variation among genets for seed production (Table 2), with the genet term alone accounting for about half of the variation in seed production among ramets (57% and 47% in the nutrient and shade experiments, respectively).
There was no indication that resistance entailed an allocation cost. Specifically, in none of the environmental treatments was a genet’s resistance to gall formation significantly related to its seed production in the absence of gall-fly oviposition (Fig. 4). An allocation cost of resistance would have been evident as a negative regression coefficient, but the only coefficient approaching significance ($P = 0.09$) was in the shade-stressed treatment, where resistance was positively correlated with seed production ($r = 0.34$).

Cost–benefit analysis of resisting vs tolerating galls

The average cost to a goldenrod ramet for tolerating *Eurosta* (i.e. the fitness reduction caused by the gall) was 1734 seeds (or 7.1% of seed production; Table 3). However, this cost varied substantially across the three environmental treatments. Tolerance cost the largest absolute number of seeds in the low-stress environment, but because these ramets produced such a large number of seeds, it was actually a low relative cost (4.4% of the total seed production). In relative fitness terms, tolerating a gall cost the least in the nutrient-stressed environment (2.8%) and the most in the shade-stressed environment (14.2%).

The cost to plants of resisting *Eurosta* (i.e. not forming a gall due to the hypersensitive necrotic response) was related to two factors: the probability of apex damage from necrosis, and the number of seeds lost due to that damage. The average cost to resisting *Eurosta* (not forming a gall) was 1883 seeds (or 7.3% of the total seed production; Table 3). This cost also varied across environmental treatments. Apical damage cost the most in terms of absolute seed loss in the low-stress environment (10 528 seeds). However, because both the probability of apical damage and the cost in terms of relative seed loss were the lowest in the low-stress environment, the cost to resistance was quite low (2.6% of total seed production). The cost of apical damage in terms of relative seed loss was similar for shade-stressed and nutrient-stressed ramets. However, because the probability of apical damage was greater in shade-stressed ramets, the cost of resistance was greater for shade-stressed than for nutrient-stressed ramets (Table 3).

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**Table 2** Results of ANOVAs of the effects of plant genet, environmental stress and *Eurosta* herbivory (gall treatment) on per-ramet seed production of tall goldenrod (*Solidago altissima*)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df num.</th>
<th>df den.</th>
<th>Mean square</th>
<th>F-ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Nutrient-stress experiment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Block</td>
<td>1</td>
<td>149</td>
<td>127.64</td>
<td>0.2202</td>
<td>0.64</td>
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<tr>
<td>Genet</td>
<td>25</td>
<td>24.997</td>
<td>889.25</td>
<td>7.0136</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>1</td>
<td>33.273</td>
<td>1220.039</td>
<td>11.6782</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gall treatment</td>
<td>2</td>
<td>149</td>
<td>5059.32</td>
<td>8.7276</td>
<td>0.0003</td>
</tr>
<tr>
<td>Fertilizer × Genet</td>
<td>25</td>
<td>149</td>
<td>1267.35</td>
<td>2.1862</td>
<td>0.0021</td>
</tr>
<tr>
<td>Fertilizer × Gall</td>
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<td>149</td>
<td>44.7728</td>
<td>0.0772</td>
<td>0.93</td>
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<tr>
<td>Error</td>
<td>149</td>
<td></td>
<td>579.7</td>
<td></td>
<td></td>
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<tr>
<td>(b) Shade-stress experiment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Block (Shade)</td>
<td>2</td>
<td>177</td>
<td>2144.01</td>
<td>3.7672</td>
<td>0.025</td>
</tr>
<tr>
<td>Genet</td>
<td>25</td>
<td>25</td>
<td>6848.94</td>
<td>5.8476</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Shade</td>
<td>1</td>
<td>3.341</td>
<td>54 588.5</td>
<td>21.8019</td>
<td>0.015</td>
</tr>
<tr>
<td>Gall treatment</td>
<td>2</td>
<td>177</td>
<td>3326.78</td>
<td>5.8454</td>
<td>0.0035</td>
</tr>
<tr>
<td>Shade × Genet</td>
<td>25</td>
<td>177</td>
<td>1171.24</td>
<td>2.0580</td>
<td>0.0037</td>
</tr>
<tr>
<td>Shade × Gall</td>
<td>2</td>
<td>177</td>
<td>337.835</td>
<td>0.5936</td>
<td>0.55</td>
</tr>
<tr>
<td>Error</td>
<td>177</td>
<td></td>
<td>569.13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Block, genet and interactions involving genet were considered random-effects factors. Seed numbers were square-root transformed before analysis. Denominator mean squares were synthesized from linear combinations of the mean squares of other terms to give the same expectation as the numerator mean square under the null hypothesis using JMP-IN 4.0.4 (SAS Institute, Cary, NC, USA).

![Fig. 4](image-url) Absence of allocation costs of resistance in the three environmental treatments. The points represent genet-means of seed production per tall goldenrod (*Solidago altissima*) ramet that was not exposed to *Eurosta solidaginis* on the y-axis, vs genet-means of resistance (percentage of ovipunctured ramets that did not form a gall) on the x-axis. The lines represent slopes of regressions of genet-mean seed production on resistance. X’s and dashed line, ramets in the control group (high nutrients and ambient light); circles and dotted line, ramets in the nutrient-stressed group; triangles and solid line, ramets in the shade-stressed group. A negative slope would indicate the presence of an allocation cost of resistance, but none of the regression lines was significantly different from zero.

Allocation costs of resistance

There was no indication that resistance entailed an allocation cost. Specifically, in none of the environmental treatments was a genet’s resistance to gall formation significantly related to its seed production in the absence of gall-fly oviposition (Fig. 4). An allocation cost of resistance would have been evident as a negative regression coefficient, but the only coefficient approaching significance ($P = 0.09$) was in the shade-stressed treatment, where resistance was positively correlated with seed production ($r = 0.34$).
Discussion

Fitness impact of Eurosta solidaginis

This study involved the most precise accounting to date of the impact of Eurosta solidaginis on the sexual reproduction of its host, Solidago altissima. Averaged across environmental treatments, the presence of a ball gall reduced seed production by 7.1%. This result corresponds well with the results of an energy budget analysis that determined that 7% of a galled ramet’s production is used in the formation of galls and insect production (Stinner & Abrahamson, 1979). However, the galler impact was much less dramatic than the 46% reduction in seed production estimated by Hartnett & Abrahamson (1979) in a field population. In that study, Eurosta galling also reduced seed mass by 22% and seed germination success by 67%. The combined evidence shows that ball galls substantially depress the performance of S. altissima (Abrahamson & Weis, 1997), and suggests that the plants could be under strong selection for increased resistance to galling.

Mechanism of resistance

Resistance to a herbivore can be achieved in two main ways: either by escaping the herbivore entirely (i.e. antixenosis) or, once attacked, by limiting the amount of damage through toxins or physical deterrents (i.e. antibiosis) (Painter, 1958; Smith, 1989). In our study, we forced oviposition on ramets, and thus differences in resistance among plants are due to antibiosis – assuming that ovipuncturing generally led to oviposition, as shown in prior work on this system (Anderson et al., 1989). A hypersensitive necrotic response is a common mechanism of antibiosis against pathogens, and it may be a more widespread resistance mechanism against insects than is generally recognized, particularly for relatively sessile herbivores such as gallers (Fernandes, 1990; Fernandes & Negreiros, 2001; Garza et al., 2001; Fernandes et al., 2003). The hypersensitive response proved to be a potentially very effective resistance mechanism in our study, as four of the 26 S. altissima genets did not form a single gall (out of a total of 54 ovipunctured ramets).

Despite the effectiveness of the hypersensitive response, there was a wide range in its expression among genets. While four genets were completely resistant, the most susceptible genet formed galls on 79% of its ramets. This range in variation among genets is consistent with previous studies of these species (Abrahamson & Weis, 1997). In two separate field surveys of 38 S. altissima genets (Anderson et al., 1989) and 117 S. altissima genets (McCrea & Abrahamson, 1987), the proportions of ovipunctured ramets that formed galls ranged from 0% to >80% across genets. This variation shows that S. altissima populations are well below their potential maximum resistance, which suggests that the evolution of the hypersensitive necrotic response is constrained by costs.

Costs of resistance

We found no evidence in this study that allocation costs were responsible for constraining goldenrod’s evolution of resistance to E. solidaginis. The regression of genet mean fitness (seed production in the absence of ovipunctures) on resistance level (1 minus the proportion of ramets forming galls when ovipunctured) did not differ significantly from zero in the nutrient-stressed, shade-stressed or low-stress control treatments. The lack of an allocation cost is perhaps not surprising because the necrotic response is induced by the presence of a larva. In the ramets that were not ovipunctured, the necrotic response would not be expressed, and thus ramets may not pay the cost associated with the necrotic response.

Nevertheless, the hypersensitive necrotic response may entail pleiotropic costs that are not strictly allocation costs. Previous work with S. altissima suggested that genets that display high levels of the hypersensitive necrotic response when attacked may tend to grow more slowly (even when not attacked) than genets with a low level of the necrotic response (McCrea & Abrahamson, 1987; Abrahamson et al., 1988). If this is the case, then alleles that cause the necrotic response may have negative pleiotropic effects on vigor (Abrahamson & Weis, 1997). If this sort of pleiotropic cost was involved in the hypersensitive response, then we should see a negative relationship between resistance and fitness in the absence of the gall flies. However, we did not see this negative relationship, the results of our study clearly indicate the absence of such a pleiotropic cost, even among already nutrient- or shade-stressed ramets.

A related type of internal cost of resistance is autotoxicity, wherein chemicals induced to poison or deter herbivores also harm the plant. The hypersensitive necrotic response could come at a cost of autotoxicity if enough of the apical meristem cells are killed to halt vertical growth of the stem. Indeed, our study

Table 3

<table>
<thead>
<tr>
<th>Environmental treatment</th>
<th>Mean number of seeds per ramet:</th>
<th>Percentage ovipunctured ramets with apex damage</th>
<th>Cost of defense type (% loss of seeds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>If not exposed to gall flies</td>
<td>Lost due to a gall</td>
<td>Lost if apex is damaged</td>
</tr>
<tr>
<td>Low stress (control)</td>
<td>84 373</td>
<td>37 23</td>
<td>10 528</td>
</tr>
<tr>
<td>Nutrient stress</td>
<td>13 352</td>
<td>36 8</td>
<td>29 09</td>
</tr>
<tr>
<td>Shade stress</td>
<td>78 20</td>
<td>11 12</td>
<td>16 08</td>
</tr>
<tr>
<td>Column means</td>
<td>35 182</td>
<td>17 34</td>
<td>50 15</td>
</tr>
</tbody>
</table>
indicates a substantial risk of autotoxicity related to the hypersensitive response. Across all ovipunctured ramets, 26% formed no gall but suffered apical death due to a strong necrotic response. There was substantial among-genet variation for autotoxicity, with 4–81% of the ovipunctured ramets suffering apical death across the 26 genets. The fitness cost of apical death was substantial across the environmental treatments, with nutrient-stressed, shade-stressed and low-stress control ramets respectively producing 22, 21 and 14% fewer seeds than the corresponding undamaged, ungalled ramets.

The risk of autotoxicity was strongly influenced by the environment in which the plants were growing. Specifically, ovipunctured nutrient-stressed ramets were nearly twice as likely as ovipunctured high-nutrient ramets to suffer apical death. High-nutrient ramets grew more rapidly, with taller and thicker stems than nutrient-stressed plants. It is likely that a smaller proportion of the high-nutrient ramets’ meristem cells are sacrificed, and that the apex grows beyond the region of necrosis more quickly. Similarly, ovipunctured shaded ramets were 2.4 times more likely than ovipunctured ambient-light ramets to suffer apical death. While shaded ramets grow taller, their stems are substantially thinner. Thus, necrosis could affect a greater proportion of the cross-sectional area of the apex in shaded ramets than in ambient-light ramets.

Constraints on the evolution of resistance

The central evolutionary question is whether the cost of autotoxicity is sufficient to negate the benefit of the hypersensitive necrotic response and thus constrain the evolution of this important resistance mechanism. In other words, would a plant do just as well to tolerate a gall’s presence as it would to resist its formation? The fitness cost to tolerating (i.e. not resisting) E. solidaginis can be quantified as the reduction in seed production caused by the gall. The cost to resisting is determined by the probability of autotoxicity multiplied by the seed reduction caused by apex death. Averaged across the three environmental treatments in this study, the cost of tolerance (1734 seeds) was roughly equal to the cost of resistance (0.375 x 5015 seeds = 1881 seeds). Therefore, the autotoxicity cost of resistance may keep the trait at an intermediate level in S. altissima populations. However, this cost alone may not explain why there is so much variation in resistance among genets.

The maintenance of genetic variation in resistance may be better explained by a shift in the relative costs and benefits of resistance among environments (Stinchcombe & Rausher, 2002; Fornoni et al., 2004b). That is, resistance may be favored under some conditions while tolerance may be favored under others (Fornoni et al., 2004a; Hakes & Cronin, 2012; Turley et al., 2013). Under the low-stress control treatment in this study, the absolute numbers of seeds a ramet lost to galls and to autotoxicity were both high. However, because the probability of autotoxicity was low, resistance cost only about half as much as tolerance. Therefore, increased resistance should be expected to be favored by selection in low-stress, high-resource environments.

In our nutrient-stressed treatment, the risk of autotoxicity was nearly twice as great as under the control conditions, and the fitness cost of autotoxicity was about eight times greater than the cost of tolerating a gall. Consequently, resistance cost nearly three times as many seeds as tolerating a gall, and selection would be expected to favor tolerance over resistance in nutrient-stressed environments.

Finally, in the shade-stressed conditions, the relative cost of autotoxicity compared to the cost of a gall was not as great as in the other treatments, but the probability of autotoxicity was the highest. As a result of the high autotoxicity risk, the cost of resisting was 21% greater than the cost of a gall in the shade-stress treatment, and thus selection should favor tolerance over resistance in shaded environments.

In summary, our results suggest that selection should act to increase S. altissima’s necrotic resistance response to E. solidaginis under benign, high-resource environments. However, due to high autotoxicity costs, this type of resistance is relatively more expensive under nutrient- and shade-stressed conditions, and thus selection should act to decrease resistance in more marginal, stressful environments. Because resource levels of this mid-successional plant will vary across populations and over time, there is likely to be a selective mosaic for resistance to E. solidaginis (Thompson, 1999; Fornoni et al., 2004b). Such a spatial and temporal selective mosaic would allow the long-term persistence of genetic variation for resistance within and among S. altissima populations.

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Author contributions

M.J.W. designed and performed the research, coordinated the collection of data, analyzed and interpreted the data, and drafted the manuscript. W.G.A. provided leadership of the research program within which the current work was executed, offered guidance on all aspects of the work and contributed to the writing of the manuscript.

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**Supporting Information**

Additional Supporting Information may be found online in the Supporting Information tab for this article:

**Fig. S1** Overview of the design of the nutrient-stress and shade-stress experiments.

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