Title: Building a barrier: investigating the defensive role of induced trichome production in herbicide exposed plants

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Summary

- Induced plant defense is commonly hypothesized to be an adaptive response to environmental heterogeneity reflecting phenotypic costs and benefits. While various studies have explored the adaptability of induced trichomes in response to biotic factors, we have limited understanding of whether induced trichomes can be an adaptive defense strategy in the presence of novel anthropogenic environmental stressors, such as herbicide exposure.
- Using annual invasive weed velvetleaf (*Abutilon theophrasti*), we explore whether induced trichome production contributes to herbicide resistance and determine its impact on growth and fitness in the presence and absence of glyphosate (active ingredient in "Roundup").
- We observed trichome traits (proportion of polymorphs and density) and found a significant tradeoff between constitutive and inducible strategies for total trichome density. We identified positive correlations between induced total trichome density and herbicide resistance as well as induced branched trichomes and herbicide resistance. Additionally, our selection analysis revealed positive linear selection acting upon induced increase of total trichome production and correlative selection favoring high induced trichome production and intermediate growth.
- Overall, our study indicates that induced trichome production may evolve as an adaptive defense strategy in agroecosystems and identifies significant constraints impacting the evolution of induced trichome defense.

Keywords: abiotic stressor, agroecosystems, herbicide exposure, induced plant defense, trichome production, phenotypic plasticity

Introduction

Phenotypic plasticity, the ability of a genotype to express various phenotypes as a response to environmental heterogeneity (Sultan and Stearns 2005), is a proposed mechanism by which plant species may persist amidst rapid environmental change (Valladares et al 2007). Although it is well established that phenotypic plasticity can evolve in plant populations (Baldwin 1998, Agrawal 1998, Karban and Baldwin 2007), the evolutionary constraints on adaptive plastic responses under a diversity of environmental conditions remain understudied. Because ecological systems are currently experiencing anthropogenic environmental change at unprecedented rates (Parmesan 2006), examining the potential for adaptation *via* plastic responses – and by extension, the evolutionary forces that mediate plasticity in nature – is vital.

Induced plant defenses include chemical and/or structural plant modifications subsequent to experiencing damaging agents such as herbivores, pathogens or chemicals. These responses are commonly thought to be an adaptive form of plastic defense and are conventionally studied through the framework of optimal defense theory. Optimal defense theory posits that 1) allocation to defense may incur a fitness cost since such allocation diverts resources away from growth and reproduction, and 2) plant populations should allocate to forms of defense that maximize fitness (McKey 1974, Rhoades 1979). Thus, an often-considered prediction is that the evolution of defense traits is mediated by fitness costs, with the balance between the costs and benefits of defense responsible for the observed level of defense in nature (Simms and Rausher 1987). Another prediction stemming from this theory is that tradeoffs in the form of a negative correlation may exist between constitutive (always present) and inducible (plastic) forms of defense (Rhoades 1979). This is because multiple types of defense would be redundant and either may incur costs; individuals that have both forms of defense could incur twice the cost compared to an individual with only one form of defense (Maurico et al 1997). Here, we test these predictions to understand the evolutionary constraints and adaptive potential of plastic forms of plant defense. We specifically do so by investigating defense strategy tradeoffs, genetic variation, and fitness costs/benefits for the plasticity of plant trichomes in the context of the stressors of agricultural systems.

Plant trichomes, which are hair-like epidermal cells that can directly sense and respond to external stimuli (Matsumura et al 2022), have historically been studied as an anti-herbivore defense trait (Doss et al 1987, Haberlandt 1914, Agren and Schemske 1993, Westerbergh and Nyberg 1995, Dalin and Bjorkman 2003), with a number of studies evaluating trichomes as an induced, plastic form of defense given herbivore damage (Agrawal et al 1999, Karban et al 1999, Tollrian and Harvell 1999). There is also growing evidence that induced trichome phenotypes can aid in defense against abiotic stressors such as radiation (Tattini et al 2000, Yamasaki et al 2007), drought (Boughalleb and Hajlaoui 2011), and elevated CO_2 (Karowe and Gribb 2011). Little is known, however, about the role of induced trichomes in adaptation to agricultural regimes, and in particular to the use of herbicides. Given intensified herbicide use (Benbrook 2016), and evidence that herbicides can drift to off-target ecological areas (Kleijn and Snoeijing 1997, Marrs et al 1989, De Snoo and Van der Poll 1999, Egan and Mortensen 2012, Hwang et al 2022), determining if unintended herbicide exposure can act as an agent of selection on forms of plant defense that serve multiple purposes, like trichomes, is crucial.

Abutilon theophrasti, hereafter velvetleaf, is a common weed of agricultural crops covered in multiple types of trichomes, giving its leaves a 'velvety' feel. There are four polymorphs of trichomes on the surface of its leaves, including two multicellular types that are glandular (called peltate and capitate) and two unicellular types that are non-glandular (single and branched) (Sterling and Putnam 1987). While we know that glandular trichomes have the ability to synthesize and secrete flavonoids as chemical defense (Sterling et al 1987), limited work has explored the defensive role for the non-glandular polymorphs in this species. Further, velvetleaf has evolved reduced susceptibility to herbicides that are often used in agricultural regimes, including atrazine (Anderson and Gronwald 1991) and glyphosate (Hartzler and Battles 2001), but the mechanisms underlying this reduced susceptibility are unknown. Thus, for both of these reasons – *i.e.*, variation in trichome types and reduced herbicide susceptibility – velvetleaf provides a unique study system with which to address questions about the evolution of trichomes as a potential type of plant defense to herbicide.

In past work, we found positive correlations between branched trichomes and glyphosate resistance, indicating that this trichome type may serve as an herbicide resistance trait in

velvetleaf (Johnson and Baucom 2023). We further showed positive selection on branched trichomes in the presence of the herbicide, indicating that herbicide exposure could lead to adaptive evolution of this trichome type. We also observed that trichome phenotypes exhibited high plasticity between growth room and field conditions, and reasoned that, in addition to constitutive trichomes, induced trichomes may likewise play a role in providing a line of defense to herbicide exposure in field conditions. In this work, we investigate trichome plasticity as a potential adaptive defense strategy, and, in accordance with optimal defense theory, examine potential constraints on the evolution of induced trichomes. We address the following questions: Is there a tradeoff between induced (activated by damage in the field) and constitutive (without damage, measured in the growth room) trichome phenotypes, representing a potential constraint on the evolution of defense? Do induced trichome phenotypes serve as an herbicide resistance trait? Are there fitness costs and benefits associated with trichome plasticity, and/or tradeoffs between this form of defense and growth or reproduction?

Materials and Methods

Study organism

Velvetleaf is an invasive annual native to Asia and is frequently found in and around corn, cotton, and soybean fields globally (Warwick and Black 1985). First introduced to Pennsylvania and Virginia in the late 17th century, velvetleaf was originally cultivated as a commercial fiber crop (Spencer 1984). Despite its commercial success in other regions, the fiber industry in the US was not able to use velvetleaf effectively, leading to the lack of management and rapid growth in and around agricultural fields. Today, this annual species has become one of the most detrimental weed species between 32º and 45º N latitude, particularly in southwestern Canada and the United States (Warwick and Black 1985).

Velvetleaf is a highly self-pollinating, hexaploid species with 2n = 42 chromosomes, and with high levels of fixed heterozygosity as a result of polyploidy (Warwick and Black 1985). Seed production ranges from 700 to 17,000 seeds per individual (Winter 1960; Chandler and Dale 1974; Hartgerink and Bazzaz 1984; Warwick and Black 1985), and seed dormancy can last up to 50 years (Warwick and Black 1988). Studies on velvetleaf accessions in North America reveal

phenotypic variation that correlates with latitudinal environmental heterogeneity. For instance, southern populations found in North America produced fewer, larger seeds with lower rates of seed dormancy when compared to northern populations (Warwick and Black 1985). This species has also been shown to exhibit resistance or decreased susceptibility to several herbicide classes including triazine (Ritter 1986, Anderson and Gronwald 1991) and glyphosate (Hartzler and Battles 2001).

In addition to resistance to herbicides, velvetleaf also has traits that reduce the potential for herbivory. These traits include glandular trichomes on the plant surface, which serve as chemical defense structures via the synthesis and storage of flavonoids such as anthocyanin, quercetin, kaempferol, and myricetin (Morris and Wang 2013). The glandular morphs are multicellular structures that include peltate trichomes, which contain 4 - 5 cells, and capitate trichomes, which contain 12 - 15 cells (Sterling 1987, Fig 1). Velvetleaf also has non-glandular trichomes, which serve as physical defense structures by shielding the plant body. The non-glandular morphs are unicellular structures that include single trichomes, which grow perpendicular to the plant surface, and branched trichomes, which grow 4 - 8 arms (Sterling 1987, Fig 1). Here, we report findings from a growth room and field experiment to investigate the adaptive value of induced trichome traits (proportion of polymorphs and total density) in response to herbicide exposure as a defense strategy in velvetleaf.

Growth Room Experiment

We imaged trichomes on the leaf surface of plants grown in the growth chamber to capture the constitutive trichome production of velvetleaf maternal lines, and we compared these estimates to that of the same maternal lines grown in field conditions to capture induced trichomes. Correlations between inducibility, estimated as the absolute difference between damaged and control groups, and constitutive resistance, estimated from the same control group, has been shown to produce spurious correlations (Traw 2002, Morris et al 2006). Thus, estimating constitutive trichomes from control individuals in the growth room ensures that constitutive estimates are not confounded by the induced estimates, and *vice versa*. This approach also ensures independence of sampling/measurement errors for the two control groups (growth room

and field) and reduces the likelihood of spurious negative correlations as the independent measurements are expected to produce a correlation of zero in the absence of a tradeoff.

In fall 2017, we collected seeds from eight velvetleaf populations around soybean fields in Dexter, MI (SFig 1). We scarified and planted 550 seeds (4 replication of 130 maternal lines) in a randomized design of 164 ml cone-tainers (1.5in diameter, Stuewe and Sons inc., Tangent Oregon) in the growth room. We rotated plants within flats weekly. Plants were kept on a 12/12 hr light-dark regime at a temperature of 25°C. Once plants began to reach reproductive maturity, roughly 10 weeks after germination, we captured images of trichomes within a $3mm^2$ area right of the abaxial midrib at 10x magnification, using a confocal laser scanning microscope (TCS SP5; Leica Microsystems CMS GmbH, D-68165 Mannheim, Germany). We applied an excitation wavelength of 488 nm and 512 x 512-pixel resolution. Using imaging software, Fiji ImageJ version 3.0 (Schindelin et al. 2012), we created a macro plug-in to recognize and highlight trichome structures. We then manually counted the total number of each type of trichome (single, branched, capitate, peltate) per $3mm^2$ segment.

Field Experiment

We conducted a field experiment to measure induced trichomes in field conditions and in response to herbicide application. We generated seeds for this experiment by selecting and self-pollinating 40 maternal lines from the growth room experiment. We planted scarified velvetleaf seeds in a randomized block design comprised of two treatments (herbicide and control). Three replicate individuals per maternal line were planted in each treatment/block combination for a total of 720 seeds (40 maternal lines x 2 treatments x 3 blocks x 3 replicates). When plants reached an average height of 11 cm, roughly 5 weeks after germination, we applied glyphosate at 550g/ha ai to the herbicide treatments, as a previous study revealed genetic variation for reduced susceptibility at this application dose (Johnson and Baucom 2023). We recorded height measurements to estimate growth rate two weeks after planting and once again when we collected fitness estimates, approximately 12 weeks after planting.

As an annual species, velvetleaf seed count indicates the success for which individual genetic material may be passed on to subsequent generations, thus we elected to use seed count as an

estimate of fitness. Plants that germinated but did not survive herbicide exposure were given a fitness of zero. To estimate herbicide damage in the field, two weeks after herbicide application we recorded the number of yellowing leaves and divided that by the total number of leaves per individual. Because velvetleaf begins to shed its leaves during seed maturation, we choose this metric to estimate herbicide damage rather than biomass so that fitness data could also be collected. Herbicide resistance was defined operationally as the inverse of herbicide damage (1 minus the proportion of yellowing leaves).

Due to low germination rates and mortality following herbicide application in the field, there were 25 maternal lines remaining for which induced trichomes could be estimated (i.e. represented in both treatments). Once plants began to flower, approximately 10 weeks after germination, we collected one leaf from 2-3 randomly chosen plants per maternal line in each treatment for trichome imaging (n = 130), applying the same method as above. Along with measuring total trichome density, we calculated the proportion of each trichome polymorph by dividing the absolute number of each trichome structure by the total trichome density recorded in each $3mm^2$ segment. We estimated the weekly growth rate as the difference in height measured 2 weeks after germination and 12 weeks after germination divided by 10.

Data Analysis -

Phenotypic plasticity in trichome production

We conducted all statistical analyses in R (version 3.4.2, R Development Core Team). We ran a series of univariate models to assess the potential for plasticity in trichome production in different environments. Our specific environments were 1) controlled conditions in the growth room, 2) field conditions in the control (non-herbicide) environment, and 3) field conditions when exposed to glyphosate. For each model, the response variable was one of the observed trichome traits (density and polymorph proportions) and the fixed effect was the environment (*i.e.* growth room, field-control, and field-herbicide). We performed subsequent post-hoc tests using Tukey to detect differences between environments.

Constitutive trichomes and trichome inducibility

Maternal line means were calculated for total constitutive trichome estimates collected from the growth room and for absolute trichome inducibility in the field when exposed to damage from herbicide ($|\text{trichome}_{\text{herbicide treatment}} - \text{trichome}_{\text{control treatment}}|$). We performed Pearson's correlations test to determine if there was a significant tradeoff between the two. In order to test if there was a relationship between constitutive trichome estimates and the magnitude of inducibility, we also calculated the net difference in total trichome density ($\text{trichome}_{\text{herbicide treatment}} - \text{trichome}_{\text{control treatment}}$). To determine if there was a different relationship for maternal lines that were positively induced versus negatively inducted, we performed Pearson's correlations test separately for each direction.

Genetic variation underlying induced trichome traits

We tested for genetic variation underlying induced trichome traits to determine if trichome induction would be expected to respond to selection and evolve. To do so, we fit the following model for each trait:

$$y = u + \text{treatment} + (1|\text{ maternal line}) + \text{treatment} \times (1|\text{ maternal line}) + e$$

where each respective trichome traits (total density, and the proportion of branched, single, peltate, and capitate) was the response variable, u is the intercept or mean of the model, treatment was a fixed effect, maternal line was a random effect, maternal line by treatment interaction was a random effect, and e is the error term. Our specific effect of interest was the interaction of maternal line with treatment, since such an interaction would indicate that trichome traits of maternal lines responded differently to the control and herbicide environments and thus implicate adaptive plasticity. As such, the remaining analysis was performed on trichome traits with significant maternal by treatment effects. For each trait, we first removed the effects of block by performing a regression of the trait with the block effect as the sole predictor variable, and then used the residuals in each subsequent analysis of variance. We determined the significance of predictor variables using the stats package (Chambers and Hastie 1992) to generate *F*-statistics for the fixed effects. We used the lmerTest package (Kuznetsova et al 2017) to perform a log-likelihood ratio test for each random effect.

Herbicide resistance and induced trichomes

As the cost of inducing resistance is more likely to be related to the change in resistance, rather than the induction ratio (i.e. mean resistance in damaged plants divided by the mean resistance in control plants) and the ratio of the increase in resistance (i.e. the mean resistance in the damage treatment minus the mean resistance in the control treatment divided by the mean resistance in control plants) (Keinänen et al. 1999, Lombardero et al. 2000, Gianoli 2002, Traw 2002, Morris 2006), we calculated induced trichomes in field settings as the mean difference between damaged (i.e. herbicide treatment) and control (i.e. control treatment) states for each maternal line in the field experiment. As such, this measurement produced a positive number when trichomes increased (i.e., induced resistance) in response to herbicide and a negative number when trichomes decreased (i.e., induced susceptibility).

We elected to perform the remaining analysis on induced total density, and induced changes in the proportion branched and capitate trichomes because these three traits exhibited significant genetic variation (see Results). We estimated Pearson correlation coefficients to determine if induction of these traits had a significant relationship with herbicide resistance and tested the significance of the correlation coefficient using the stats package.

Genotypic selection for induced trichomes

We performed genotypic selection analysis to determine if trichome induction in response to herbicide exposure is under selection and to assess if there was a cost when compared to control conditions. We estimated differentials to measure selection on induced trichome density, proportion branched and capitate trichomes in the presence and absence of herbicide. Because the proportion of branched trichomes and capitate trichomes are not independent of total trichome density, we estimated selection differentials rather than gradients. We estimated selection differentials (S) by performing univariate regressions of relative fitness in each trait separately which captures total (indirect and direct) selection acting upon each trait. Relative fitness was calculated as the final seed count per individual divided by mean seed count for each treatment.

Inducible defenses are expected to come at the expense of plant growth as a form of cost (Herms and Mattson 1992), thus, we explored if there was correlative selection acting on the interaction between growth rate and induced trichomes. We performed non-linear selection analyses (γ) containing trichome traits and growth rate as linear terms, quadratic terms, and the cross-product of the focal trichome trait and growth rate. We assessed the potential of quadratic or correlative selection by doubling the quadratic regression coefficients. Significant quadratic terms would indicate non-linear (disruptive or stabilizing) selection acting upon growth rate or induced trichome traits, whereas significant correlative selection would indicate selection favoring a combination of the focal traits.

Results

Trichome production in *Abutilon theophrasti* is highly plastic, with significant changes in trichome density and the proportion of each polymorph produced according to environment. When compared to the growth room, individuals grown in the field exhibited significant differences in the patterns of trichome production. Overall trichome density ($F = 123.95$ $p < 0.001$, SFig 2) and the proportion of unicellular trichomes (single: $F = 14.55$, $p < 0.001$; branched: $F = 235.90$, $p < 0.001$, SFig 2) increased in field conditions, whereas the proportion of multicellular glandular trichomes (capitate: $F = 105.62$, $p < 0.001$; peltate: $F = 235.88$, $p < 0.001$, SFig 2) decreased in field conditions.

When comparing between the control (non-herbicide) environment and herbicide environment in the field, plants exposed to herbicide exhibited fewer total trichomes (*i.e.* reduced trichome density: SFig **2a**). We found no evidence for changes in the proportions of the four trichome polymorphs between control and herbicide environments (SFig **2b-e**). However, induced trichome production varied by maternal line when plants were exposed to herbicide (STable 1). We identified genetic variation in induction of the proportion of branched trichomes (maternal line by treatment effect: $\chi^2 = 3.92$, $p = 0.048$, Table 1, Fig 2), and the proportion of capitate trichomes (maternal line by treatment effect: $\chi^2 = 5.64$, $p = 0.018$, Table 1, Fig 2). We also found significant maternal line by treatment interaction for total trichome density ($\chi^2 = 10.05$, $p = 0.002$, Table 1, Fig 2).

Constitutive trichomes and trichome inducibility

A significant tradeoff between constitutive and inducible trichomes would indicate a constraint on investment into plant defense. We found a negative correlation between constitutive trichomes measured in the growth room and absolute trichome inducibility in the field ($r = -0.438$, $p = 0.041$, Fig 3), indicating the presence of a trade-off between constitutive and induced defense strategies in this species. We next subset the data to examine the correlation between constitutive and positively induced trichomes (i.e., families that exhibited more trichomes in the field) or negatively induced trichomes (families that exhibited fewer trichomes in the field). We found a negative relationship between constitutive trichomes and positively induced trichomes, and a positive relationship between constitutive trichomes and negatively induced trichomes, but neither correlation was significant (positively induced trichomes and constitutive trichomes: $r = -0.580$, $p = 0.228$, negatively induced trichomes and constitutive trichomes: $r = 0.353$, $p = 0.179$). This indicates that the degree of relatedness, but the direction, between constitutive and inducible trichomes is influenced in part by both the genetic and environmental background.

Herbicide resistance and induced trichomes

We investigated if there was a significant relationship between induced trichomes and herbicide resistance (1 – proportion herbicide damage), which would indicate that induced trichomes act as an herbicide resistance trait. We found that lines that exhibited a greater induction of branched trichomes and a greater induction of total trichome density exhibited higher levels of herbicide resistance ($r = 0.44$, $p = 0.035$, Fig **4a**; $r = 0.43$, $p = 0.040$, Fig **4c**). There was no evidence of a relationship between the proportion of capitate trichomes and herbicide resistance ($r = -0.37$, $p = 0.084$, Fig **4b**).

Genotypic selection for induced trichomes

In the absence of herbicide, we found no evidence for selection acting upon any induced trait (branched: $S = -0.03$, $p = 0.976$; capitate: $S = -0.39$, $p = 0.698$, total density: $S = -0.01$, $p = 0.99$; STable 2). In the presence of herbicide, however, we found negative selection for induced capitate trichomes ($S = -2.13$, $p = 0.045$, Fig 5, STable 2) and positive selection for induced

trichome density (S = 2.56, p = 0.018, Fig 5, STable 2). We found no evidence of selection on induced branched trichomes (S = 0.010, p = 0.925, Fig 5, STable 2) in the presence of herbicide.

In the absence of herbicide, we identified significant correlative selection acting branched trichomes (γ = 6.13, p = 0.006, STable 3), in that genotypes with the highest growth rate and a greater ability to alter the proportion of branched and capitate trichomes exhibited highest fitness. However, there was no evidence of correlative selection between growth rate and induced total density in this environment (γ = -0.87, p = 0.669, STable 3).

In contrast, in the presence of herbicide, there was evidence for correlative selection between growth rate and induced total density (γ = 5.71, p = 0.010, STable 3), in that genotypes with the highest fitness exhibited intermediate growth and high levels of induced total density (Fig 6). There was no evidence of correlative selection acting upon growth rate and either induced branched (γ = -0.77, p = 0.070, STable 3) or induced capitate (γ = -2.58, p = 0.091, STable 3) trichomes in the presence of herbicide.

Discussion

The goal of this study was to determine if phenotypic plasticity in trichomes is an adaptive response to herbicide (glyphosate) exposure and to examine the potential for constraints on its evolution. We explored two key expectations of the optimal defense theory: first that the cost and benefits of defense, which are apparent in the absence and presence of a damaging agent, respectively, reflect a tradeoff between defense and reproduction (Rhoades 1979, Zangerl and Bazzaz 1992, Gavrilets and Scheiner, 1993), and second that inducibility exists on a continuum inverse to constitutive defense such that a trade-off between the two forms of defense are present.

In the broadest sense, plastic responses cause real-time modifications of resource allocation and can result in a range of defense strategies. For example, plants can induce phytochemicals (Baldwin and Schmelz 1994, Textor and Gershenzon 2009) and increase in physical defenses such as thorns and trichomes (Young 1987, Milewski et al 1991, Gonzales et al 2008). Of the

research on trichomes, ample work has examined environmental factors that induce trichome production to include: herbivory (Baur et al 1991, Agrawal 1999, Rautio et al 2002, Dalin and Bjorkman 2003), pathogens (Zhang et al 2020), water availability (Nagata et al 1999, Bosu and Wagner 2014, Vanhoutte et al 2017), UV radiation (Höglund and Larsson 2005), elevated CO_2 (Karowe and Grubb 2011) and temperature (Ehleringer 1982, Perez-Estrada 2000, Shibuya et al 2016). However, few studies have identified such factors as selective agents and subsequently determined the adaptive significance of induced trichome production in response to exposure to them (Barto and Cipollini 2005). Here, we discuss our results conducted on velvetleaf (*Abutilon theophrasti*) as it relates to induced trichome production in response to herbicide exposure experienced in agro-ecosystems.

Induced trichome production as an herbicide resistance trait

Most plants have developed the ability to perceive and respond to external stimuli (Braam, 2005), and recent studies indicate that plant trichomes can directly sense external forces in anticipation of threats (Peiffer et al 2009, Matsumura et al 2022). Plant trichomes have been hypothesized as a contributor to herbicide resistance (Devine et al 1992, Baucom 2019), but few studies have empirically tested this hypothesis. While we have previously shown that the proportion of branched trichomes is positively correlated to the level of herbicide resistance in this species (Johnson and Baucom 2023), suggesting this trait may act as a resistance trait, the potential that trichome plasticity may also function as a defense trait had not been previously considered. The present study indicates that velvetleaf maternal lines can induce an increase or decrease in total trichome density and in the proportion of trichome types in response to herbicide exposure. Our results revealed a positive relationship between herbicide resistance and an induced increase of total trichome density as well as induced increase in the proportion of branched trichomes, indicating that an increase in trichome production in conjunction with an increase in the proportion of branched trichomes can serve as a form of structural defense against herbicide exposure.

While we do not know the mechanistic basis of this positive correlation, we hypothesize two potential reasons for this relationship. First, it is possible that genotypes that are more generally defended are likewise able to induce trichomes when damaged. In this scenario, which we

imagine as agnostic to the particular damaging agent, the plant experiences stress that then triggers increased trichome production in genotypes that are better defended in a broad range of ways. Second, damage from an abiotic source, and specifically a chemical like an herbicide could trigger the production of trichomes that contain vacuoles, allowing for increased sequestration of the chemical into the vacuoles prior to general detoxification. As trichome vacuoles can to occupy up to 95% of the total volume (Gutiérrez-Alcalá et al 2000), increased branched trichomes may be particularly advantageous because this trichome polymorph often has the greatest vacuole volume potential (Calvert et al 1985). Alternatively, genes that influence trichome development in *A. thaliana* have known pleiotropic effects that impact other physiological functions (Bird and Gray 2003, Kirik et al 2004), thus it is possible that the induced trichomes we observed in velvetleaf could be linked to a response not measured in our experiment. While uncovering the exact mechanism underlying this relationship was outside of the scope of this study, further research is needed to determine if the induction in plant trichomes in more resistant genotypes is due to being more defended in general, have the ability to sequester herbicide for detoxification purposes, pleiotropically related to an unknown cause that we did not capture, or a combination of any of these explanations.

Costs and benefits of trichome plasticity

The adaptive significance of damage-induced changes to trichome production are dependent on the benefits and costs of the phenotypic change (Dalin et al 2008). Positive correlations between induced increases in trichome traits and herbicide resistance suggests that plants would benefit from the induction of trichomes as long as the fitness benefits of this induction outweigh the costs of induction. Our linear selection analysis revealed that in the presence of herbicide there was positive selection acting upon the induced increase of total trichome density, but no evidence of selection acting upon the induced increase of proportion branched trichomes. This indicates that there are fitness benefits associated with the induced increase of total trichome density.

In the event of significant fitness cost, the pattern of selection would reveal fitness peaks in the absence of herbicide that correspond with the absence of the resistance character in question (*i.e*. induced total trichomes or induced increase of proportion branched trichomes) (Mauricio 1998).

While many models assume that fitness costs increase linearly with allocation towards defense (Rhoades 1979, Simms and Rausher 1987), our results revealed no evidence of selection acting upon any of the induced trichome traits in the absence of herbicide, suggesting a lack of fitness costs. This finding aligns with several past studies in which costs for induced resistance had not been detected (Brown 1988, Simms 1992, Karban 1993, Mole 1994), but it also conflicts with other work that has identified costs of induced defenses (Tally et al 1999, Heil et al 2000, Cipollini 2002). Some explanations for the lack of costs may be that costs are too small, or that the costs may only be detectable in highly competitive environments (Simms 1992, Karban 1993). Here, we find that for induced trichome density, the net pattern of selection is positive, such that the benefits associated with trichome induction outweigh any cost.

Other constraints on trichome plasticity

Plastic responses are expected to evolve when environmental cues provide a predictable indicator of appropriate adaptive phenotypes that exhibit genetic variation (Padilla and Adolf 1996; Reed et al. 2010). Thus, one constraint on the evolution of plastic defense responses would be the lack of genetic variation underlying such phenotypes. In this work we identified significant maternal line variation for induced total trichome density, as well as induced changes in the proportion of branched and capitate trichomes, indicating that these plastic trichome traits have the potential to evolve. While past work from other researchers has identified genetic variation in density, glandular, non-glandular trichome polymorphs (Agrawal 2002, Holeski 2007, Ogran et al 2019), our study is one of few designed to detect genetic variation for induced trichome production for multiple trichome traits.

In addition to lack of genetic variation, one type of potential constraint on the evolution of plant defense could be in the form of a trade-off between different types of defense. In this work we investigated the potential for a trade-off between constitutive and induced trichomes. We found an inverse relationship between trichomes produced in growth room conditions (constitutive trichome production), and trichomes induced by herbicide exposure in the field (induced trichome production). This result indicates that there are limitations to investing resources into plant defense, in alignment with both predictions of optimal defense theory and with studies demonstrating trade-offs associated with the induction of defenses given either herbivory (Traw

2002) or mechanical damage (Zangerl and Berenbaum 1990, Lewinsohn et al 1991, Traw and Bergelson 2003). While it is important to note that induced trichome responses to herbicide will likely depend on chemical formula, just as induced trichome responses to herbivory are dependent on herbivore species (Chambliss and Jones 1966, Da Costa and Jones 1971), our results indicate that the evolution of trichome plasticity in response to glyphosate is constrained by limitations on resource allocation toward defense.

Past work tells us that in some cases induced plant defense can directly hamper reproductive output (Agrawal 1999, Redman 2001), and in other cases can directly hamper plant growth (Siemens 2002, Hermosa et al 2013). As such, we explored the possibility that allocation to induced defenses could come at a cost to plant growth. Following optimal defense theory, we predicted that in the presence of herbicide, selection should favor genotypes that allocated away from growth and toward induced defense, whereas in the absence of herbicide selection should favor genotypes that exhibited high plant growth and low levels of induced defense. We found different responses according to the type of trichome phenotype we considered. In the presence of herbicide, for total trichome density, we identified correlative selection favoring induced increases in the total number of trichomes and intermediate growth rates, indicating that induced trichomes come at the expense of increased growth. In contrast, in the absence of herbicide, we found no evidence of correlative selection acting upon induced total trichome density and growth rate, suggesting that plant growth is not limited in the absence of damage when trichome production is not induced.

In comparison, while we did not identify correlative selection acting on growth rate and either the proportion of branched and capitate trichomes in the presence of herbicide, in the absence of herbicide we found evidence of correlative selection in favor of both high growth rate and high inducibility for these trichome traits. This indicates that genotypes with the highest growth rate and high plastic ability to alter the proportion of trichome polymorphs have the highest fitness in the absence of herbicide. Overall, these results suggest that in the presence of herbicide, genotypes with intermediate growth and high plasticity in trichome production, but not plasticity in the proportion of polymorphs, exhibit the highest fitness. These selection results align with past studies that indicate inducibility is negatively associated with species growth (Jacobsen

2022). Thus, while we did not find costs on induced trichomes in terms of fitness, the pattern of correlative selection in the presence of herbicide indicates a cost in terms of plant growth. We speculate that the trade-off between plasticity in trichome production and growth in the presence of herbicide is reflected in the negative correlation between induced and constitutive trichome production we observed.

Conclusion

Over the past four decades, much has been learned about the importance of trichomes in modulating leaf boundary dynamics, and recent developments suggest that we may be underestimating the eco-evolutionary significance of these plant appendages. As agriculture is one of the main global drivers of environmental change, adaptive trichome plasticity may play a key role in the persistence of plants in novel conditions. The current study examines the adaptive significance of induced trichome production as a reflection of the cost-benefit ratio associated with herbicide damage. Our results provide evidence of a fitness benefit for induced trichome production in the presence of herbicide, demonstrating that induced responses can serve an adaptive defensive function against herbicide. It also provides evidence of evolutionary constraints in terms of tradeoffs between defense strategies (i.e. inducibility versus constitutive defense) and correlative selection acting upon induced trichome production and reduced plant growth, indicating limiting factors that may impede, but not prevent, adaptive trichome plasticity. Future research should explore the functional variation of trichome polymorphs and the potential for transgenerational trichome plasticity in agro-ecosystems.

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Conflict of Interest

The authors declare no conflict of interest.

Author contributions

N.M.J and R.S.B. contributed to the experimental design, writing, and editing of this manuscript.

Data Availability Statement

The data underlying this article are available in Github, at https://github.com/niajohnson1/HerbicideInducedTrichomes

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Figures

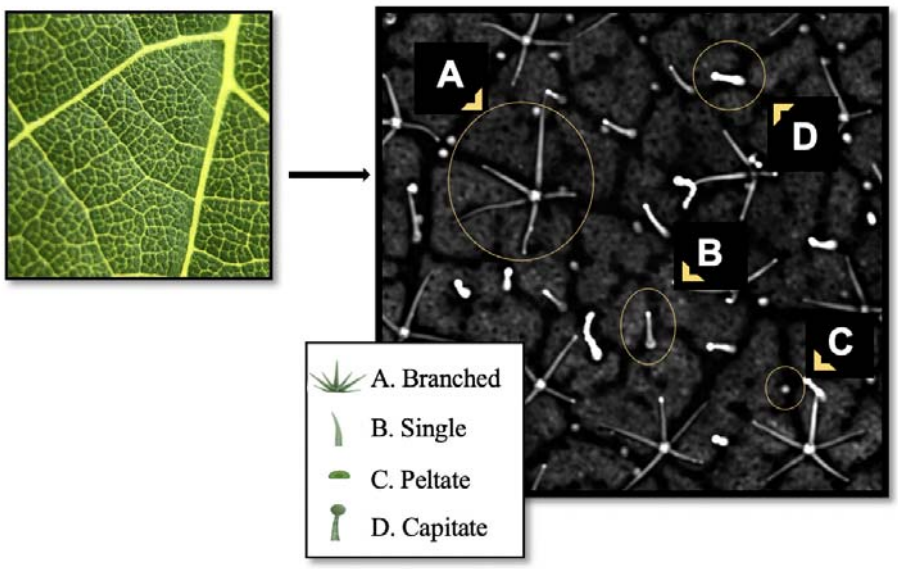

Figure 1. Trichome structures present on the adaxial surface of *Abutilon theophrasti* leaves using confocal imaging (x10). A) Branched trichomes are unicellular structures with 4-8 appendages. B) Single trichomes are unicellular unbranched structures. C) Peltate trichomes are multicellular glandular structures made up of 4-5 cells. D) Capitate trichomes are multicellular glandular structures made up of 12-15 cells. Density is calculated as the total number of all trichomes captured in the image.

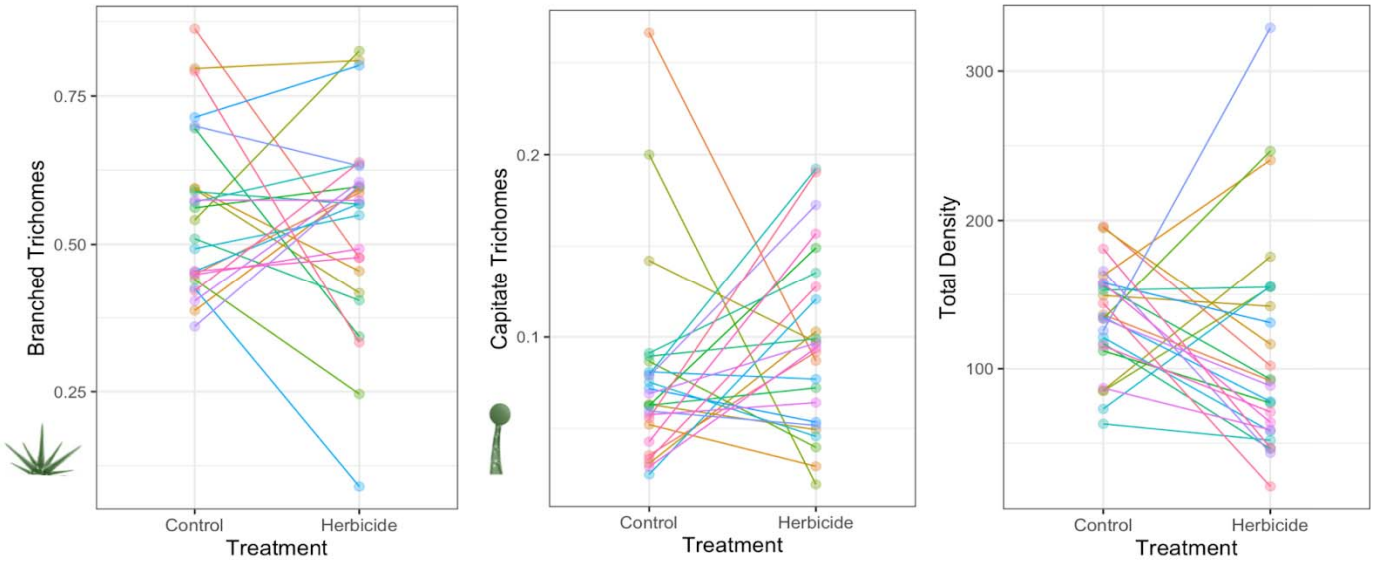

Figure 2. Illustration of genetic variation for induced trichome phenotypes in *Abutilon theophrasti* maternal lines grown in field conditions in either the control (non-herbicide environment) or herbicide present environment. Shown are the significant maternal line by treatment effects for the proportion branched trichomes ($\chi^2 = 3.92$, $p = 0.048$), the proportion capitate trichomes ($\chi^2 = 5.64$, $p = 0.018$), and total trichome density ($\chi^2 = 10.05$, $p = 0.002$).

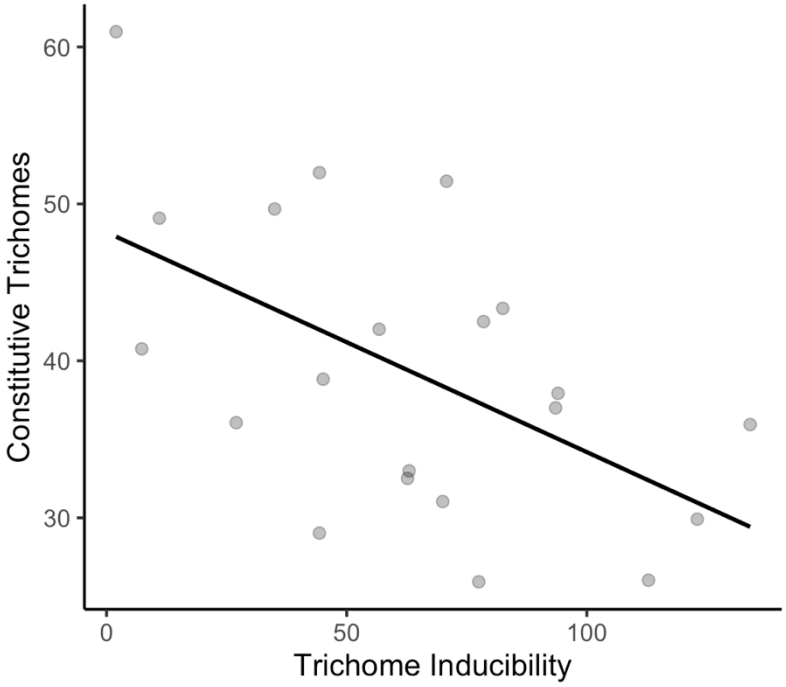

Figure 3. Relationships between *Abutilon theophrasti* constitutive trichomes and trichome inducibility in response to herbicide exposure. Pearson's correlation test indicates a significant and strong negative relationship between the two defense strategies ($r = -0.438$, $p = 0.041$). Data points represent maternal line means. Constitutive trichomes were measured as the total density of trichomes observed in the growth room, and trichome inducibility was measured as the absolute difference between damaged (herbicide present) and control states of maternal lines observed in the field.

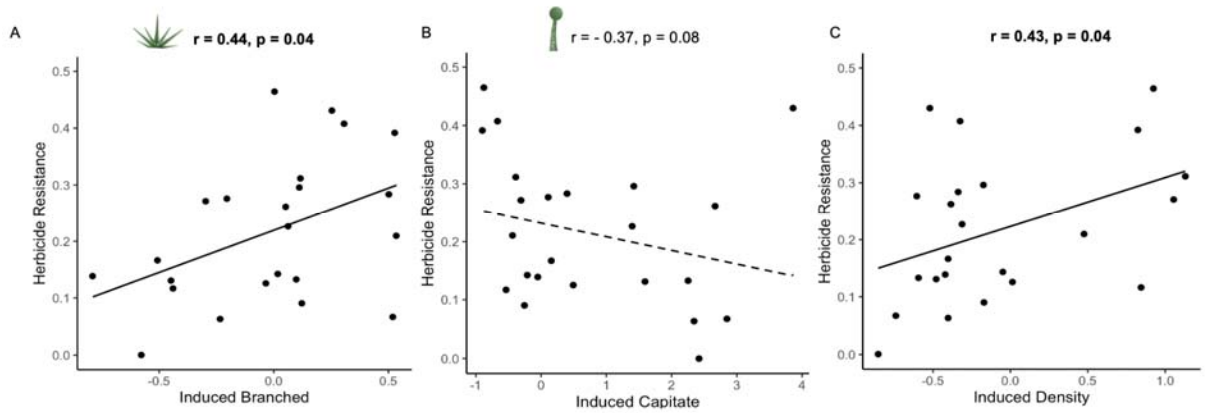

Figure 4. Relationships between herbicide resistance and A) induced proportion branched and B) induced proportion capitate and C) induced total trichome density for *Abutilon theophrasti*. Shown are Pearson's correlation coefficients; significant correlations are indicated in bold. Data points represent maternal line means. Induced trichome traits were measured as trichomes $_{\text{damaged}}$ – trichome $_{\text{control}}$, and herbicide resistance was measured as 1 – proportion of leaf yellowing.

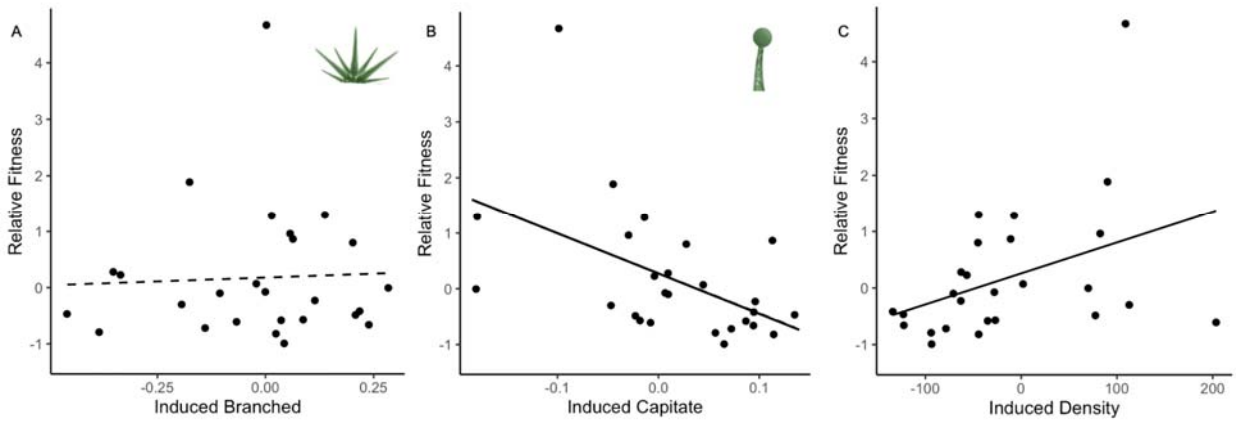

Figure 5. The relationship between relative fitness and A) induced proportion branched (*S* = 0.10, p = 0.925) and B) induced proportion capitate (*S* = -2.13, p = 0.045) and C) induced total trichome density (*S* = 2.56, p = 0.018) in *Abutilon theophrasti*. Solid lines represent selection differentials in the presence of herbicide. Data points represent maternal line means; induced trichome traits were measured as damaged – control states, and herbicide resistance was measured as 1 – proportion of leaf yellowing.

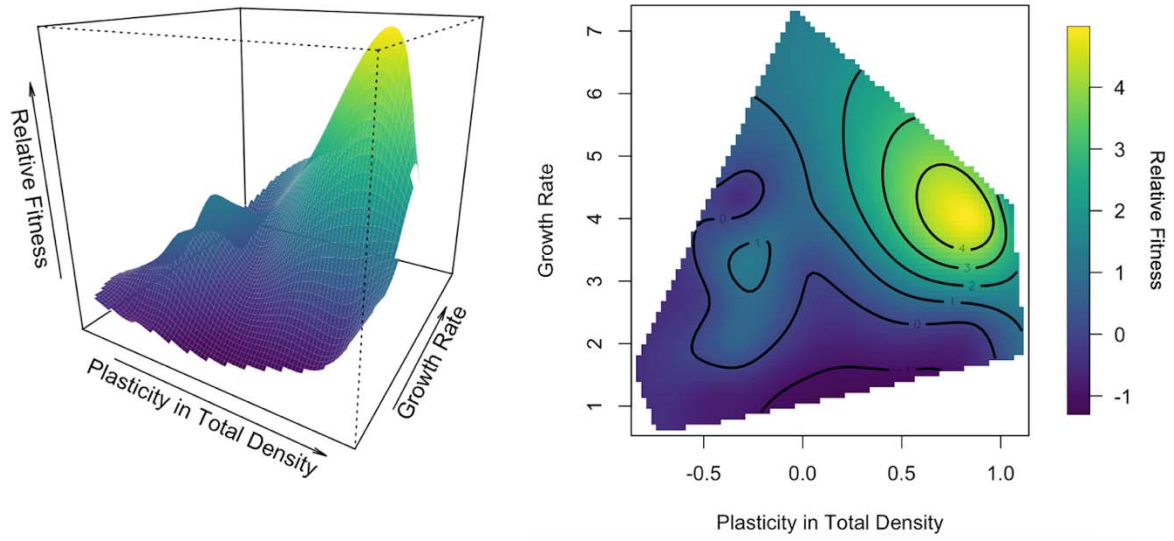

Figure 6. Fitness surface for correlative selection acting upon *Abutilon theophrasti* induced total trichome density and growth rate in the presence of herbicide (γ = 5.71, p = 0.010), tested using non-linear selection gradients (multivariate selection). Relative fitness is depicted by the color gradient; yellow is the highest fitness, blue is the lowest fitness, and green is intermediate.

Tables

	Maternal Line x Treatment Effects		
	χ2	df	p - values
Proportion Branched	**3.92**	**1**	**0.048**
Proportion Single	3.26	1	0.071
Proportion Capitate	**5.64**	**1**	**0.018**
Proportion Peltate	0.71	1	0.399
Total Density	**10.05**	**1**	**0.002**

Table 1. Results from the test for genetic variation in *Abutilon theophrasti* showing the effects of maternal line by treatment on trichome traits (proportion branched, single, capitate, peltate, and total density) captured in the field. Significant effects are indicated in boldface. The full ANOVAs are presented in STable 1.