Eco-Evolutionary Ramifications of Herbicide-Driven Plant Modifications on Plant-Herbivore Interactions in Modern Agricultural Landscapes

by

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Dedication

This dissertation is dedicated to my mother, Alesia, my father, Elton, and my brothers Christopher and Jamil, who had confidence in me from beginning to end as the first person in our family to earn a Ph.D.

Figure 0 - 1. Illustration of dissertation study system.

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Abstract

Natural populations evolve in response to biotic and abiotic changes in their environment, which lead to wide variation in defense traits that can shape species interactions. Often, variation in defense is a result of complementary or conflicting strategies for dealing with external stressors. Agricultural systems can introduce novel stressors via herbicide exposure to non-crop plant systems surrounding fields. Of the various plant defense strategies, trichomes, or hair-like appendages on the outermost boundary, are known to play a critical role in how these sessile organisms interact with their environment. While herbicide drift is known to produce a variety of toxic effects in plants, little is known about its impact on plant-herbivore interactions and the evolution of plant defense strategies. Thus, my dissertation addresses the overarching question: *Does herbicide drift impact plant-herbivore interactions and the evolution of plant trichomes?*

Using the annual invasive velvetleaf (*Abutlion theophrasti*) as the focal species, I conducted various field, greenhouse, and growth room experiments to explore the potential effects of herbicide drift. In the first data chapter (Chapter 2) of this dissertation, I investigate the impact of dicamba, a novel herbicide known to travel particularly far distances, on plant-herbivore interactions. The findings from this multi-year study revealed a significant increase in the phloem feeding silverleaf whitefly (*Bermisia tabaci*) abundance on plants exposed to herbicide. Here, I identified a significant phenotypic tradeoff between whitefly resistance and herbicide resistance. I also found evidence that dicamba drift significantly increased leaf chlorophyll content (mg/cm2) which positively affected whitefly abundance.

In the second (Chapter 3) and third (Chapter 4) data chapters of this dissertation, I assessed whether glyphosate, a traditional herbicide found in “Roundup”, acts as an agent of selection on induced trichome traits (polymorphs and density) and whether variation observed in trichome traits represents dual or conflicting roles against herbicide and herbivory damage. I identified positive correlations between induced total trichome production and herbicide resistance as well as induced branched trichomes and herbicide resistance. The selection analysis revealed positive linear selection acting on induced trichome production and correlative selection favoring high induced trichome production and intermediate plant growth. In the third data chapter, I found that having a high proportion of branched trichomes not only had a positive effect on herbicide resistance, but it also had a positive effect on chewing herbivory resistance.

In brief, my dissertation demonstrates that herbicide exposure can alter plant-herbivore interactions mediated by modified plant phenotypes. My thesis demonstrates that herbicide drift can create unintended reservoirs for agricultural pests like phloem feeding whiteflies that are attracted to chlorophyll content. It also provides novel evidence that herbicide is a selective agent on induced trichome production and demonstrates that branched trichomes can contribute synergistically to herbicide and chewing herbivory resistance, serving as a dual structural form of resistance reducing plant injury. While responses are expected to be species and herbicide dependent, collectively, this work reveals that herbicide exposure to non-target plant systems can significantly alter species interactions and community dynamics in habitats located at the agro-ecological interface.

Chapter 1 Introduction

For nearly 10,000 years, humans have been modifying Earth's landscape for agriculture (Denham and White 2007), providing essential commodities such as food, fiber, and biofuels. In order to meet the demands of rising human population growth, however, the extent, pace, and impact of agriculture has significantly accelerated over the last three hundred years (Ellis et al 2010). Agricultural lands currently occupy one-third of the Earth's ice-free surface area, making it the largest biome on the planet (Ellis and Ramankutty 2008). One of the main forces for agricultural expansion and intensification has been the introduction of new, innovative technologies, such as herbicides designed to target and kill unwanted plant species that threaten crop yields (Osteen and Fernandez-Cornejo 2016). Consequently, modern agricultural practices have become a leading environmental threat driving global change (Campbell 2017).

Herbicide application can result in drift, or unintended migration, to neighboring non-agricultural lands (Freemark and Boutin 1995) at concentrations lower than the application dose (Carlsen et al. 2006). This phenomenon has been linked to unexpected impacts, including declines in pollinator visits (Bohnenblust et al 2013, Bohnenblust et al 2016), reduced arthropod abundance (Egan et al 2014) and changes to plant community composition (Iriart, Baucom, and Ashman 2021). While it is evident that herbicide drift can have a host of effects, few studies have explored how herbicide drift may influence current and future species interactions. As such, my thesis investigates the ways in which herbicide drift may disrupt plant-insect interactions and considers the evolutionary implications of herbicide exposure on such interactions. Specifically, I address the following questions: Does herbicide application impact herbivore behavior? Is there a trade-off between herbivory resistance and herbicide resistance? Is there evidence of selection acting on herbicide resistance and/or herbivory resistance, or correlative selection acting on the interactions of these focal traits? Does herbicide induce traits conventionally viewed as herbivory resistance (trichomes)? And if so, is herbicide an agent of selection? Do trichomes perform dual or conflicting defense roles given exposure to herbicide and herbivory? Does herbicide alter selection patterns on induced trichome production when compared to control settings?

Understanding plant defense

Plant-herbivore interactions are responsible for initial movement of energy and nutrients across systems and play a key role in maintaining ecosystem function (Lavorel and Garnier 2002). For this reason, it is crucial to study how novel environmental factors, like herbicide drift, may disrupt established dynamics. Herbivores are known to consume up to 30% of primary production in grasslands and up to 60% in seaweed forest (Hay 1991), which can negatively impact plant fitness (Simms and Rauscher 1987). As a result, many species have developed traits that reduce herbivore damage and increase survival and/or reproductive success – i.e., resistance traits (Fritz and Simms 2012). However, the evolution of such traits is often constrained by the expense of diverted resources from other functions such as future growth, reproduction, and resistance against other external stressors (Coley et al 1985, Simms and Triplett 1994).

Understanding the processes that inform plant-herbivore interactions has been a major goal of evolutionary ecologists, and many of the long-standing models predict tradeoffs under diverse conditions (Rhoades 1979, Coley, Bryant and Chapin 1985, Herms and Mattson 1992). Tradeoffs refer to the consequence of reducing the quality or quantity of one factor in exchange for increasing or obtaining another factor in order to maximize effectiveness under given conditions (Roff and Fairburn 2007). In Origin of Species (1859) Darwin explains, “The whole organism is so tied together that when slight variations in one part occur, and are accumulated through natural selection, other parts become modified.” As such, balancing or trading between multiple advantageous outcomes (or strategies) is considered a defining feature of life (Futuyma & Moreno 1988). Tradeoffs are often paid in the currency of fitness, which often impose evolutionary constraints (Stearns 1989) that can influence plant-herbivore interactions in nature. The potential for constraints on the evolution of defense in the form of either trait trade-offs or fitness costs is a major theme that I examine in each of my chapters.

Furthermore, while we are starting to understand how plant defense strategies evolve in response to herbivory (Agrawal 2007, Orians and Ward 2010, Turley et al 2013), much less is known about how plant defense evolves in response to multiple simultaneous stressors. Consequently, there may exist a tradeoff in the evolutionary importance of various types of resistance. For instance, the development of herbicide resistance may be more beneficial to the survival of natural populations over herbivory resistance if there is greater selection pressure imposed by herbicides. This may occur because herbicides can significantly reduce the plant’s ability to reproduce, making it more detrimental to plant fitness than herbivory. Alternatively, populations exposed to both agents of damage may in time develop a single resistant strategy that offers protection from both types of stressors. Thus, my thesis seeks to understand the impacts herbicide stress can have on plant defense and the dynamics of plant-herbivore interactions.

Herbicide Usage

Glyphosate (active ingredient in "Roundup"), applied to agroecosystems in 37 countries annually, is the most used herbicide world-wide (Heap and Duke 2018). As a post-emergence herbicide, glyphosate is absorbed by plant leaves and roots. Its damaging effects stem from inhibition of the target enzyme, 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS) in the chloroplast, and the subsequent blockage of the shikimate pathway (Bentley and Haslam 1990). By disrupting the shikimate pathway, glyphosate inhibits the ability to synthesize aromatic amino acids as well as the production of many secondary metabolites (Malik et al 1989). As such, my second data chapter (Chapter 3) and my third data chapter (Chapter 4) examine impacts from this long-standing herbicide on the evolution of plant defenses.

Increased resistance to common herbicides such as glyphosate has led to the rise in applications of novel classes of herbicides. One such herbicide is dicamba (3,6-dichloro-2-methoxybenzoic acid) (Behrens et al 2007). Dicamba is a synthetic auxin that mimics the deformative and growth-inhibiting effects caused by the overdose of the natural auxin, indole-3-acetic acid (IAA). The deregulation of plant growth by auxin herbicides are caused by 3 phases: first by a stimulation phase in the leaves, second by the inhibition of growth of root and stem cells, then lastly, the accelerated foliar senescence by the destruction of membrane and vascular integrity. (Grossmann 2010). As a herbicide reported to drift particularly far away (Behrens and Lueschen 1979), my first data chapter (Chapter 2) explores the potential impacts of dicamba drift on plant-herbivore interactions.

Study System

I conducted various experiments on the annual invasive Abutilon theophrasti, commonly referred to as velvetleaf. First introduced to Pennsylvania and Virginia from China in the late 17th century, velvetleaf was cultivated as a fiber crop for the purpose of building rope, bags, and fishnets. Despite its common use in Asia, 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 corn (Zea mays) and soybean (Glycine max) fields around southwestern Canada, Europe, and the United States (Spencer 1984). Studies in South Dakota (Scholes et al 1995) and Pennsylvania (Werner et al 2004) suggest that velvetleaf can account for maximum annual losses of 33% and 37% on corn and grain, respectively. Annual herbicide cost of this species has been estimated at $55.00/ha in corn fields alone (Werner et al 2004), with an estimated 82.5 million acres of corn fields being treated with herbicide within the US (USDA 2021). The success of this competitive ruderal is largely due to reproductive plasticity including self-pollination capabilities (Warwick and Black 1985), prolific seed production (can produce >8,000 per plant), seed dormancy for 50+ years (Spencer 1984), chemically defended seed coats (Paszkowski and Kremer 1988), and reduced susceptibility to herbicides (Murphy and Lindquist 2002).

Velvetleaf is a highly self-pollinating, hexaploid species with 2n = 42 chromosomes, and with high levels of fixed heterozygosity as a result of polyploidy. Studies on velvetleaf accessions in North America reveal variation in life history strategies that correlate with the latitudinal range and environmental conditions of populations. For instance, southern populations found in North America produced smaller amounts of larger seeds that were less dormant than northern populations (Warwick and Black 1985). Velvetleaf has also been shown to exhibit resistance or decreased susceptibility to multiple herbicide classes including atrazine (Anderson and Gronwald 1991) and glyphosate (Hartzler and Battles 2001). A study conducted on glyphosate mechanisms of toxicity in velvetleaf showed that glyphosate, which travels through the phloem, translocates from source structures, such as old leaves, to sink structures, such as new leaves, stems, and root tips. These patterns of translocation from source to sink structures may contribute to the diversity in the degree of herbicide susceptibility and resistance in velvetleaf, as reduced translocation and sequestration has been identified as resistance mechanisms which often combine with other mechanisms such as target-protein mutations and gene amplifications (Sammons and Gaines 2014).

In addition to resistance to herbicides, velvetleaf has traits that aid in the resistance to herbivory. These traits include trichomes on the plant surface, which produced the velvet-like feeling from which the common name originates. Velvetleaf produces four polymorphs. Two are multicellular, glandular forms that synthesize flavonoids chemicals in leaf cell vacuoles such as anthocyanin, quercetin, kaempferol, and myricetin, (Morris and Wang 2013). The glandular types include peltate trichomes, which are globular structures composed of 4-5 cells, and capitate trichomes, which are stalked structures composed of 12-15 cells. There are two non-glandular trichomes – single and branched, which are structures that grow perpendicular to the plant surface, or single cell structures consisting of 4-8 arms, respectively (Figure 1). The combination of these trichome polymorphs serve as a physical and chemical resistance against herbivory (Sterling et al 1987). There is also growing evidence that trichomes can aid in defense against abiotic stressors such as radiation (Tattini et al 2000), drought (Boughalleb and Hajlaoui 2011), and elevated CO_2 (Karowe and Gribb 2011). Although it has been hypothesized as a potential herbicide resistance mechanism by limiting absorption into the plant body (Devine et al 1992, Baucom 2019), little is known about the role of trichomes in agricultural regimes. As a species exhibits resistance to both herbicides and herbivory, velvetleaf is a powerful study system to explore the interplay of resistance strategies to these two types of damage causing agents and investigate the evolutionary constraints of plant defense.

Thesis overview

In chapter 2, I perform a multi-year study to investigate the impact of dicamba drift on plant-herbivore interactions at the intersection of natural and managed ecosystems. Following dicamba application on velvetleaf, I observed and recorded herbivory damage and herbivore abundance in the field. I find that plants exposed to dicamba drift had a significant increase in the abundance phloem feeding silverleaf whitefly (Bermisia tabaci). Further, I identify tradeoffs between herbicide resistance and herbivory resistance in year two of this study. In order to investigate the cause of the increased whitefly abundance, I conducted a follow-up greenhouse study in which I measured leaf chlorophyll content after dicamba exposure. This work reveals a positive relationship between whitefly abundance and chlorophyll content.

In chapter 3, I focus on plant traits with known importance for herbivore resistance – trichomes, or hair-like epidermal plant cells. I perform a series of experiments to uncover whether induced trichome production contributes to herbicide (glyphosate) resistance and determine its adaptive significance. I observe maternal line averages of constitutive trichomes and compare it to trichome inducibility in the field. I identify a tradeoff between lines that have highly constitutive trichome defenses and lines that have highly induced trichome defenses. I also observe induced trichome traits (polymorphs and density) in response to glyphosate exposure. I find that herbicide resistance was correlated with induced total trichome production and induced increase of branched trichomes. I also find that selection favored induced total trichome production and intermediate plant growth.

In chapter 4, explore the potential for dual or conflicting roles in trichome traits as it relates to herbicide and herbivory resistance. Here, I expose velvetleaf to glyphosate to observe trichome traits (polymorphs and density) associated with both herbicide and herbivory resistance and determine if glyphosate exposure alters the pattern of selection on trichome traits. I find that a greater proportion of branched trichomes was correlated with both herbicide and herbivory resistance. In addition, I find that glyphosate imposes marginally positive selection on branched trichomes and reveal that glyphosate is an agent of selection on velvetleaf trichomes.

Finally, in Chapter 5, I synthesize the results from all three of my data chapters and discuss future directions needed to address remaining gaps in our knowledge. Additionally, I include a series of supplemental figures and tables to accompany each chapter.

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Chapter 2 Dicamba Drift alters Plant-Herbivore Interactions

2.1 Abstract

Natural populations evolve in response to biotic and abiotic changes in their environment, which shape species interactions and ecosystem dynamics. Agricultural systems can introduce novel conditions via herbicide exposure to non-crop habitats in surrounding fields. While herbicide drift is known to produce a variety of toxic effects in plants, little is known about its impact on non-target wildlife species interactions. In a two-year study, we investigated the impact of herbicide drift on plant-herbivore interactions with common weed velvetleaf (*Abutlion theophrasti*) as the focal species. The findings reveal a significant increase in the phloem feeding silverleaf whitefly (*Bermisia tabaci*) abundance on plants exposed to herbicide at drift rates of 0.5% and 1% of the field dose. We also identified a significant phenotypic tradeoff between whitefly resistance and herbicide resistance in addition to whitefly resistance and relative growth rate in the presence of dicamba drift after increasing the populations grown in year two. In a follow-up greenhouse study, we found evidence that dicamba drift at 0.5% of the field dose significantly increased average chlorophyll content (mg/cm^2) along with a positive correlation between whitefly abundance and chlorophyll content. Overall, these findings suggest herbicide exposure to non- target communities can significantly alter herbivore populations; a response that can potentially impact biodiversity and community dynamics of weed populations found at the agro-ecological interface through shifts in energy and nutrients cycling.

Keywords: agroecology, eco-evolution, ecology, herbicide drift, plant-insect interactions, plasticity

2.2 Introduction

Biotic and abiotic interactions influence species assemblage and evolution throughout ecosystems (Thompson 1999, Klanderud et al. 2015). Often a source of atypical abiotic factors, agricultural practices are largely dependent on pesticide use for increased crop yield and labor efficiency (Gianessi 2013). Despite the economic benefits of herbicides for weed control, however, their use can lead to unintentional impacts on non-target wildlife if the herbicide ‘drifts’ or migrates outside of the target area during or after the initial application (Freemark and Boutin 1995, Carlsen et al. 2006). Though herbicide drift is known to produce a variety of toxic effects on non-target vegetation (Marrs et al. 1991, Fletcher et al. 1996, Marrs and Frost 1997, Gove et al. 2007, Boutin et al. 2014, Cederlund 2017), and can lead to significant plant phenotypic and compositional changes (Iriart, Baucom, and Ashman 2020), few studies have evaluated how herbicide drift may disrupt or influence the interactions between plants and other community members such as pollinators and herbivores.

Weeds and other non-crop plants found at the edges of agricultural fields serve as important reservoirs of insect biodiversity (Egan and Mortensen 2012), which provides crucial ecosystem services for agriculture such as pollination and pest control (Daily 1997). The plant-insect interactions that occur at the agro-ecological interface are key determinants for the movement of energy and nutrients as well as drivers of ecological and evolutionary dynamics (Ehrlich and Raven 1964, Futuyma and Agrawal 2009). However, weed communities at the agro-ecological interface can be exposed to herbicide drift rates between 0.1% and 5% of the normal field application rate of herbicide (Cessna et al. 2005), and we currently understand very little about how this drift may influence ecological interactions and potentially lead to subsequent evolutionary responses. For example, does herbicide drift change herbivore abundance or damage, and/or lead to altered patterns of plant investment in resistance to either herbicide or herbivore damage?

One herbicide known for non-target damage is dicamba (3,6-dichloro-2-methoxybenzoic acid). Dicamba is a broadleaf selective synthetic auxin that is on an upwards trajectory in agriculture due to the increased adoption of dicamba-tolerant crops (USDA-ERA, 2019) and is evident by a 600% increase of use in the US between 2014 and 2019 (USGS - NAWQA, 2021). Dicamba is absorbed by plant leaves and mimics the deformative and growth-altering effects caused by overdoses of the natural plant auxin, indole-3-acetic acid (IAA) (Grossman 2010). Dicamba drift can cause a shift in plant and arthropod diversity (Egan et al. 2014), delay the onset of flowering, reduce the number of flowers, and even reduce the amount of pollinator visits (Bohneblust et al. 2016). However, we have very little information on how dicamba drift may impact plant-herbivore interactions. A broad expectation is that exposure to a synthetic auxin, such as dicamba, could alter plant allocation towards growth and defense, and potentially lead to increased vulnerability to herbivores, in line with growth-defense tradeoff framework (Coley, Bryant, Chapin 1985). Recent work has shown that exposure to drift rates of herbicides like dicamba can lead to the evolution of reduced herbicide sensitivity (Vieria et al. 2020) and recurrent selection of low dose herbicides can lead to non-target site resistance (Busi et al. 2013), however, there is little understanding of how this process may influence selection on allocation towards herbivory defense.

Here we examined the impact of dicamba drift on plant herbivory, dominated by naturally occurring populations of the phloem feeding silverleaf whitefly (*Bermisia tabaci*) in the field, using the crop weed velvetleaf, *Abutilon theophrasti*. First introduced to the United States from Asia in the late 17th century, this annual species is now one of the most common broad-leaved species in and around corn and soybean agricultural fields located in Canada, Europe, and the United States (Spencer 1984). While past work in Amaranthus hybridus has shown that lines with evolved herbicide resistance (defined by survival given application of herbicide at the recommended field dose) can exhibit reduced herbivory resistance depending on light availability (Gassman 2005), there is a striking lack of information on how such phenotypic tradeoffs evolve when populations are exposed to drift rates under natural field conditions. We performed field experiments to address this gap in our knowledge, and specifically asked the following questions: Does the application of dicamba drift influence herbivory in the common weed *Abutilon theophrasti* (velvetleaf)? Does the amount of herbicide damage, which reflects resistance to herbicide, influence the amount of herbivory damage, which reflects resistance to herbivory? (i.e., is there a trade-off between these two forms of defense in the presence of dicamba drift)? Is either herbicide or herbivore resistance negatively correlated with relative growth rate, indicating a growth- defense tradeoff? Is there evidence of selection acting directly on herbicide resistance, herbivory resistance, or relative growth rate or evidence of correlative selection acting on the interactions of these focal traits? Does herbicide drift impact leaf chlorophyll levels, and if so, does that correlate with whitefly abundance?

2.3 Methods

Field Experimental Design – We performed two consecutive field experiments to characterize the extent of herbicide drift damage and herbivory response in *A. theophrasti*. In the first experiment, we planted replicate seeds from 23 maternal lines originally sampled from a single population (Dexter, MI) in the summer of 2018. We increased the number of populations from 1 to 8 for a total of 50 maternal lines in the second field experiment (Appendix S1: Fig S1), including the 23 maternal lines from the originally sampled population and 2-5 maternal lines from the subsequently sampled populations. All seeds were collected in the Fall prior to each summer in and around soybean fields located in Dexter, MI.

In the first field experiment (2018), we scarified and planted 276 total seeds in a randomized block design (23 maternal lines x 3 treatments x 2 replications x 2 blocks) with each treatment randomized within each block, and two replicate plants per maternal line present within each treatment/block combination. Our three experimental treatments were two levels of a drift exposure (1% and 0.5% of the recommended field dose of dicamba (3,6-dichloro-2-methoxybenzoic acid) (2.8 g ae/ ha dicamba, 5.6 g ae/ha dicamba) and water as the control. For the second field experiment (2019), we scarified and planted 450 total seeds in a randomized block design with the same treatments as the previous field experiment (50 maternal lines x 3 treatments x 3 blocks). Treatments were randomized in each block, and one replicate of each maternal line was present within each treatment/block combination. We recorded leaf count, height, and largest leaf width for all plants biweekly in both experiments. We recorded flower count weekly and sampled all seeds at the end of the season for an estimate of fitness. Five weeks after seed germination, when the average plant height was 11 cm tall, we applied 1% and 0.5% dicamba to plants in each respective treatment with a hand-held pressurized sprayer for roughly 3 seconds which ensured adequate coverage of the entire plant.

Herbicide Damage – We assessed herbicide damage two weeks following herbicide application by recording the number of leaves exhibiting visual deformation (curling or cupping), and the proportion of plant damage was determined by evaluating the number of leaves with a cupping or curling pattern divided by the total number of leaves.

Herbivory Damage – To estimate resistance to herbivory in 2018, we assessed both physical damage from chewing insects and the abundance of phloem-feeding insects (dominated by silverleaf whiteflies). Because there was little evidence of chewing insects, only whitefly abundance was assessed in 2019. We assessed chewing herbivory damage five weeks after herbicide application using the imaging software, Fiji ImageJ version 3.0 (Schindelin et al. 2012). To do this, we collected 3 leaves at random from each plant and scanned them using the Canon CanoScan L110. To obtain the total surface area, we converted each image to a binary format and used the particle analysis tool which quantifies total pixel number into centimeters. We then used a macro plug-in to place a grid with 0.1cm2 spacing over each image to estimate the amount of chewing herbivore damage per leaf.

Visual estimates of whitefly abundance were sampled in both field experiments by selecting 10 leaves at random per plant. Because most whiteflies feed and oviposit at the same location (Van

Lenteren and Noldus 1990), estimates of percentage of leaf area covered by whitefly oviposits were captured by visually partitioning each leaf into quarters and evaluated for each section, as suggested in Johnson et al 2015. We estimated whitefly abundance on a scale from 0 – 5 (adapted from Banks 1954). A score of zero meant no whitefly oviposits were present, one represented 1 - 20% leaf coverage by whitefly oviposits, two represented 21 – 40% leaf coverage, three represented 41 - 60% leaf coverage, four represented 61 - 80% leaf coverage, and five represented 81 - 100% leaf coverage.

Photosynthetic carbon dioxide response – To determine if there was a link between herbicide resistance and photosynthetic response to dicamba drift, we estimated photosynthetic efficiency by examining variation in photosynthetic carbon dioxide response curves (A-Ci) using the Li-Cor 6400 portable photosynthesis system (Open System Vers, 4.0, Li-Cor, Inc., Lincoln NE) in 2018. During gas exchange measurements, we maintained cuvette conditions at a photosynthetic photon flux density (PPFD) of 1500 µmol/ m2/s, air flow rate at 500 µmol/s, and leaf chamber block temperature being 30°C to match ambient air temperature. In order to measure the gas exchange, we first set the CO2 concentration reference at 400 µmol/mol, and we maintained the leaf under such conditions for ten minutes for adaptation and stabilization of leaf photosynthesis. We set a loop of changing reference CO2 concentration at 400, 300, 200, 100, and 50µmol/mol. We controlled CO2 concentration in the cuvette with a CO2 mixer across this series and measurements were recorded after equilibration to a steady state. We then set another loop of changing reference CO2 concentration at 400, 600, 800, 1000, 1300, and 1500 µmol/mol and again logged at each iteration. Between each group of measurements, we set the reference CO2 concentration back to 400 µmol/mol for 5 minutes. These measurements were made four to five weeks after herbicide application, within a ten-day period. We recorded these data on a leaf area of 2 cm2, from 2 leaves on each of three plants from each treatment. For plants growing in drift treatments, we included one leaf that developed prior to herbicide application (Leaf 1), and one leaf that developed after herbicide application which as a result exhibited a deformation in shape (Leaf 2). The purpose of taking measurements on leaves with different times of development was to investigate if potential herbicide sequestration impacts photosynthetic efficiency of leaves exhibiting damage, potentially impacting herbivore resistance mechanisms for the entire plant. Measurements for each leaf per plant were taken on the same day.

Greenhouse Experimental Design – With the goals of understanding treatment effects on whitefly abundance (i.e. impacts on host-plant selection), we conducted a greenhouse experiment in the summer of 2021. We planted 6 replicates of 20 maternal lines randomly selected from 5 of the populations used in the 2019 field experiment totaling 240 seeds (20 maternal lines x 6 experimental replicate seeds x 2 treatments). Half of the plants were directly treated with dicamba at 0.5% of the field dose, while the other half was treated with water 4 weeks following planting. In preparation for herbicide application, plants were placed in rows outside of the greenhouse in order to prevent herbicide drift. We subsequently applied the herbicide solution in the same manner as in the field experiments and relocated the plants back into the greenhouse once they had visually dried. We elected to treat plants with the herbicide one week prior to the field experiment due to observed accelerated growth in the greenhouse. Because whiteflies are common pests in many greenhouses, and the origin of the whitefly population in the field had previously been connected to crops transplanted from the greenhouse in plots adjacent to our own, we elected to allow whiteflies to naturally migrate to velvetleaf plants without manipulation or introduction.

Chlorophyll Content – We estimated whitefly abundance with the same methodology as above. Since whitefly host selection has previously been linked to leaf wavelength emissions (Husain and Trehann 1940, Mound 1962), using the atLEAF Plus Digital Chlorophyll Meter, we measured the chlorophyll levels (mg/cm2) of 3 leaves per plant chosen at random and calculated the average for each plant.

2.4 Data Analysis

Herbivory Response to Drift

We conducted all statistical analyses in R studio (version 3.4.1, R Development Core Team). For each type of herbivory measurement in the field experiments (chewing damage and whitefly abundance), we performed analysis of variance to determine whether herbivory resistance differed in response to dicamba application for each year separately. Prior to performing ANOVAs, we transformed response variables using Tukey's Ladder of Powers in the rcompanion package (Mangiafico 2016) to correct for non-normality of residuals. In 2018 (n = 118), we fit the following mixed linear model using lmer function of the lmer4 package (Bates et al. 2011):

y = u + treatment + block + (treatment x block) + (1|maternal line) + (1|maternal line x treatment) + e where, y, the response variable, is chewing damage or whitefly abundance, u is the intercept or mean of the model, treatment and block are fixed-effect terms, maternal line and maternal by treatment are random effect terms. In 2019 (n = 240), we nested maternal line into population and added population as a random effect to the existing model: y = u + treatment + block + (treatment x block) + (1|population) + (1|population: maternal line) + (1|population: maternal line) x treatment + e. We determined the significance of the predictor variables using F-statistics for the fixed effects with Kenward-Roger to estimate the degrees of freedom and used a log-likelihood ratio test to estimate $\chi 2$ for the random effect. We then used a Welch two sample t-test to determine if there were significant differences between the two dicamba drift treatments.

Plant Performance in Response to Drift

To determine how drift treatments influenced plant performance in the field, we ran separate mixed linear models as described above by year. In 2018, we fit separate models for each plant trait (height, leaf count, leaf width, and flower number): y = u + treatment + block + (treatment x block) + (1|maternal line) + (1|maternal line x treatment) + e; where each trait was the response variable, block, treatment, and block by treatment interactions were fixed effects, and maternal line, and maternal by treatment interactions were random effects. In 2019, we nested maternal line into population and added population as random effects to the existing model: y = u + treatment + block + (treatment x block) + (1|population: maternal line) + (1|population: maternal line) x treatment + (1|population) + (1|population) x treatment + e. We transformed each trait again using Tukey’s Ladder of Powers to meet the assumptions of normality. We performed separate analyses for each year because preliminary analysis revealed a significant difference in plant growth between years.

Additionally, photosynthetic carbon dioxide response means were analyzed for 2018 using a Kruskal-Wallis test with treatment as the independent variable. We performed exact Wilcoxon rank sum tests on photosynthetic carbon dioxide response means of individuals grown in each drift treatment separately to determine if time of leaf development impacted photosynthetic carbon dioxide response among leaves with and without deformation by including leaf developmental time as the independent variable.

Genetic Variation for Herbicide Resistance

We examined herbicide resistance for each year separately and were explicitly interested in determining if there was evidence for genetic variation for resistance to herbicide drift via a population or maternal line by treatment interaction. We fit the following separate mixed linear models for proportion of plant damaged from herbicide: y = u + treatment + block + (treatment x block) + (1|population: maternal line) + (1|population: maternal line) x treatment + (1|population) + (1|population) x treatment + e; where each type of resistance was the response variable, block, treatment, and block by treatment interactions were fixed effects, and maternal line, maternal by treatment interactions, population, and population by treatment interactions were random effects. We determined if there was evidence of genetic variation for herbicide resistance and whitefly resistance by performing log-likelihood ratio tests to estimate $\chi 2$ for maternal, maternal line by treatment, population, and population by treatment interactions for each resistance type. Finally, because we are interested in potential growth-defense trade-offs, we used a similar mixed linear model to examine genetic variation in relative growth rate.

Operational Definition of Resistance

We define herbicide resistance operationally as 1 minus the proportion of leaves exhibiting damage from the herbicide, which was observed as yellowing and curling/deformation of leaves. Because there was limited damage from chewing herbivores, we elected to focus on phloem-feeding herbivory, dominated by whiteflies. Whitefly resistance was defined as 1 minus the amount of whitefly larvae coverage per individual (as described previously).

Phenotypic Correlations

In order to test for correlations between herbicide resistance and whitefly resistance (defined operationally as above), we performed Pearson's correlation test. We examined correlations for each experimental year separately.

Phenotypic Selection

In accordance with the Lande–Arnold approach (e.g., Arnold et al. 2001; Arnold 2003; Hereford et al. 2004), we used phenotypic measurements to quantify natural selection on whitefly resistance, herbicide resistance and relative growth rate. Here, we report selection gradients rather than differentials to understand whether direct selection is acting on individual traits by controlling for any indirect selection present. Relative fitness was calculated as the final seed count per individual divided by mean seed count for each treatment per year (2018: n = 118;

2019: n = 240). We estimated linear (directional) selection gradients (β) within drift environments (0.5% and 1% the field dose of dicamba) by performing multiple linear regressions of relative fitness on whitefly resistance, herbicide resistance, relative growth rate (calculated as change in leaf count plus change in height divided by the number of weeks spent growing) and their interactions separately for each year. We also estimated nonlinear selection gradients (γ) in a full model that included linear terms, quadratic terms, and the cross-product terms of focal traits. Quadratic regression coefficients were doubled to estimate nonlinear selection gradients. Nonlinear selection gradients examined the potential for selection on phenotypic variance of a trait (quadratic selection) or phenotypic covariance (correlated selection) between focal traits, as there is evidence of relationships between plant stress, defense, and growth (Huot et al. 2014, Zust and Agrawal 2017). For all selection analysis we mean standardized focal traits (i.e., subtracted the mean and divided by the standard deviation) and used untransformed values of relative fitness. Furthermore, we estimated linear and nonlinear selection on whitefly resistance in the absence of herbicide drift in order to determine whether herbicide altered the pattern of selection. We compared selection gradients between the two treatment types using an ANCOVA to perform a regression for relative fitness on whitefly resistance, treatment, and their interaction.

Greenhouse Experiment - Chlorophyll Content

To investigate the effects of dicamba on chlorophyll content in the greenhouse, we performed analysis of variance on the plants grown in the greenhouse. We fit the following simple linear model: y = u + treatment + e; where, y, the response variable, is chlorophyll average, u is the intercept or mean of the model, and e is the error term. In order to test for correlations between chlorophyll averages and whitefly abundance, we performed Pearson's correlation test.

2.5 Results

Herbivory Response to Drift Exposed Host

We found no evidence that dicamba drift influenced the amount of chewing damage experienced by *A. theophrasti* (Fig 1a; Appendix S1: Table S1) but we did find a significant increase in whitefly abundance on dicamba treated plants (F-value = 12.01, p-value < 0.01, Fig 1b; Appendix S1: Table S1). This effect was also present in the second field season in which we included five more *A. theophrasti* populations (F-value = 5.30, p-value < 0.01, Fig 1c; Appendix S1: Table S1). Both levels of dicamba drift -- 0.5% and 1% of the suggested field dose -- exhibited higher whitefly abundance than the controls. Plants exposed to 0.5% dicamba had significantly higher whitefly abundance than 1% dicamba treatments across both years (2018: t = 2.74, p < 0.01, Fig 1b; 2019: t = 2.06, p = 0.04, Fig 1c).

Plastic Response to Herbicide Drift on Plants

We examined several plant traits in response to dicamba drift in order to determine how drift impacts velvetleaf growth and physiology, which may underscore herbivory responses. Across both experimental years, we found that leaf count increased following the application of dicamba drift (Treatment effect for 2018: F-value = 28.01, p-value < 0.01, Fig 2; Table S2; 2019: F-value = 5.11, p-value < 0.01, Fig 2; Appendix S1: Table S3), whereas leaf width decreased significantly in 2018 but not 2019 (Treatment effect: 2018, F-value = 8.25, p-value < 0.01, Fig

2; Appendix S1: Table S2; 2019: F-value = 0.79, p-value = 0.46, Fig 2; Appendix S1: Table S3), which may be a result of significant population variation in 2019 for leaf width ($\chi 2 = 5.11$, p-value = 0.02, Appendix S1: Table S3). Both plant height and flower count decreased as a result of dicamba drift in both experiments (Treatment effect: Height 2018: F-value = 220.56, p-value < 0.01; Flower Count 2018: F-value = 7.28, p-value < 0.01, Fig 2; Appendix S1: Table S2; Treatment effect: Height 2019: F-value = 2.84, p-value = 0.06; Flower Count 2019: F-value = 6.92, p-value < 0.01, Fig 2, Appendix S1: Table S3). Typically, plant leaf number was greater, and height and flower count were lower in the 1% dose of dicamba drift compared to the 0.5% dose (Fig 2). We found evidence for block effects and block by treatment effects in the first field experiment across most phenotypic traits (Appendix S1: Table S2), but these effects were less evident in the second field experiment (Appendix S1: Table S3). Finally, we found no evidence for maternal line or population effects associated with dicamba drift exposure, indicating that at least in this sample of 50 maternal lines, there was no evidence that the plastic response for height, leaf count, leaf width, and flower count varied genetically.

Photosynthetic carbon dioxide response (A-Ci, umol/m2), which was examined in the 2018 experiment, differed across treatments, indicating that dicamba drift significantly impacts photosynthetic carbon dioxide response overall (x2 = 37.605, p < 0.001; Appendix S1: Fig S2). Within drift treatments, we found evidence that time of leaf development significantly impacts A-Ci curves (0.5%: W = 696, p < 0.001; 1%: W = 360, p < 0.001; Appendix S1: Fig S2). In general, leaves exposed directly to 0.5% of the field dose of dicamba exhibited higher A/Ci responses than their counterparts that developed after dicamba treatment (Appendix S1: Fig S2).

In contrast to the lower dosage, leaves treated directly with 1% of the field dose of dicamba exhibited lower A/Ci responses than their counterparts that developed after dicamba exposure (Appendix S1: Fig S2). This result indicates high amounts of photosynthetic plasticity in response to different dicamba drift levels, potentially associated with altered herbicide translocation and metabolism contributing to resistance to drift damage.

Chlorophyll Content

In the greenhouse, we uncovered a significant treatment effect on chlorophyll levels (mg/cm2), where plants exposed to dicamba at drift levels had on average higher chlorophyll levels than the controls ($F = 4.56$, $p = 0.03$, Fig 3a). We also identified a significantly positive correlation between average chlorophyll values and whitefly abundance ($r = 0.25$, $p < 0.001$, Fig 3b), suggesting that chlorophyll content impacts whitefly host-selection on velvetleaf.

Genetic variation for whitefly resistance, herbicide resistance, and relative growth rate

We did not detect evidence of maternal line, maternal line by treatment, population, or population by treatment effects on whitefly resistance, herbicide resistance or relative growth rate in either year (Appendix S1: Table S4; Appendix S1: Table S5). This suggests there is not significant genetic variation in the populations we sampled from, likely due, at least in part, to the low number of maternal lines included in the study.

Phenotypic correlations

We did not detect evidence of phenotypic correlations between any of the focal traits in 2018 (i.e. herbicide resistance, whitefly resistance, and relative growth rate), but in 2019 we found evidence of a moderately strong negative phenotypic correlation between herbicide resistance and whitefly resistance within drift environments ($r = -0.32$, $p > 0.001$). This suggests that both types of resistance may be indirectly impacting one another. We also found a negative correlation between whitefly resistance and relative growth rate in the presence of drift but not in its absence in 2019 (drift environment: $r = -0.22$, $p = 0.02$; control environment: $r = -0.07$, $p = 0.58$), indicating a tradeoff between growth and herbivory defense when exposed to herbicide drift.

Phenotypic selection

In 2018, we did not identify evidence of linear selection on herbicide resistance nor whitefly resistance (herbicide resistance: $\beta = -0.19$, $p = 0.57$; whitefly resistance: $\beta = 0.08$, $p = 0.30$; Table S4) in the herbicide drift environment, though we did identify selection acting on relative growth rate ($\beta = 1.71$, $p < 0.001$; Appendix S1: Table S4). We further detected marginal evidence of quadratic selection acting on whitefly resistance ($\gamma = -0.19$, $p = 0.09$, Appendix S1: Table S4) and marginal correlative selection acting on whitefly resistance and relative growth rate ($\gamma = 0.82$, $p = 0.07$, Table S4). In 2019, we again found no evidence of linear selection on herbicide resistance and whitefly resistance in the herbicide drift environment and identified positive selection on relative growth rate (whitefly resistance, $\beta = 0.002$, $p = 0.99$; herbicide resistance, $\beta = 0.05$, $p = 0.72$; relative growth rate, $\beta = 2.17$, $p < 0.001$; Appendix S1: Table S5). Patterns of correlative selection differed in 2019 compared to the 2018 experiment in that we detected marginal evidence of correlative selection acting on herbicide resistance and relative growth rate ($\gamma = 0.66$, $p = 0.08$, Appendix S1: Table S5). Overall, though we found a significant negative phenotypic correlation between resistance to herbicide and whitefly herbivory, indicating a trade-off between the two types of resistance (at least in 2019) but we found no evidence of correlative selection acting on the two traits in either year (2018: $\gamma = 3.36$, $p = 0.14$; Appendix S1: Table S4; 2019: $\gamma = 0.29$, $p = 0.46$; Appendix S1: Table S5).

2.6 Discussion

Ecological effects -- dicamba drift increases whitefly abundance and alters plant morphology and physiology

Our study provides the first evidence that dicamba drift can increase phloem feeding herbivore abundance in the field, a finding that should be of concern to agriculture more broadly given the negative effects of whiteflies on crops (e.g., viral transmission mediated by whitefly - Hogenhout 2008, Legg et al. 2014, Ning et al. 2015, Sundararaj et al. 2017, Moodley 2019). In two separate field experiments, velvetleaf exposed to dicamba drift showed higher whitefly abundance in comparison to their control counterparts. These findings indicate dicamba drift can increase susceptibility to phloem feeding herbivorous insects such as whitefly, which is aligned with previous work showing increased abundance of English green aphids feeding on dicamba treated barley in a glasshouse experiment (Hintz 1971).

Additionally in the second year of our study, we found evidence of significant phenotypic tradeoffs between whitefly resistance and herbicide resistance as well as between whitefly resistance and relative growth rate in the presence of drift. Growth rate has long been linked to investment towards herbivory defense, suggesting that when resources are limited competition favors fast-growing plant species that allocate less towards herbivore defenses (Coley, Bryant, and Chapin 1985). In the literature, this is evident by positive correlations between growth rate and herbivory damage (Coley 1987, Cebrian and Durate 1994, Fine et al. 2006) and negative correlations between biomass accumulation and defense chemicals, such as salicylic acid (Meyer et al. 2007), a plant hormone specifically involved in defense against phloem-feeding silverleaf whiteflies (Kempema 2007). Such negative correlations suggest that pools of herbivore defenses can be depleted as strong growth occurs under certain environmental conditions. While our results reveal no correlation between growth rate and herbicide resistance, a significant correlation between growth rate and whitefly resistance when under drift induced stress supports the growth-defense tradeoff expectations of depleted reservoirs of herbivore defenses in the presence of high growth.

Because plant architecture is known to influence herbivore performance and abundance (Jaenike 1978, Haysom and Coulson 2004, Schlinkert et al. 2015), we assessed the morphological and physiological response of velvetleaf to dicamba drift. While our results indicate dicamba at drift levels stunts velvetleaf height, this synthetic auxin also increased the number of leaves produced, although they were typically smaller in size compared to non-sprayed control plants. Additionally, we examined the impact of dicamba on A/Ci response curves, as photosynthetic capabilities are linked to natural auxin presence (McAdam et al. 2007) and are a key driver for plant metabolism. The pattern of A/Ci response differed between drift treatments and time of leaf development, indicating a high amount of photosynthetic plasticity which varied over herbicide exposure and time. These findings align with a recent study that showed dicamba exposure initially decreased photosynthesis by 22% in Palmer amaranth, which then improved over time (Browne et al. 2020). Moreover, there was no evidence of maternal line nor population variation for morphological or physiological responses to herbicide drift, suggesting a high level of plasticity among all traits measured across individuals.

Natural auxins are involved in chlorophyll accumulation (Yuan et al 2018). However, the addition of synthetic auxins, such as dicamba applied at 50% of the field dose (280g ae) causes greater than 70% reduction in chlorophyll content (Turgut 2007). Our results show that dicamba applied at drift rates (0.5% the field dose, i.e., 2.8g ae) can increase the average amount of chlorophyll in plants which could impact host- selection of whitefly. These findings are consistent with recent studies in which whitefly species laid more eggs on leaves with higher chlorophyll and higher nitrogen (Park et al. 2009, Tsueda 2014) due to the highly linked nature of chlorophyll content and chloroplast thylakoids, which represent a large proportion of leaf nitrogen content (Evans 1983, Evans 1989), but conflicts with past work reporting whitefly attraction to yellow plants (Mound 1978, Van Lenterern and Noldus 1990). This suggests that color alone may not be the primary indicator for whitefly host-selection with every species. Although further studies on this dynamic are warranted, our results suggest that one mechanism behind the increase of whiteflies on dicamba-drifted plants is due to the auxin-induced increase in chlorophyll content of leaves.

Evolutionary effects -- correlated evolution between resistance and relative growth rate

In addition to morphological and physiology responses, we also examined the potential that resistance to both dicamba drift and whitefly damage could evolve within these populations and investigated the potential for correlative selection between traits since we identified significant negative phenotypic correlations between herbicide resistance and whitefly resistance along with whitefly resistance and relative growth rate in 2019. In 2018, we detected marginal significance for correlative selection acting on whitefly resistance and relative growth rate. Correlative selection was also marginally detected in 2019 between relative growth rate and herbicide resistance in the presence of drift. While this could imply populations may evolve to an optimum fitness at intermediate values of relative growth rate, whitefly resistance and herbicide resistance, we did not uncover evidence of genetic variation within these populations. We further did not uncover significant maternal line variation for resistance traits, likely due to high amounts of plasticity within this species and/or an insufficient number of individuals per maternal line per treatment. Environment may also be a factor as previous studies in Amaranthus hydrius have shown that the fitness cost of herbicide resistance associated with increased herbivore susceptibility can be environmentally dependent (Gassmann 2005). Although we did not detect maternal line variation, more work here should be done investigating selection acting on the relationship between herbicide resistance, whitefly resistance, and relative growth rate with a greater number of maternal lines from more distant populations and environments under consideration.

2.7 Conclusion

Herbicide drift from synthetic auxins can shift herbivorous insect host-selection at the agro-ecological interface. Such modifications of herbivore behavior have the potential to impact plant community composition and nutrient dynamics (Schowalter 2006, Belovsky & Slade 2000) and may likewise, reciprocally influence insect herbivore populations. Increases in insect herbivore abundance may result in positive feedbacks where elevated consumption increases nutrient cycling and thus stimulates insect population growth, which can directly affect the abundance of other members of the community such predators and pollinators (Forkner & Hunter 2000, Hunter 2001, Ceulemans 2017).

Furthermore, our finding that dicamba drift significantly increases whitefly abundance on velvetleaf populations could mean dicamba-treated weeds act as a reservoir for whitefly populations, potentially leading to negative impacts on agricultural yields. If our results are applicable more broadly, plants exposed to dicamba drift in nature may be preferred host for whitefly populations. As agricultural pests that colonize more than 600 host plants globally (Byrne and Bellows 1991), whiteflies are known vectors for transmitting over 70% of the world’s plant viruses (Hogenhout 2008). Given the projected expanded use of dicamba, there is a clear and urgent need to examine to what extent community dynamics may shift as a result of dicamba use, especially for communities existing at the intersection of natural and managed vegetative systems.

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2.9 Figures

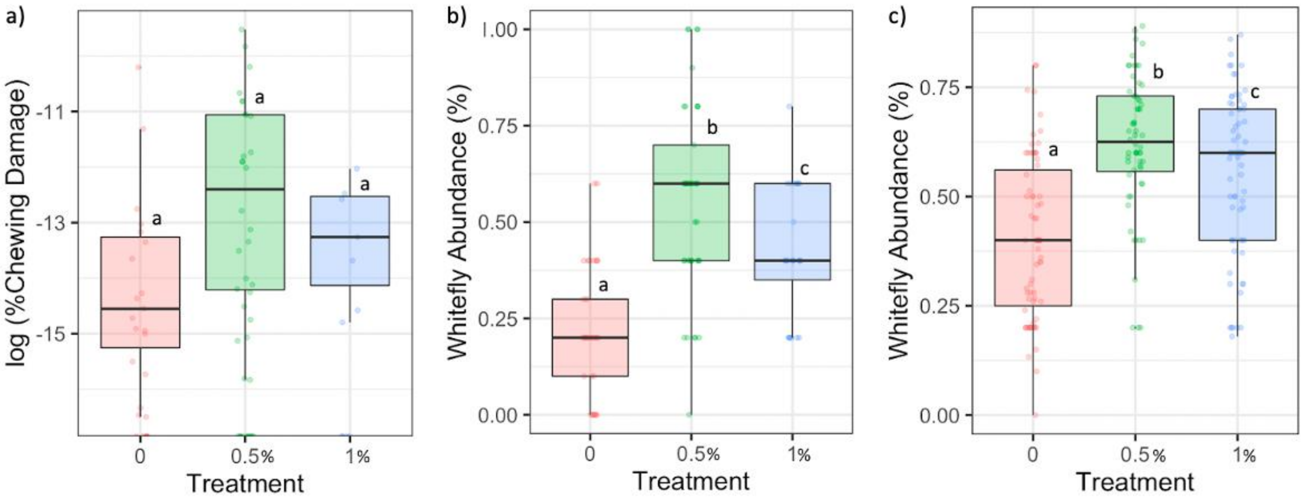

Figure 2-1. Chewing damage (a) and whitefly abundance (b and c) measurements in response to dicamba drift: 0% field dose (peach), 0.5% field dose (green), and 1% field dose (blue). a) Chewing herbivory damage summarized by dicamba treatment from the first field experiment in 2018. Damage was measured as area with chewing damage/total leaf surface area. Treatment effect on whitefly abundance summarized by treatment in both the b) 2018 and c) 2019 field experiments. Whitefly abundance was measured as visual estimates of percent larvae area/total leaf surface area. Each graph illustrates median values and confidence intervals.

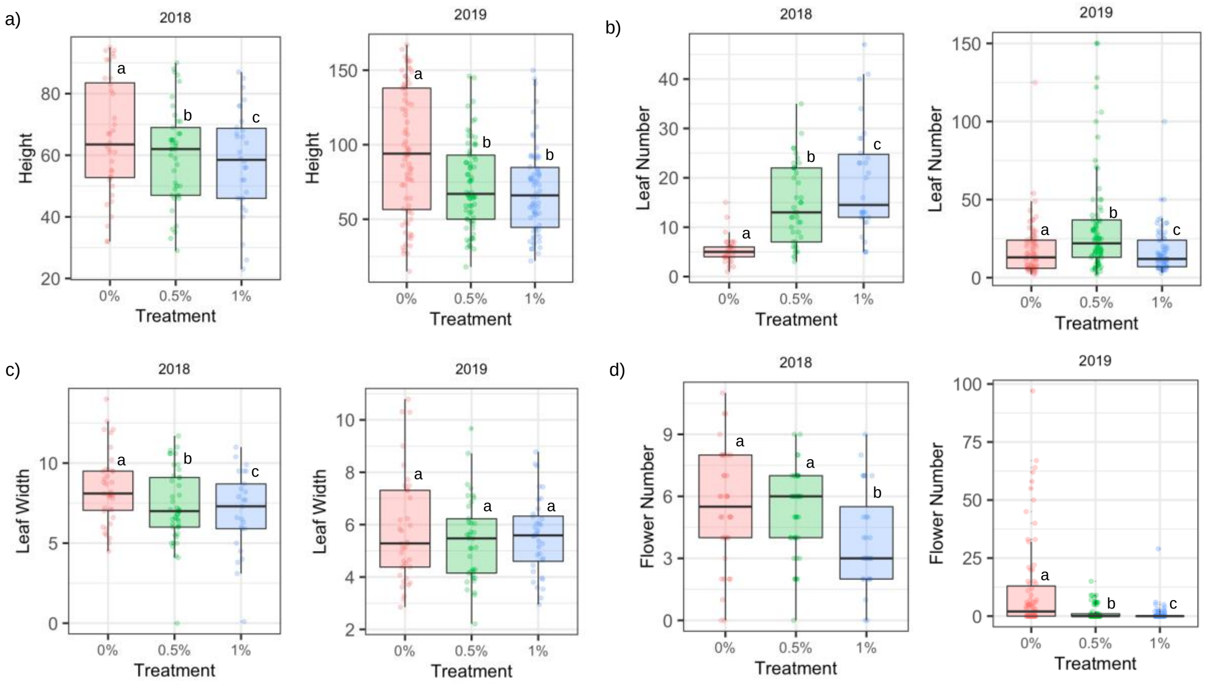

Figure 2-2. Plant size measurements in response to dicamba drift environments: 0% field dose (peach), 0.5% field dose (green), and 1% field dose (blue). Shows treatment effect for both years on velvetleaf traits: a) height b) leaf count c) leaf width and d) flower count. Each graph illustrates median values and confidence intervals.

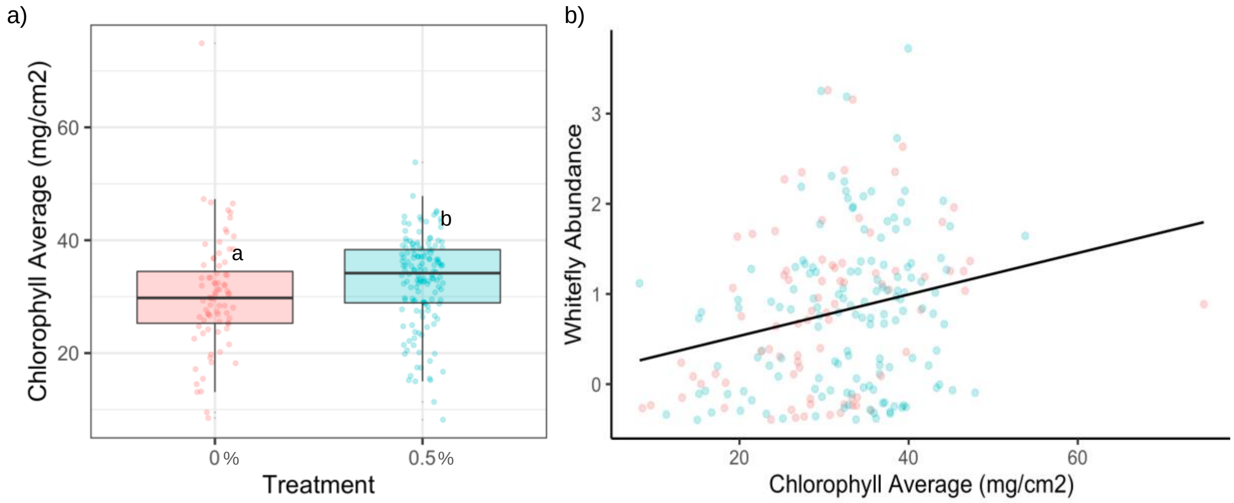

Figure 2-3. Greenhouse Experiment a) Average chlorophyll content per individual in response to dicamba application of 0.5% of the field dose (F = 4.56, p = 0.03). b) Relationship chlorophyll average and whitefly abundance estimated as Pearson product-moment correlation; Control treatment (peach) r = 0.34, p = 0.002; Drift treatment (blue) r = 0.21 p = 0.01. Each graph illustrates median values and confidence intervals.

Chapter 3 Building a Barrier: Investigating the Defensive Role of Induced Trichome Production against Herbicides

3.1 Abstract

Phenotypic plasticity is ubiquitous and considered a defining feature of life. Induced plant defense is commonly hypothesized to be an adaptive response to environmental heterogeneity reflecting phenotypic costs and benefits. In accordance with optimal defense theory, selection is expected to favor defensive strategies that maximize resource allocation towards growth and reproduction. 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. Here, 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 the herbicide "Roundup"). Over a series of experiments conducted on annual invasive weed (*Abutilon theophrasti*), we observed trichome traits (proportion of polymorphs and density) and examined potential constraints on adaptability. We found a significant tradeoff between constitutive (always present) and inducible (plastic) strategies for total trichome production. We identified positive correlations between induced total trichome production 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 velvetleaf as a response to herbicide exposure in agroecosystems and identifies significant constraints on resource allocation impacting the evolution of induced trichome defense.

Keywords: agroecosystems, phenotypic plasticity, plant defense, abiotic stressor, herbicide resistance

3.2 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 defense responses, which include chemical or structural changes in plants following exposure to damaging agents such as herbivores, pathogens or chemicals, are commonly hypothesized to be an adaptive form of plastic defense response and are typically studied in 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 of optimal defense theory is that defense trait evolution 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 optimal defense 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: Do induced trichome phenotypes serve as an herbicide resistance trait? Is there a tradeoff between induced (activated by damage in the field) and constitutive (without damage in the growth room) trichome phenotypes, representing a potential constraint on the evolution of defense? Are there fitness costs and benefits associated with trichome plasticity, and/or tradeoffs between this form of defense and growth or reproduction?

3.3 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). 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). 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.

3.4 Data Analysis

Constitutive trichomes and trichome inducibility

We conducted all statistical analyses in R (version 3.4.2, R Development Core Team). 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.

Variation among induced trichomes

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:

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

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. For each trait, we removed the effects of block by performing a regression of the trait on block, and then used the residuals in each 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.

3.5 Results

Constitutive trichomes and trichome inducibility

A significant tradeoff between constitutive and inducible trichomes would indicate a constraint on investment into plant defense. Pearson's correlation test revealed a negative correlation between constitutive trichomes captured in the growth room and absolute trichome inducibility in the field (r = -0.546, p = 0.010, Fig 1), indicating the presence of a trade-off between constitutive and induced defense strategies in this species. Although there was a difference in direction for the relationship between constitutive trichomes and positively induced trichomes (r = -0.580, p = 0.228) when compared to for the relationship between constitutive trichomes and negatively induced trichomes (r = 0.353, p = 0.179), neither relationship was statically significant.

Variation among induced trichomes

Trichome production generally decreased when exposed to herbicide, but induced responses varied by maternal line. We identified genetic variation in induction the proportion of branched trichomes (χ^2 = 3.94, p = 0.047, Table 1, Fig 2), and the proportion of capitate trichomes (χ^2 = 5.33, p = 0.021, Table 1, Fig 2). We also found significant maternal line by treatment interaction for total trichome density (χ^2 = 9.73, p = 0.002, Table 1, Fig 2).

Herbicide resistance and induced trichomes

We investigated if there was a significant relationship between induced trichomes and herbicide resistance (1 – proportion herbicide damage). We found that lines that exhibited a greater induction of branched trichomes and a greater induction of total trichomes density exhibited higher levels of herbicide resistance (r = 0.44, p = 0.037, Fig 3A; r = 0.41, p = 0.050, Fig 3C). There was no evidence of a relationship between the proportion of capitate trichomes and herbicide resistance (r = -0.27, p = 0.220, Fig 3B).

Genotypic selection for induced trichomes

In the absence of herbicide, we found no evidence for selection acting upon any induced trait (branched: *S* = -0.22, p = 0.826; capitate: S = -0.38, p = 0.707, total density: S = 0.50, p = 0.625; STable 1). In the presence of herbicide, however, we found negative selection for induced capitate trichomes (*S* = -2.78, p = 0.012, Fig 4, STable 1) and positive selection for induced trichome density (*S* = 2.88, p = 0.009, Fig 4, STable 1). We found no evidence of selection on induced branched trichomes (*S* = 0.23, p = 0.824, Fig 4, STable 1) in the presence of herbicide.

In the absence of herbicide, we identified significant correlative selection acting on growth rate and induced proportion of capitate (γ = 4.91, p = 0.024, STable 2) and branched trichomes (γ = 4.50, p = 0.036, STable 2), 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 = -0.34, p = 0.869, STable 2).

In contrast, in the presence of herbicide, there was evidence for correlative selection between growth rate and induced total density (γ = 5.33, p = 0.016, STable 2), in that genotypes with the highest fitness exhibited intermediate growth and high levels of induced total density (Fig 5). There was no evidence of correlative selection acting upon growth rate and either induced branched (γ = -0.12, p = 0.953, STable 1) or induced capitate (γ = -2.68, p = 0.197, STable 2) trichomes in the presence of herbicide.

3.6 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: resistance to herbivory (Baur et al 1991, Agrawal 1999, Rautio et al 2002, Dalin and Bjorkman 2003), resistance to 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 defense 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 triggers 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 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, due to 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 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 types of 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. 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 the optimal defense hypothesis, we predicted that in the presence of herbicide, selection should favor genotypes that allocated 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 allocation cost. 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 several 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.

3.7 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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3.9 Figures

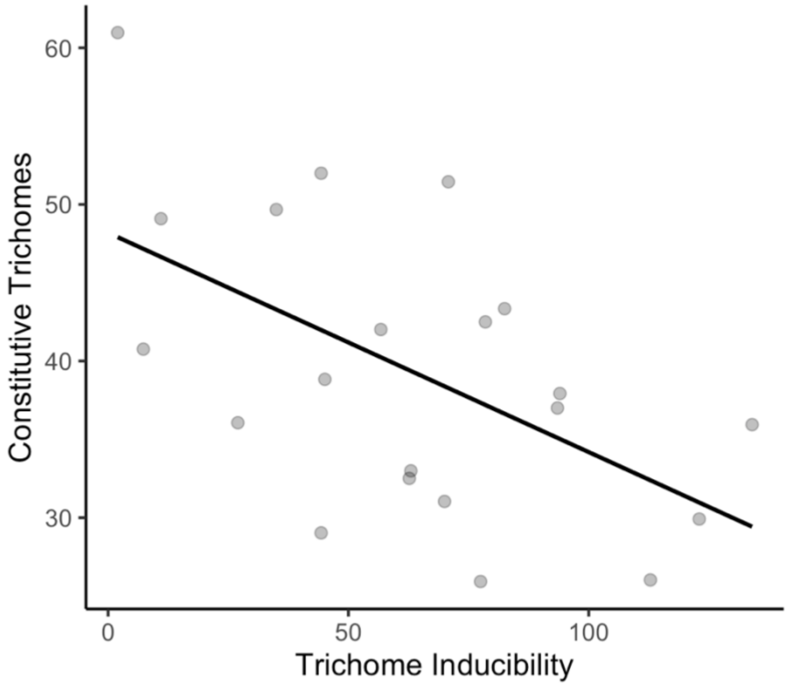

Figure 3-1. Relationships between *Abutilon theophrasti* constitutive trichomes and trichome inducibility in response to herbicide exposure. Pearson's correlation test indicates a negative relationship between the two defense strategies (r = -0.546, p = 0.010). Data points represent maternal line means; 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 and control states of maternal lines observed in the field.

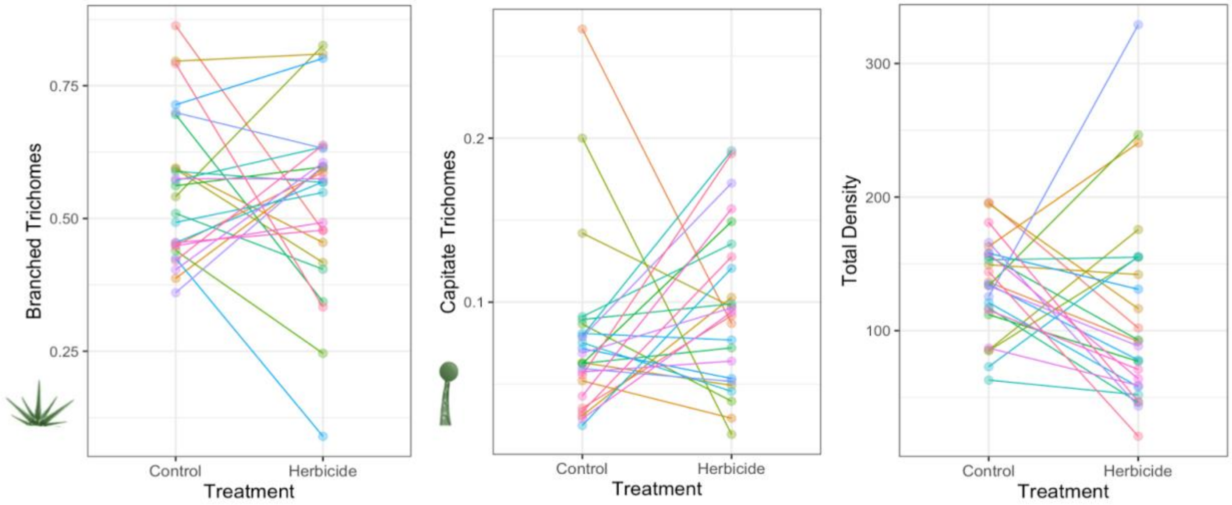

Figure 3-2. Illustration of genetic variation for *Abutilon theophrasti* maternal line by treatment effects tested using chi statistics on the proportion branched trichome ($\chi^2 = 3.94$, $p = 0.047$), proportion capitate trichome ($\chi^2 = 5.33$, $p = 0.021$), and total trichome density ($\chi^2 = 9.73$, $p = 0.002$).

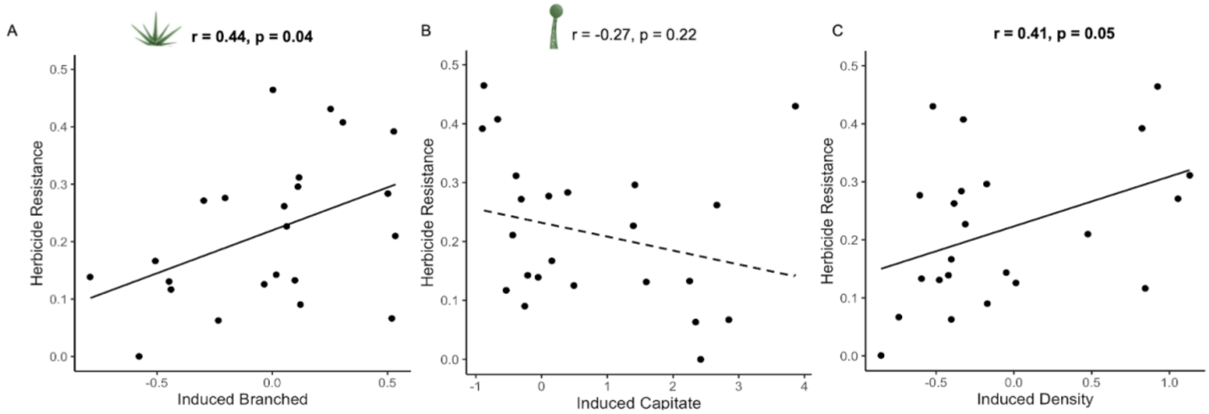

Figure 3-3. Relationships between herbicide resistance and A) induced proportion branched and B) induced proportion capitate and C) induced total trichome density for *Abutilon theophrasti*. Shown are Pearson's correlation coefficients; significant correlations are indicated in bold. Data points represent 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.

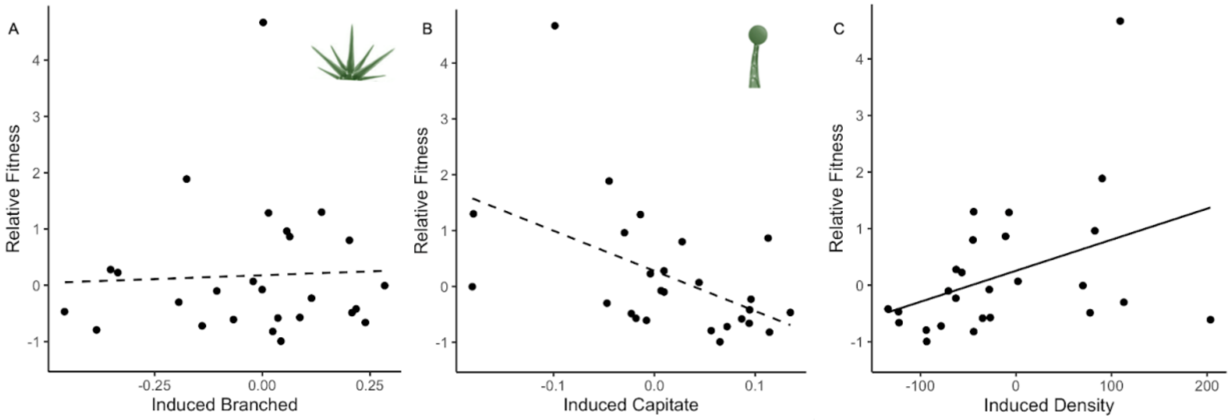

Figure 3-4. The relationship between relative fitness and A) induced proportion branched (S = 0.23, p = 0.824) and B) induced proportion capitate (S = -2.78, p = 0.012) and C) induced total trichome density (S = 2.88, p = 0.009) in *Abutilon theophrasti*. Solid lines represent selection differentials in the herbicide treatment. 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.

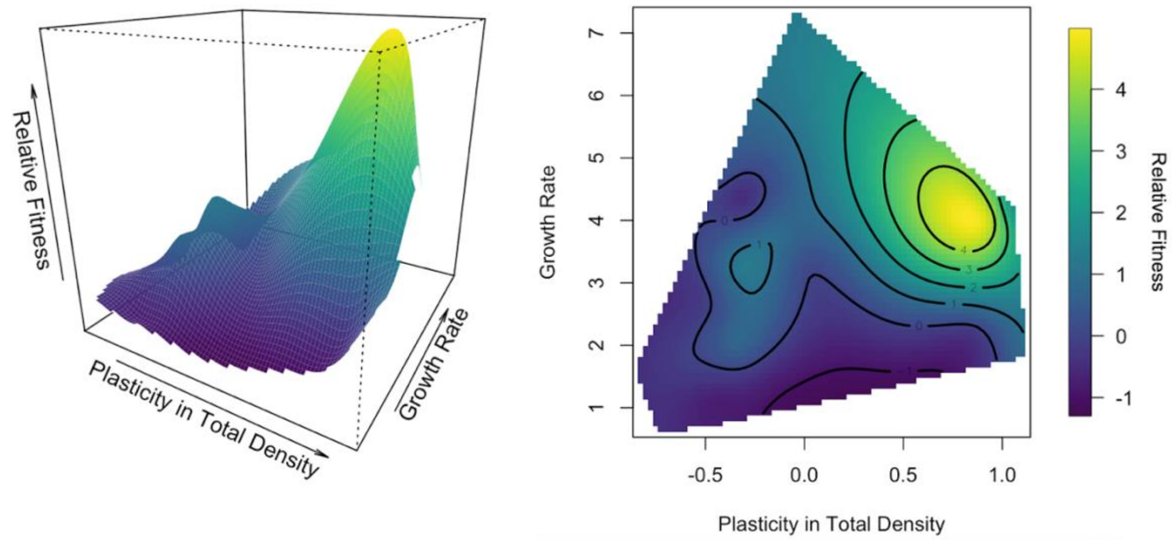

Figure 3-5. Fitness surface for correlative selection acting upon *Abutilon theophrasti* induced total trichome density and growth rate in the presence of herbicide ($\gamma = 5.33$, $p = 0.016$), tested using non-linear selection gradients (multivariate selection). Relative fitness is depicted by the color gradient; yellow is the highest fitness, blue is the lowest fitness, and green is intermediate.

3.10 Table

	Maternal Line x Treatment Effects		
	$\chi 2$	df	p - values
Proportion Branched	**3.93**	**1**	**0.047**
Proportion Single	3.29	1	0.069
Proportion Capitate	**5.33**	**1**	**0.021**
Proportion Peltate	0.33	1	0.568
Total Density	**9.72**	**1**	**0.002**

Table 3-1. Results from the test for genetic variation in *Abutilon theophrasti* using chi statistics values showing the effects of maternal line by treatment variation on trichome traits (proportion branched, single, capitate, peltate, and total density) captured in the field. Significant effects are indicated in boldface.

Chapter 4 The Double Life of Trichomes: Understanding their Role in Herbivory and Herbicide Resistance

External features of organisms often serve as the first line of defense in their immediate environments. Trichomes are hair-like appendages on plant surfaces that can defend against damaging agents such as pathogens, herbivores, and UV radiation. However, it is currently unknown if the variation observed in trichome traits represents dual or conflicting roles against different types of stressors. Here, we assess whether trichomes serve as an herbicide resistance trait and how it coincides with the conventionally studied defensive strategy of herbivory resistance. In a series of experiments, we exposed the annual invasive velvetleaf (*Abutilon theophrasti*) to glyphosate (active ingredient in the herbicide "Roundup") to investigate whether trichome traits (polymorph and density) are linked to herbicide resistance and to test whether herbicide influences selection on plant trichomes. We found that an increased proportion of branched trichomes positively impacted herbicide resistance as well as chewing herbivory resistance. We also found evidence that glyphosate imposes marginally positive selection on branched trichomes in velvetleaf. Overall, our results indicate that branched trichomes can contribute to both herbicide and chewing herbivory resistance, serving as a dual structural form of resistance reducing plant injury. Our results suggest that herbicide exposure can alter the composition of plant trichomes, potentially impacting trichome-mediated defenses against various external stressors.

4.1 Introduction

Organisms cope with many types of external stressors, which can lead to wide variation in defensive traits. Often, this trait variation is a result of complementary or conflicting strategies for dealing these stressors (Simms 1992, Koricheva 2001, Fornoni et al 2004) and the relationships between defense traits and fitness in any given environment. Of the various types of defense strategies in plants (chemical toxicity, low digestibility, leaf toughness, etc.) one of the most important may be the multifaceted traits that make up the landscape of the physical boundary, since, once encountered, this boundary acts as the first line of defense and plays a critical role in how plants interact with their environment.

A key form of defense found across a range of plants on the outermost boundary are hair-like appendages referred to as trichomes (derived from the Greek word for hair: trichos). These unicellular or multicellular structures can create complex networks that respond to external cues (Werker 2000). While traditionally viewed as a defensive strategy against herbivory (Levin 1973, Stipanovic 1983, Southwood 1986), trichomes also play a protective role against other biotic stressors such as pathogens (Shepard et al 2005, Gao et al 2017) and abiotic stressors such as UV-B radiation, ground-level ozone, and drought (Yan et al 2012, Li et al 2018, Bickford 2016). Leaf trichomes have also been shown to play a role in the detoxification of heavy metals by way of compartmentalization (Küpper et al. 2000, Marmiroli et al 2004, McNear 2013) and secretion (Psaras et al 2000).

The many potential functions of trichomes likely results from different selection pressures acting simultaneously on trichome phenotypes, including their morphology and density. For example, trichomes may serve as an herbivory resistance trait, but the evolution of a particular trichome phenotype that optimizes herbivory resistance may come at the cost of increased susceptibility to other agents of damage like UV, drought, or herbicide. In this case, trichome phenotypes would play a ***conflicting*** role in plant defense, as selection for one function (*i.e.*, to reduce the fitness effects of herbivory) may contradict a different function leading to increased susceptibility to another type of damage (*i.e.*, increased susceptibility to herbicide). Alternatively, selection for increased resistance to one agent of damage may likewise lead to increased resistance to a different agent of damage, thus serving a ***dual*** defensive role (Agrawal and Fishbein 2006).

Here, we evaluate the dual or conflicting role that trichomes may serve when defending a plant to multiple stressors. The agents of damage we consider are herbivory and herbicide, both of which natural populations of plants regularly experience in agricultural ecosystems. We do so using the weed *Abutilon theophrasti*, commonly referred to as velvetleaf because of the dense layer of trichomes that blanket the surface of its leaves. This species is known for being covered in distinct polymorphs of trichomes (branched, single, peltate, and capitate; Sterling 1978), which may each play different functional roles in plant defense (Levin 1973). Furthermore, the species has evolved reduced susceptibility to multiple herbicide classes, including triazine and glyphosate (Anderson and Gronwald 1991, Hartzler and Battles 2001), but the mechanisms underlying this reduced susceptibility are presently unknown. While leaf surface morphology can influence herbicide distribution and absorption (Hess and Falk 1990, Hess 2018), the most well-studied herbicide resistance mechanisms are biochemical rather than structural (reviewed in Gaines et al 2020). Thus, despite their implication as a possible herbicide resistance mechanism (Devine et al 1992, Baucom 2019), it remains unknown if trichomes can serve as a form of structural defense against herbicide. Furthermore, just as trichome traits are subject to selection from herbivory (Valverde et al 2001) and UV or drought (Steets et al 2010, Tang et al 2020), herbicide may act as an agent of selection on trichomes. To our knowledge, however, this hypothesis has never been formally tested by examining selection on trichome traits in the presence and absence of herbicide.

To test whether trichomes are undergoing conflicting or parallel selection from different stressors, we ask the following questions: 1) Do trichomes serve as a defense trait to herbivory and/or herbicide in *A. theophrasti*? A positive correlation between trichome traits and either herbicide or herbivory resistance would provide evidence that trichomes serve as a defense trait to either respective agent of damage. We next ask, 2) Do trichomes perform a dual or conflicting defense role given exposure to herbicide and herbivory? A positive correlation between trichomes and both herbivory and herbicide resistance would provide evidence that trichomes serve a dual defensive role, whereas a positive correlation with one form of resistance but a negative correlation with the other would suggest a conflicting role. Finally, we ask 3) Does herbicide act as an agent of selection on trichome traits? Evidence of positive selection on a particular trichome phenotype would indicate that herbicide is selecting for that phenotype, whereas negative selection would indicate that herbicide is selecting against that phenotype. While manipulative experiments in other plant systems show convincing evidence that plant herbivores act as selective agents on trichomes (Mauricio and Rausher 1997), to our knowledge no such relationship has been established for trichomes and herbicide.

4.2 Methods

Study Organism

Abutilon theophrasti Medicus, (Malvaceae) is an invasive annual native to Asia and is frequently found in and around corn (*Zea mays*) and soybean (*Glycine max*) fields. In contrast to its historic past as a fiber crop for early North American colonists, *A. theophrasti* is presently a major weed species in North America as well as other temperate regions between 32º and 45º N latitude. Studies in South Dakota (Scholes et al 1995) and Pennsylvania (Werner et al 2004) suggest that velvetleaf can account for maximum annual losses of 33% and 37% on corn and grain, respectively. Annual herbicide cost of this species has been estimated at $55.00/ha in corn fields alone (Werner et al 2004), with an estimated 32.75 million ha of corn fields being treated with herbicide within the US (ISAAA, 2017). The success of this competitive ruderal is largely due to reproductive plasticity including self-pollination capabilities (Warwick and Black 1988), prolific seed production (can produce >8,000 per plant), seed dormancy for 50+ years (Spencer 1984), and reduced susceptibility to herbicides (Sattin et al 1992).

Abutilon theophrasti gets its common name, velvetleaf, from the velvety feeling produced by a soft, dense layer of trichomes on the plant surface. Velvetleaf produces four trichome types. Two are multicellular, glandular forms that synthesize and secrete metabolites. Two are unicellular and non-glandular trichomes. The glandular types include peltate trichomes, which are globular structures composed of 4-5 cells, and capitate trichomes, which are stalked structures composed of 12-15 cells. There are two non-glandular trichomes – single and branched, which are structures that grow perpendicular to the plant surface, or single cell structures consisting of 4-8 arms, respectively (Figure 1). As a species that possesses the major trichome classifications observed in nature, velvetleaf is a powerful system to study the role of trichome-mediated defense.

We consider trichome phenotypes in two main ways – first and following typical evolutionary ecology studies (Kauffman and Kennedy 1989, Soetens et al 1991, Gianfagna et al 1992, Strauss et al 2003, Castillo et al 2013, Zhou et al 2018), we measured the total density of trichomes present on the adaxial surface of the leaf. Second, we examined the proportion of trichome types (branched, single, peltate, capitate) present on the adaxial surface to account for potential investment in different trichome types across individuals. We characterized the level of herbicide (glyphosate) resistance across *A. theophrasti* populations in a growth room and used this information to determine whether the recommended (1,000 g/ha ai) or an alternative herbicide dose would be appropriate for identifying variation in a field experiment. In the field, we assessed the potential for correlations between trichome phenotypes (total density, proportion of each trichome type) and the level of herbicide and herbivory resistance and examined the pattern of selection on trichome phenotypes in the presence and absence of herbicide.

Growth Room Experiment

To evaluate the level of resistance among natural populations, we collected seeds from eight velvetleaf populations in the fall of 2017 from various soybean fields around Dexter, MI (SFigure 1), and conducted a growth room experiment in the spring of 2019 using these field collections. The growth room design consisted of 6 treatments, including five levels of glyphosate (550g/ha ai, 770g/ha ai, 1125g/ha ai, 2000 g/ha ai, and 3000 g/ha ai (N-(phosphonomethyl) glycine3) Roundup® Herbicide (RU) (Monsanto Company, St. Louis, MO) and water as the control. We scarified and planted 3,120 total seeds in a randomized design of 164 ml cone-tainers (1.5in diameter, Stuewe and Sons inc., Tangent Oregon) in the growth room (130 maternal lines x 6 treatments x 4 replications) with each maternal line randomized within each treatment. We rotated plants within flats weekly. Plants were kept on a 12/12 hr light-dark regime at a temperature of 25°C. Four weeks after seed germination, when the average plant height was 11 cm tall, we sprayed glyphosate on individuals using a handheld CO_2 sprayer, ensuring adequate coverage in each respective treatment. We harvested each plant six weeks after herbicide application to evaluate survival and the median lethal dosage (LD50), defined as the dosage of glyphosate required to kill half of the individuals for each population. To estimate herbicide resistance, we dried samples at 70°C for 3 days to get a measurement for dried biomass, a common metric for measuring herbicide resistance (Jacobs et al 1988, Vila-Aiub et al 2009, Kumar and Jha 2015).

Field Experiment

In the summer of 2019, we conducted a field experiment to determine if there was a relationship between trichome traits and herbicide or herbivory resistance and to examine whether herbicide exposure alters the pattern of selection on trichomes. We generated seeds for this experiment by self-pollinating 40 maternal lines from the populations used as controls in the growth chamber experiment. We scarified and planted these seeds in a randomized block design with each treatment randomized within each block, and three replicate plants per maternal line present within each treatment/block combination (40 maternal lines x 2 treatments x 3 blocks x 3 replicates = 720 individuals total). Five weeks after seed germination, when the average plant height was 11 cm, we applied glyphosate at 550 g/ha ai to the glyphosate treatment, as the growth room experiment revealed the most genetic variation at this application dose. This herbicide dosage was informed by the growth room study in which the 550 g/h ai treatment exhibited the most genetic variation between populations (see SFigure 2) and is a dose that natural populations might experience given herbicide drift. We collected leaves from a subset of plants for the trichome analysis (see below) and collected seeds from all plants at the end of the field season to estimate relative fitness of this annual species. Moreover, individuals that germinated but did not survive herbicide exposure were given a fitness of zero.

Trichome Phenotyping – We sampled the adaxial surface on one leaf from 2-3 haphazardly chosen plants from each maternal line and each treatment environment (herbicide/no herbicide) one week after flowering began (n = 130). We immediately brought the leaves back to the laboratory where they were preserved at 3.3°C until processing. Using a confocal laser scanning microscope (TCS SP5; Leica Microsystems CMS GmbH, D-68165 Mannheim, Germany), we captured images of leaf trichomes and quantified traits from a $3mm^2$ diameter subsection collected from the lower right quadrant of each leaf. We used a Plan Apochromat 10x/0.75 objective lens with an excitation wavelength of 488 nm and 512 x 512-pixel resolution. The confocal laser scanning microscope produced 2D image slides at 1μm increments on the z-axis plane of focus. We reconstructed these 2D sections into 3D illustrations and analyzed these images using imaging software, Fiji ImageJ version 3.0 (Schindelin et al. 2012). We applied a macro plug-in we created to recognize trichome structures and manually took counts of each type of trichome structure. Subsequently, we calculated the proportion of each trichome type by dividing the absolute number of the structure by trichome density (*i.e.*, total number of trichomes per 1 cm diameter segment). Because raw values of plant traits are more likely to be affected by a variety of factors such as size, age, and environmental conditions not captured in our study, we elected to present the proportions of trichome types rather than the raw counts as they provide a more reliable indicator of adaptive strategy. For example, the ratio of leaf area to plant biomass is a better indicator of plant photosynthetic capacity than the absolute leaf area or plant biomass alone. Here, we report total trichome density and the proportions of trichome polymorphs to understand the functional role of trichome traits as it captures the overall landscape pattern on the leaf surface.

Herbicide Damage – We measured herbicide damage in the field as the number of yellowing leaves two weeks following herbicide application and converted this to the proportion of the plant damaged by dividing the number of damaged leaves by the total number of leaves. We choose this metric for herbicide resistance for the field experiment because velvetleaf begins to release its leaves during seed maturation, thus it was not feasible to gather both biomass and fitness data.

Herbivory Damage – To estimate resistance to herbivory in the field, we quantified physical damage from chewing insects on all individuals one week before herbicide application. Thus, herbivory estimates were determined for all plants but only in the absence of herbicide. To do this, we selected 3 leaves at random from each individual (620 individuals x 3 leaves = 1,860 total leaves examined) and photographed them completely attached to the plant in the field. We used the imaging software, LeafByte (Getman-Pickering et al 2020) to calculate the area of leaf tissue consumed by herbivores. We edited the photographs by cropping and removing background noise, then uploaded them into LeafByte. To obtain the total leaf surface area along with total chewing herbivory damage, we standardized an 8cm x 8cm area around each image allowing the software to accurately capture measurements.

Operational Definition of Herbicide and Herbivory Resistance

We define herbicide resistance operationally as 1 minus the proportion of leaves exhibiting damage from the herbicide. Similarly, we define herbivory resistance as 1 minus the average amount of chewing damage across each of the 3 leaves per individual.

4.3 Data Analysis

Growth room study - Assay for herbicide resistance

We conducted all statistical analyses in R (version 3.4.2, R Development Core Team). A dose-response curve was fitted to the survival data for each of the populations, using the method of generalized log-logistic models with the binomial data implemented by the drc package (Ritz et al 2015). We estimated the logarithm of the dose which killed 50% of the plants for each population using the dose.p function from the MASS package (Venables and Ripley 2002). To uncover if there was genetic variation for herbicide resistance (measured as biomass) by treatment in the growth room, we fit the following mixed linear model using lmer function of the lme4 package (Bates et al. 2015):

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

where herbicide resistance was the response variable, u is the intercept or mean of the model, treatment was a fixed effect, maternal line nested within population was a random effect, and e is the error term. We first standardized biomass within herbicide treatments by the control and the significance of effects was determined using the lmerTest package (Kuznetsova et al 2017). Resistance was log + 1 transformed to meet the assumptions of the model.

Field experiment - Correlations and variation among trichome traits

To determine if trichome traits were correlated, we performed Pearson's correlation tests on the raw values of each of our trichome traits (single, branched, peltate, capitate, and total density).

To test for genetic variation for trichome traits, we fit the following model for each trait:

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

where each respective trichome trait was the response variable, u is the intercept or mean of the model, treatment was a fixed effect, maternal line nested within population was a random effect, and e is the error term. For each trichome trait, we removed the effects of block by performing a regression of the trait on block, and then used the residuals in each 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.

Trichome relationship to herbicide and herbivory resistance

Because the proportion of branched trichomes and capitate trichomes exhibited significant genetic variation (see Results) and because total trichome density is the most conventional trichome trait tested in the literature, we elected to perform the remaining analysis on these three traits. To determine if trichomes influence herbicide resistance in the field, we performed analysis of variance (ANOVA) by fitting a model for herbicide resistance:

$$y = u + \text{trichome trait} + e$$

where, y, the response variable, is herbicide resistance, trichome trait (proportion of branched, capitate, or total density) as the dependent variable, and e is the error term. We determined the significance of the predictor variables using *F*-statistics for trichome characteristics using the stats package described above.

We performed a similar analysis of variance (ANOVA) to determine whether patterns of herbivory resistance were altered by trichome phenotype. We fit regressions of herbivory resistance on the trait in question:

$$y = u + \text{trichome trait} + e$$

Prior to analysis, the herbivory metric was log transformed to improve normality. Because herbicide damage was only captured in the herbicide treatment and herbivory estimates were collected prior to herbicide application, treatment was excluded from the models. Preliminary analyses also indicated that there were not significant maternal line effects. We thus elected to exclude these effects from our final models.

Phenotypic Selection

We performed phenotypic selection analysis (Lande and Arnold 1983) to determine if herbicide alters selection on trichome traits. We estimated differentials to measure total selection on the proportion of branched trichomes and trichome density and gradients to measure direct selection on each. Relative fitness was calculated as the final seed count per individual divided by mean seed count for each treatment. Phenotypic traits were standardized to a mean of zero and variance of one prior to analysis. We estimated selection differentials (S) by performing univariate regressions of relative fitness on each trait separately as a measurement of both indirect and direct selection acting on each trait. We estimated linear selection gradients (β) using models only containing the linear terms as a measurement of direct selection on each trichome trait. Non-linear selection gradients (γ) were estimated using a full model containing linear terms, quadratic terms, and the cross-products terms of the focal trichome traits. By doubling the quadratic regression coefficients, we were able to examine the potential of quadratic (disruptive or stabilizing) selection or correlative selection (acting on cross-products).

Moreover, we used an ANCOVA to determine whether herbicide application significantly altered the pattern of selection on differentials. To do this, we performed a univariate regression of relative fitness on the trait in question, treatment, and their interaction, in which a significant interaction would indicate that herbicide significantly alters selection. We similarly tested whether linear and quadratic selection were significantly altered by treatment.

4.4 Results

Growth room study - Assay for herbicide resistance

Analysis of dose response curves revealed that the median lethal dosage (LD50) of glyphosate among populations was 740.63 g/ha ai, which is below the recommended field dose of 1,000 g/ha ai. We detected evidence of population differences in standardized biomass post-herbicide application ($\chi^2 = 5.01$, $p = 0.025$, SFigure 2B), indicating significant variation for glyphosate susceptibility. We found that the highest LD50 was population 4 with 1000 g/ha ai, indicating reduced susceptibility may be evolving in this species (SFig 1A). Notably, however, we did not find evidence of maternal line variation for glyphosate resistance ($\chi^2 = 0.00$, $p = 1.000$).

Field experiment - Correlations and genetic variation among trichome traits

We found that total trichome density was positively correlated with all trichome types except for capitate trichomes (branched: $r = 0.72$, $p < 0.001$; single: $r = 0.71$, $p < 0.001$; peltate: $r = 0.55$, $p < 0.001$). We also found that peltate trichomes were positively correlated with single trichomes ($r = 0.42$, $p < 0.001$) and branched trichomes ($r = 0.26$, $p = 0.002$, SFigure 3).

We examined the potential for genetic variation in trichome traits to determine which traits may evolve in response to selection from the herbicide. We detected significant maternal line variation for proportion branched ($\chi^2 = 3.96$, $p = 0.047$, STable 1) and capitate trichomes ($\chi^2 = 3.91$, $p = 0.048$, STable 1) but not the other trichome types/traits (single: $\chi^2 = 2.82$, $p = 0.092$; peltate: $\chi^2 = 0.75$, $p = 0.387$; density: $\chi^2 = 1.02$, $p = 0.313$; STable 1).

Trichome relationship to herbicide and herbivory resistance – We examined the potential for a relationship between trichome traits and the level of herbicide resistance to determine if either total trichome density, the proportion of branched or the proportion capitate trichomes on the leaf surface may act as an herbicide resistance trait. We found that the proportion of branched trichomes was positively correlated to the level of herbicide resistance (F = 5.87, p = 0.019; Fig. 2), but that total trichome density (F = 0.00, p = 0.956; Fig. 2) and the proportion of capitate trichomes (F = 0.13, p = 0.719; Fig. 2) showed no such relationship. We found that the proportion of branched trichomes had a positive effect on herbivory resistance (F = 10.20, p = 0.002, Fig. 2). Capitate trichomes (F = 0.03, p = 0.235, Fig. 2) and total density of trichomes (F = 0.22, p = 0.789, Fig. 2) were not correlated to our measure of herbivory resistance.

Phenotypic selection–We examined phenotypic selection differentials in both the control and herbicide treatments to determine if the pattern of selection on trichome traits was altered by the presence of herbicide. For the proportion of branched trichomes, the ANCOVA revealed that herbicide application significantly altered the pattern of selection (F = 8.20, p = 0.005, Table 1, Fig 3), with evidence for positive selection on the proportion of branched trichomes in the presence of herbicide (S = 2.05, p = 0.045, Table 1, Fig 3) and evidence for negative selection on this trait in control conditions (S = -1.98, p = 0.052, Table 1, Fig 3). Under control conditions, trichome density was under positive selection (S = 2.62, p = 0.011, Table 1, Fig 3), whereas there was no evidence of significant selection in the presence of herbicide (S = 1.77, p = 0.082, Table 1, Fig 3). There was no evidence of selection acting on the proportion of capitate trichomes in the presence (S = -1.28, p = 0.207) or absence of herbicide (S = -1.60, p = 0.112).

Linear selection gradients were similar to the selection differentials. We identified significant positive linear selection favoring increased trichome density in the absence of herbicide (β = 2.06, p = 0.043, Table 2) but marginally positive selection in the presence of herbicide (β = 1.38, p = 0.06, Table 2). We also found that the proportion of branched trichomes was under positive selection in the presence of herbicide (β = 2.16, p = 0.036, Table 2), and that herbicide exposure significantly altered the pattern of selection to negative on the proportion of branched trichomes (β = -1.96, p = 0.053, ANCOVA: F = 8.75, p = 0.004, Table 2). We found no evidence of selection on the proportion capitate trichomes in either the presence (β = -0.34, p = 0.622, Table 2) or absence of herbicide (β = -0.86, p = 0.393, Table 2). We did not identify evidence of quadratic selection acting upon branched trichomes, trichome density, or their interaction in the presence or absence of herbicide (STable 2).

4.5 Discussion

The goal of our study was to determine if trichome traits exhibit dual or conflicting roles in plant defense when faced with different forms of damage. Using the common weed *Abutilon theophrasti*, we found positive correlations between the proportion of branched trichomes and both herbicide and herbivory resistance, indicating that trichomes can serve a dual defensive role by acting as both an herbivory and herbicide resistance trait. We also showed that herbicide acts as an agent of selection on the proportion of branched trichomes. Our findings expand current knowledge of how plant trichome phenotypes may evolve in response to multiple stressors and highlight the importance of studying trichome traits beyond that of trichome density.

Branched trichomes in plant resistance

Though the relationship between trichomes and herbivory resistance has been well studied, to our knowledge, this is the first time that trichomes have been considered as a potential herbicide resistance trait. While we do not know how branched trichomes might function as an herbicide resistance trait, we hypothesize they may either store the herbicide or reduce its contact with the leaf surface. For example, it is possible that herbicide might be compartmentalized into trichome cell walls and vacuoles, like the compartmentalization of heavy metals into the trichomes of heavy-metal tolerant serpentine plants (Cobbett 2000, Küpper et al 2000, Broadhurst et al 2004, Marmiroli et al 2004). In support of this idea, branched trichomes have a greater vacuole volume than other trichome types (Calvert et al 1985) as the vacuole occupies 90% - 95% of the total trichome volume (Gutierrez-Alcalcaet at al 2000). Alternatively, increased amounts of branched trichomes on leaves may provide a more complex network of interweaving appendages, acting as a cohesive umbrella of sorts that holds herbicide droplets on the upper epidermal layer due to surface tension and reducing absorption into the plant altogether (*i.e.,* providing a barrier that reduces herbicide damage in the plant body, Baucom 2019).

Our study also demonstrates that the proportion of branched trichomes is correlated to chewing herbivory damage such that a higher proportion of branched trichomes is positively related to higher herbivory resistance. These results align with work in cotton showing that accessions with the highest amount of branched trichomes have the greatest resistance to spider mites (Kamel and Elkassaby 1965). Somewhat surprisingly, we did not find an association between chewing herbivory resistance and total trichome density in velvetleaf; in this way our results differ from findings from other plant species, in which individuals with a greater density of trichomes evade herbivory damage more successfully than individuals with lower densities of trichomes (Doss et al 1987, Webster et al 1994, Romeis et al 1999).

There are several possible ways in which a higher proportion of branched trichomes may mitigate damage from a range of herbivorous species. Trichomes make it difficult for the insect to access the plant body, may cause entrapment and inflict injury (Dalin et al 2008, Szyndler 2013), and inhibit herbivore growth and development via post-ingestive effects (Shanower 2008, Kariyat et al 2017). Branched trichomes in particular cover a larger surface area than other trichome shapes and may either entrap or impede insects. In our field study, Japanese emerald beetles and grasshoppers were the most common herbivorous insects feeding on velvetleaf, thus, these large insect herbivores may prefer plants with a lower proportion of branched structures to avoid their negative effects.

Dual defensive function

Environmental heterogeneity is thought to be a primary mechanism maintaining phenotypic variation (Hedrick 1986). Under this premise, we might expect defense traits that contribute to resistance against traditional forms of damages such as herbivory in one environment to differ or conflict with traits that contribute to resistance against novel forms of damage in another environment such as herbicide exposure. Instead, we find that branched trichomes are serving a dual defensive role in that they are mutually contributing to herbivory resistance and herbicide resistance. An important caveat to our study is that we did not reduce or remove herbivory when examining the potential for a relationship between trichome traits and the level of herbicide resistance. Thus, if herbivory induces an increase in the proportion of branched trichomes prior to herbicide exposure, the positive relationship we identified between the level of resistance and the proportion of branched trichomes may simply be due to induced effects from herbivore damage. While this is an important caveat of our study, a trait may offer a fitness advantage and be classified as defensive without identifying its primary function (Strauss and Agrawal 1999). In *Verbascum thapsus*, for example, trichomes on younger leaves have been identified as a defense against grasshopper herbivory but also protects against water loss in high temperatures (Woodman and Fernandes 1991). In the context of agricultural fields, plants would experience herbivory and herbicide spraying, such that even if herbivory induces a higher proportion of branched trichomes, the higher proportion present on leaves as a result would still be functionally relevant for subsequent herbicide exposures.

Adaptive significance of trichome traits

Given the importance of trichomes in mitigating effects from numerous abiotic and biotic stressors, it is important to understand the processes that promote variation in trichome traits, since selection imposed by one stressor may impact adaptation to another. We examined the potential for selection on trichome traits and focused on the proportion each of branched and capitate trichomes, since these traits exhibited genetic variation. Although we did not detect significant variation in trichome density for the populations in this study, we included analysis of trichome density since density is known to be a highly variable character in several other species (Heinz and Zalom 1995, Roy et al 1999, Valverde et al 2001).

Interestingly, our analyses revealed that selection on trichome traits differed by herbicide treatment. In the absence of herbicide, we found evidence of negative selection on branched trichomes and in the presence of herbicide we uncovered marginally positive selection on branched trichomes. This result suggests that herbicide acts as an agent of selection on the proportion of branched trichomes. Additionally, in the absence of herbicide, we found significant positive selection on trichome density and marginal positive selection on this trait in the presence of herbicide, suggesting that there is another environmental factor selecting for trichome density. We note, however, that we did not identify genetic variation in trichome density in our study population, meaning that we would not expect a response to this selection. Further, for both trichome traits, we found that the selection gradients showed the same results as the differentials indicating that selection is directly acting on these traits in the presence of herbicide, and there were no indirect effects acting on either trait. Finally, there was no evidence of correlative selection acting upon trichome density and the proportion of branched trichomes in either environment.

4.6 Conclusions

A major objective of plant evolutionary ecology has been to understand how defense strategies differ and are maintained over time and space (Feeney 1976). Though it has been proposed that the evolution of specific assortments of traits may be associated with particular environmental factors (Grime 1977, Chapin et al 1993), this framework has rarely been applied to explore the potential adaptive significance of trichome composition in complex environments (Escobar-Bravo et al 2019, Karabourniotis et al 2020). We explored how the proportion of trichome types, beyond the traditional classification of glandular and non-glandular, can provide novel insight into plant defense strategies when faced with conventional and modern damaging agents in agro-ecosystems. Moreover, in identifying variation of trichome landscapes among individuals and populations, we present an approach that may help us to better understand the evolution of plant defense.

Overall, our study reveals that branched trichomes contribute both to herbicide resistance and herbivory resistance in *A. theophrasti*. The results also suggest plants exposed to glyphosate could become better defended against chewing herbivores, since glyphosate imposes positive selection on the proportion of branched trichomes a plant exhibits, which in turn increases resistance to herbivory. If such evolution occurred, it could influence herbivore population growth, habitat choice, and subsequent multi-trophic interactions. As such, future studies should investigate whether trichome composition correlates with distance from agricultural fields and herbivore abundance over long timescales. In summary, our findings are that changes in trichome composition against one stressor may impact defense against other stressors. These changes could also have cascading effects upon other aspects of the food web. Understanding the functionality of trichomes as structural defensive traits may help us better predict ecological evolution of communities most responsive to global environmental change.

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4.9 Figures

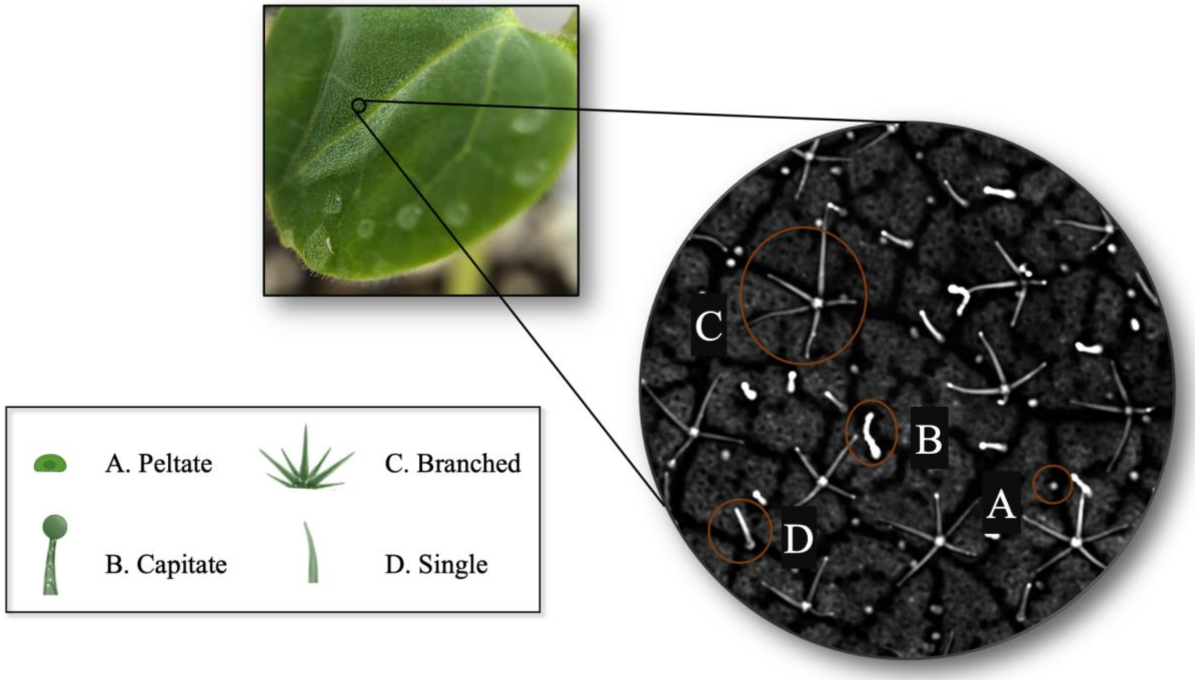

Figure 4-1. Characterization of the trichomes present on adaxial surfaces of Abutilon theophrasti leaves using confocal imaging (x10). A) Peltate trichomes are multicellular glandular structures made up of 4-5 cells. B) Capitate trichomes are multicellular glandular structures made up of 12-15 cells.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

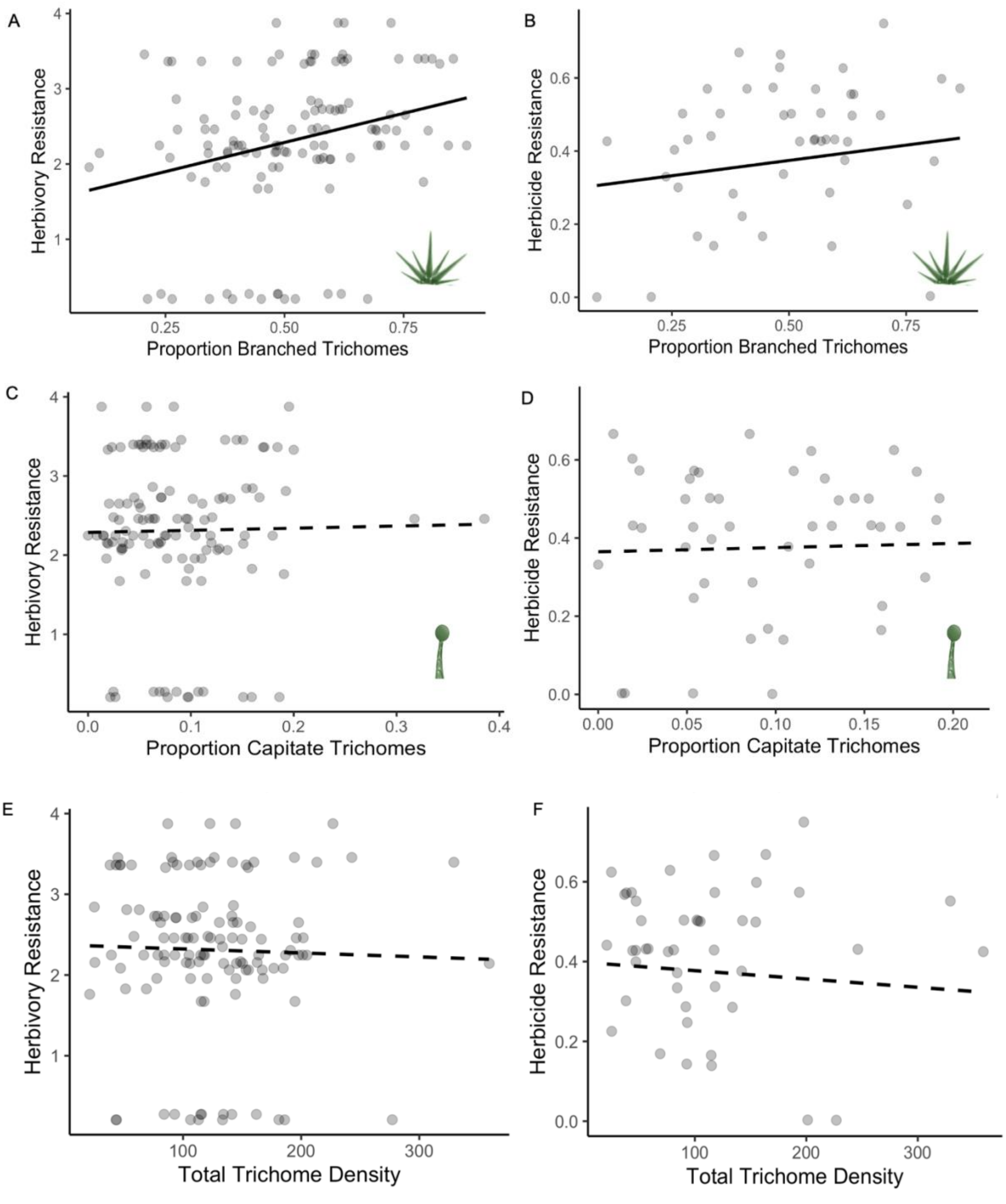

Figure 4-2. Relationships between the proportion of each trichome type or trichome density with resistance to herbivory and herbicide. A) The relationship between the proportion of branched trichomes and herbivory resistance (F = 10.20, p = 0.002) and B) proportion branched trichomes and herbicide resistance (F = 5.82, p = 0.019). C) The relationship between the proportion of capitate trichomes and herbivory resistance (F = 0.03, p > 0.05) and D) proportion of capitate trichomes and herbicide resistance (F = 0.64, p > 0.05). E) The relationship between total

trichome density and herbivory resistance ($F = 0.22$, $p > 0.05$) and F) total trichome density and herbicide resistance ($F = 0.09$, $p > 0.05$). Datapoints represent individual plants; herbivory resistance was measured as log-transformed 1 – amount of chewing herbivory damage, and herbicide resistance was measured as 1 – proportion of leaf yellowing.

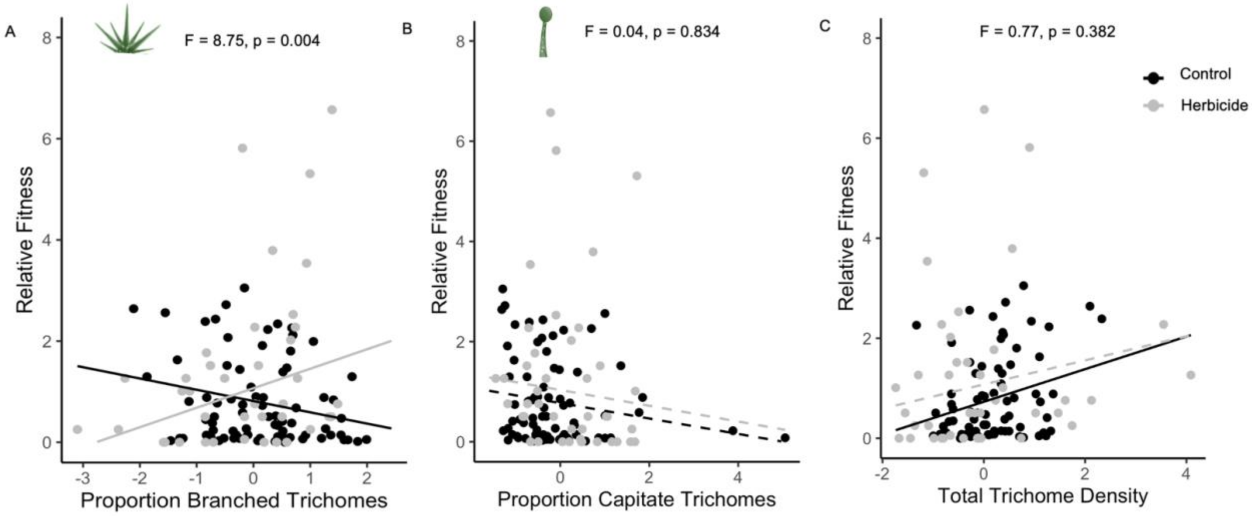

Figure 4-3. The relationship between relative fitness and A) the proportion branched trichomes, B) proportion capitate trichomes, C) and total trichome density in the presence (grey) and absence (black) of herbicide in Abutilon theophrasti in field conditions. Solid lines represent significant selection differentials in each treatment environment and F-statistics show the treatment by trait interaction from the ANCOVA.

4.10 Tables

Phenotypic Selection (*S*) Differentials							
	Control			Herbicide			**F-values (from ANCOVA)**
Trait	*S*	SE	p-value	*S*	SE	p-value	
Proportion Branched	**-1.98**	**0.10**	**0.052**	**2.05**	**0.22**	**0.045**	**8.20**
Proportion Capitate	-1.60	0.09	0.11	-1.28	0.29	0.207	0.77
Total Density	**2.62**	**0.13**	**0.011**	1.77	0.20	0.082	0.01

Table 4-1. Total selection (S) on velvetleaf trichome traits (proportion branched, proportion capitate, and total trichome density). Treatments are the absence (control) and presence of herbicide. Shown are selection differential values, standard errors, and p-values for traits in each treatment. The F-value indicates the treatment by trait interaction from an ANCOVA, and significant effects are indicated in bold.

Linear Selection (β) Gradients							
	Control			Herbicide			**F-values (from ANCOVA)**
Trait	β	SE	p-value	β	SE	p-value	
Proportion Branched	**-1.96**	**0.10**	**0.053**	**2.16**	**0.23**	**0.036**	**8.75**
Proportion Capitate	-0.86	0.09	0.39	-0.34	0.33	0.63	0.02
Total Density	**2.06**	**0.14**	**0.042**	1.38	0.24	0.17	0.07

Table 4-2. Direct (multivariate) selection acting on trichome traits (proportion branched, proportion capitate, and total trichome density) in the absence and presence of herbicide. Shown are linear (β) gradient values, standard errors, and p-values in each treatment. F-values are from the ANCOVA analysis testing the effect of herbicide treatment on selection gradients. Significant effects are indicated in boldface.

Chapter 5 Discussion and Future Directions

The objective of this dissertation is to expand our understanding of how herbicide exposure can impact plant-herbivore interactions facilitated through modified plant phenotypes with velvetleaf (*Abutilon theophrasti*) as the focal species. First, I examined how plant responses to a novel herbicide, dicamba, impacted herbivory. I then explored how a long-used herbicide, glyphosate, impacts potential plasticity and evolutionary trajectories of plant trichomes, a known herbivory defense trait. Combined, the results stemming from this thesis provides novel insight into how short-term plant-herbivore interactions are impacted by herbicide exposure and how long-term communities at the agro-eco interface may evolve.

Overall, my dissertation is one of the first to examine evidence for herbicide impacts on plant-herbivore interactions and the evolution of plant defense. In addition, this novel work reveals that plant trichomes can serve as an herbicide resistance trait. My thesis provides an insightful basis for considering how plant defense can evolve in response to one of the major global change drivers, agricultural intensification. Addressing additional gaps in our understanding of the evolution of plant-herbivore interactions will allow us to make predictions for how to best manage ecosystems that neighbor agricultural fields.

What do dicamba-mediated shifts teach us?

Communities at the edges of crops engineered to be herbicide tolerant can frequently be exposed to drift rates generally between 0.1% and 5% of field application rates (Cessna et al 2005). As a result of the introduction of dicamba tolerant crops and the increased use of dicamba in agriculture, dicamba drift symptoms have been reported in numerous crop systems, including cotton and soybean, as well as non-crop systems, including state parks and residential landscapes (ERS- USDA 2019). Given the projected increased use of this “next generation” herbicide (Wechsler et al 2019), it is vital to address the unknowns regarding the unintended consequence of drift. In **Chapter 2** I addressed this gap by examining the impact of dicamba drift on plant herbivory. Findings from this work indicate that drift-mediated changes can have subsequent effects on local herbivore populations. Specifically, I found that dicamba exposed velvetleaf had significantly higher herbivore presence dominated by naturally occurring populations of the phloem feeding silverleaf whitefly (*Bermisia tabaci*) compared to the control counterparts. I found evidence of a negative correlation between herbicide resistance and whitefly resistance in the second year of this study, suggesting that tradeoffs in defense may be environmentally regulated. While I identified marginally significant correlative selection acting on whitefly resistance and growth rate in the first year, as well as herbicide resistance and growth rate in the second year, there was no evidence of genetic variation within the populations studied. This suggests that if there is significant variation for either type of resistance and growth rate occurring in other natural populations, dicamba drift could act as a novel selection pressure influencing evolutionary trajectories.

A follow-up greenhouse experiment revealed that dicamba exposure at drift rates can increase the overall amount of chlorophyll found in the leaves. Interestingly, chlorophyll content was positively correlated with whitefly colonization. These findings align with past work which revealed that some whitefly species choose to colonize and lay more eggs on leaves with higher chlorophyll content and nitrogen levels (Park et al 2009, Tsueda 2014). Further, dicamba is a synthetic auxin that acts by mimicking natural phyto-auxins responsible for plant growth (Grossman 2010) and chlorophyll accumulation (Yuan et al. 2018).

While the deformative effects of dicamba drift are well-documented (ERS- USDA 2019), a major takeaway of this work is that dicamba drift can cause increased chlorophyll accumulation in leaves. In my work this response was linked to a greater abundance of whiteflies, a consecutive response observed over multiple experiments. Because the chloroplast thylakoids that produce chlorophyll represent a large proportion of nitrogen content found in leaves (Evans 1983, Evans 1989), it is also possible that dicamba drift impacts the nutrient quality of plant leaves. As higher nitrogen content can reduce herbivore developmental time (Bentz and Larew 1992, Jauset et al 2000), further research is needed to examine if faster whitefly developmental time is also linked to the increased abundance observed on dicamba exposed individuals. Moreover, leaf nitrogen is also correlated with host-selection in a number of herbivorous insects (reviewed in Mattson 1980). Future studies should explore whether the phenomenon of higher colonization rates and/or faster developmental time is observed in other species.

What do glyphosate-mediated shifts teach us?

Glyphosate has been the world's most commonly used herbicide in agricultural systems for the past three decades (Andert et al 2019). With dozens of weedy species evolving glyphosate resistance (Heap and Duke 2017), this herbicide has become a consistent and expanding selective agent (Powles 2008, Londo et al 2010). Though leaf surface morphology has been known to influence herbicide distribution (Hess and Falk 1990), there has been limited work to explore the role the outermost boundary of plants can play in herbicide resistance. Trichomes (derived from the Greek word for hair: trichos) are hair-like epidermal projections which can serve as the first line of defense for plants against external stressors such as herbivory (Levin 1973), UV radiation (Karabourniotis et al 1995), and drought (Ning et al 2016). In **Chapter 3** I investigate potential evolutionary constraints and adaptive value of trichome plasticity in response to glyphosate exposure. In my dissertation, I examined assumptions of the long-held theory of optimal defense (Rhoades 1979) to understand fitness benefits, costs, and constraints on trichome phenotypic plasticity that may shape its evolution.

To uncover potential adaptive significance of trichomes, I measured the total density of trichomes as well as the proportion of trichome types (branched, single, peltate, capitate) present on the adaxial surface of the leaves to account for potential variation in investment of trichome types across individuals. Phenotypic plasticity was measured as the difference of trichome traits observed in the presence and absence of glyphosate exposure for each genetic line of velvetleaf in this study. I found positive correlations between glyphosate resistance and induced total density of trichomes as well as glyphosate resistance and induced increase of the proportion of branched trichomes. These results suggest that induced total density of trichomes and induced increase of the proportion of branched trichomes can contribute to glyphosate resistance in this species. While identifying the mechanism of resistance was outside of the scope of this study, one possible explanation could be that plant trichomes are sequestering herbicide toxins. In support of this hypothesis, branched trichomes are known to have the largest vacuole storage capacity (Calvert et al 1985) such that an increased proportion of branched trichomes may offer a particular advantage over the other trichome polymorphs. Moreover, several heavy metal tolerant species are known to exhibit this method of detoxification *via* trichomes (Harada and Choi 2008, Sarret et al 2009, Quinn et al 2010). Though additional work is necessary to uncover whether trichomes can sequester herbicide toxins, these results suggest that plant trichomes may provide a novel mechanism of herbicide resistance *via* sequestration.

In exploration of evolutionary constraints, I found tradeoffs between constitutive trichomes and trichome inducibility in response to glyphosate exposure. This result indicates a limitation on resource allocation towards trichome defense, which can impede, but not prevent the evolution of adaptive trichome plasticity. Further, I found significant genetic variation for induced increase in total trichome density and correlative selection acting upon induced increase in total trichome density associated with reduced plant growth rate in the presence of herbicide exposure. While I did not find significant 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. Therefore, if glyphosate remains a consistent selective agent in agro-ecosystems, velvetleaf populations can evolve to have higher induced trichome production which comes at the expense of high growth rates. The crucial highlights from this work are that induced trichome production contributes as a mechanism for herbicide resistance and that herbicide actively selects for induced trichome production.

Core findings from Chapter 3 align with recent work which suggests that we might have been undervaluing the ecological importance of trichomes, as conventional knowledge considers these structures as a herbivory resistance trait. From radiation protection to heavy metal accumulation, we now have evidence that trichomes can contribute to a suite of functions. While this area of research is growing, there remains scant understanding of whether trichome functions work in tandem or in opposition with one another. In **Chapter 4** I explore if trichomes play a dual or conflicting role in herbicide and herbivory resistance.

Heterogeneous environmental stressors are thought to be a primary force in maintaining phenotypic variation (Hedrick 1986). Under this premise, we might expect defense traits that contribute to resistance against herbivory to differ or conflict with traits that contribute to resistance against novel forms of damage such as herbicide exposure. **Chapter 4** shows that individuals with a higher proportion of branched trichomes exhibit both lower chewing herbivory damage and lower glyphosate damage. These results demonstrate that branched trichomes can serve a dual defensive role by acting as both an herbivory and glyphosate resistance trait in velvetleaf. Moreover, I found that glyphosate exposure alters the pattern of selection on trichome traits and selects for a greater proportion of branched trichomes. Together chapters 3 and 4 indicate that individuals with a higher proportion of branched trichomes or with the ability to induce a greater proportion of branched trichomes have an advantage in the presence of glyphosate exposure. Alternative to the toxin-to-trichome sequestration hypothesis, it is also possible that genes informing trichome development may be linked to a different response not measured in this work. Future studies should work to uncover the exact processes involved in glyphosate resistance and herbivory resistance observed in this species.

What can we infer about the evolution of plant-herbivore interactions at the agro-eco interface?

Eco-evolutionary dynamics are driven by interactions between the environment and phenotypes. Research tells us that modern agricultural practices mediate some of the most well-documented environmental changes with its widespread use of herbicides (Fletcher 1956, Whiteside and Alexander 1960, Wilkinson et al 1969, Marrs and Frost 1997, Rashid et al 2010, Relyea 2012, Van Bruggen et al 2018). If the results of this dissertation are applied more broadly, populations at crop edges exposed to dicamba may experience an increase in the abundance of herbivorous species that host-select via chlorophyll content such as whiteflies. These findings should be of particular concern to agriculture given that 70% of the world's plant viruses are known to be transmitted by whiteflies (Hogenhout 2008). Additionally, populations at crop edges exposed to long-standing glyphosate may evolve to have a greater number of individuals with inducible trichomes as well as greater proportion of branched trichomes. This subsequent evolution may deter herbivorous chewing insects in the short-term and lead to insect adaptations in the long-term. Further, as dicamba and glyphosate are often applied in sequence with one another, the eco-evolutionary dynamics of communities at the agro-eco interface may experience significant long-term changes in plant-herbivore interactions.

As the upward trend of agricultural intensification continues (Tilman et al 2011), my work demonstrates that not only can herbicide exposure impact individual community members, but it can also influence species interactions. Through direct modifications to plant quality and physiology, I show that herbivore dispersal and behavior can be significantly altered. These effects on herbivore dispersal and behavior also have the potential to reciprocally impact plant community composition and nutrient dynamics (Hunter 2001, Belovsky & Slade 2000). As such, there remains a critical need to understand how herbicide induced shifts may influence the intricate balance of ecological interactions and subsequent evolutionary responses. Future research should explore the overall impacts herbicide exposure has on the interactions of multi-trophic community members to include additional plant and herbivore species, predators, and pollinators.

Closing remarks

My dissertation demonstrates that plant-herbivore interactions can be profoundly transformed by modern agriculture practices, yet there is still much to uncover how such changes influence other ecological interactions and subsequent evolutionary responses. As one of the main drivers of global change, agricultural expansion and intensification has, and will likely continue to contribute to incremental change. From what is already known, it is clear we must urgently find a balance in agriculture to feed a growing human population while preserving the natural world. Though herbicides enable increased agricultural yields, my work uncovers how herbicide exposed natural systems can be impacted presently and may evolve in the future. Some believe the path forward is to convert to completely organic (pesticide-free) agriculture, while others believe there should be a tiered process of pest management with pesticides as the last resort. Regardless of the approach, most agree that adoption of eco-evolutionary principles, which promote ecosystem balance, in decision-making is necessary for the health of the entire system, including the ecological and social/economic dimensions. As new technologies are developed, my work shows that we must prioritize solutions in which food production becomes more sustainable for the longevity of both natural and human systems.

5.1 References

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Appendices

Appendix S1. Supplementary Figures and Tables for Chapter 2

Herbivory Response	Chewing Damage (2018)		Whitefly Abundance (2018)		Whitefly Abundance (2019)	
Fixed Effects:	F	p	F	p	F	p
Treatment (df = 2)	0.906	0.410	**12.014**	**< 0.001**	**5.300**	**0.006**
Block 2018 (df = 1) 2019 (df = 2)	1.236	0.270	1.656	0.201	1.356	0.261
Treatment X Block 2018 (df = 2) 2019 (df = 4)	0.109	0.897	0.363	0.697	1.076	0.370
Random Effects:	χ2	p	χ2	p	χ2	p
Population (df = 1)					0.000	1.000
Maternal Line (df = 1)	0.498	0.480	0.000	1.000	0.535	0.464
Maternal Line X Treatment (df = 1)	0.000	1.000	0.000	1.000	0.000	1.000

Table S1- 1. Influence of herbicide treatment on chewing damage for 2018 and whitefly abundance for both years, analyzed using F-statistics values showing effects of treatment, block, treatment by block interactions, and likelihood ratio test statistics (x^2) showing maternal line variation on herbivory measurements. In 2019 maternal lines are nested within populations. Significant values are expressed in boldface.

2018								
Trait	Height		Leaf Count		Leaf Width		Flower Count	
Fixed Effects:	F	P	F	P	F	P	F	P
Treatment (df = 2)	**220.555**	**< 0.001**	**28.012**	**< 0.001**	**8.246**	**0.001**	**7.284**	**0.001**
Block (df = 1)	0.278	0.599	**7.627**	**0.007**	**6.151**	**0.014**	**5.421**	**0.022**
Treatment X Block (df = 2)	**60.804**	**< 0.001**	**15.714**	**< 0.001**	**3.554**	**0.032**	**4.287**	**0.016**
Random Effects:	χ2	P	χ2	P	χ2	P	χ2	P
Maternal Line (df = 1)	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000
Maternal Line X Treatment (df = 1)	0.000	1.000	1.986	0.159	0.000	1.000	0.054	0.816

Table S1- 2. 2018 influence of herbicide treatment on velvetleaf traits, analyzed using F-statistics values showing the effects of treatment, block, treatment by block interactions, and likelihood ratio test statistics **(χ2)** maternal line and maternal line by treatment interactions on variation of plant phenotypes. Significant values are expressed in boldface.

2019								
Trait	Height		Leaf Count		Leaf Width		Flower Count	
Fixed Effects:	F	P	F	P	F	P	F	P
Treatment (df = 2)	2.844	0.061	**5.113**	**0.007**	0.791	0.458	**6.916**	**0.001**
Block (df = 2)	0.144	0.866	0.700	0.498	2.398	0.096	1.313	0.271
Treatment X Block (df = 4)	0.551	0.699	0.506	1.697	1.763	0.188	0.470	0.758
Random Effects:	χ2	P	χ2	P	χ2	P	χ2	P
Population (df = 1)	0.000	1.000	0.632	0.427	**5.112**	**0.024**	0.397	0.528
Maternal Line (df =1)	0.000	1.000	0.000	1.000	0.000	1.000	0.166	0.684
Maternal Line X Treatment (df = 1)	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.986

Table S1- 3. Influence of herbicide treatment on velvetleaf traits, analyzed using F-statistics values showing the effects of treatment, block, treatment by block interactions, and likelihood ratio test statistics **(χ2)** showing population, maternal line, population by treatment interactions, and maternal line by treatment interactions on variation of plant phenotypes. Maternal lines were nested within populations. Significant values are expressed in boldface.

2018								
Trait:	β	SE	P	γ	SE	P	r	P
Whitefly Resistance	0.081	0.077	0.299	-0.191	0.055	0.087	0.060	0.622
Herbicide Resistance	-0.191	0.330	0.565	3.464	1.056	0.107	-0.123	0.174
Relative Growth	**1.715**	**0.218**	**< 0.001**	**3.912**	**0.558**	**< 0.001**	**0.750**	**< 0.001**
Whitefly Resistance X Herbicide Resistance				0.712	0.334	0.291		
Whitefly Resistance X Relative Growth				0.816	0.222	0.072		
Herbicide Resistance X Relative Growth				3.364	1.109	0.136		

Table S1- 4. 2018 Selection analysis showing direct selection on focal traits: whitefly resistance, herbicide resistance, and relative growth rate. Linear (β) ($R^2 = 0.510$; $p < 0.001$) and quadratic (γ) ($R^2 = 0.609$; $p < 0.001$) selection gradients with associated standard errors (SE) and P-values (P). The (r) column represents correlation coefficients between trait and fitness, estimated as Pearson product- moment correlations. Significant values are expressed in boldface.

			2019					
Trait:	β	SE	P	Y	SE	P	r	P
Whitefly Resistance	0.002	0.190	0.990	0.232	0.230	0.526	-1.56	0.098
Herbicide Resistance	0.052	0147	0.722	-0.133	0.116	0.759	-0.004	0.955
Relative Growth	**2.169**	**0.245**	**< 0.001**	0.774	0.353	0.363	**0.694**	**< 0.001**
Whitefly Resistance X Herbicide Resistance				0.285	0.190	0.455		
Whitefly Resistance X Relative Growth				-0.818	0.390	0.297		
Herbicide Resistance X Relative Growth				1.328	0.373	0.078		

Table S1- 5. 2019 Selection analysis showing direct selection on focal traits: whitefly resistance, herbicide resistance, and relative growth rate. Linear (β) ($R^2 = 0.453$; $p < 0.001$) and quadratic (γ) ($R^2 = 0.466$; $p < 0.001$) selection gradients, and total selection with associated standard errors (SE) and P-values (P). The (r) column represents correlation coefficients between trait and fitness, estimated as Pearson product- moment correlations. Significant values are expressed in boldface.

Trait	Relative Growth Rate		Herbicide Damage	
Fixed Effects:	F	P	F	P
Treatment (df = 2)	**2.431**	**0.10**	**76.464**	**< 0.001**
Block 2018 (df = 1)	7.971	0.005	107.667	1.000
Treatment X Block 2018 (df = 2)	**5.239**	**0.007**	100.918	0.340
Random Effects:	χ2	P	χ2	P
Maternal Line (df = 1)	0.000	1.000	0.000	1.000
Maternal Line X Treatment (df = 1)	0.000	1.000	0.000	1.000

Table S1- 6. 2018 Influence of treatment and block on relative growth, and herbicide damage, analyzed using F-statistics values showing the effects of treatment, block, treatment by block interactions, and likelihood ratio test statistics **(χ2)** showing, maternal line, and maternal line by treatment interactions on variation of plant phenotypes. Significant values are expressed in boldface.

Trait	Relative Growth Rate		Herbicide Damage	
Fixed Effects:	F	P	F	P
Treatment (df = 2)	0.031	0.969	**46.730**	< **0.001**
Block (df = 2)	0.323	0.724	0.006	0.994
Treatment X Block (df = 4)	0.259	0.904	**5.982**	< **0.001**
Random Effects:	χ2	P	χ2	P
Population (df = 1)	0.000	1.000	0.190	0.663
Population X Treatment (df = 1)	0.479	0.488	2.300	0.129
Maternal Line (df = 1)	0.000	1.000	0.000	1.000
Maternal Line X Treatment (df = 1)	0.000	1.000	0.000	1.000

Table S1- 7. 2019 Influence of treatment and block on relative growth, and herbicide damage, analyzed using F-statistics values showing the effects of treatment, block, treatment by block interactions, and likelihood ratio test statistics **(χ2)** showing population, maternal line, population by treatment interactions, and maternal line by treatment interactions on variation of plant phenotypes. Significant values are expressed in boldface.

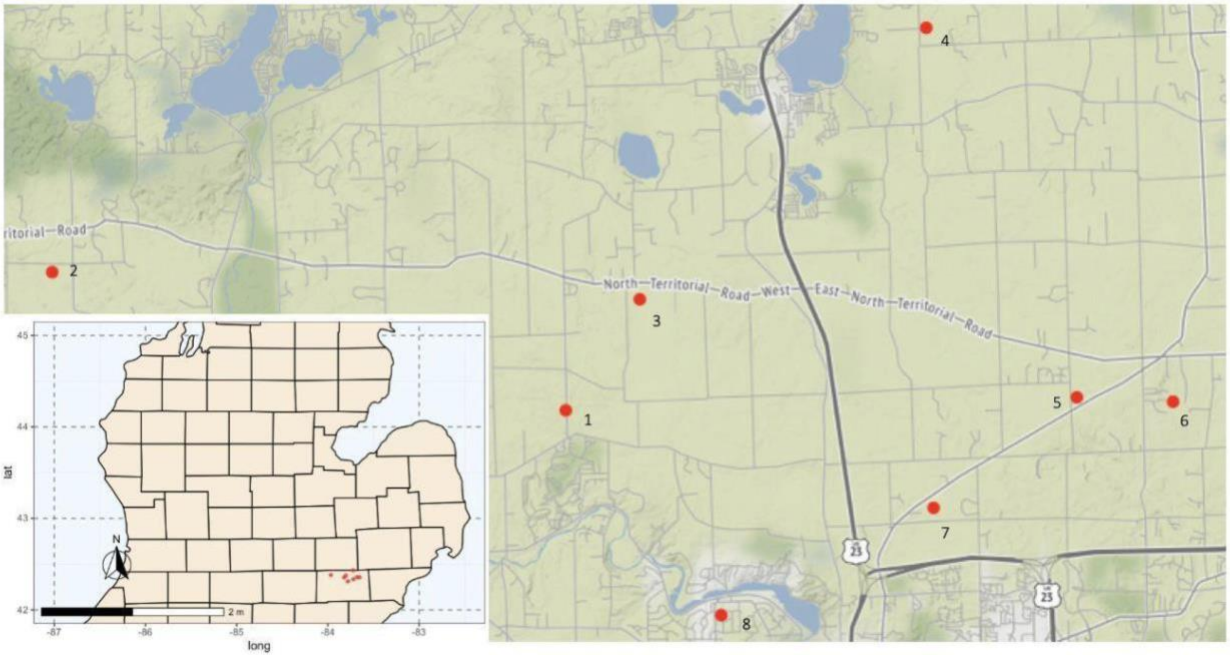


Figure S1- 1. Locations of velvetleaf populations sampled and used for this study. In 2018, the field experiment was conducted with only population one, while in 2019 the sample size was increased to all eight populations. Populations 3, 4, 6, 7, and 8 were used in the 2021 greenhouse experiment.

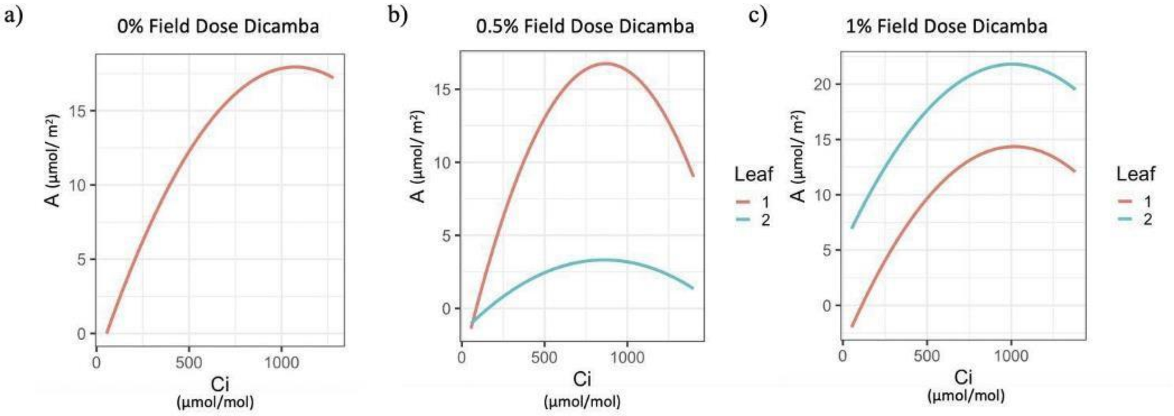


Figure S1- 2. Photosynthetic carbon dioxide response curves by drift environment. a) A-C_i curves measured on leaves grown without drift exposure b) Comparison of A-C_i curves measured on leaves that developed before drift exposure (Leaf 1) and after drift exposure (Leaf 2) at 0.5% field dose. c) and at 1% field dose.

Appendix S2. Supplementary Figures and Tables to Chapter 3

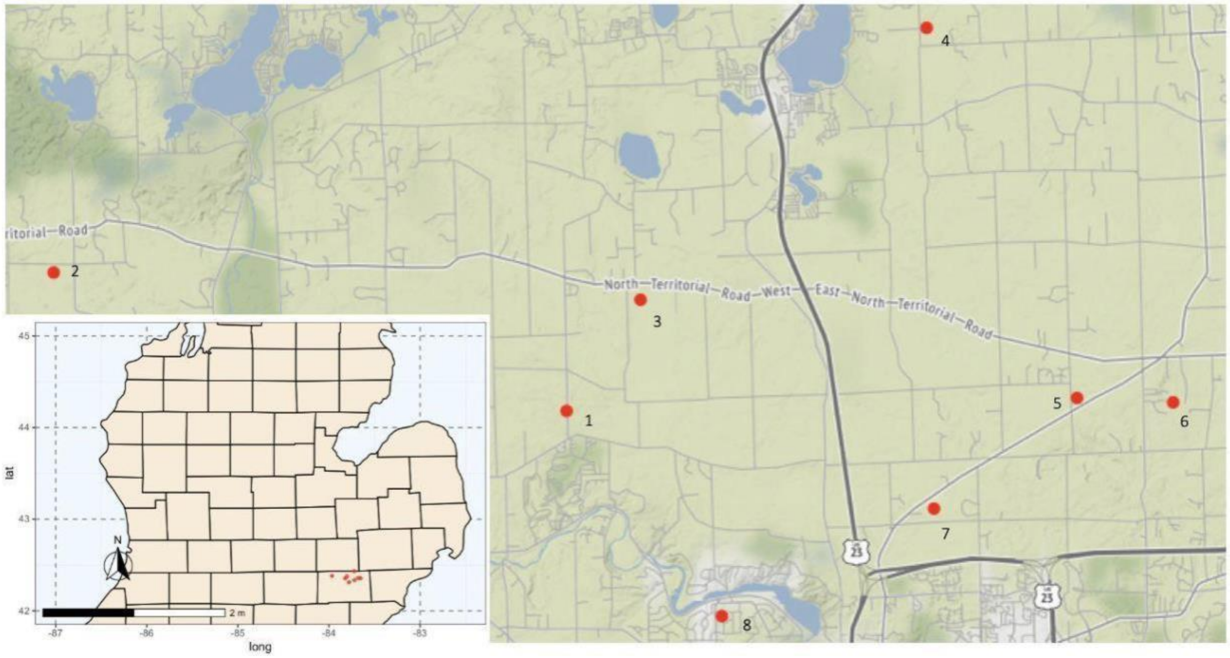


Figure S2- 1. Locations of Abutilon theophrasti populations sampled and used for this study.

PHENOTYPIC SELECTION (*S*) DIFFERENTIALS

	Control			Herbicide		
Trait	*S*	SE	p-value	*S*	SE	p-value
Induced Branched	-0.22	0.15	0.826	0.23	0.47	0.824
Induced Capitate	-0.38	0.04	0.707	-2.78*	0.11*	0.012*
Total Induced Density	0.50	0.09	0.625	2.88**	0.37**	0.009**

Table S2- 1. Total selection (S) on Abutilon theophrasti induced trichome traits (proportion branched, proportion capitate, and total density) in the absence and presence of herbicide. Shown are selection differential values, standard errors, and p-values for traits in each treatment. Significant effects are indicated with asterisks: *P < 0.05, **P < 0.01, ***P < 0.001.

NON-LINEAR SELECTION (*Γ*) GRADIENTS

Trait	Control			Herbicide		
	γ	SE	p-value	*γ*	SE	p-value
Induced Branched X Growth Rate	4.91*	0.21*	0.024*	-0.12	1.24	0.953
Induced Capitate X Growth Rate	4.50*	0.04*	0.036*	-2.68	0.27	0.197
Induced Density X Growth Rate	-0.34	0.14	0.869	5.33*	0.54*	0.016*

Table S2- 2. Correlative selection (γ) on Abutilon theophrasti induced trichome traits (proportion branched, proportion capitate, and total density) and growth rate in the absence and presence of herbicide. Shown are selection differential values, standard errors, and p-values for traits in each treatment. Significant effects are indicated with asterisks: *P < 0.05, **P < 0.01, ***P < 0.001.

Appendix S3. Supplementary Figures and Tables to Chapter 4

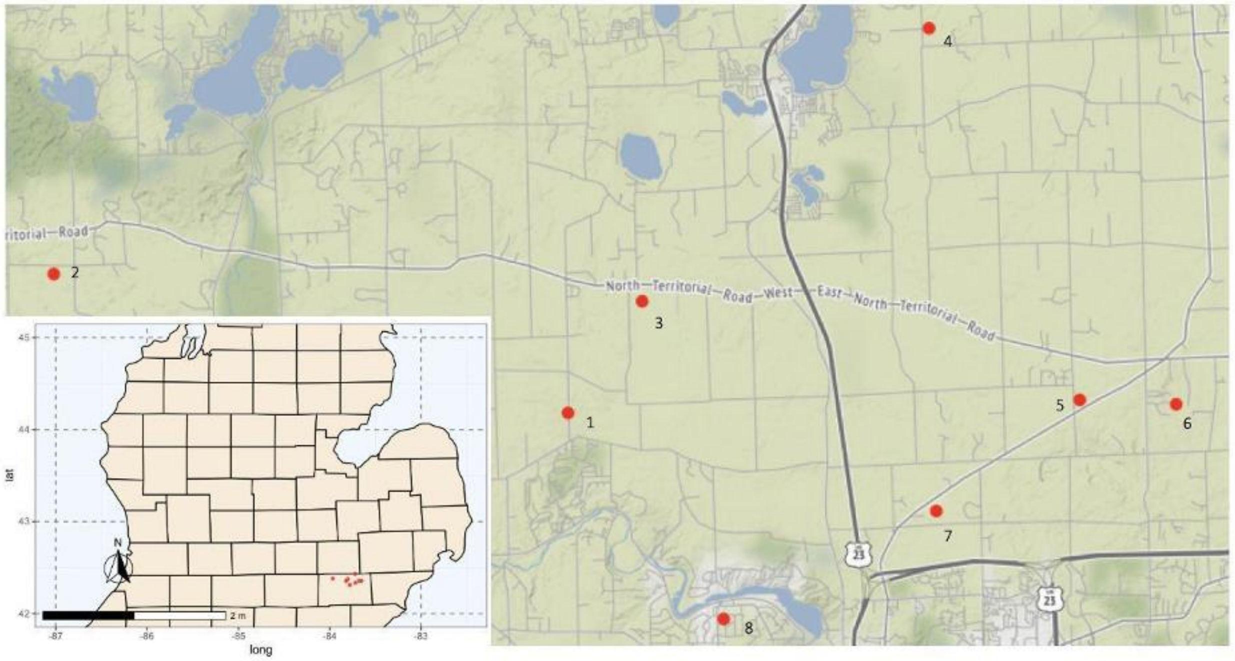
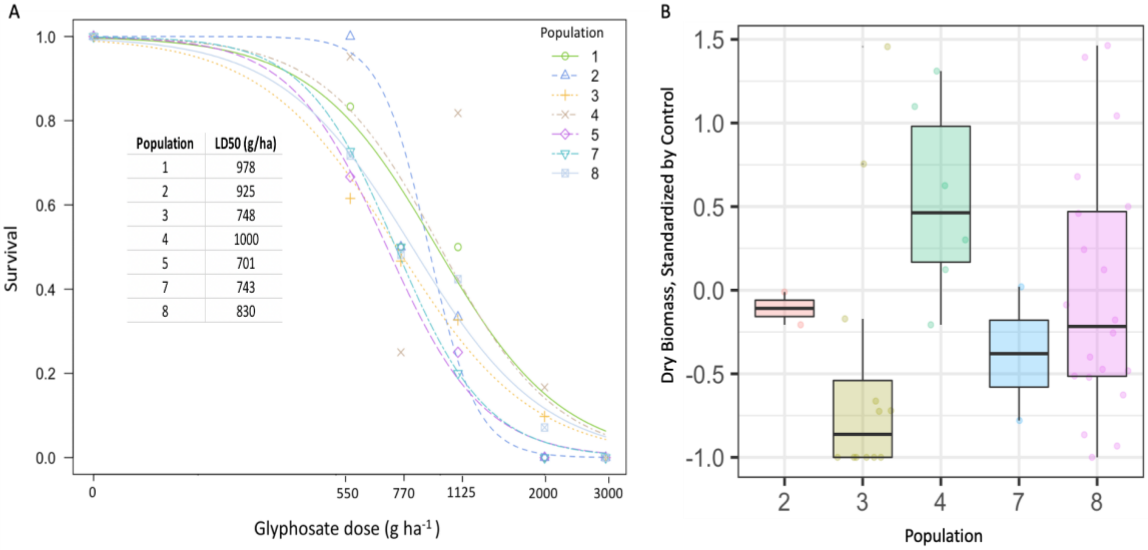


Figure S3- 1. Locations of velvetleaf populations sampled and used for this study.



Population	LD50 (g/ha)
1	978
2	925
3	748
4	1000
5	701
7	743
8	830

Figure S3- 2. A. Dose-survival response relationships of 8 Abutilon theophrasti MI populations after treatment with glyphosate at 550g/ha ai, 770g/ha ai, 1125g/ha ai, 2000 g/ha ai, and 3000 g/ha ai measured in the growth room experiment. Shown are median lethal dosage (LD50) among the populations sampled. B. Bar graph shows the variation of dry biomass at the dose 550g/ha ai standardized by the controls across populations 2, 3, 4, 7, and 8. We determined the fixed treatment effects (F = 51.92, p > 0.001) using Type III Sums of Squares and the significance of the random population effects using chi-squared test ($\chi^2 = 5.01$, p = 0.025).

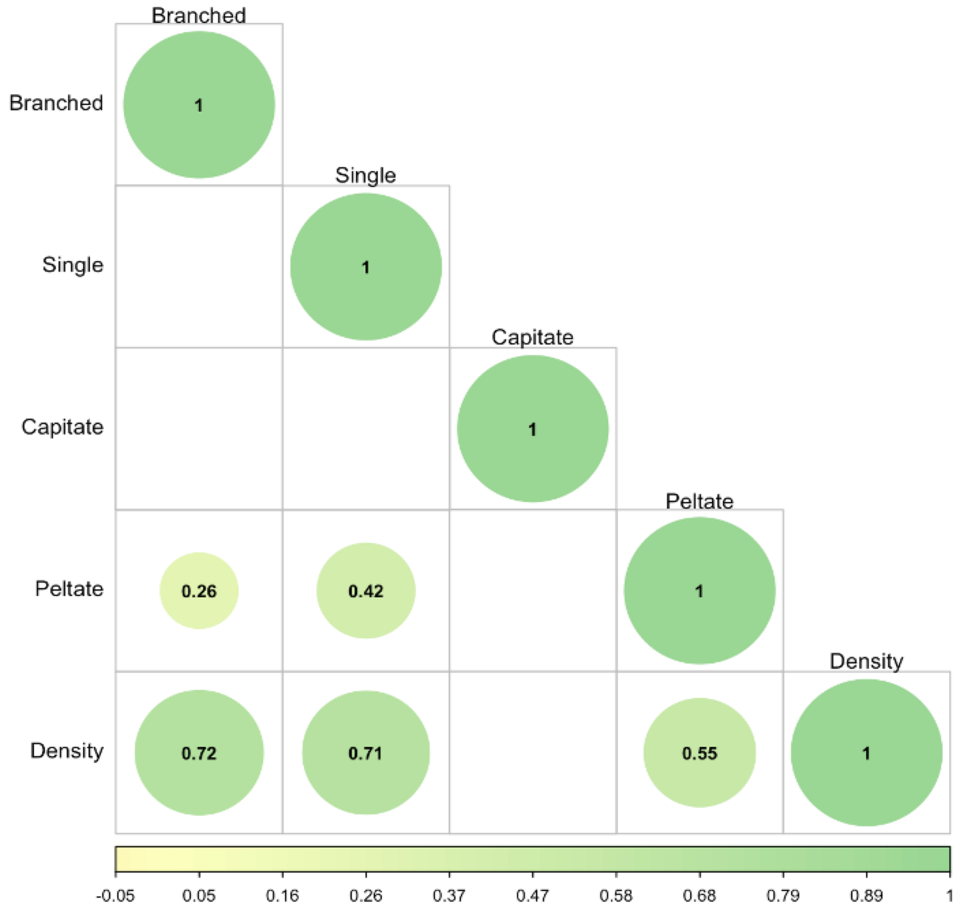


Figure S3- 3. Pearson correlations describing the correlation between trichome traits represented by color gradients of green to yellow (1 to -1). The presence of a circle indicates a significant trait correlation. Circle size indicates p-value.

	Maternal Line			Population		
	χ2	df	p-value	**χ2**	df	p-value
Branched	**3.96**	**1**	**0.047**	0.00	1	1.000
Single	2.82	1	0.092	0.00	1	1.000
Capitate	**3.19**	**1**	**0.048**	0.00	1	1.000
Peltate	0.75	1	0.387	0.00	1	1.000
Density	1.02	1	0.313	0.39	1	0.531

Table S3- 1. Results from a test for genetic variation using chi statistics (χ^2) values showing the effects of maternal line and population variation on trichome traits (proportion branched, single, capitate, peltate, and trichome density) captured in the field. Significant effects are indicated in boldface.

Quadratic Selection (*γ)* Gradients							
	Control			Herbicide			**F-values (from ANCOVA)**
Trait	*γ*	SE	p-value	*γ*	SE	p-value	
Proportion Branched	1.89	0.54	0.349	-0.26	1.94	0.898	0.01
Proportion Capitate	-0.97	0.28	0.63	0.59	2.56	0.769	0.02
Total Density	0.38	0.92	0.85	0.27	1.75	0.895	1.24
Proportion Branched x Proportion Capitate	-1.34	0.17	0.51	-1.67	3.12	0.41	0.13
Proportion Capitate x Density	-1.22	1.33	0.55	2.32	4.01	0.257	0.52
Proportion Branched x Density	0.68	0.98	0.737	0.62	3.81	0.76	0.15

Table S3- 2. Direct (multivariate) selection acting on trichome traits (proportion branched, proportion capitate, density, and their interactions) in the absence and presence of herbicide. Shown are the quadratic (γ) gradient values, standard errors, and p-values in each treatment. F-values are from the ANCOVA analysis testing the effect of herbicide treatment on selection gradients. Significant effects are indicated in bold.