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TITLE: Herbivory defense in *Mimulus guttatus* (yellow monkeyflower): consequences for generalist and specialist herbivores

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ABSTRACT

HERBIVORY DEFENSE IN MIMULUS GUTTATUS (YELLOW MONKEYFLOWER): EFFECTS FOR GENERALISTS AND SPECIALISTS

Abstract

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Herbivory defense in *Mimulus guttatus* (yellow monkeyflower): consequences for generalist and specialist herbivores

by

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Abstract

Plants have undergone strong selection from herbivory, resulting in diverse physical and chemical defenses. Chemical defenses deter generalist herbivores, but are often tolerated by specialists. *Mimulus guttatus* (yellow monkeyflower) is a model genetic system with ecological relevance and has physical and chemical defense mechanisms. To examine the effects of these defenses on herbivores, we conducted feeding trials with *M. guttatus* and larvae of the specialist herbivore *Junonia coenia* (buckeye caterpillar) and the generalist herbivore *Grammia incorrupta* (woolly bear caterpillar). Specialist growth rate significantly exceeded generalist growth rate. In addition, number of trichomes, a physical defense of *M. guttatus*, showed a significant inverse relationship with specialist herbivore growth. In our research, specialists grew most efficiently on *M. guttatus*, but generalists were most resistant to physical defense.

Introduction

Herbivory has exerted strong selection pressures on natural plant populations over both ecological and evolutionary time scales, leading to the great diversity of plant defenses we observe today (Berenbaum & Zangerl 2008). Plant species that experience a wide array of herbivory pressures are likely to have evolved a variety of defenses. Individual plants may have diverse, non-redundant types of defense, which generally reflect historical selection pressures on the plant species (Agrawal & Fishbein 2006). Physical defenses, such as trichomes or thorns, can prevent herbivores from moving freely across the plant surface or from establishing a good feeding position on a plant; secondary compounds (phytochemicals not involved in plant primary metabolism) can act as feeding deterrents, reduce digestibility of plant material, or act as toxins (Adler et al. 1995). While generalist herbivores are usually negatively affected by secondary compounds, specialist herbivores have evolved to cope with specific chemical defenses and may even use them as feeding stimulants and/or sequester them for their own defense (Adler et al. 1995).

In our research, we examine the defense of the plant *Mimulus guttatus* (yellow monkeyflower) and the performance two herbivore species, a specialist and a generalist. *Mimulus guttatus*, an herbaceous plant found in open, wet habitats across western North America, has emerged as a model system for studies of ecological and evolutionary functional genomics (McKay & Stinchcombe 2008; Wu et al. 2008). It possesses all of the traits required for a model plant system (i.e., short generation time, extensive genetic variability, amenability to greenhouse and field studies, comprehensive genomic resources; Wu et al. 2008). Finally, *Mimulus* is becoming an important system for the study of species interactions (Carr & Eubanks 2002; Holeski 2007; Holeski et al. 2010). Defense traits in *M. guttatus* include trichomes and secondary compounds (primarily phenylpropanoid glycosides), both of which possess substantial genetic variation, a prerequisite for their evolution, within and between natural populations (Holeski 2007; Holeski et al. 2010; Ivey et al. 2009).

We conducted comparative feeding trials on *M. guttatus* using the specialist herbivore *Junonia coenia* (common buckeye) and the generalist herbivore *Grammia incorrupta* (woolly bear caterpillar). **The results of this project examine: 1) which herbivore performs the best on *M. guttatus*, and 2) the effects of trichomes on each herbivore's performance.**

Methods

We conducted feeding trials with *M. guttatus* plants derived from 8 natural populations, chosen to represent natural genetic variation in trichome density and secondary chemical production (Holeski et al. 2012, in review). These experimental plants were grown in standard greenhouse conditions, as described in Holeski et al. (2010). To form the experimental populations, we used a crossing design across several plant generations (in the greenhouse) to create multiple full-sibling families within each population; each experimental population was directly derived from seeds collected from a particular natural population. We used four full-sibling families within each population and 8 individuals per family for a total of 256 plants. This experimental design allowed us to evaluate the effects of genetic variation in defense on herbivores. We first did feeding trials with a specialist herbivore species, and then repeated the entire trial with the same set of plants and a generalist herbivore species.

Herbivores: We used the specialist herbivore species *Junonia coenia* (larvae; buckeye caterpillar); this herbivore feeds on several plant species (including *M. guttatus*). All of these species contain iridoid glycosides and/or phenylpropanoid glycosides (PPGs) (Bowers & Stamp 1997). The PPG verbascoside acts as a feeding stimulant for buckeye larvae (K. Darrow & M.D. Bowers, unpublished data), and iridoid glycosides are feeding and oviposition stimulants (Adler et al. 1995; Bowers & Stamp 1997). Buckeye larvae sequester iridoid glycosides (and possibly PPGs) for defense against predation (Bowers & Stamp 1997). In our second feeding trial, we will use the generalist herbivore species *Grammia incorrupta,* the woolly bear caterpillar. *G. incorrupta* larvae are polyphagous herbivores which feed on a wide variety of plant families (Smilanich et al. 2011).

Feeding trials and corresponding analyses: In our feeding trials, we used leaves from the same developmental stage across plants. The feeding trial leaf was removed from the plant, weighed, and the petiole was placed in a water pic. The leaf and water pic were then placed in a petri dish. One 1st instar larva (within 8 hours of larval emergence from the egg and no previous feeding) was weighed and placed on each leaf. Each buckeye larva was allowed to feed for 8 days and each woolly bear larva fed for 10 days. After that time, the larvae were removed, dried, and weighed, along with remaining leaf material. The principal results examined were the amount of leaf eaten (dry weight) and amount of weight gain by larvae (dry weight). A subset of additional, similar leaves was analyzed to give the ratio of wet to dry weight, used to calculate the dry weight of leaf eaten. Insect performance was defined as average larvae weight gain (dry weight) per day of feeding.

At the same time that the feeding trial leaf was removed from the plant, we removed the opposite leaf (growing from the same node) from the plant and assessed trichome density using a dissecting microscope (4x magnification; trichome density = trichomes on basal, abaxial side of leaf; Holeski 2007). Each of the leaves was then flash frozen in liquid nitrogen and stored in the freezer (-20°C) until they were vacuum freeze-dried and ground. At a later date these ground leaves will be analyzed by high performance liquid chromatography (HPLC) to quantify and identify the PPGs present.

When the feeding trials were complete, we statistically analyzed our data. Prior to analysis, we determined whether each response variable was normally distributed. We assessed the individual effects of each independent variable (trichomes, population, herbivore) on the response variable (larvae growth rate, amount of food consumed) using analysis of variance (one-way ANOVA) or linear regression.

Results

Unless otherwise stated, all data were found to be normally distributed. It should also be noted that for both herbivore species, no significant variation in initial larvae wet weight was found by one-way ANOVA ($p>0.2$ for both species). Because of this and the miniscule magnitude of initial wet weights, initial dry weights were deemed negligible and not included in calculation of larval growth.

Herbivore growth rates by population: Buckeye caterpillar growth rates were compared among populations of *M. guttatus* using one-way ANOVA. Buckeye caterpillars showed significantly different performance among populations of *M. guttatus* ($F=5.7$, $df=7$, $p<0.001$; Figure 1).

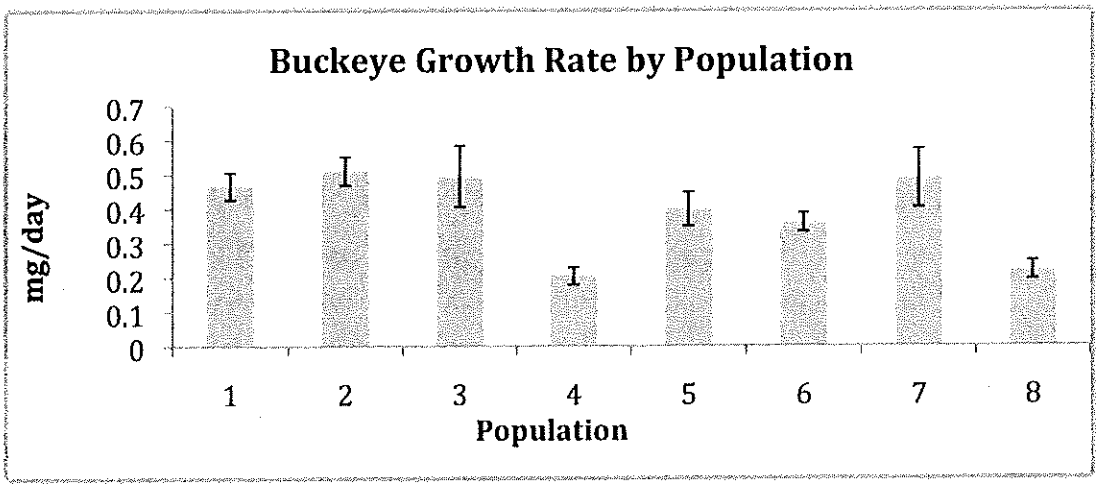


Figure 1 Individual dry weight gain per day for buckeye caterpillars feeding on each population of *M. guttatus*, labeled arbitrarily. Growth rates differ significantly by population ($p < 0.001$). Error bars indicate standard error of the mean.

Similarly, woolly bear caterpillar performance was compared by population. Woolly bear growth rates differed significantly by population of *M. guttatus* (one-way ANOVA; $F=12.2$, $df=7$, $p<0.001$; Figure 2).

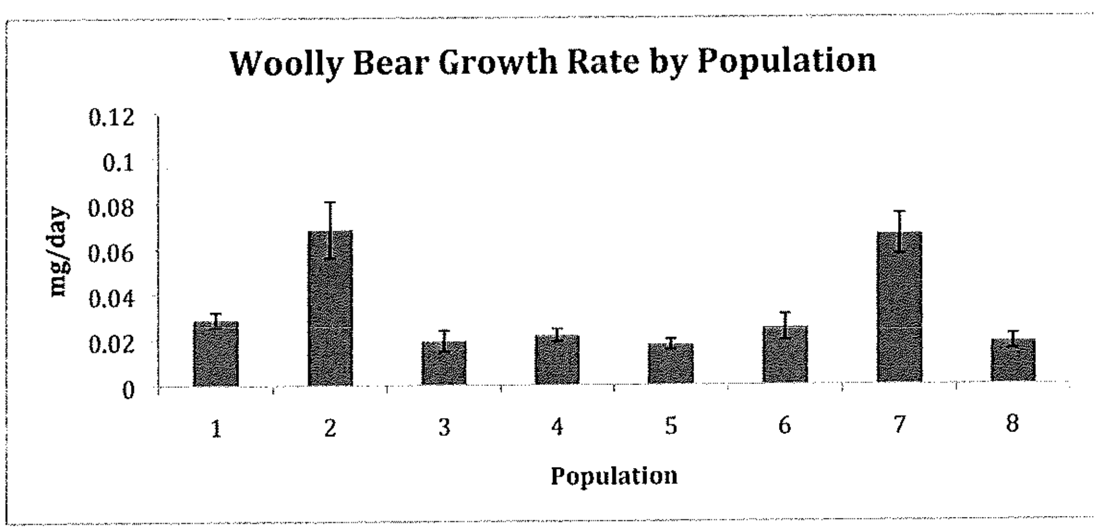


Figure 2 Individual dry weight gain per day for woolly bear caterpillars feeding on each population of *M. guttatus*, labeled arbitrarily. Growth rates differ significantly by population **($p<0.001$)**. Error bars indicate standard error of the mean.

Overall herbivore growth rates: Overall growth rates were then compared between the two species. It was found that the growth rate of buckeye larvae greatly exceeded that of woolly bear larvae (one-way ANOVA, $p<0.001$, Figure 3).

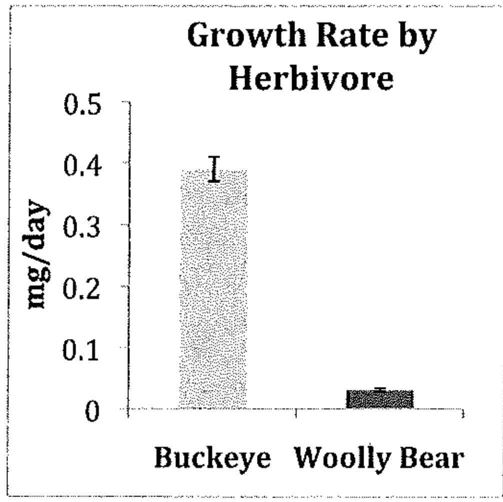
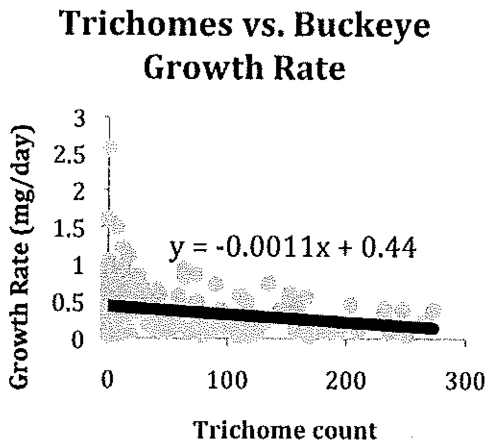


Figure 3 Individual dry weight gain in mg per day by species of herbivore. One-way ANOVA returned significant results (**$p < 0.001$**). Error bars indicate standard error of the mean.

Trichomes by population: Levels of trichome defense were as also compared across *M. guttatus* populations (one-way ANOVA). Note that trichome density did not significantly differ between leaves in the same leaf pair for a subset of trial leaves (one-way ANOVA). Trichome density did indeed vary by population ($p<0.001$).

Herbivore growth rates vs. trichomes: The relationship between trichome density and growth rate of each herbivore was analyzed by linear regression. In both cases, herbivore growth rate decreased with increasing trichome density (Figure 4). However this relationship was significant only for buckeyes ($F=13.2$, $df=1$, $p<0.001$, $R^2=0.06$), while the woolly bear relationship was less pronounced ($F=3.1$, $df=1$, $p=0.080$, $R^2=0.03$).



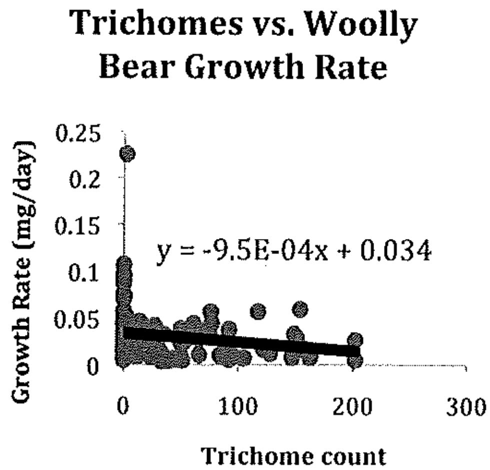


Figure 4 Trichome count is plotted against dry weight gain per day for both buckeye and woolly bear caterpillars. The lines resulting from standard linear regression analysis show a negative relationship, which is significant for buckeye caterpillars alone.

Leaf material consumed: The dry weight of leaf material consumed by each herbivore was also analyzed. Results suggest that buckeye larvae do not eat significantly more *M. guttatus* than woolly bear larvae (p=0.48, one-way ANOVA).

Discussion

Herbivore growth rates and trichomes by population: Both herbivore species examined here exhibited different growth rates on different populations of *M. guttatus*. Each population of *M. guttatus* is known to differ in defense profile. Because the purpose of plant defense is to reduce herbivore performance (Adler et al. 1995), these findings suggest that the difference in herbivore performance by population is a result of the population-level variation in defense.

Overall herbivore growth rates: As expected, the specialist buckeye caterpillar showed higher performance than the generalist woolly bear caterpillar when feeding on *M. guttatus*. This is evidenced by its significantly greater growth rate. This is consistent with the generally accepted notion that specialist herbivores adapted to tolerate specific secondary compounds will perform better than generalists on a plant containing those specific secondary compounds (Adler et al. 1995).

Herbivore growth rates vs. trichomes: The fact that herbivore performance decreased overall for each herbivore with increasing trichome density is consistent with the fact that trichomes inhibit the growth of small herbivores (Adler et al. 1995). However, we found that this relationship is significant only for the specialist buckeye caterpillar. If this finding holds true in general for specialist/generalist performance, it suggests that generalist herbivores are better able to tolerate physical defenses than specialists. Perhaps this can be explained by physical defense varying less between plants than the diverse range of chemical defense. Physical defense often impedes insect mandibles (Massey and Hartley 2009), and thus can be overcome by physical adaptations, such as larger, stronger feeding parts, for example. This may make different physical defenses easier to adapt to than chemical defenses, which require much more specific adaptations to overcome. If this is the case, then generalists have physical adaptations to tolerate physical plant defense while still feeding on a wide variety of plant species.

Leaf material consumed: Amount of leaf tissue consumed by larvae was underestimated in this experiment. This is likely due to exposure of dried leaves to humidity and incomplete cleaning of frass from the leaves after completion of the feeding trial, artificially increasing the final dry weight of the leaf. To correct this problem, in the future we recommend a more thorough method to remove frass before the leaves are dried. It appears that in this case, buckeye larvae consumed more leaf material than woolly bear larvae, but this relationship is not significant. Because the difference in growth rate between the two herbivores is strikingly evident, this brings up the question of efficiency. Because the specialist buckeyes outperformed but did not necessarily outconsume the generalist woolly bears, this suggests that the difference in performance is due to a difference in efficiency of converting food to biomass. This also suggests that the chemical defense of *M. guttatus* acts not by inhibiting feeding so much as by inhibiting conversion of plant material to herbivore biomass. The results of this experiment suggest that future research should be directed toward the efficiency of conversion of food to biomass for these organisms.

We are still awaiting the results of our chemical analyses, to be performed in the coming months. These results will give insight into the effects of the specific secondary compounds of *M. guttatus* on herbivore performance.

Plants, the major source of energy for most organisms on the planet, devote great deals of energy to developing diverse physical and chemical defenses, which herbivores in turn spend energy tolerating (Agrawal & Fishbein 2006; Berenbaum & Zangerl 2008). In gaining information on herbivory by specialists and generalists, we can better understand the interactions that drive the structure of ecosystems and the evolution of the organisms therein.

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