Title

Linking Plant Trait Variation to Arthropod Community Ecology From an Ecological Perspective

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Linking Plant Trait Variation to Arthropod Community Ecology From an Ecological Perspective

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Biological Sciences

by

Tyler Paul Zarubin

Dissertation Committee:
Professor Kailen Mooney, Chair
Professor Travis Huxman
Professor Sarah Kimball

2024

DEDICATION

“I am fain to compare myself with a wanderer on the mountains who,
not knowing the path, climbs slowly and painfully upwards
and often has to retrace his steps because he can go no further—
then, whether by taking thought or from luck, discovers a new track
that leads him on a little till at length…he reaches the summit”

Hermon von Helmholtz

To those who helped illuminate the path when I knew not where to go,
encouraged me to continue climbing when I grew tired,
and walked with me as I retraced my steps time and again,
I dedicate this body of work.

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ACKNOWLEDGEMENTS

Outside of my close friends and family, four people have taken a chance on me that came with great risk, both to themselves and to their respective organizations. Kailen, you are one of those four. You took me on despite all the constraints that entailed and gave me an opportunity that has fundamentally shaped my life. For that I will forever be grateful.

I am indebted to my committee members, Sarah and Travis, for investing so much in me. The ways you have challenged me to look at plants, arthropods, ecological processes differently have transformed the ways I think and act as an ecologist for the better.

To the Department of Natural Sciences and the Dean's office of the School of Arts and Sciences at Concordia University Irvine, thank you for the flexibility, support (both in the field and the classroom), and consistent encouragement that you provided as it gave me the time, the space and the continued motivation needed to finish the race.

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FIELD OF STUDY

Plant mediated structuring of arthropod communities

ABSTRACT OF THE DISSERTATION

Linking Plant Trait Variation to Arthropod Community Ecology
From an Ecological Perspective
by
Tyler Paul Zarubin
Doctor of Philosophy in Biological Science
University of California, Irvine, 2024
Professor Kailen Mooney, Chair

In this study, we utilized a suite of plant trait-based approaches towards understanding plant-arthropod interactions from an ecological perspective. Through laboratory and field-based assays on 14 species of woody shrubs native to the Coastal Sage Scrub ecosystem, we compared variance in plant resistance to herbivory and in non-defensive plant traits to variance in components of associated arthropod communities. Our analyses revealed the following: (1) plant resistance to herbivory assessed in a lab bioassay was overall a poor predictor of herbivore density in the field though it did provide insight into arthropod herbivore dynamics for several plant species and over time; (2) plant species varied strongly in predator abundance but this did not correlate with herbivore densities, suggesting a limited role for predators as a from of indirect defense for plants ; and (3) variance in non-defensive plant traits strongly correlated with variance in associated arthropod density and community composition. Taken together, our findings indicate that plant-arthropod interactions on ecological time scales in realistic ecological are driven more by traits presumably evolved for other purposes than by aspects of direct and indirect defense that are tyically the focus of studies on plant-herbivore evolutionary ecology. While these other traits may be the dominant drivers of herbivore abundance, we speculate that aspects of plant

defense may still have weaker, hard to detect effects that act to mediate these interactions over evolutionary time scales. While these other traits may be the dominant drivers of herbivore abundance, we speculate that aspects of plant defense may still have weaker, hard to detect effects that act to mediate these interactions over evolutionary time scales.

INTRODUCTION

The interactions between terrestrial plants and their associated herbivores comprise a foundational relationship that shapes ecological communities. Plants represent over 75% of global biomass and play a critical role in fixing carbon into an organic form necessary for most organisms while herbivores, particularly those within the phylum Arthropoda, are the primary consumers of these energy-rich organic molecules. These antagonistic interactions, then, are consequential to not only plants and their associated herbivores, but to entire ecological communities.

Plants employ a diverse array of defensive strategies to resist herbivory; strategies that can be categorized into direct or indirect defenses. Direct defenses include structural components like thorns and leaf trichomes, and chemical components such as cardenolides, which affect the fitness of herbivores directly. Indirect defenses, such as herbivore-induced plant volatiles or extrafloral nectaries, recruit natural enemies of herbivores reducing the impact of herbivory on plants indirectly. The effect of these defensive strategies on herbivory in plants is proximally detectable by measuring plant traits known to covary with increases in direct or indirect defense. These trait-based approaches are commonly used methods of investigating the relationships between plant defense and herbivory across a wide range of ecosystems.

Polyphagous herbivores, which can consume plants across multiple taxa, are attractive candidates for trait-based approaches assessing plant direct defense, especially in settings with diverse plant taxa. Laboratory bioassays using these generalist herbivores provide a common currency to evaluate and compare plant resistance among various species that likely differ in defensive strategies. While these bioassays have been extensively used to quantify plant direct defense, few studies have linked laboratory findings with levels of herbivory in natural settings leaving the applicability of these assays in modeling actual herbivory in the field in question. The investigation of indirect defenses and their effect on plant-herbivore interactions utilizing trait-based approaches is often limited to studies of highly co-evolved mutualisms or simplified plant-herbivore-predator studies in highly simplified and tightly controlled settings. Though much progress has been made in understanding the impact of indirect defenses on plant-herbivore interactions, there is a limited understanding of the dynamics of these relationships in realistic ecological settings. Lastly, much of the research on plant defense to herbivory using trait-based approaches has focused primarily on plant defense traits and largely ignored non-defensive plant traits. Given the insight that non-defense traits give us into how plants respond to and mediate interactions with their abiotic environment, it is not out of the question that these traits may also provide insight into plant-arthropod interactions.

In this study, we sought to address the complex interplay between plant traits and arthropod communities through three distinct but interconnected research questions. First, we investigated the correlation between plant direct resistance to the generalist herbivore, *Spodoptera exigua* and natural herbivore density in the field to understand the applicability of these bioassays as a proxy for plant direct defense with actual herbivore density. Second, we examined variations in herbivore abundance and two proxy measures of indirect plant defense: predatory arthropod abundance and bird attack rates to understand how these measures of indirect defenses correlate with herbivore densities. Finally, we explored how variance in six non-defensive plant traits correlate with arthropod community diversity categorized by both feeding guild and taxonomic order/suborder. By integrating the findings from these three studies, we provide a holistic view of how plant traits—both defensive and non-defensive—correlate with arthropod community composition.

CHAPTER 1

PLANT SPECIES AND INTERANNUAL VARIATION COMPLICATE THE USE OF PLANT DIRECT RESISTANCE ASSAYS TO PREDICT HERBIVORE DENSITY IN THE FIELD

ABSTRACT

Where plant direct resistance to herbivores is often presumed to be a dominant driver of herbivory, many additional factors may drive herbivore abundance, including predators, herbivore-herbivore interactions and the abiotic environment. The relationship between plant resistance measured in isolation of such factors, and herbivore density under natural conditions is rarely explored. We compared the growth response of *Spodoptera exigua* reared on 13 different plant species to actual herbivore density on those species in natural settings across three years to understand the general applicability of lab-based bioassays to ecological dynamics. Our findings show that the growth response of *Spodoptera exigua* in the lab bioassay did not consistently correspond to herbivore density in the field. Within plant species, the lab bioassay was predictive of herbivore density through time for only a small subset of plant species whereas among species, the lab bioassay was not predictive of herbivore density through time. Finally, the bioassay was predictive of aggregate herbivore density in the plant community, but only in the third year of the study. Overall, we conclude that though plant resistance measured in isolation is a weak predictor of herbivore density under natural conditions, ... [BLEND WITH REST] a plant species-by-species level, they do have potential to offer insight into aggregate ecological dynamics for the whole plant community, especially if paired with the measurement of other traits that mediate herbivore density.

INTRODUCTION

The interactions between terrestrial plants and the herbivores that consume them comprise one of the most fundamental sets of forces responsible for shaping ecological communities (Deteier 1954, Fraenkel 1959, Ehrlich and Raven 1964, Jaenike 1990). Plants have primacy in terrestrial ecosystems as they make up over 75% of total global biomass and fix carbon into the organic form that the majority of organisms require for their energy supply. The primary consumers of the energy-rich organic molecules plants create are herbivores belonging to the phylum Arthropoda, a phylum that contains the largest number of species and is estimated to constitute over 50% of total terrestrial animal biomass (Bar-On et al. 2018). Given this critical role that plants play in supporting food webs and the outsized abundance of arthropods (most of which are herbivores) that make up these food webs, an understanding of plant-herbivore interactions is essential to an understanding of plants and, by extension, the communities that they support. Because plant-herbivore interactions are antagonistic, they constitute a strong selective force that shapes both plant defensive strategies and herbivore responses to these defenses. The push and pull of these opposing forces, then, is understood to be a major driver in plant evolution and consequential in shaping entire ecological communities (Agrawal 2011, Agrawal et al. 2012, Kant et al. 2015, Hahn et al. 2019).

Plants employ a diverse array of defensive strategies for survival (War et al. 2012). Direct plant defenses, which include both structural and chemical components such as thorns, leaf trichomes, or cardenolides, hinder herbivory by directly interfering with the ability of herbivores to consume plant tissue. Indirect plant defenses, on the other hand, function to hinder herbivory indirectly either by the recruitment of natural enemies to herbivores such as parasitoids or predators via herbivore-induced plant volatiles or extrafloral nectaries or through life history strategies such as deciduousness or dormancy to escape herbivory entirely

(Chen 2008, Mooney et al. 2012, Aljbory and Chen 2018). This ability to fine-tune defensive strategies is thought to be a selective advantage since any plant defense is assumed to be costly and the arthropod herbivores a plant will encounter are likely to be diverse in both feeding strategies and population size. Taken together, these observations have allowed for the holistic classification of plants according to defensive syndromes (Coley et al. 1985, Power 1992, Stamp 2003, Agrawal and Fishbein 2006, Hahn and Maron 2016), or overall defensive strategies, which seek to describe not only the diversity of defensive traits plants employ but, more importantly, how these traits cooperate in allowing plants to defend against an ever-changing composition of herbivores.

Plant defense is often measured under simplified laboratory conditions that allow for the isolation of individual plant trait effects on defense, while controlling for the stochasticity of realistic ecological settings. Of these laboratory-based methods of assessing plant defense, bioassays are particularly useful, especially when assessing plant defense across multiple species. In these bioassays, the performance (e.g., growth, survival) of polyphagous herbivores reared on plant tissue in controlled laboratory settings is used as a proxy to evaluate and compare the resistance of unrelated plants that may deploy fundamentally different defenses, which are otherwise difficult to compare quantitatively. Although laboratory bioassays with dietary generalists have been used extensively to quantify plant direct defense (Rodriguez-Saona and Trumble 1999, Champion and Laplaze 2020, Mielke and Gasperini 2020, Phambala et al. 2020), there are few studies that link the findings of these lab bioassays with actual levels of herbivory in realistic ecological settings (Underwood et al. 2002, Kempel et al. 2011). This has limited our understanding of how herbivore resistance measured in isolation relates to herbivore resistance (i.e. herbivore densities) in realistic ecological settings where both biotic and abiotic factors such as precipitation and predation are simultaneously at work (Stenberg and Muola 2017).

In this study, we investigated how measuring plant resistance to herbivory via the growth of a generalist herbivore reared on specific tissues under laboratory conditions (i.e. *S. exigua* growth rate) relates to herbivore densities in the field across a taxonomically diverse community of plant species. For three years, we assayed growth rates of the dietary generalist, *S. exigua*, reared on leaf tissue from 14 species of plants belonging to the Coastal Sage Scrub community in a laboratory setting. We then compared these lab results to the abundance of naturally occurring herbivores on these same plants in the field. In doing so, we document the contribution of plant direct resistance to *S. exigua* with overall patterns of herbivore densities under realistic ecological conditions.

METHODS

Common garden site preparation

The study was performed on a 1/10 hectare experimental garden located on the campus of Concordia University Irvine (33°39'24.1"N 117°48'32.3"W). The site, previously an unused, south-westerly facing slope dominated by Brassica nigra made up primarily of Alo clay soil (www.usda.gov), was prepared for planting by removing all existing flora, plowing with a 26" disc harrow to homogenize soil, and installing a 4ft tall perimeter fence to prevent incursion of rodent herbivores. Twenty plots were then constructed, each measuring 6m x 5m with 1m perimeter borders and covered with both water-permeable weed barrier and 2-inch jute mesh to prevent weed growth and mitigate water runoff. Weed barrier was removed in February of 2022 after seedlings were established.

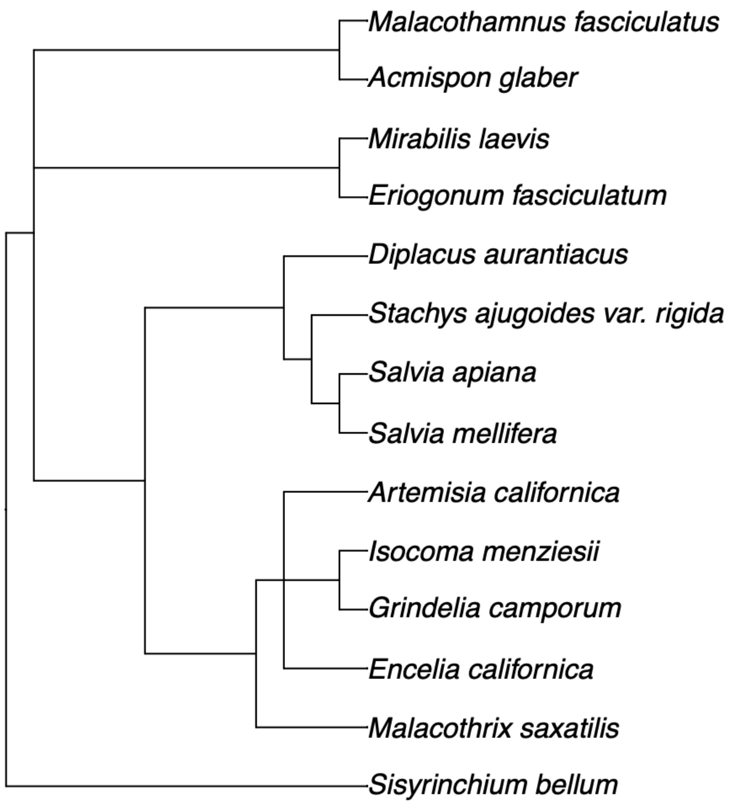

FIGURE 1.1. Phylogenetic tree of CSS perennial and forb shrubs used in the experiment using data obtained from the Phylomatic database (Webb and Donoghue 2005).

Plant propagation, transplanting and growth

Select perennial forbs and shrubs from diverse taxa and native to the Coastal Sage Scrub (CSS) ecosystem (Figure 1.1) were grown from seed (S&S Seeds, Carpinteria, CA). Seeds first underwent species-specific dormancy breaking protocols (unpublished data, Table 1.1) before being planted in a 2:1:1 peat moss:cedar bark:sand mixture and grown in a climate-controlled greenhouse 25c for 1 year. 1 seedling per species per plot was transplanted in February of 2020 and watered to establish throughout the Summer of 2020. Plots were weeded but otherwise left undisturbed throughout the length of the experiment. Plants were assessed annually at the time of sampling during peak growth season (Table 1.2).

TABLE 1.1. Plant species and seed dormancy breaking protocols.

Species	Dormancy Breaking Protocol
Acmispon glaber	Mechanical scarring
Artemisia californica	None
Encelia californica	None
Eriogonum fasciculatum	None
Grindelia camporum	None
Isocoma menziesii	None
Malacothamnus fasciculatus	None
Malacothrix saxatilis	None
Mimulus (Diplacus) aurantiacus	5% liquid smoke
Mirabilis laevis	None
Salvia apiana	None
Salvia mellifera	None
Sisyrinchium bellum	1.5 month cold stratification (4℃)
Stachys ajugoides var. rigida	None

TABLE 1.2. Yearly plant survival data from 2021-2023 from starting populations of N= 20 planted in 2020.

Species	2021	2022	2023
Acmispon glaber	16	10	2
Artemisia californica	20	20	20
Encelia californica	17	17	17
Eriogonum fasciculatum	16	15	15
Grindelia camporum	16	8	1
Isocoma menziesii	16	14	13
Malacothamnus fasciculatus	16	14	12
Malacothrix saxatilis	17	15	12
Mimulus (Diplacus) aurantiacus	19	18	18
Mirabilis laevis	20	18	17
Salvia apiana	17	16	15
Salvia mellifera	20	18	17
Sisyrinchium bellum	18	14	8
TOTAL	228	197	167

Laboratory Bioassays

Laboratory bioassays were conducted from leaves collected in the field annually for every living plant from 2021-2023 during peak growth season (April-May) with the generalist herbivore, *Spodoptera exigua*, using a modified method from that described previously (Nell and Mooney 2019). Briefly, *S. exigua* eggs (Benzon Research) were incubated for 48 hours at 29℃ to hatch. Four larvae were placed onto a 3% agar petri dish with fresh leaves from each plant. Larvae and leaves were checked once per day for a total of 10 d. Leaves were replaced with fresh material once after day 5 with additional leaf changes as needed to ensure larvae had unlimited leaf material available. At the end of 10 d, all surviving larvae for each plant were counted, massed and averaged to obtain the average *S. exigua* biomass per plant, herein referred to as *S. exigua* growth rate.

Arthropod Sampling

Arthropods were sampled annually for every living plant from 2021-2023 through vacuum-sampling (Nell and Mooney 2019). At peak plant growth (April-May), an electric vacuum (3.5 hp Rigid model # HD06001) fitted with a fine mesh bag in the nozzle was used to sample each plant for 3 minutes or the entire plant, whichever came first. Care was taken during sampling to minimize both injury to the plants and the amount of leaf litter collected. Mesh bags were immediately placed on ice then transferred to -20℃ for long-term storage that same day.

Arthropod Sorting and Classification

In these samples, vacuumed plant material was exhaustively checked for arthropods with the naked eye using fine-tipped paint brushes and entomological forceps, with arthropods placed in 70% ethanol for later identification. Arthropod herbivores were classified into one of five orders/suborders (Sternorrhyncha, Auchenorrhyncha, or Thysanoptera) and assigned to one of two feeding guilds (Phloem sap and cell content feeders or Chewing feeders) based on feeding behavior. Over the three years of the study, a total of 55,689 arthropods were classified, of which 70% were herbivores (unpublished data). Since sampling efforts were standardized across all plants, herbivore counts were used as a proxy for herbivore density.

Data analysis

We assessed the relationship between *S. exigua* growth rate and herbivore density for each plant in each year (N_{2021} = 228; N_{2022} = 197; N_{2023} = 167) for a total of 592 replicate samples with which lab-based resistance assays could be compared with herbivore densities in the field. These analyses were conducted across a range of scales as described below. In each case, we constructed a series of generalized linear mixed models to examine the relationship between *S. exigua* growth rate and herbivore density in the field. All analyses were conducted using the glmmTMB package (Brooks et al. 2017) in RStudio (Posit team 2024) with log-transformations performed on both *S. exigua* growth rate and herbivore density in order to meet assumptions of normally distributed residuals. In our models, "Plot" refers to the specific plot (N = 20) a plant was found in and "Plant" refers to the individual plant within those plots.

Within-species analysis

We first analyzed the relationship between variation in plant species and variation in *S. exigua* growth rate and herbivore density separately (Equation 1.1a, 1.1b).

(1.1a) log(Avg. S.exigua biomass) ~ Species + (1|Plot) + (1|Plant) + (1|Year)

(1.1b) log(Herbivore density) ~ Species + (1|Plot) + (1|Plant) + (1|Year)

We then tested for variation in the relationship between *S. exigua* growth and herbivore density within species across all years of the study. In addition to the fixed effects by themselves, the interaction between species and *S. exigua* growth on herbivore density was included to determine how differences in species affect the relationship between *S. exigua* growth and herbivore density (Equation 1.2).

(1.2) log(Herbivore density) ~ log(*S. exigua* growth rate) + Species + log(*S. exigua* growth rate)*Species + Year + (1|Plot) + (1|Plant)

Based upon a significant interaction between species and *S. exigua* growth on herbivore density (see Results), we then ran separate analyses for each species (Equation 1.3).

(1.3) log(Herbivore density) ~ log(*S. exigua* growth rate + Year + (1|Plot) + (1|Plant)

Among-species level analysis

Next, we investigated relationships among species. Using annual species means for both *S. exigua* growth rate and herbivore density, we tested for the effect of *S. exigua* growth rate on herbivore density (Equation 1.4). We included year as a fixed effect to account for inter-annual variation and species as a random effect to account for repeated measures of the same species across multiple years.

(1.4) Mean log(Herbivore density) ~ mean log(*S. exigua* growth rate) + Year + (1|Species)

Community level analysis

We concluded with analyses at the level of the ecological community to examine broad-scale relationships between *S. exigua* growth rate and herbivore abundance irrespective of (i.e. controlling for) differences between species. In these analyses, species (N = 13) was treated as a random effect.

First, we analyzed annual changes in *S. exigua* growth rate and herbivore density separately (Equations 1.5a, 1.5b). Then, *S. exigua* growth rate and herbivore density relationships were analyzed across years by looking at the effect of *S. exigua* growth rate, Year, and the interactive effect of *S. exigua* growth rate and year (Equation 1.6).

(1.5a) log(*S. exigua* growth rate) ~ Year + (1|Species) + (1|Plot) +(1|Plant)

(1.5b) log(Herbivore density) ~ Year + (1|Species) + (1|Plot) + (1|Plant)

(1.6) log(Herbivore density) ~ log(*S. exigua* growth rate) + Year + log(*S. exigua* growth rate) * Year + (1|Species) + (1|Plot) + (1|Plant)

Because we found a significant *S. exigua* * Year interaction (see Results), relationships were also examined separately for each year (Equation 1.7).

(1.7) log(Herbivore density) ~ log(*S. exigua* growth rate) + (1|Species) + (1|Plot) + (1|Plant)

RESULTS

Within-species analysis

S. exigua growth rates and herbivore abundance showed noticeable differences between species year on year as well as across all years of the study (Figure 1.2). Linear mixed model analyses confirmed this demonstrating that variance in plant species significantly affected variance in both herbivore density and *S. exigua* biomass for each year of the study as well as across all years of the study (Table 1.3).

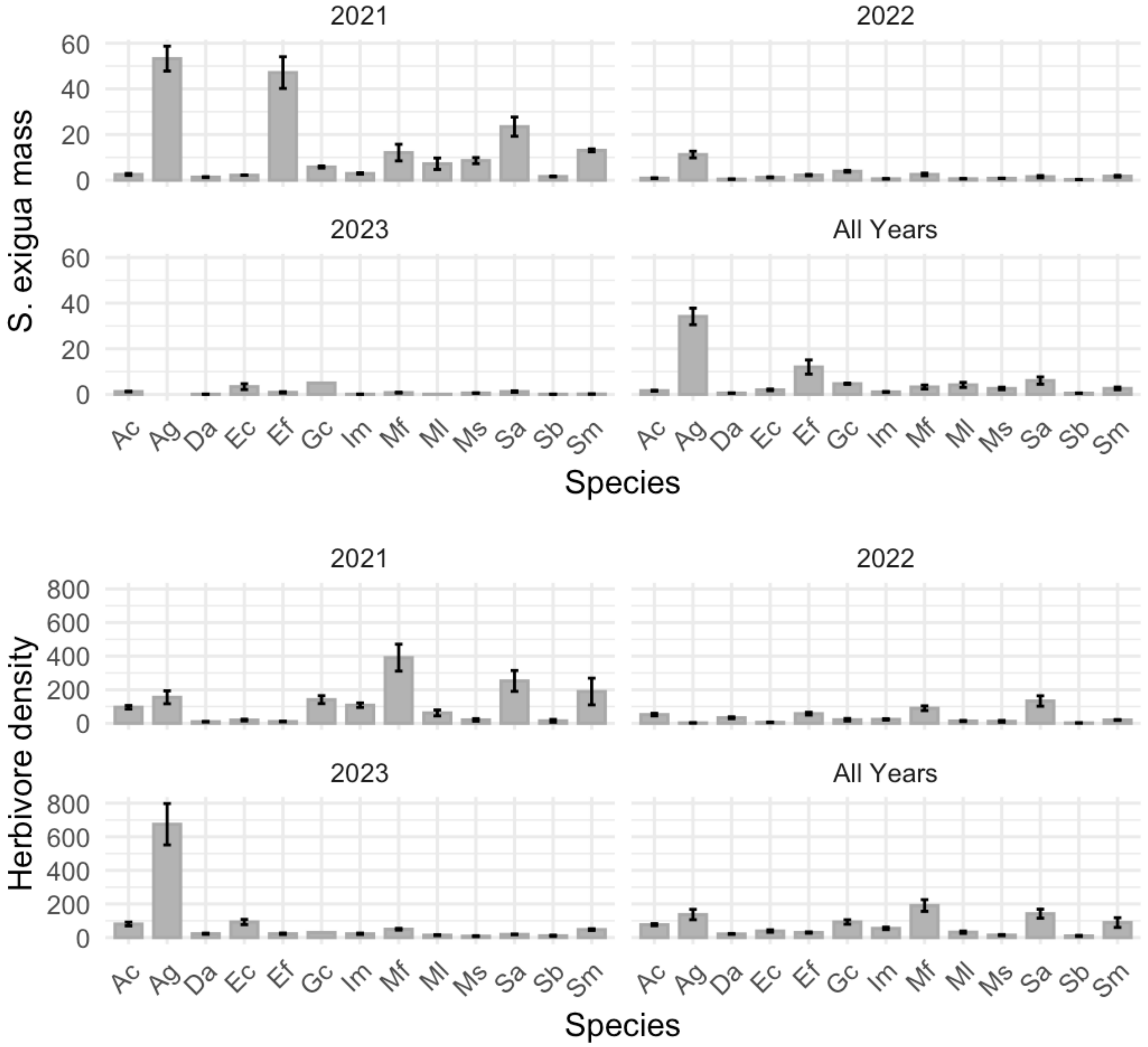

FIGURE 1.2. Least square means ± SD of *S. exigua* growth rate and herbivore density by species for each year and across all years of the study.

TABLE 1.3. Effect of variation in plant species on variation in *S. exigua* growth or herbivore density for each year of the study and across all years.

Response variable	**Year**	**Chi sq**	**Df**	**P-value**	**Marginal R^2**
log(Avg. *S. exigua* growth rate)	2021	201.36	12	**< 0.001**	0.71
	2022	169.7	12	**< 0.001**	0.59
	2023	38.47	11	**< 0.001**	0.35
	All	198.08	12	**< 0.001**	0.26
Herbivore abundance	2021	239.13	12	**<0.001**	0.51
	2022	320.74	12	**<0.001**	0.63
	2023	265.00	12	**<0.001**	0.62
	All	296.77	12	**<0.001**	0.32

Marginal R^2 represents the variance explained by the fixed effect of species alone

There was a significant species-by-*S. exigua* growth rate interaction ($p < 0.001$; Table 1.4), showing that the relationship between *S. exigua* growth rate and herbivore density varied among species. Subsequent species-level analyses showed that species varied widely in their relationships of S.exigua and herbivore density. Five of the 13 species showed significant relationships (Table 1.5) with four of those being positive relationships and one being negative.

TABLE 1.4. Relationship between *S. exigua* and herbivore density between species and across years.

Fixed effect	P-value	Marginal R^2
log(Avg. *S. exigua* growth rate)	0.51	0.05
Species	**< 0.001**	0.32
Year	**< 0.001**	0.42
log(Avg. *S. exigua* growth rate) * Species	**< 0.001**	0.06

Marginal R^2 represents the variance explained by the fixed effect of species alone

TABLE 1.5. Species-level relationships between *S. exigua* and herbivore density.

Species	P-value	Marginal R^2	Estimate
Acmispon glaber	**< 0.01**	0.07	-0.62
Artemesia californica	0.67	0.07	0.07
Diplacus aurantiacus	0.88	0.10	-0.60
Encelia californica	0.49	0.05	-0.10
Eriogonum fasciculatum	**< 0.01**	0.03	0.33
Grindelia camporum	0.24	0.25	0.67
Isocoma menziesii	0.43	0.71	-0.14
Malacothamnus fasciculatus	**< 0.05**	0.34	0.21
Malacothrix saxatillis	**< 0.05**	0.18	0.84
Mirabilis laevis	0.86	0.24	-0.04
Salvia apiana	0.91	0.22	0.01
Salvia mellifera	**< 0.001**	0.09	0.39
Sisyrinchium bellum	0.37	0.03	0.36

Marginal R^2 represents the variance explained by the fixed effect of species alone

Among-species analysis

Analyses based on species means within each year (*N* = 39; 13 species x 3 years) found no significant relationship between species means for *S. exigua* growth rate and herbivore density (Figure 1.3, Table 1.6).

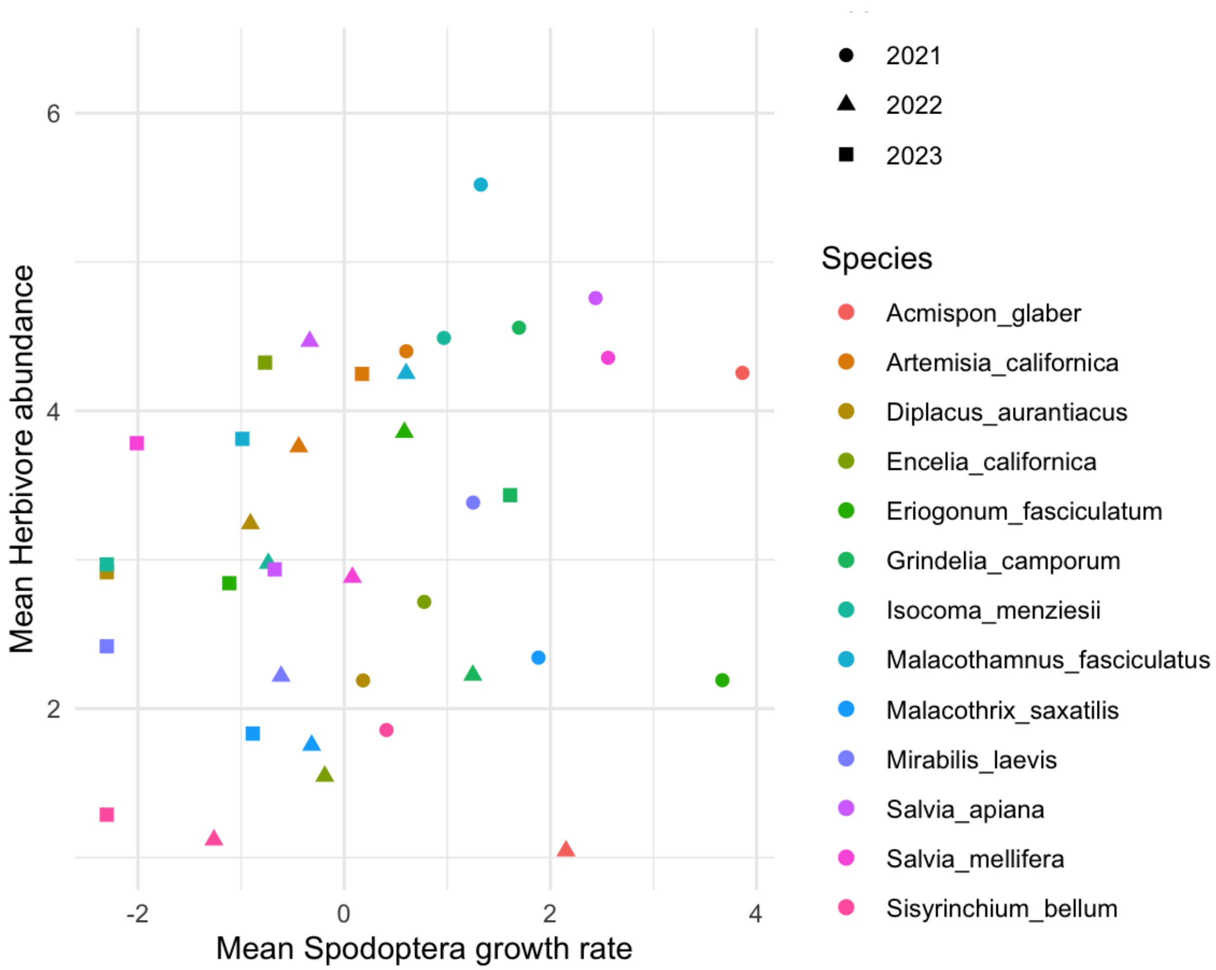

FIGURE 1.3. Species means of *S. exigua* growth rate and herbivore abundance across years. Annual means of *S. exigua* growth rate and herbivore density were plotted for each species.

TABLE 1.6. Relationship between species means of *S. exigua* and herbivore density across years.

Fixed effect	**P-value**	**Marginal R^2**
Mean log(Avg. *S. exigua* growth rate)	0.71	0.07
Year	0.10	0.09

Marginal R^2 represents the variance explained by the fixed effect of species alone

Community-level

S. exigua growth rate significantly decreased each year, demonstrating an increase in plant resistance ($p < 0.001$; Figure 1.4, Table 1.7). Though the year also had a significant effect on herbivore density ($p < 0.001$; Table 1.7), this variation did not correspond to interannaual variation in the lab-based assay with *S. exigua* growth rate with the highest in 2021 and the lowest in 2022 (Figure 1.5). Assessing the associations among all 592 samples, there was no significant relationship between *S. exigua* growth rate and herbivore density (Figure 1.5, Table 1.8a). There was, however, an interactive effect of *S. exigua* growth and year on herbivore density ($p < 0.05$; Table 8a). Tests within each year showed a significant positive association in 2023 ($p < 0.05$) but no relationship in 2021 or 2022 (Table 8b, Figure 5).

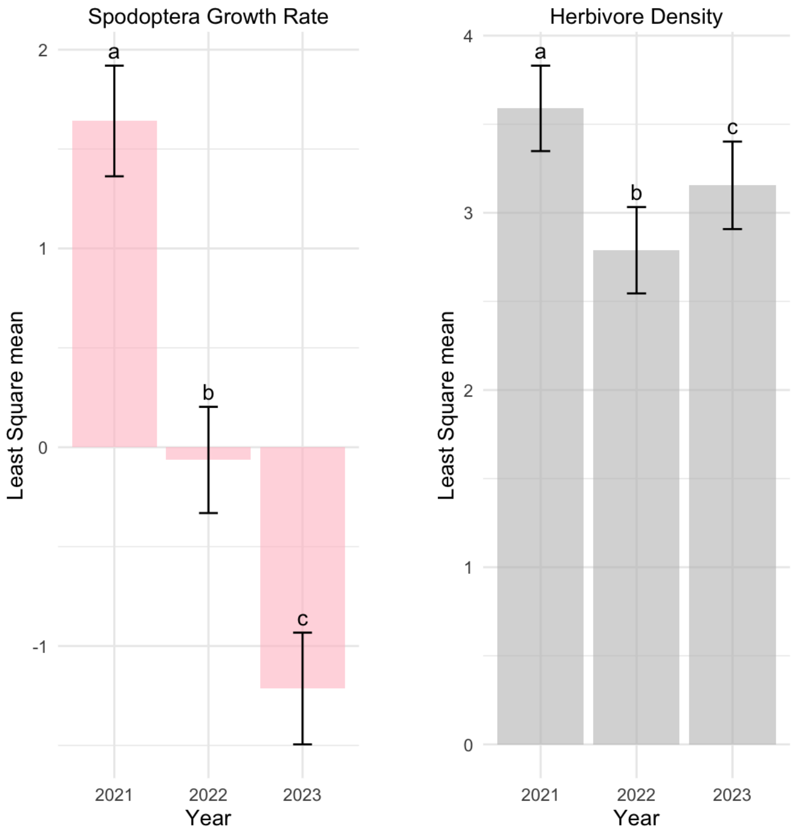

FIGURE 1.4. Year to year variation in *S. exigua* growth rate and herbivore density. Least square means with standard errors for the log of average *S. exigua* growth rate and log of Herbivore density were plotted for each year. Lowercase letters indicate significance of post-hoc Tukey comparisons between each year.

TABLE 1.7. Community-level analysis of the effect of year on *S. exigua* growth rate and herbivore density.

Response variable	**Fixed effect**	**P-value**	**Marginal R^2**
S. exigua growth rate	Year	**< 0.001**	0.38
Herbivore density		**< 0.001**	0.05

Marginal *R*^{2} represents the variance explained by the fixed effect of species alone

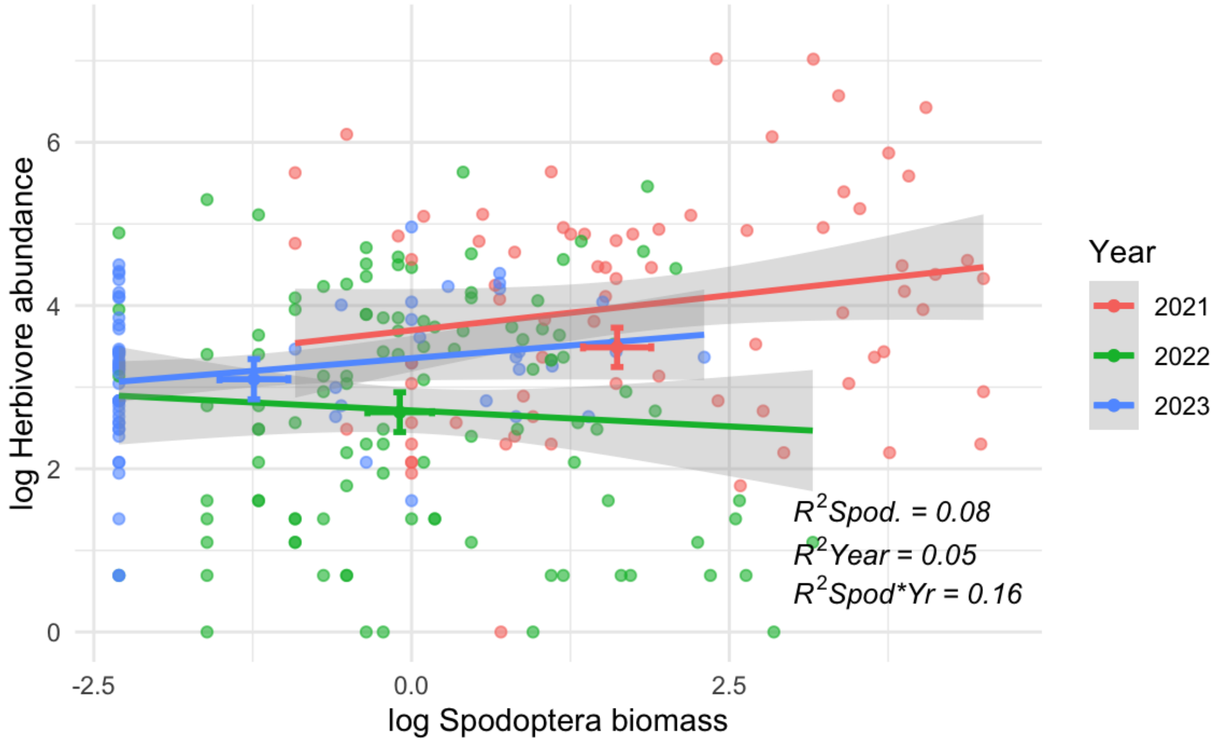

FIGURE 1.5. Community-level relationships between *S. exigua* growth rate and herbivore density across years. *S. exigua* growth rate was plotted against herbivore density for all data and grouped according to year. Least square means with standard errors for both *S. exigua* growth rate and herbivore density were included for comparison.

TABLE 1.8. Statistical results of the relationship between Herbivore density ~ *S. exigua* growth rate. Panel (a) is the full model including all years whereas panel (b) examines each year separately based on the *S. exigua* * Year interaction.

(a)

Fixed effect	**P-value**	**Marginal R^2**
log(Avg. *S. exigua* growth rate)	0.47	0.08
Year	**<0.001**	0.05
log(Avg. *S. exigua* growth rate) * Year	**<0.05**	0.16

(b)

Fixed effect	**Year**	**P-value**	**Marginal R^2**
log(Avg. *S. exigua* growth rate)	2021	0.49	0.006
	2022	0.62	0.002
	2023	**<0.05**	0.052

Marginal R^2 represents the variance explained by the fixed effect of species alone

DISCUSSION

Plant resistance to *S. exigua* in a lab bioassay did not consistently correspond to herbivore density in the field leading us to conclude that laboratory bioassays are unreliable predictors of naturally occurring herbivore densities and, by proxy, direct resistance in the realistic ecological settings. Nevertheless, for a subset of plant species, the lab bioassay were strongly correlated with herbivore densities. Year on year plant resistance in laboratory bioassays decreased and was correlated with herbivore densities in the last year of the study. Taken together, we speculate that differences in plant species correlations between laboratory bioassays and herbivore density imply the importance of other factors besides direct resistance such as abiotic effects or indirect defense that may influence herbivore density in natural conditions. By pairing laboratory bioassays with other measurements of whole-plant communities that are thought to mediate herbivore density, we speculate that these bioassays can serve as an integral measure into understanding the forces that shape herbivore density across ecological communities.

Of the 13 plant species in the study, just three (*M. fasciculatus, M. saxatillis, S. mellifera*) displayed moderate to strong relationships between *S. exigua* growth rates and herbivore densities with the remaining 10 plant species showing no significant relationships (Table 1.5). Interestingly, these same three plant species had some of the highest relative growth rates and lowest C:N ratios in the study (data not shown). Furthermore, two of these plant species (*M. fasciculatus & S. mellifera*) were in the upper quartile of both total and chewing herbivore densities (Figure 1.2, unpublished data). It should be noted, though, that *S. exigua* is a dietary generalist leaf chewer; a feeding strategy that does not map well onto the vast majority of naturally occurring herbivores observed in our experimental system. Of the total herbivores collected over the three years of the study (39,739), only 1% of those were chewing herbivores with the rest being sap-feeders (unpublished data). Plant resistance to *S. exigua*, then, may not have widespread relevance in this ecosystem. Taken together, these observations lead us to speculate that the utility of these bioassays as a predictive tool for naturally occurring herbivores in plant species is limited by optimal ranges in variance for plant traits and/or arthropod community composition.

Other bottom-up, top-down and intraguild effects present in the field but absent from the laboratory bioassays likely played a role in mitigating the ability of the bioassay to consistently predict herbivore density. During the course of the study, interannual patterns in precipitation steadily increased with 144 mm of total precipitation in 2021 to 345 mm of total precipitation in 2023 (unpublished data). These interannual changes likely impacted plant productivity and, by extension herbivore density by altering the timing of leaf growth and senescence; changes that were not controlled for since arthropod collection and *S. exigua* bioassays were performed at the same time each year. Density-dependent effects, such as intraguild competition between herbivores or predator responses to increased prey density may have likewise shifted herbivore densities in ways not directly linked to plant quality. It is worth mentioning, though, that some sources of variation were controlled for such as plant age (same chronological age at time of planting) and neighbor effects and edge effects (randomized arrangement of species within plots) so the lack of observable relationships for most of the species was a bit surprising.

Scaling up, our analyses revealed no significant relationships at the level of intraspecific variation across all years of the study (Table 1.6, Figure 1.3). At the community level, the relationship between *S. exigua* growth rate and herbivore density was significant, but only in the third year of the study which is also the year corresponding to the lowest overall *S. exigua* growth rate (Figure 1.4, Table 1.8b). Since *S. exigua* is a non-native dietary generalist, it may be that its utility as a predictor of natural herbivore density is temporally constrained by the age or phenology of the plant given that plants were 1.5 year old seedlings when planted and underwent substantial growth throughout the duration of the study (unpublished data). Additional years of data will be necessary to determine if *S. exigua* growth rates and naturally occurring herbivore densities covary to the point of establishing a predictive relationship.

It is interesting that some of the strongest associations observed between *S. exigua* performance in the lab and herbivore density in the field were seen not at the level of variation among species but nevertheless occurred within some individual species. One would assume that as you increased in scale from the species to the community, the noise from intra and interspecies variation would diminish if broad-scale trends were operating in the ecosystem as we had hypothesized. The opposite seems to hold true here and suggests that the factors which affect herbivores, both the non-native generalist and the naturally occurring, are tied to individual species and do not extend to the community level.

In conclusion, the dietary generalist *S. exigua* is a poor predictor by itself of naturally occurring herbivore density in our experimental system, contradicting our hypothesis that plant resistance, in part, exhibits a universal currency capable of defense against a diverse array of herbivorous arthropods. While overall there is no strong pattern, *S. exigua* does seem to be a good predictor of naturally occurring herbivore density for some species such as *M. fasciculatus, M. saxatillis,* and *S. mellifera* suggesting the potential for a universal currency in plant defense for these three species. Given the likely impact that other factors play in mediating herbivore density, we argue for the inclusion of bioassays into a comprehensive approach for future research; one that utilizes laboratory bioassays in conjunction with other top-down and bottom-up measures that mediate herbivore density.

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CHAPTER 2

THE EFFECT OF SPECIES VARIATION IN INDIRECT HERBIVORE DEFENSE ON HERBIVORY

ABSTRACT

Plants defend against herbivores utilizing a multidimensional approach that includes both direct and indirect defenses. Indirect defenses against herbivory involve the recruitment of predators and are presumed to result in a reduction of herbivory by reducing herbivore abundance. The ecological importance of predators is well established, and plant control over predators as an evolved defense is well studied in highly specialized and co-evolved systems. Less well understood is whether plants exert any influence over predators in unspecialized systems, and the importance of such effects in driving herbivore density. Using a common garden approach, we measured predator density among 13 plant species over three years in order to (i) assess to what extent plant species vary in the abundance of unspecialized predators, and (ii) whether such variation in generalized predator was in turn associated with variation in herbivore density. Plant species varied in the abundance of predatory arthropods while controlling for herbivore abundance, i.e. the density of predators relative to herbivores, but not in visitation by insectivorous birds. Furthermore, there was no correlation between the either predator group and herbivore abundance. Overall, the effects of predatory arthropods on herbivory under realistic ecological settings may be weak because herbivores are constrained other factors that affect either predator or herbivore abundance such as predator-predator interactions or abiotic factors.

INTRODUCTION

In order to effectively defend against a diverse array of herbivores, plants utilize a multidimensional approach that includes both direct and indirect defenses (Agrawal 2011). Direct defenses work to limit herbivory by either imposing structural barriers to herbivores (e.g. thorns, trichomes, cuticles) or impairing the physiology of the herbivore as they consume plant tissue (e.g. tannins, flavonoids). Indirect defenses, on the other hand, work to recruit the natural enemies of herbivores as bodyguards. Examples include the release of volatile organic compounds (VOC's) to attract parasitoid wasps, domatia and extrafloral nectaries to feed and house predatory arthropods, and facilitation of bird foraging through alterations in branching architecture (Chen 2008, War et al. 2012, Aljbory and Chen 2018). Indirect defenses, then, are predicted to reduce overall herbivore abundance thus imparting a fitness benefit to plants (Pearse et al. 2020).

The clearest evidence for the reduction in herbivory due to indirect effects has been observed in the case of highly co-evolved interactions between ants and plants. For example, certain Acacia species contain modified swollen thorns, or domatia, that provide habitat for the ants which in turn protect the plant from phytophagous insects (Janzen 1966). In Acacia species where this ant/plant mutualism is present, the density of phytophagous insects on shoots of Acacia cornigera was significantly higher across all metrics measured. Similar ant-mediated effects on herbivore density were seen in tropical species with strong ant-plant mutualisms (Heil et al. 1999, Dyer et al. 2001).

Despite the evidence of reduction in herbivory from predatory arthropods for these highly co-evolved plant-ant mutualisms and the ways in which plants mediate these mutualisms, (i) it is unknown whether plants might have similar control over densities of generalized predators and, (ii) if so, whether such plant-mediated variation in predators effects herbivore density relative to other factors such as direct defense, abiotic effects, etc. (Kessler and Heil 2011, Ali and Agrawal 2012, Fernández De Bobadilla et al. 2022, Kessler et al. 2023, Marquis and Whelan 1996, Pearse et al. 2020, Price et al. 1980) .

In considering the effect of predatory arthropods on herbivore abundance, there is also cause to consider how the interaction between different components of indirect defense may impact herbivore abundance (Mooney et al. 2010). Much of this understanding comes from studies on different groups of predators that prey on the same herbivore as well as predator-predator interaction studies (Losey and Denno 1998, Denno and Lewis 2009, Sanders et al. 2011, Nell and Mooney 2019). Together, they suggest that both competition between predator groups for shared prey (herbivores) and intraguild predation may alter the net effect of predators on their shared prey (Polis and Holt 1992, Rosenheim et al. 1993, Holt and Polis 1997).

In this study, we investigated whether there was variation in predator recruitment among plant species, suggesting the possibility for plant control and adaptive evolution of indirect defense, and the importance of such plant-mediated variation in driving herbivore densities. To do so, we characterized among-species variation in generalized predatory arthropod abundance and bird attack rates. In addition to this, we also examined the patterns of variation among these proxy measures of indirect defense. Specifically, we asked the following questions: 1) Do plant species differ in herbivore abundance and proxy measures of indirect defense from two groups of generalist predators; 2) if so, does variation in these proxy measures of indirect defense correlate with herbivore abundance; and 3) within these proxy measures of indirect defense, are there correlations suggestive of competition or intraguild predation among these predatory groups. By addressing these questions, our study evaluates the extent to which indirect defense may impact herbivore abundance in realistic ecological settings.

METHODS

Common garden site preparation

The study was performed on a 1/10 hectare experimental garden located on the campus of Concordia University Irvine (33°39'24.1"N 117°48'32.3"W). The site, previously an unused, south-westerly facing slope dominated by non-native Brassica nigra and made up primarily of Alo clay soil (www.usda.gov), was prepared for planting by removing all existing flora, plowing with a 26” disc harrow to homogenize soil, and installing a 1.5 meter tall perimeter fence to prevent incursion of rodent herbivores. Twenty plots were then constructed, each measuring 6m x 5m with 1m perimeter borders and covered with both water-permeable weed barrier and 5 cm jute mesh to prevent weed growth and mitigate water runoff. Weed barrier was removed in February of 2022 after seedlings were established.

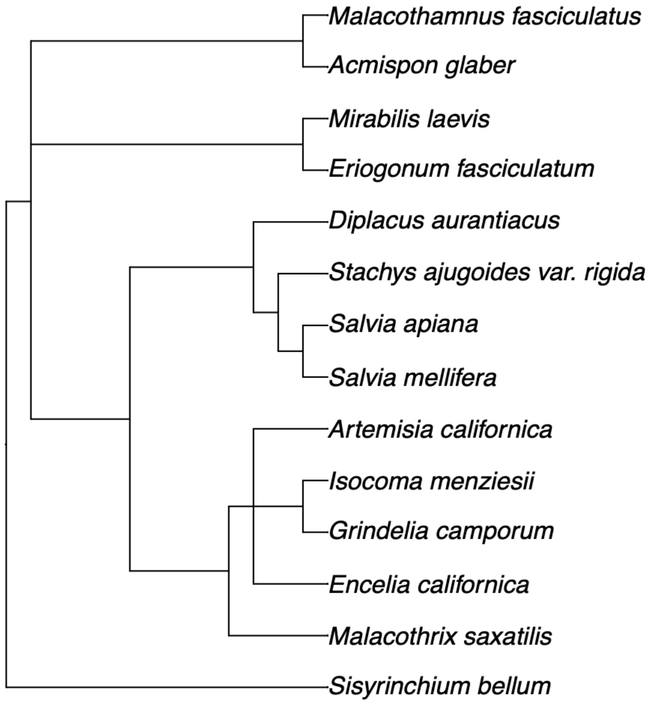

FIGURE 2.1. Phylogenetic tree of CSS perennial and forb shrubs used in the experiment using data obtained from the Phylomatic database (Webb and Donoghue 2005).

Plant propagation, transplanting and growth

Select perennial forbs and shrubs from diverse taxa and native to the Coastal Sage Scrub (CSS) ecosystem (Figure 2.1) were grown from seed (S&S Seeds, Carpinteria, CA). Seeds first underwent species-specific dormancy breaking protocols (unpublished data, Table 2.1) before being planted in a 2:1:1 peat moss:cedar bark:sand mixture and grown in a climate-controlled greenhouse 25c for 1 year. 1 seedling per species per plot was transplanted in February of 2020 and watered to establish throughout the Summer of 2020. Plots were weeded but otherwise left undisturbed throughout the length of the experiment. Plant survival was recorded annually at the time of sampling during peak growth season (Table 2.2).

TABLE 2.1. Plant species and seed dormancy breaking protocols.

Species	Dormancy Breaking Protocol
Acmispon glaber	Mechanical scarring
Artemisia californica	None
Encelia californica	None
Eriogonum fasciculatum	None
Grindelia camporum	None
Isocoma menziesii	None
Malacothamnus fasciculatus	None
Malacothrix saxatilis	None
Mimulus (Diplacus) aurantiacus	5% liquid smoke
Mirabilis laevis	None
Salvia apiana	None
Salvia mellifera	None
Sisyrinchium bellum	1.5 month cold stratification (4℃)
Stachys ajugoides var. rigida	None

TABLE 2.2. Yearly plant survival data from 2021-2023 from starting populations of N= 20 planted in 2020.

Species	2021	2022	2023
Acmispon glaber	16	10	2
Artemisia californica	20	20	20
Encelia californica	17	17	17
Eriogonum fasciculatum	16	15	15
Grindelia camporum	16	8	1
Isocoma menziesii	16	14	13
Malacothamnus fasciculatus	16	14	12
Malacothrix saxatilis	17	15	12
Mimulus (Diplacus) aurantiacus	19	18	18
Mirabilis laevis	20	18	17
Salvia apiana	17	16	15
Salvia mellifera	20	18	17
Sisyrinchium bellum	18	14	8
TOTAL	228	197	167

Clay Caterpillar Assay

Using green non-hardening plasticine modeling clay, 5 cm long and 5 mm wide artificial caterpillars were formed to mimic lepidopteran larvae (Dean et al. 2024), attached with super glue to branches on each plant and assessed for bird, other vertebrate, and invertebrate attacks each week for a period of 4 weeks beginning in April of 2022. Because plant branching architecture and heterogeneous prey distributions may influence bird foraging behavior, two caterpillars were deployed on each plant: one on the interior of the plant and one on the exterior. Interior caterpillars were glued on bare branches in the center of the plant between .5m and 1.5m from the ground whereas exterior caterpillars were glued on the ends of branches 15-30 cm from the branch tip. The shape and size of impressions left in the clay were used to identify the attacker whereas missing caterpillars were not recorded. Attacked or missing caterpillars were replaced with unmarked caterpillars glued to the same location after each week.

Arthropod Sampling

Arthropods were sampled annually for every living plant from 2021-2023 through vacuum-sampling (Nell and Mooney 2019). At peak plant growth (April-May), an electric vacuum (3.5 hp Rigid model # HD06001) fitted with a fine mesh bag in the nozzle was used to sample each plant for 3 minutes or the entire plant, whichever came first. Care was taken during sampling to minimize both injury to the plants and the amount of leaf litter collected. Mesh bags were immediately placed on ice then transferred to -20℃ for long-term storage that same day.

Arthropod Sorting and Classification

In these samples, vacuumed plant material was exhaustively checked for arthropods with the naked eye using fine-tipped paint brushes and entomological forceps, with arthropods placed in 70% ethanol for later identification. Arthropod herbivores were classified into one of five orders/suborders (Juvenile Lepidoptera, Orthoptera, Sternorrhyncha, Auchenorrhyncha, or Thysanoptera) and assigned to one of two feeding guilds (Phloem sap and cell content feeders or Chewing feeders) while arthropod predators were classified into one of five orders (Aranea, Acarina, Hymenoptera Vespidae, Mantodea, Neuroptera). Over the three years of the study, a total of 55,689 arthropods were classified, of which 70% were herbivores.

Data analysis

All data were analyzed using RStudio (Posit team 2024) with generalized linear mixed models constructed with the glmmTMB package (Brooks et al. 2017) and correlation analyses performed using the corrplot package (Wei and Simko 2017).

Among-species variation in direct and indirect effects

We first established whether variation among species (N = 13) was significant for herbivore density (Equation 2.1), indirect defense from predatory arthropods (i.e. predator density, Equation 2.2), and indirect defense from birds (i.e. clay caterpillar attack rates, Equation 2.3). To account for interannual variation, year was included as an additional fixed effect for Equations 1 and 2. To account for residual variance in our models, individual plants ("Plant") and the plot they were planted in ("Plot") were included as random effects for Equations 2.1-2.3. For the analysis of predatory arthropods (Equation 2.2), we sought to control for the influence of herbivore abundance on predatory arthropod abundance and thus use plant species residual variation in predatory arthropod abundance to account for effects on predatory arthropods transmitted via variation in herbivore abundance. We performed AIC model comparisons of linear and quadratic effects of herbivores on predators, determining that the relationship was curvilinear (see Results). For the analysis of indirect defense from birds (i.e. clay caterpillar attacks, Equation 2.3), we specified a binomial distribution, included the fixed effect of caterpillar position (interior or exterior), and added the random effect of individual caterpillar to account for resampling over a 4-week period.

(2.1) log(Herbivore count) ~ Species + Year + (1|Plot) +(1|Plant)

(2.2) log(Predator count) ~ Species + poly(log(Herbivore count), 2) + Year + (1|Plot) + (1|Plant)

(2.3) log(Herbivore count) ~ Species + Caterpillar position + (1|Plot) + (1|Plant) + (1|Caterpillar)

Correlations between direct and indirect effects

We next examined the significance of relationships between herbivore density, predatory arthropod density and bird attacks on clay caterpillars among species to test for the effects of indirect defense measures on herbivore abundance. We calculated species means (herbivore counts and bird attacks) or least square means (predator counts) for each untransformed fixed effect (Table 2.3). Using these species means, we then created a correlation matrix to evaluate the significance of each pairwise relationship.

RESULTS

Herbivore abundance and indirect defenses among species

The fixed effect of year was significant in all analyses ($p < 0.001$ for all cases), demonstrating strong interannual variation for herbivores, predatory arthropods and birds. Herbivore abundance differed significantly among species indicating strong species-level variation ($p < 0.001$, Table 2.3). For predatory arthropods, the effect of herbivore density on predators was significant ($p < 0.001$) with a quadratic effect providing superior fit as compared to a linear model (Δ AIC = 3.274, Figure 2.2). Having accounted for the effects of herbivores on predators, there was still significant plant species variation in predator density ($p < 0.001$, Table 2.3). In contrast, there was no detectable effect of plant species on bird attacks ($p = 0.81$, Table 2.3).

TABLE 2.3. Species means for herbivore abundance, and indirect defense from predatory arthropods and birds.

Species	**Herbivore abundance**	**Residual Predator Abundance**	**Bird Attack**
Acmispon glaber	137	6.89	0.13
Artemesia californica	76.61	6.55	0.17
Diplacus aurantiacus	21.43	8.76	0.15
Encelia californica	38.81	5.37	0.14
Eriogonum fasciculatum	29.83	6.62	0.16
Grindelia camporum	92.67	6.05	0.16
Isocoma menziesii	54.63	10.38	0.2
Malacothamnus fasciculatus	190.75	5.21	0.09
Malacothrix saxatillis	14.56	5	0.09
Mirabilis laevis	31.81	2.83	0.13
Salvia apiana	142.13	4.95	0.15
Salvia mellifera	89.18	5.81	0.13
Sisyrinchium bellum	9.82	4.26	0.14
Species effect significance:	**< 0.001**	**< 0.001**	0.81

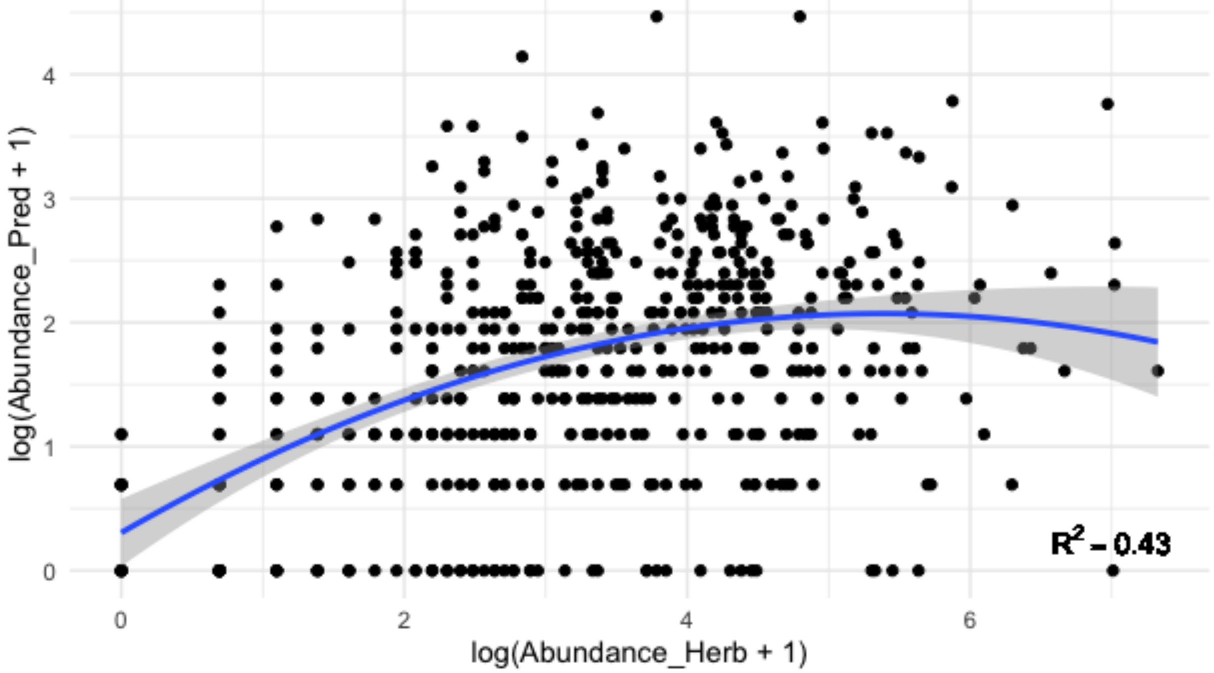

FIGURE 2.2. Polynomial relationship between Herbivorous and Predatory arthropod density across all species.

Correlations between herbivore density and indirect plant defense

Pairwise correlations between measures of herbivore density and indirect plant defense (predatory arthropod density, bird attacks) showed no significant relationships ($p = 0.93$, $p = 0.46$, respectively; Figure 2.3). This indicates that effects of indirect defense from predatory arthropod and birds as measured have marginal effects on herbivore abundance. Interestingly, a significant positive relationship between predatory arthropods and bird attacks was seen ($p < 0.05$, Figure 2.3) despite no variation in bird attacks between species (Table 2.3).

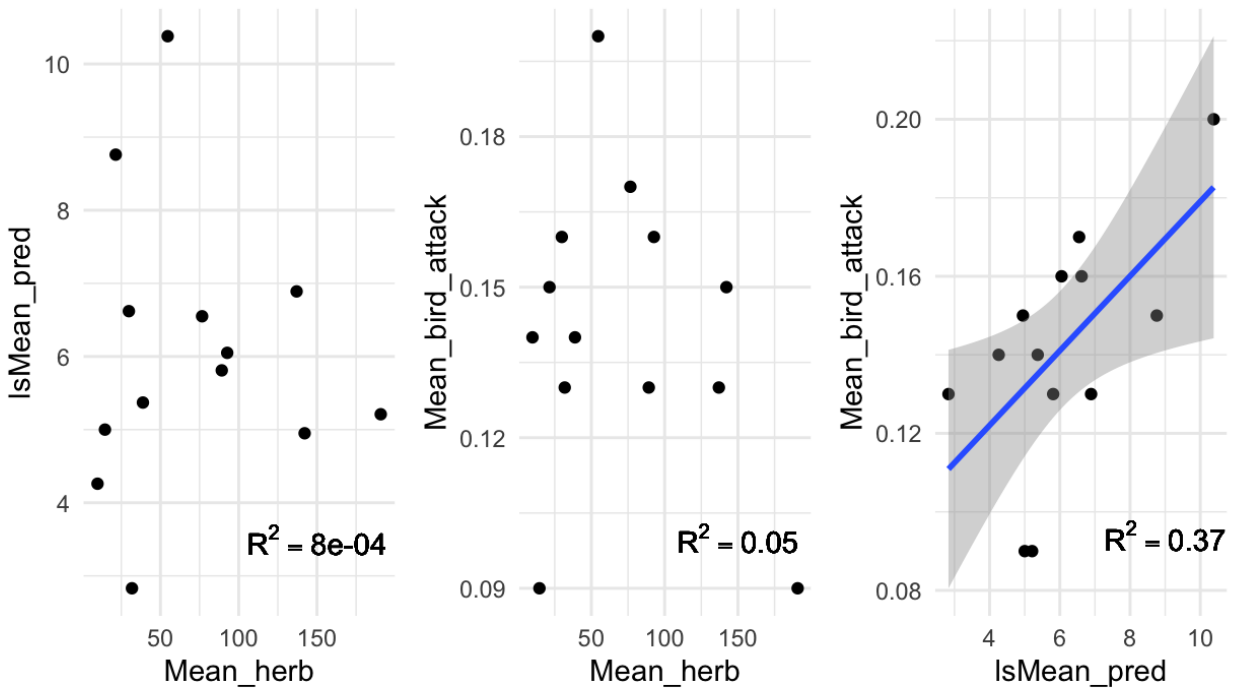

FIGURE 2.3. Relationships between herbivore density and indirect effects of predator arthropods and birds. Points represent species-level means or least-square means (N = 13).

DISCUSSION

There was significant variation among species in the density of predatory arthropods, demonstrating the possibility that plant traits can influence these generalized predators and the potential for adaptive evolution to employ these predators as a form of indirect defense. On the other hand, there was no statistically detectable difference among species in visitation by insectivorous birds and may demonstrate the existence of limits in a plants ability to influence different predator groups. Despite the variation in these generalist predatory arthropods among species, herbivore density showed no correlation indicating that indirect defense from predatory arthropods and birds on herbivore density was marginal in realistic ecological settings (Figure 2.4). While species variation in indirect defense from birds was not significant, there was nevertheless a significant positive correlation with indirect defense from predatory arthropods. This positive association is surprising given the expectation that birds would not discriminate between predatory arthropods and herbivores, having similar effects on both groups of arthropods (Mooney et al. 2010). Regardless of this positive association, the effects of birds and predatory arthropods on herbivores were both negative in line with predictions (Figure 2.4).

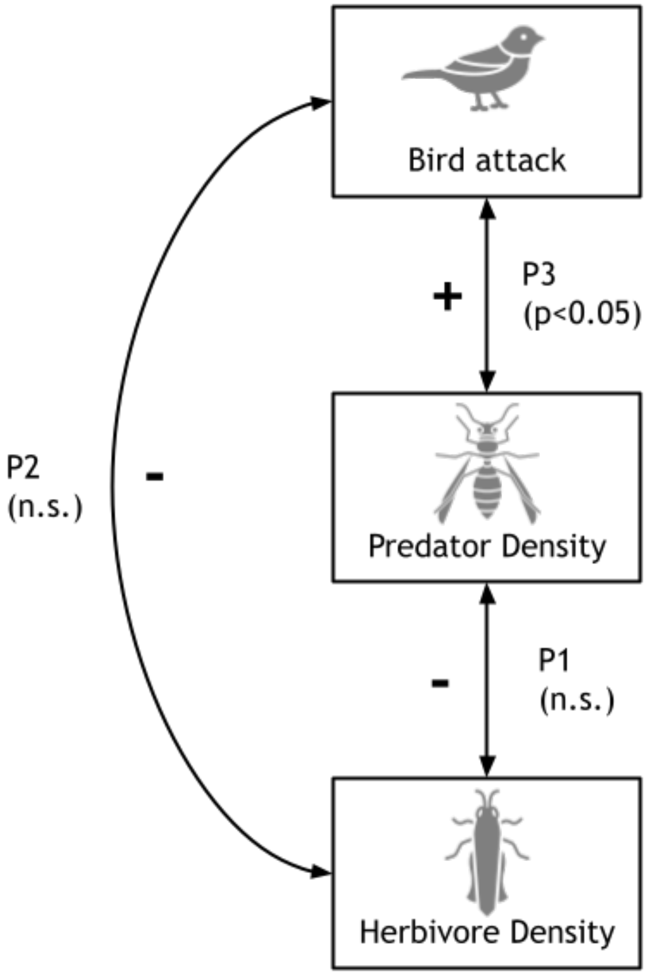

FIGURE 2.4. Significance and magnitude of relationships between measures of herbivore density and indirect defense from birds (clay caterpillar attacks) and predatory arthropods (predator density).

We interpret this variation in predatory arthropods and birds among plant species to be indicative of variation in levels of indirect defense. Variation within and among plant species in levels of both direct defense as well as indirect defense from highly specialized interactions is well established (Janzen 1966, Heil et al. 1999, Fernandes and Negreiros 2001, De Moraes et al. 2001, Clissold et al. 2004, Goussain et al. 2005, Harris et al. 2006, Chen et al. 2007), however variation in indirect defense from generalist predators is less clear (Fernández De Bobadilla et al. 2022). Here we demonstrate that plants do vary in densities of generalist predatory arthropods; an observation that documents the possibility of adaptive evolution in plants providing at least some control over measures of indirect defense.

Despite variation in indirect defense from predatory arthropods, plants did not demonstrate predicted correlations between herbivore density and predatory arthropod density. We think there are plausible reasons for this given the realistic ecological settings of the experiment. First, it may be that defense from predatory arthropods is not strong enough to demonstrate a significant correlation with herbivore abundance (Agrawal and Fishbein 2006). Second, other factors affecting either herbivore or predatory arthropod density such as intraguild competition may be masking the effect predatory arthropods have on herbivores (Denno and Finke 2006). For example, given the strong inter- and intra-annual changes in temperature and precipitation in the Coastal Sage Scrub ecosystem, abiotic effects on plant fitness may be having a much stronger effect on herbivore abundance that that of predatory arthropods. Alternatively (or concomitantly), predator-predator competition for herbivores may be reducing the overall impact of predatory arthropods on herbivores.

It is puzzling that despite variation in herbivore density and indirect defense from predatory arthropods, as well as the significant positive correlation seen between birds and predatory arthropods, indirect defense from birds did not vary among plant species (Figure 2.4). Since herbivores and predatory arthropods together comprised the bulk of arthropods among all species and most birds from observational surveys were insectivorous (data not shown), we would have predicted that birds would covary according to arthropod densities. Several reasons may explain these results, the most likely of which is that the use of clay caterpillars to measure indirect defense from birds may not be ecologically relevant to our system as juvenile lepidoptera comprised only 1% of total arthropods collected over the three years of the study (unpublished data).

Collectively, these findings are important in understanding of how relationships between communities of plant-associated organisms impact overall diversity in this ecosystem. Herbivores and generalist predatory arthropods are not distributed evenly among plant species; an observation we assume, at least in part, is driven by bottom-up effects of these species on their associated communities across multiple trophic levels. The drivers of this variation, however, remain unclear at this time. Given the broad phylogenetic diversity of plants studied, it may be that this variation was mediated by plant trait differences across lineages. Understanding the identify and relative role, then, of underlying plant traits on herbivore density should help us to better understand the relationship between plant community composition and the composition of associated communities at higher trophic levels.

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CHAPTER 3

CORRELATIONS BETWEEN NON-DEFENSIVE PLANT TRAITS AND ARTHROPOD COMMUNITY COMPOSITION

ABSTRACT

While efforts to explain variation in herbivore abundance have focused on plant defense traits, little is known how variance in non-defensive plant traits that mediate plant responses to its abiotic environment may simultaneously affect arthropod communities. In this study, we examined 14 plant species native to the Coastal Sage Scrub ecosystem and assessed how variation in six non-defensive plant traits correlate with arthropod community composition. We utilized constrained distance-based canonical correspondence analysis (db-CCA) and paired ordination plots to investigate these relationships both quantitatively and qualitatively. Our results revealed that variation in non-defensive plant traits were related to variation in arthropod community composition with distinct clusters of arthropod groups associating with specific plant trait gradients. These findings suggest that non-defensive plant traits may influence associated arthropod communities. This research advances our understanding of plant-arthropod interactions by providing evidence that plant traits traditionally associated with non-defense processes may also have substantive effects on arthropod community composition.

INTRODUCTION

Plants shape both ecosystem-level processes and the composition of associated communities (Kant et al. 2015, Calixto et al. 2021). In both cases, plant trait-based approaches have found great success in elucidating how plants mediate these ecosystem and community-structuring effects (Hairston et al. 1960, MacArthur and MacArthur 1961, Whitham et al. 2003, Lal 2004, Agrawal 2011, Pan et al. 2011, Schlesinger and Bernhardt 2013, Lamarre et al. 2016, Abdala-

Roberts et al. 2017). Despite these advances, studies examining plant responses to ecosystem-level processes and plant effects on mediating associated communities have operated largely as independent and isolated lines of inquiry with little attention paid to any overlap (Chapin et al. 1993, Agrawal and Fishbein 2006, Caldwell et al. 2016, Carvajal et al. 2019). Consequently, there is limited understanding of how and under what conditions plant responses to ecosystem-level processes affect associated communities and vice-versa.

Traits historically associated with ecosystem-level processes but absent from their impact on associated arthropod communities are specific leaf area (SLA), relative growth rate (RGR), leaf carbon concentration (%C), leaf nitrogen concentration (%N) and leaf percent water content (PWC), herein referred to as non-defensive plant traits. These traits mediate plant responses to water availability or resource allocation and energetic tradeoffs (LES) (Wright et al. 2004, Lambers and Oliveira 2019). For example, drought-tolerant plants might have reduced specific leaf area and lower leaf water content to minimize water loss, higher leaf carbon concentrations to increase leaf toughness and resistance to herbivory, and lower leaf nitrogen concentrations from decreased photosynthetic rates (Chapin et al. 2011, Iqbal et al. 2020, Farooq et al. 2024). These correlations have led to the discovery of higher-level patterns or spectrums whereby plants adapted to similar abiotic conditions demonstrate similar relative values across non-defensive plant traits; patterns that have provided the theoretical framework for predicting how plants relate and respond to their physical environment (Brodribb and Holbrook 2003, Wright et al. 2004, Bartlett et al. 2012, Reich 2014, Díaz et al. 2016).

Similarly, research on plant-insect, and in particular plant-herbivore, interactions have revealed sets of traits that mediate plant defense while largely ignoring their impact on ecosystem-level processes. Concentrations of plant defensive compounds, trichome density, plant structural complexity, specific leaf area and leaf concentrations of carbon or nitrogen are all examples of plant defense traits that have been shown to work synergistically in affecting arthropod community composition (Agrawal 2000, Kant et al. 2015, Barbour et al. 2015, Abdala-Roberts et al. 2017, Harrison et al. 2018, Nell and Mooney 2019), and have given rise to theoretical frameworks such as the Resource Availability Hypothesis, Optimal Defense Theory, and the Growth-Differentiation Balance Hypothesis that seek to explain and predict plant-herbivore interactions (Coley et al. 1985, Herms and Mattson 1992, Stamp 2003). Despite the significant scholarship in how these plant defense traits mediate associated communities and the overlap a subset of these plant defense traits have with non-defensive functions, research on plants and their associated arthropod communities has given little consideration as to how non-defensive leaf traits may consequently affect arthropod community composition (Huberty and Denno 2004, Maron and Crone 2006, Agrawal et al. 2010).

In this study we characterized how non-defensive plant traits correlate with variation in associated arthropod communities. To do so, we used a common garden to compare inter-specific variation in these non-defensive plant traits with associated arthropod community diversity among 14 plant species native to the Coastal Sage Scrub ecosystem of Southern California. By evaluating the relationships between non-defensive plant traits and arthropod community composition across multiple plant species over multiple years, we expand the scope of our understanding on how plant affect arthropod community composition.

METHODS

Common garden site preparation

The study was performed on a 1/10 hectare experimental garden located on the campus of Concordia University Irvine (33°39'24.1"N 117°48'32.3"W). The site, previously an unused, south-westerly facing slope dominated by *Brassica nigra* made up primarily of Alo clay soil (www.usda.gov), was prepared for planting by removing all existing flora, plowing with a 26" disc harrow to homogenize soil, and installing a 4ft tall perimeter fence to prevent incursion of rodent herbivores. Twenty plots were then constructed, each measuring 6m x 5m with 1m perimeter borders and covered with both water-permeable weed barrier and 2 inch jute mesh to prevent weed growth and mitigate water runoff. Weed barrier was removed in February of 2022 after seedlings were established.

Plant propagation, transplanting and growth

Select perennial forbs and shrubs from diverse taxa and native to the Coastal Sage Scrub (CSS) ecosystem (Figure 3.1) were grown from seed (S&S Seeds, Carpinteria, CA). Seeds first underwent species-specific dormancy breaking protocols (unpublished data, Table 3.1) before being planted in a 2:1:1 peat moss:cedar bark:sand mixture and grown in a climate-controlled greenhouse 25c for 1 year. 1 seedling per species per plot was transplanted in February of 2020 and watered to establish throughout the Summer of 2020. Plots were weeded but otherwise left undisturbed throughout the length of the experiment. Plants were assessed annually at the time of sampling during peak growth season (Table 3.2).

TABLE 3.1. Plant species and seed dormancy breaking protocols.

Species	Dormancy Breaking Protocol
Acmispon glaber	Mechanical scarring
Artemisia californica	None
Encelia californica	None
Eriogonum fasciculatum	None
Grindelia camporum	None
Isocoma menziesii	None
Malacothamnus fasciculatus	None
Malacothrix saxatilis	None
Mimulus (Diplacus) aurantiacus	5% liquid smoke
Mirabilis laevis	None
Salvia apiana	None
Salvia mellifera	None
Sisyrinchium bellum	1.5 month cold stratification (4°C)
Stachys ajugoides var. rigida	None

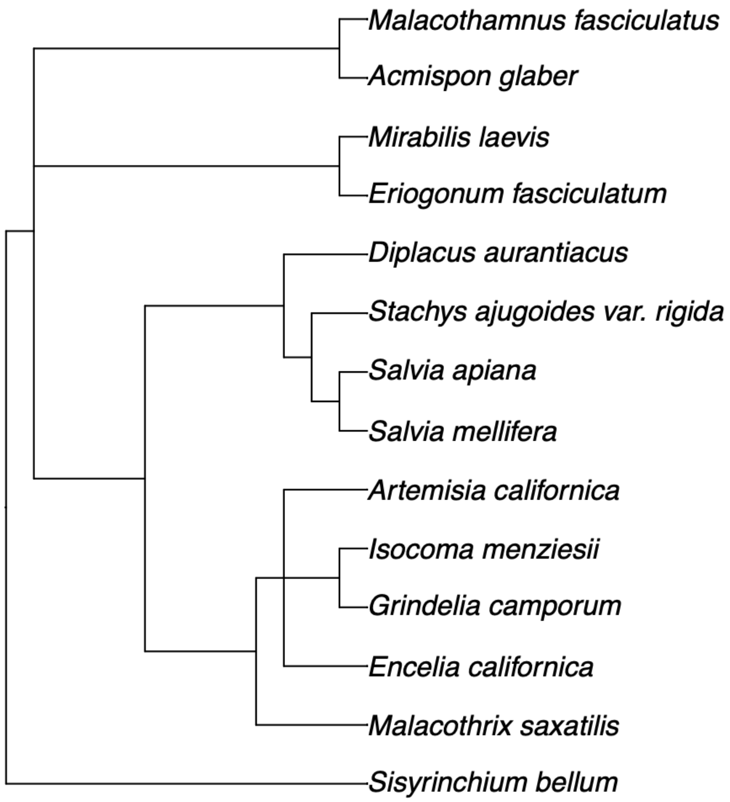


FIGURE 3.1. Phylogenetic tree of CSS perennial and forb shrubs used in the experiment using data obtained from the Phylomatic database (Webb and Donoghue 2005).

TABLE 3.2. Yearly plant survival data from 2021-2023 from starting populations of *N= 20* planted in 2020.

Species	2021	2022	2023
Acmispon glaber	16	10	2
Artemisia californica	20	20	20
Encelia californica	17	17	17
Eriogonum fasciculatum	16	15	15
Grindelia camporum	16	8	1
Isocoma menziesii	16	14	13
Malacothamnus fasciculatus	16	14	12
Malacothrix saxatilis	17	15	12
Mimulus (Diplacus) aurantiacus	19	18	18
Mirabilis laevis	20	18	17
Salvia apiana	17	16	15
Salvia mellifera	20	18	17
Sisyrinchium bellum	18	14	8
Stachys adjugoides var. rigida	19	12	11
TOTAL	**247**	**209**	**178**

Relative growth rate (RGR)

Above-ground plant dry biomass was estimated at peak growth in May of 2021 and 2023 using a branch-biomass estimation method (Mooney, unpublished data). Briefly, two representative branches for each perennial species were selected from individuals not part of the study area. The number of branches to account for the total volume of each plant was estimated. Representative branches were then dried for 72h at 70°C, weighed, and multiplied by branch counts to determine an estimate of above-ground biomass for each plant. RGR was then calculated as RGR = (ln M_2 – ln M_1)/(t_2 – t_1) where M_1 and M_2 are plant dry biomass at 2021 and 2023, respectively, and T_1 and T_2 are 2021 and 2023, respectively.

Plant leaf traits

Leaf samples for percent water content (PWC), Specific leaf area (SLA) and carbon/nitrogen isotope analysis were collected in May of 2023 during peak growth and immediately processed. For PWC, leaves were soaked in deionized water overnight at 4°C to obtain mass saturation and weighed. Leaves were then dried for 72h at 70°C and weighed again. The ratio of saturated mass to dry mass was calculated to determine PWC. To measure SLA, fresh leaves were immediately imaged, dried for 72h at 60°C, then weighed. The surface area of each sample was measured using ImageJ software (Rasband 2012). SLA was calculated as cm^2 x g^{-1} dry weight. For carbon and nitrogen isotope analysis, leaves were dried for 72h at 60°C then finely ground in liquid nitrogen. Samples were analyzed for percent composition of Carbon-13 and Nitrogen-15 using isotope ratio mass spectrometry (IRMS).

Arthropod sampling, sorting and classification

Arthropods were sampled annually for every living plant from 2021-2023 through vacuum-sampling (Nell and Mooney 2019). At peak plant growth (April-May), an electric vacuum (3.5 hp Rigid model # HD06001) fitted with a fine mesh bag in the nozzle was used to sample each plant for 3 minutes or the entire plant, whichever came first. Care was taken during sampling to minimize both injury to the plants and the amount of leaf litter collected. Mesh bags were immediately placed on ice then transferred to -20℃ for long-term storage that same day.

In these samples, vacuumed plant material was exhaustively checked for arthropods with the naked eye using fine-tipped paint brushes and entomological forceps, with arthropods placed in 70% ethanol for later identification. Arthropods were classified into feeding guilds then further into taxonomic orders/suborders (Table 3.3). Over the three years of the study, a total of 55,689 arthropods were classified. Since sampling efforts were standardized across all plants, herbivore counts were used as a proxy for herbivore density.

TABLE 3.3. Arthropod feeding guild and taxonomic order classifications

Feeding guild	Taxonomic order/suborder
Herbivore	Auchenorrhyncha Sternorrhyncha Lepidoptera (juvenile) Orthoptera Thysanoptera
Predator	Aranaea Acarina Heteroptera Hymenoptera (Vespidae) Neuroptera Mantodea
Omnivore	Coleoptera Diptera Hymenoptera (Formicidae)
Palynivore	Hymenoptera (Apoidea) Lepidoptera (adult)
Detritivore	Psocoptera Collembola

Data analysis

The relationships between variance in species, variance in plant traits, and variance in arthropod community composition were assessed for each plant across each year *($N_{2021} = 247$; $N_{2022} = 209$; $N_{2023} = 178$)* for a total of 634 replicate samples. Generalized linear mixed models were conducted using the glmmTMB package (Brooks et al. 2017) and visualized using the ggplot2 package (Wickham 2016). The Vegan package (Oksanen et al. 2001) was used for all PERMANOVA analyses as well as for principal coordinate (PCoA), and distance-based redundancy analyses (db-CCA).

Plant traits and arthropod community composition

Each plant trait was assessed independently to quantify the effect of plant species identity on plant trait variance using generalized linear mixed models with “plot” and “plant” included as random factors (Equation 3.1).

(3.1) Plant trait ~ Species + (1|Plot) + (1|Plant)

The effect of plant species on arthropod community composition was assessed from the guild-level. Means of herbivores, predators and total arthropods pooled across all years of the study were calculated for each plant. Generalized linear mixed models were then performed for each guild separately with plant, plot and year as random effects (Equation 3.2).

(3.2) Arthropod guild ~ Species + (1|Plot) + (1|Plant) + (1|Year)

To assess how variance in plant species affected the variance in all plant traits or all arthropod orders simultaneously, we next performed permutational multivariate analysis of variance (PERMANOVA) tests. Since there was considerable variance among plant traits values and arthropod order counts, we first transformed both datasets to normalize variable ranges and allow each variable to contribute more equally to the distance matrix calculations in our

PERMANOVA analyses. Z-transformations were performed to standardize the scale of our plant trait dataset while for arthropod data, we pooled arthropod counts across all years for each plant then calculated percent composition of each arthropod order to the whole. PERMANOVA analyses with 10,000 permutations were then run for plant trait variance (Equation 3.3a) and arthropod order variance (Equation 3.3b) separately. For the plant trait PERMANOVA, a matrix of pairwise Euclidean dissimilarities was calculated whereas for arthropod order PERMANOVA, Bray-Curtis dissimilarities were used. Principal coordinates analysis PCoA coordinates were then plotted in two dimensions for each analyses separately with points reflecting centroids of either plant trait variance or arthropod order variance for each species. Plant traits and arthropod orders that strongly influenced the overall result ($P < 0.05$; $R^2 > 0.1$) were displayed as vectors with length scaled to R^2.

(3.3a) Arthropod order 1, Arthropod order 2, … Arthropod order N ~ Species

(3.3b) Plant trait 1, Plant trait 2, … Plant trait N ~ Species

Linking plant traits to arthropod community composition

Since variance in both plant traits and arthropod community composition were strongly influenced by differences in species, we next analyzed the multivariate relationships between plant traits and arthropod community composition using a distance-based canonical correspondence analysis (db-CCA) with constrained ordinations (Equation 3.4). db-CCA is an ordination method that assumes a unimodal as opposed to linear relationship between predictor and response variables. Pairwise Bray-Curtis dissimilarities for arthropod community composition between species were used in a principal coordinate analysis constrained by plant traits and plotted in two dimensions with centroids for each plant species. Influential plant traits and arthropod orders ($P < 0.05$; $R^2 > 0.1$) were included as vectors with magnitude scaled to R^2 value to visualize which plant traits were most important for driving arthropod community composition and which arthropod orders were most influenced by these plant traits.

(3.4) Arthropod order 1, Arthropod order 2, … Arthropod order N ~ Plant trait 1, Plant trait 2, … Plant trait N (dbcca)

RESULTS

Non-defensive plant traits and arthropod community composition.

All non-defensive plant traits measured varied significantly among plant species ($P < 0.001$, $R^2 > 0.4$ for all traits; Figure 3.2, Tables 3.4, 3.5). Among-species variance in arthropod community composition according to feeding guild showed similar results, with significant among-species variance in herbivore, predator and total arthropod density ($P < 0.001$, $R^2 > 0.3$ for all guilds; Figure 3.3, Table 3.6). When arthropod community composition was reclassified according to taxonomic order/suborder, similar patterns of variance were found across plant species (PERMANOVA, $P < 0.001$; Table 3.7). Plant species variation in arthropod community composition was most strongly associated with variation in the densities of Heteroptera ($P < 0.001$, $R^2 = 0.35$), Auchenorrhyncha ($P < 0.001$, $R^2 = 0.74$), Sternorrhyncha ($P < 0.001$, $R^2 = 0.94$), Thysanoptera ($P < 0.001$, $R^2 = 0.45$) and to a lesser extent Hymenoptera (Formicidae) ($P < 0.001$, $R^2 = 0.15$, Table 3.8).

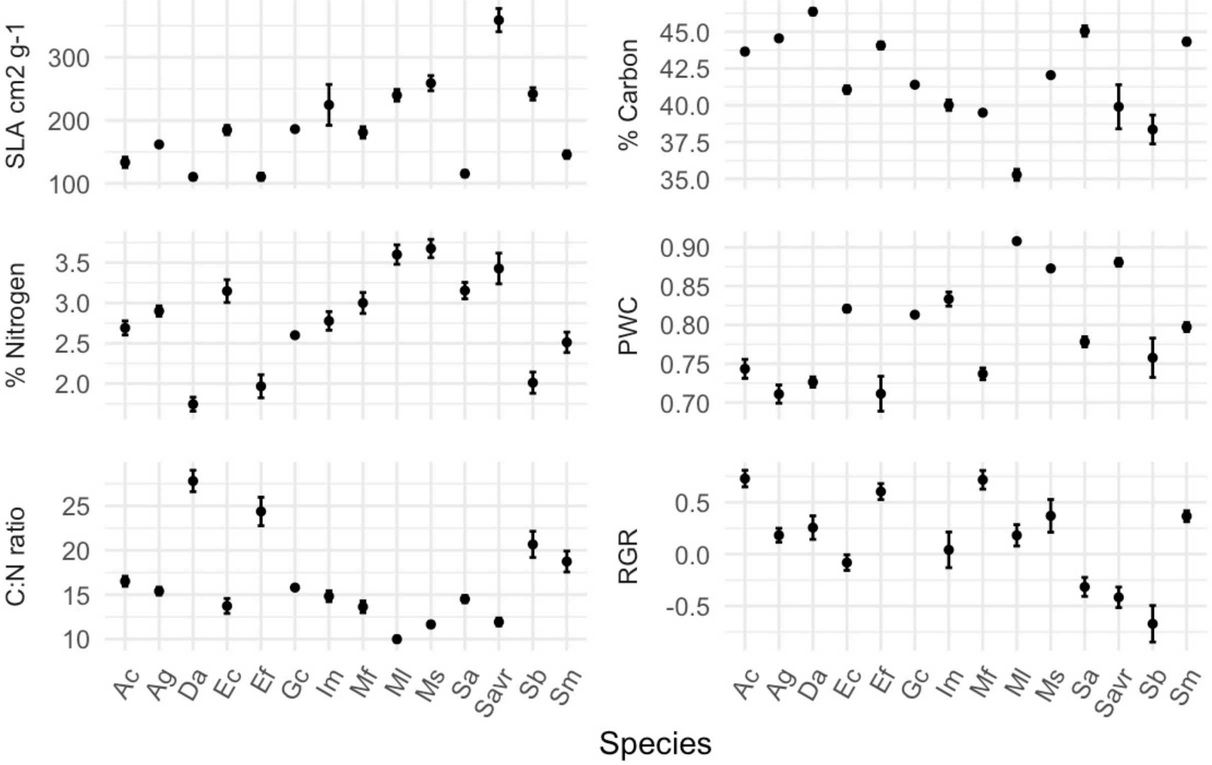


FIGURE 3.2. Plant species variation in specific leaf area (SLA), percent carbon (% Carbon), percent nitrogen (% Nitrogen), percent water content (PWC), carbon-to-nitrogen ratio (C:N ratio), and relative growth rate (RGR). Least square means of plant traits (±SE) are shown for each species (see methods for details). Initials represent the first letters of plant *Genus* and *species*, respectively (See Table 3.2 for species list).

TABLE 3.4. Summary statistics for species-level means of non-defensive plant traits.

Species	SLA	% C	% N	PWC	C:N Ratio	RGR
A. glaber (Ag)	161.80	44.55	2.90	0.71	15.40	0.18
A. californica (Ac)	133.41	43.65	2.69	0.74	16.52	0.73
D. aurantiacus (Da)	110.33	46.37	1.74	0.73	27.81	0.26
E.californica (Ec)	184.60	41.06	3.15	0.82	13.73	-0.08
E. fasciculatum (Ef)	110.55	44.07	1.97	0.71	24.37	0.60
G.camporum (Gc)	186.09	41.40	2.60	0.81	15.80	Na
I.menziesii (Im)	224.52	40.01	2.78	0.83	14.82	0.04
M. fasciculatus (Mf)	180.83	39.50	3.00	0.73	13.63	0.72
M. saxatilis (Ms)	258.90	42.05	3.68	0.87	11.65	0.37
M. laevis (Ml)	239.73	35.28	3.60	0.91	9.99	0.18
S. apiana (Sa)	115.43	45.05	3.15	0.78	14.51	-0.32
S. mellifera (Sm)	145.63	44.32	2.51	0.80	18.74	0.37
S. bellum (Sb)	241.95	38.36	2.01	0.76	20.67	-0.67
S. adjugoides (Savr)	358.85	39.90	3.43	0.88	11.93	-0.42

TABLE 3.5. Results from generalized linear mixed effects (GLMM) models examining the significance of plant species-level differences in non-defensive plant trait variance. See equation 3.1 for details.

Plant trait	Chi Sq	Chi *df*	P value	Marginal R^2	Conditional R^2
SLA	355.35	13	<0.001 *	0.69	0.85
PWC	332.02	13	<0.001*	0.66	0.83
RGR	130.24	13	<0.001*	0.43	0.72
% Carbon	404.64	13	<0.001*	0.69	0.70
% Nitrogen	253.84	13	<0.001*	0.58	0.80
C:N ratio	339.80	13	<0.001*	0.66	0.79

SLA = specific leaf area, *PWC* = percent water content, *RGR* = relative growth rate, % Carbon = percent leaf carbon, % Nitrogen = percent leaf nitrogen, *C:N ratio* = *leaf* carbon-nitrogen ratio. Marginal R^2 represents the variance explained by the fixed effect of species alone, while Conditional R^2 represents variance explained by both fixed effect of species and random effects of individual plant observations and plot.

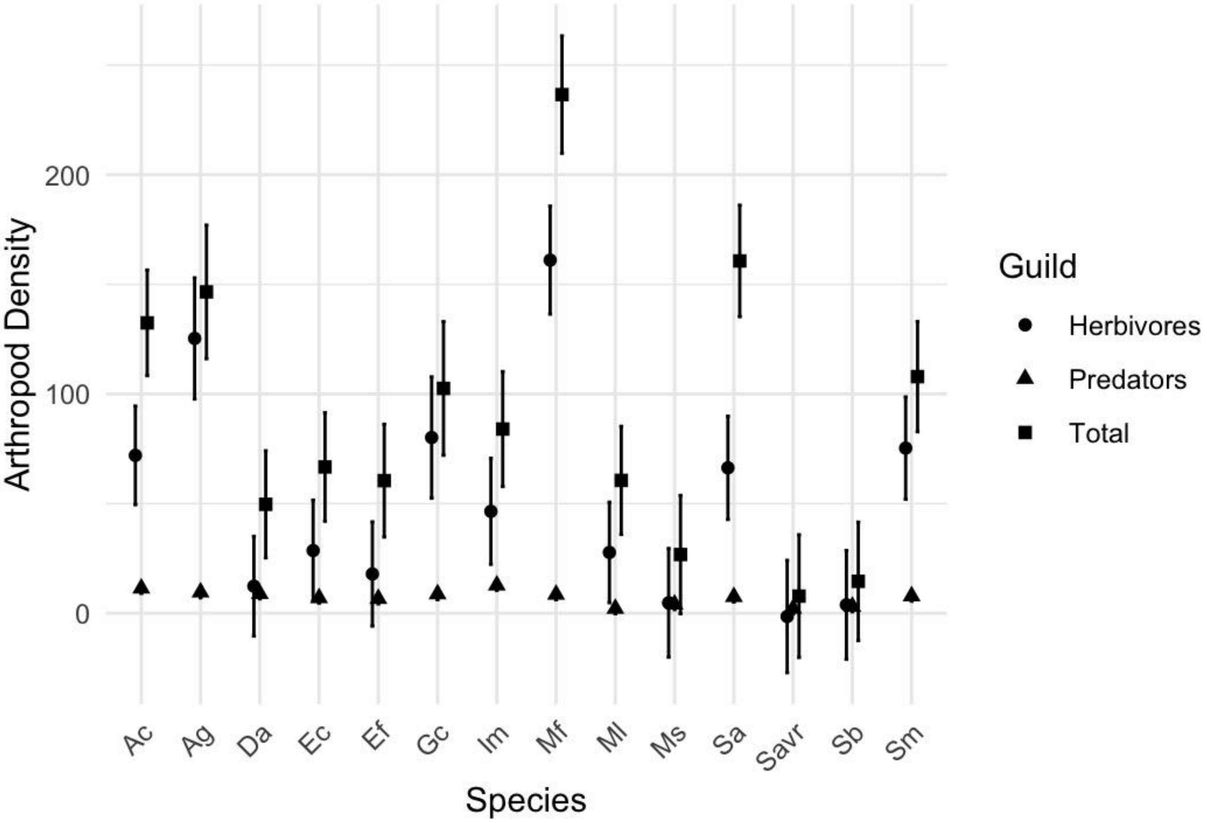


FIGURE 3.3. Plant species variation in arthropod densities. Least square means densities (±SE) for total arthropods (squares), herbivores (circles) and predators (triangles) are shown. Initials represent the first letters of plant *Genus* and *species*, respectively. See Table 3.2 for species list.

TABLE 3.6. Summary results from generalized linear mixed effects (GLMM) models examining plant species-level differences in arthropod guild composition. See equation 3.2 for details.

Arthropod guild	**Chi Sq**	**Chi *df***	**P value**	**Marginal R^2**	**Conditional R^2**
Total	126.94	13	<0.001 *	0.34	0.38
Herbivores	310.13	13	<0.001*	0.29	0.31
Predators	373.47	13	<0.001*	0.32	0.36

Marginal and Conditional R^2 represent the variance in the response variable explained by fixed effects of species alone, while conditional R^2 represents variance explained by both fixed effect of species and random effects of individual plant observations, plot, and year.

TABLE 3.7. Results from permutational multivariate analysis of variance (PERMANOVA) analysis on the significance of plant species-level differences on the variance in arthropod community composition. See equation 3.3a for details.

Effect	***df***	**Sum of squares**	**R^2**	**F**	**Pr (>*F*)**
Plant species	13	22.844	0.42	13.158	<0.001*
Residuals	237	31.652	0.58	NA	
Total	250	54.496	1.00	NA	

TABLE 3.8. Individual taxonomic orders of arthropods significantly affected by variation in plant species based on results from permutational multivariate analysis of variance (PERMANOVA) analysis on the significance of plant species-level differences on the variance in arthropod community composition. See equation 3.3a for details.

Taxonomic order/suborder	***P* value**	**R^2**
Heteroptera	<0.001 *	0.35
Auchenorrhyncha	<0.001 *	0.74
Sternorrhyncha	<0.001 *	0.94
Thysanoptera	<0.001 *	0.45
Hymenoptera (Formicidae)	<0.001 *	0.15

Plant trait mediation of arthropod community composition

Distance-based constrained canonical correspondence analysis (db-CCA) demonstrated that arthropod community composition was significantly correlated with all non-defensive plant traits combined ($P < 0.001$) as well as with the individual plant traits of % C ($P < 0.001$), PWC ($P < 0.05$), % N ($P < 0.001$), RGR ($P < 0.001$), C:N Ratio ($P < 0.001$) (Table 3.9). Constrained ordination plots demonstrated three axes of correlation between plant traits and arthropod orders: (1) C:N Ratio was positively correlated with Araneae, Hymenoptera (Vespidae), Diptera, and Coleoptera, (2) RGR was positively correlated with Thysanoptera and

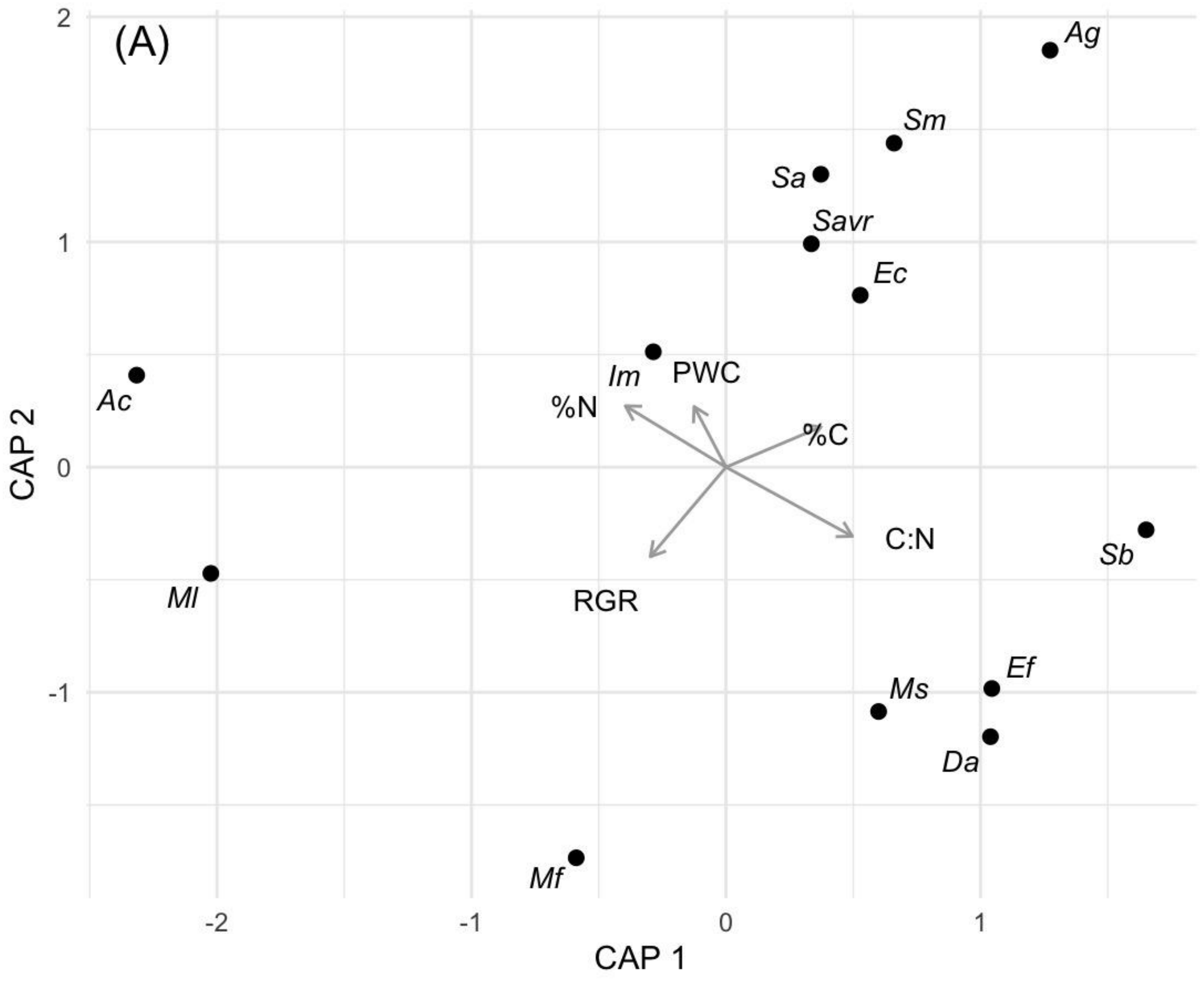
Heteroptera, and (3) PWC and % Nitrogen were positively correlated with Auchenorrhyncha and (4) % Carbon was positively correlated with Hymenoptera (Formicidae) and Sternorrhyncha (Figure 3.4, Table 3.10).

TABLE 3.9. Results from distance-based canonical correspondence analysis (db-CCA) on the correlation between non-defensive plant trait variance and variance in arthropod community composition as classified by taxonomic order/suborder among plant species.

Trait	***df***	**Sum of squares**	**F**	**Pr (>*F*)**	**R^2**
All plant traits combined	5	1.96	1.97	<0.001 *	0.13
SLA	1	0.22	1.05	0.375	0.001
PWC	1	0.64	3.11	< 0.01 *	0.04
RGR	1	0.68	3.28	< 0.01 *	0.13
% N	1	0.67	3.25	< 0.01 *	0.12
% C	1	0.60	2.90	< 0.01 *	0.09
C:N Ratio	1	0.56	2.70	< 0.01 *	0.17
Residual	146	30.14			

TABLE 3.10. Correlations between variance in individual non-defensive leaf traits and variance in individual arthropod orders/suborders based on results from distance-based canonical correspondence analysis (db-CCA). Correlations between plant traits and arthropod order/suborders are assigned to quadrants based on results from constrained ordination plots with (see Figure 3.4).

Quadrant	Arthropod Order/Suborder	Pr (>*F*)	R^2	Plant trait correlations
I (+,+)	Sternorrhyncha	< 0.001 *	0.43	↑ % C
	Hymenoptera (Formicidae)	< 0.001 *	0.19	
II (-,+)	Auchenorrhyncha	< 0.001 *	0.77	↑ %N, PWC ↓ C:N
III (-,-)	Heteroptera	< 0.001 *	0.63	↑ RGR
	Thysanoptera	< 0.001 *	0.37	↓ %C
IV (+,-)	Araneae	< 0.001 *	0.16	↑ C:N ↓ %N, PWC
	Hymenoptera (Vespidae)	< 0.001 *	0.20	
	Coleoptera	< 0.001 *	0.24	
	Diptera	< 0.001 *	0.21	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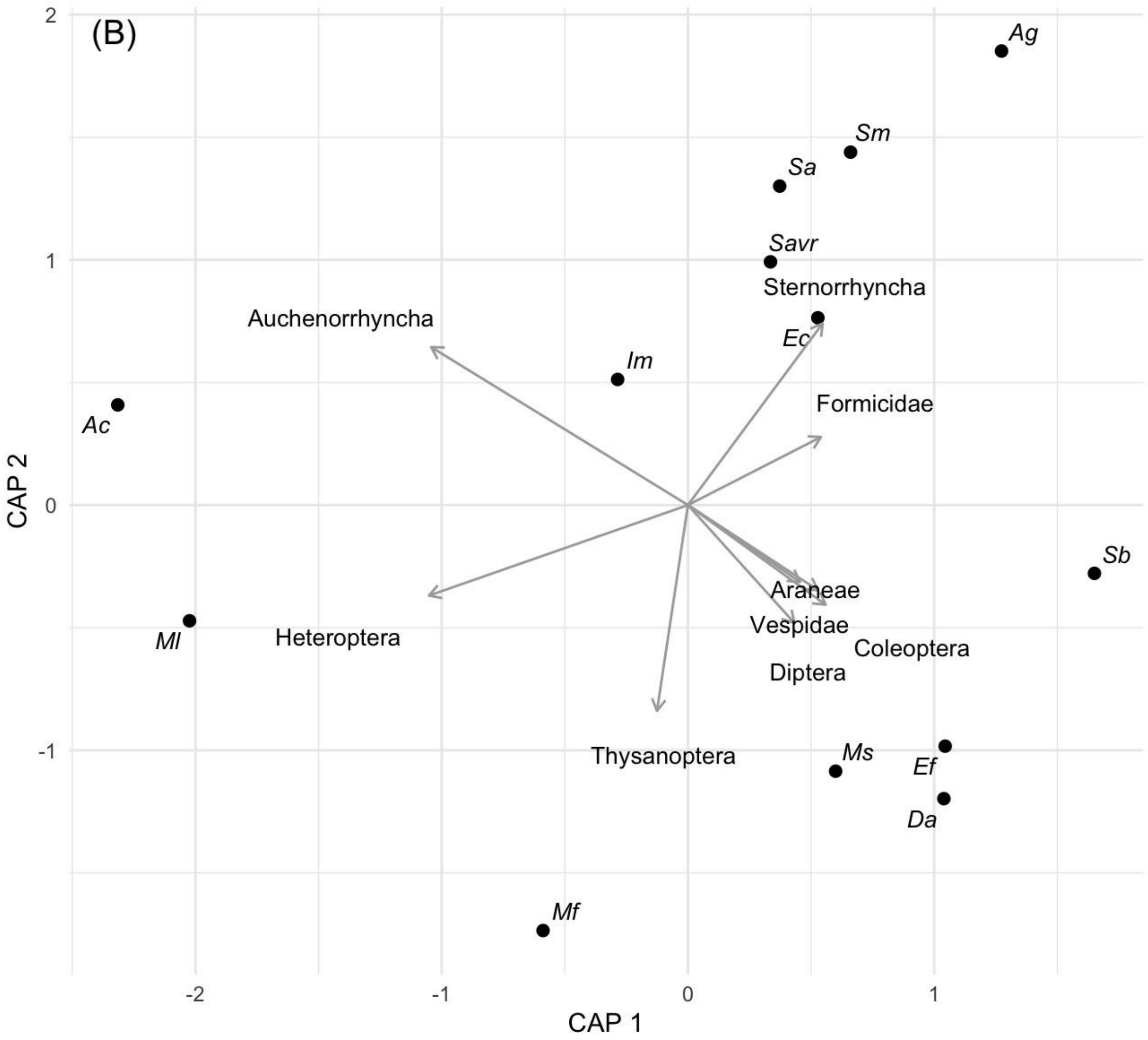

FIGURE 3.4. Ordination plots from distance-based canonical analysis (db-CCA) of plant species effects on variance in arthropod community composition when constrained by variance in non-defensive plant traits. Centroids of variance in arthropod community composition according to taxonomic order/suborder (circles) are included for each plant species (see Table 3.2). Vectors for trends in individual non-defensive plant traits (A) and arthropod orders (B) are included and scaled to reflect their relative magnitude based on R^2 values.

DISCUSSION

Among-species variance in non-defensive plant traits was strongly correlated with variation in arthropod community composition, especially sap-feeders, hinting at a broader ecological significance of these non-defensive plant traits in affecting associated biotic communities. Closer examination revealed that groups of arthropod communities showed strong associations with specific non-defensive plant traits—an unexpected finding given the limited variation in these plant traits anticipated due to strong ecological similarity among plant species. Collectively, the variation in arthropod community composition showed strong covariation with non-defensive plant traits across species suggesting potential roles for non-defensive plant traits in mediating plant associated communities.

Ordination trends among these non-defensive plant traits yielded mixed results from those predicted by plant-water relation or LES theory. Though some expected covariance was seen between RGR and SLA, we did not see the same covariance in PWC (Figure 3.4). This is somewhat surprising given the expectation that plants with more rapid tissue expansion and higher growth rates indicated by SLA and RGR, respectively, would likewise demonstrate higher leaf water contents to meet growth demands (Wright et al. 2004, Lambers and Oliveira 2019). The partially negative relationship seen between Carbon and Nitrogen concentrations were expected as was the negative relationship between Carbon concentrations and RGR. However, Nitrogen concentrations did not show strong covariance patterns with RGR as expected. Taken together, these observations suggest that while faster-growing plants had broader, flatter leaves with shorter lifespans in line with theoretical predictions, they did not display the high leaf Nitrogen concentrations expected for plants with a more resource-acquisitive growth strategy (Wright et al. 2004). One major reason for these observations is likely due to the ecological similarity among plant species. All species are native to the same Coastal Sage Scrub ecosystem, woody shrubs, and are grown in the same common garden;

similarities that likely constrained the variance in plant traits such that covariance patterns predicted from theoretical frameworks developed across fundamentally different ecosystems were weakened.

Variance in arthropod community composition when constrained by plant traits revealed three distinct clusters of covariance among arthropod orders/suborders. Cluster 1 consisted of strong covariance between ants and aphids. This finding concurs with the ant-aphid keystone interactions being recognized as a significant driver of overall arthropod community composition where these relationships are present (Wimp and Whitham 2001, Styrsky and Eubanks 2007). Ant-aphid relationships demonstrated a strong correlation with increased leaf carbon concentration, hinting at the possibility of a relationship between increased leaf carbon and increased ant/aphid densities. However, three of the five plant species that these relationships mapped onto belong to the same clade suggesting that leaf carbon concentrations may primarily be a consequence of phylogenetic similarity and have little or no relationship with changes in ant or aphid densities.

Cluster 2 consisted of other sap-feeding herbivores (thrips, true bugs, and leafhoppers) and was correlated with increased RGR, PWC and leaf Nitrogen. This cluster of arthropods was more loosely correlated with each other than ants and aphids which is unsurprising given that correlation patterns among these three groups are inconsistent and seem to depend strongly on individual feeding habits and host plant identity (Novotny and Basset 2005, Forister et al. 2015, Wardhaugh 2015). High RGR, PWC and leaf Nitrogen indicate nutrient rich leaves which may explain higher abundances of sap-feeding herbivores in this cluster (Herms and Mattson 1992, Huberty and Denno 2004, 2006), however, similar to Cluster 1, all 3 plant species that these relationships mapped onto were more closely related to each other than any of the other plant species in the study, again inferring that phylogenetic similarity may be the primary driver of covariance in these plant traits.

Predatory and leaf-chewing taxa comprise Cluster 3 and demonstrate the strongest patterns of arthropod covariance out of all clusters. These taxa correlate with high C:N ratios; an observation that is consistent with plants investing more in direct defense strategies (Coley et al. 1985). If true, this is puzzling as it infers that plants with the highest potential levels of direct defense against herbivores may also have the highest potential levels of indirect defense against herbivory from predatory and parasitic arthropods; an inference that cuts directly against elements of plant defense tradeoff theory (Agrawal and Fishbein 2006, Agrawal et al. 2010). It should also be noted that plant species mapped onto Cluster 3 showed limited phylogenetic similarity compared to Clusters 1 and 2, indicating that evolutionary forces may play a much smaller role in affecting plant trait variance for this cluster.

In conclusion, we present strong evidence for the correlation of arthropod community composition with non-defensive plant traits across multiple arid-adapted plant species in a multi-year common garden study under realistic ecological conditions. Research on plant defense has focused primarily on traits thought to have evolved in response to herbivory while largely ignoring traits that mediate a plants response to its abiotic environment. This has led to a sizable gap in how a plant's relationship with its abiotic environment may affect associated communities in general, and arthropod communities in particular (Huberty and Denno 2004). Though much research in plant-arthropod interactions has been devoted towards seeking explanations for spatial variation patterns in arthropod communities along latitudinal and elevational gradients, little attention has been given as to how non-defensive plant traits may effect spatial variation in arthropod communities (Pratt et al. 2017, Moreira et al. 2018). Collectively, our findings underscore the significant and potentially synergistic influence of non-defensive plant traits in affecting arthropod community composition. This highlights the importance of future research on plant-arthropod interactions to incorporate non-defensive plant traits as a means of better understanding how plant responses to the abiotic factors may scale up to impact associated communities.

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