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Bottom-up and Top-down Cascading Effects of Climate Change on Herbivorous Insects

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Bottom-up and Top-down Cascading Effects of Climate Change on Herbivorous Insects

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Ecology and Evolutionary Biology

by

Alma Nalleli Carvajal Acosta

Dissertation Committee:
Professor Kailen Mooney, Chair
Professor Diane Campbell
Professor Cascade Sorte

2021

DEDICATION

To my partner and best friend Aaron Tester who was always there to support me through my PhD journey.

To my mother who always believed in me and taught me to always believe in myself.

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ACKNOWLEDGEMENTS

First and foremost, I would like to acknowledge my advisor and mentor, Dr. Kailen Mooney, whose guidance and expertise were invaluable for formulating research questions and conceptualizing my research findings. Thank you for all your patience, support, and all opportunities I was given to further my research and career. I would also like to thank my committee members, Drs. Diane Campbell and Cascade Sorte, for the insightful feedback I was given throughout my studies. Your wise counsel was fundamental for my academic accomplishments and success.

I would like to acknowledge my funding sources: UC-Mexus/CONACyT, UC-Irvine Graduate Division, the Rocky Mountain Biological Laboratory, the Colorado Mountain Club Foundation, the Voth Foundation, and the National Science Foundation. Particularly, the UC-Mexus/CONACyT which was a critical source of funding during my time at UC-Irvine.

In addition, I thank current and former Mooney Lab members who provided stimulating discussions and constructive feedback on my research endeavors. Specially, Annika and Jordan, with whom I shared most of my dissertation journey. I would also like to thank my peers, in particularly John Powers who was always willing to help with methodological issues and questions. To my peer-mentor Alberto Soto, whose guidance made my transition into PhD life smoother. And to my accountability partner Greg Vose; our weekly meetings helped me stay on track for completing my dissertation on time.

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CURRICULUM VITAE

Alma Nalleli Carvajal Acosta

EDUCATION

2016-present	**Ph.D. Ecology & Evolutionary Biology,** University of California Irvine, (UC Irvine); Advisor: Kailen Mooney Dissertation: The role of intra- and inter-specific variation in host-plants traits in mediating climate change effects on insect-plant interactions.
2010-2011	**M.S. Environmental Sciences and Applied Ecology,** School of Environmental & Public Affairs, University of Nevada, Las Vegas (UNLV); Advisor: Scott Abella Research: Evaluating success of habitat restoration methods in high-elevation butterfly habitat.
2001-2006	**B.A. International Business.** Centro de Estudios Superiores del Estado de Sonora, Mexico.

Additional studies:

Summer 2017	**Organization for Tropical Studies** (OTS). Tropical Biology: An Ecological Approach. Graduate course. Costa Rica.
Fall 2015	**Instituto de Ecología** (INECOL). Graduate courses. México.
2008-2010	**College of Southern Nevada** (CSN). Undergraduate courses.
Spring 2006	**Texas A&M University** (Exchange student). Undergraduate courses.

PUBLICATIONS

Peer-reviewed publications

Published or in review

(5) **Carvajal Acosta, A. N.,** Agrawal, A. A., and Mooney, K. A. *In review*. Plant drought-adaptation traits as mediators of herbivore's drought response: phylogeny, plant physiology, and functional traits. *Functional Ecology.*

(4) **Carvajal Acosta, A. N**., and Mooney K.A. 2021. Effects of geographic variation in hostplant resources for the distribution and climate change response of a dietary specialist. *Ecosphere.*

(3) Nelson, A. S., **Carvajal Acosta, A.N**, and Mooney, K. A. 2019. Plant chemical mediation of ant behavior. *Current Opinion in Insect Science*, 32, 98-103.

(2) Garcia□Robledo, C., Horvitz, C. C., Kress, W. J**., Carvajal Acosta, A. N.,** Erwin, T. L., & Staines, C. L. 2017. Experimental assemblage of novel plant–herbivore interactions: ecological host shifts after 40 million years of isolation. *Biotropica*, 49(6), 803-810.

(1) **Carvajal Acosta, A. N.,** Abella, S. R., and Thompson, D. B. 2015. Initial vegetation response to fuel mastication treatments in rare butterfly habitat of the Spring Mountains, Nevada. *Arizona-Nevada Academy of Science*, 46(1), 6-17.

In preparation *(manuscripts available upon request)*

Carvajal Acosta, A. N., Gloss, A., Mooney, K. *In preparation*. Chemical Ecology of Plant-biotic interactions in a changing environment. *Frontiers in Ecology and Evolution.*

Carvajal Acosta, A. N., Formenti, L., Godschlax, A., Katsanis, A., Mooney K., Villagra, C. Rasmann, S. *In preparation*. The cost-benefit ratio of an Andean nursery pollination system decreases with elevation and is related to variation in flower volatiles and pollinator preferences. *Ecology.*

Commentaries

Croy, J. **Carvajal-Acosta, A. N**., Mooney, K. 2021. Regulating plant herbivore defense pathways in the face of attacker diversity. *New Phytologist.*

PROFESSIONAL PRESENTATIONS

Contributed talks: *

Presenter

Carvajal-Acosta, A.N.*. 2021. "Drought effects on plant direct and indirect chemical defenses and its consequences for tri-trophic interactions". Ecological Society of America Meeting, August 2-6, virtual meeting.

Carvajal-Acosta, A. N.* Climate change effects on tri-trophic interactions. Invited guest speaker; Dirzo lab, Stanford University. October 16, 2020.

Carvajal-Acosta, A. N.*, and Mooney K.A. 2020. Accounting for hostplant availability and quality to predict the distribution of the western monarch under climate change. Ecological Society of America Meeting, August 6-10, virtual meeting.

Carvajal-Acosta, A. N.*, and Mooney K.A. 2019. Disentangling the direct and indirect effects of climate change on tri-trophic interactions. RMBL graduate research seminar, Gothic, Colorado, USA.

Carvajal-Acosta, A. N.*, Agrawal, A., and Mooney K.A. 2018. Indirect effects of drought on herbivores mediated by host-plants traits. Ecological Society of America Meeting, August 5-10, New Orleans, Louisiana, USA.

Carvajal-Acosta, A. N.*, Agrawal, A., and Mooney K.A. 2018. Indirect effects of drought on herbivores mediated by host-plants traits. UC-MEXUS Fellow Conference. March 13-16, Washington D.C.

Carvajal-Acosta, A. N.*, Agrawal, A., and Mooney K.A. 2018. Indirect effects of drought on herbivores mediated by host-plants traits. Winter Ecology & Evolutionary Biology Graduate Student Symposium. Irvine, CA, USA.

Carvajal-Acosta, A. N.* and Mooney K.A. 2018. The Milkweed diet: keeping up with climate change. Associate of Graduate Studies (AGS) Graduate Research Symposium. Irvine, CA, USA.

Contributed posters:

Cotoras, D.*, Kulkarni S., **Carvajal-Acosta, A. N.,** Deshwal, A., Pardo, C., Powell, E., and, Schumacher, E. 2019. Some spiders from the Oak forest and Páramo of Costa Rica. OTS-REU (Research Experience for Undergraduates) program, Las Cruces, Costa Rica.

Carvajal-Acosta, A. N.* and Mooney K.A. 2016. Latitudinal adaptations in California sagebrush and associated herbivores communities. Competitive Edge Research Symposium. Irvine, CA, USA.

Godschalx A. L*, Formenti, L. Mooney, K., **Carvajal Acosta, A. N**., Villagra, C. and Rasmann, S. 2019. When herbivores pollinate: scent-fly dynamics across elevation habitats and species of *Haplopappus*. Gordon Conference of Herbivore-Plant Interactions.

FELLOWSHIPS & AWARDS

Fellowships

2020	Graduate Dean Dissertation Fellowship, UC Irvine Graduate Division. $5,000.
2019	Graduate Student Research Award, Colorado Mountain Club Foundation (CMCF). $1,000.
2019	Snyder Endowment Fellowship, Rocky Mountain Biological Station (RMBL). $500.
2018	Travel Awards, Ecology & Evolutionary Biology, UC Irvine. $500.
2017	Graduate Fellowship to attend the course “Tropical Biology: An Ecological Approach”, Organization for Tropical Studies (OTS). $700.
2016-2020	UC Mexus-CONACyT Doctoral Fellowship (Mexican Counsel for Science and Technology and UC-Mexus). $96,000 + tuition + health benefits.
2016	Competitive Edge Fellowship, UC Irvine Graduate Division. $5,000 + health benefits.
2016-2020	Provost Doctoral Fellowship, UC Irvine Graduate Division. $20,000.
2016	Diversity Recruitment Fellowship, UC Irvine Graduate Division. $5,000 + health benefits.
2016-2017	VOTH Foundation Fellowship. Ecology & Evolutionary Biology and Orange County Natural Communities Coalition collaborative research grant. Research: “Assessing coastal Sagebrush restoration success”. $10,000.
2015	CONACyT Doctoral fellowship, CONACyT (Mexican Counsel for Science & Technology). Tuition + $1,000 stipend. Declined.
2006	CONAHEC Fellowship, (Consortium for North American Higher Education Collaboration) Texas A&M University- Kingsville International Exchange program. $5,000 + tuition.
2004	XIV Summer Research Program, Undergraduate Fellowship, Mexican Academy of Science to conduct research at Mexico College (COLMEX). $1,000.

Awards

2018	Ford Foundation Predoctoral Fellowship, National Academy of Sciences, *Alternate to Honorable Mention.*
2018	Julie S. Denslow Prize Award, co-recipient, for outstanding paper in *Biotropica*: "Experimental assemblage of novel-plant-herbivore interactions: ecological shifts after 40 million years of isolation".
2010	Golden Key International Honour Society member, University of Nevada-Las Vegas.

RESEARCH, TEACHING & OTHER PROFESSIONAL EXPERIENCES

Teaching positions:

9-12/2021	Global Sustainability, Teaching Assistant, UC-Irvine. Irvine, CA.
2/2019	Entomology, Guest Lecturer, UC-Irvine. Irvine, CA.
9-12/2018	Field Biology, Teaching Assistant, UC-Irvine. Irvine, CA.
1-4/2018	Horticulture, Teaching Assistant, UC-Irvine. Irvine, CA.
9-12/2017	Global Sustainability, Teaching Assistant, UC-Irvine. Irvine, CA.
9-12/2016	Human Physiology, Teaching Assistant, UC-Irvine. Irvine, CA.
2013-2015	Substitute Teacher, Clark County School District, Las Vegas, NV

Research positions:

Winter 2018. Research Assistant, Institute of Biology at University of Neuchâtel. Santiago, Chile.
Research: Role of floral volatiles in shaping mutualistic-antagonistic interactions in the *Haplopappus* system along ecological gradients. Advisor: Dr. Sergio Rassman.

Summer 2016. Graduate Student Researcher, Summer Research program Competitive Edge at University of California, Irvine**.** Irvine, CA, USA.
Research: Latitudinal adaptations in California sagebrush and associated herbivores communities (*Artemisia californica*). Advisor: Dr. Kailen Mooney.

Fall 2015. Graduate Research Assistant at Instituto de Ecología, A.C. Xalapa, México.
Research: Delimiting the thermal limits and geographical ranges of role leaf beetles at Los Tuxtlas Biological Station. Advisor: Dr. Carlos Garcia-Robledo.

2011-2012. Research Assistant at University of Nevada, Las Vegas. Las Vegas, NV, USA.
Research: Effects of fuel-beds on Mt. Charleston blue butterfly habitat and factors influencing germination of the butterfly hostplant *Astragalus calycosus.* Advisor: Dr. Scott Abella.

Field technician positions:

2014-2016. Biology Field Technician, Bureau of Land Management. Buffalo, WY
Independently performed environmental compliance inspections in abandoned oil/gas projects and issued legal notices to ensure lands were appropriately restored.

2014. Ecology Field Technician, Colorado State University. Las Vegas, NV

Conducted field vegetation surveys for accuracy assessment of a remote sensing vegetation map at the Lake Mead National Recreation Area.

2013. Research Assistant, San Diego Zoo/ Desert Tortoise Conservation Center. Las Vegas, NV

Assisted in various lab and field operations for the conservation of the endangered desert tortoises (*Gopherus agassizii*).

2013. Botanist, Transcon Environmental. Nevada-Utah

Monitored native vegetation and weed infestations along a 400-mile petroleum pipeline installed from Salt Lake City, UT to Las Vegas, NV.

2012. Restoration Monitoring Technician, Rocky Mountain Bird Observatory. Arizona-New Mexico

Conducted vegetation surveys in restored bird wintering grasslands throughout Southern Arizona and New Mexico.

2012. Botany Field Technician, The Great Basin Institute. Las Vegas, NV

Performed rare, endemic, and butterflies' host-plant surveys in the Spring Mountains National Recreation Area (SMNRA). Collected and prepared plant specimens for herbarium. Created a vegetation and butterfly habitat maps. Prepared technical reports.

Higher Education positions:

2008-2009. Academic Advisor at College of Southern Nevada. Las Vegas, NV, USA.

Advised prospective, in-coming, and current students regarding the college admission process, financial aid application, and graduation requirements.

2009-2010. Recruitment Specialist at College of Southern Nevada. Las Vegas, NV, USA.

Recruited and guided high school students throughout the college admission process and financial aid application. Lead campus tours and host college informational session at various high schools.

ADVANCED COURSEWORK & TRANINING

2020	"Mentoring Excellence Program" UC Irvine, Irvine CA
2018	"Science Communication" class, UC Irvine, Irvine CA
2017	"Climate Modeling" Data Science Initiative Workshop, UC Irvine, Irvine, CA. Workshop for analyzing spatial-temporal datasets in R.
2016	"Advanced Topics in R" Data Science Initiative Workshop**,** UC Irvine, Irvine, CA Topics: ggplot2, dplyr, Shiny, and R Markdown.
2016	"Teaching Assistant Professional Development" UC-Irvine, Irvine, CA Workshop to improve teachings skills and classroom management techniques.
2015	Conservation and Land Management (CMLM) Workshop, Chicago Botanic Garden, Chicago IL. Topics: Crash course in identification of flora of the west, Vegetation monitoring and inventory techniques, conservation genetics, "Seeds of Success" (SOS) program, seminar: "Lessons learned from large-scale restoration projects".

2014 “Geographic Information Systems and GPS” refresher training, Bureau of Land Management. Buffalo, WY.

Topics: TerraSync for navigation, understanding geographic data, ArcMap interphase.

2014 “Defensive driving and 4-wheel drive training”. Bureau of Land Management, (online)

2014 “Substitute Teacher Certification” Clark County School District (CCSD).

Topics: learning styles, classroom management techniques, technology in the classroom, among others.

2013 San Diego Zoo Employee Training (online)

Topics: zoonic disease and Biosecurity, government regulations, fundamentals of animal training and working safety with dangerous animals.

2012 “Grass identification workshop” Audubon Research Ranch, Tucson, AZ. Rocky Mountain Bird Observatory.

2012 “ESRI training in ArcMap GIS”, The Great Basin Institute (online)

Topics: Basics of Map Projections in ArcMap GIS 10; working with annotations; basics of geographic coordinate systems; referencing data to real world locations using; and finding geographic data in ArcGIS.

First aid training:

2015 Certificate, CPR and Adult and Child First Aid– BLM, Buffalo, WY
2013 Certificate, CPR, and Adult and Child First Aid – the American Red Cross, Cedar City, UT

SYNERGISTIC ACTIVITIES

Diversity outreach:

Panelist, Diverse Educational Community and Doctoral Experience (DECADE) program “Competitive Edge”, UC Irvine, 2021
– discussed mentorship experience and strategies for the upcoming per-mentor cohort participating in the “Competitive Edge” summer research program.

Peer-mentor, Diverse Educational Community and Doctoral Experience (DECADE) program “Competitive Edge”, UC Irvine, 2020
–mentored incoming UCI PhD students participating in the “Competitive Edge” summer research program.

Mentor, Chicano Latino Staff Association (CLSA) mentorship program, UC Irvine, 2020
–mentored undergraduate minority students navigating academic and campus life.

Panelist, Minority groups in science, RMBL Diversity Committee, 2019
– discussed a variety of diversity-related topics related to academia

Co-organizer, “Reach Out Teach Out” Mooney Lab tour, UC Irvine, 2019
– hosted a high-school group of students from under representative minorities. Planned lab activities and a field tour for guest students.

Student Representative, DECADE Student Council, Ecology & Evolutionary Biology dept, UC Irvine, 2017-1018

Moderator, XXIV Congreso Anual de Mexicanistas Juan Bruce-Novoa, Spanish Department, UC Irvine, 2018
Panelist, DECADE Student Council Outreach event (x2), 2017
    – advised undergraduates regarding the graduate application process.
Mentor, Diversity Recruitment Program, Ecology & Evolutionary Biology, UC Irvine, 2017
    – mentored students from underrepresented minorities through the graduate application process.

Peer-referee:
Annals of Botany Journal, Canadian Journal of Zoology x 2*, Oikos*

Professional service:
Organizer, Ecology Group Seminar, Ecology & Evolutionary Biology, UC Irvine, 2020
    – led graduate students in the development of the Winter 2020 curriculum
Member, Graduate Student Recruitment Planning Committee, Department of Ecology & Evolutionary Biology, UC Irvine, 2018-2019
Member, "What can I do with my PhD?" Job Symposium Planning Committee, Department of Ecology and Evolutionary Biology, 2017-2018
Panelist, Graduate School Preparation Class, Sociology Department, UC Irvine, 2018
    – discuss graduate application process and related topics to undergraduate class

Memberships in professional organizations:
Ecological Society of America (2018-present)

Volunteer service:
Volunteer, "Home 4ever" animal shelter, Costa Mesa, CA (2016)
Volunteer, "St. Francis" animal shelter, Buffalo, WY (2014-2015)
Volunteer, Trail Watcher and Resource Steward programs, Lake Mead National Recreation Area (LMNRA), National Park Service, Las Vegas, NV (2012-2014)
    – attended trainings on fauna and plant identification, explored wilderness areas, reported weed infestation as well as sensitive flora and fauna sightings.
Volunteer, Seed of Success (SOS) LMNRA, National Park Service, Las Vegas, NV (2013)
    – performed cleaning, preparation, and storage of seeds used in restoration projects.
Volunteer, Sahara Mustard control program, LMNRA, National Park Service, Las Vegas, NV, (2012)
    – obtained certification and participated in Sahara Mustard (*Brassica tournefortii*) removal events.
Volunteer, Baird and Grasshopper sparrow survey. Rocky Mountain Bird Observatory (RMBO), "El Uno" Ranch, Chihuahua, Mexico (2012)

DISSERTATION ABSTRACT

Bottom-up and Top-down Cascading Effects of Climate Change on Herbivorous Insects

By

Alma Nalleli Carvajal Acosta

Doctor of Philosophy in Ecology and Evolutionary Biology

University of California, Irvine

Professor Kailen Mooney, Chair

Global climate change is widely recognized as a threat to human and natural ecosystems, yet the mechanisms underlying such impacts are poorly understood and difficult to predict. My dissertation research aims at understanding the mechanisms driving climate change impacts on herbivorous insects – key drivers of ecosystem processes – focusing on two central mechanisms: a) bottom effects mediated by hostplants resources and b) top-down effects mediated by herbivore's natural enemies. I hypothesize that accounting for variation in hostplant resources as well as their response to climate change, is essential for predicting these bottom-up and top-down effects of climate change on herbivorous insects. To test this hypothesis, I conducted three separate research projects using two well-studied systems, the milkweed (*Asclepia*s genus) and the heartleaf bittercress (*Cardamine cordifolia)*. I employed species distributions models (SDMs) to test for the role of geographic variation in hostplant availability and quality for the distribution and climate change response of the western monarch (*Danaus plexippus*), a milkweed specialist and for which hostplant quality varies among species by an order of magnitude. I found that accounting for hostplant's distributional response to climate change, but not host-quality, is essential for predicting herbivores' distribution under projected climatic conditions. Again, using the milkweed system, I tested whether variation in plant traits associated with aridity gradients determine the effects of drought on two milkweed specialists: a leaf chewer (monarch larvae; *Danaus plexippus*) and a sap feeder (oleander aphid; *Aphis nerii*). I showed that herbivore response to drought can range from positive to negative depending upon the milkweed species they feed on as well as the herbivore feeding mode. Specifically, the leaf-chewer performed better on drought-stressed milkweed species with trait values associated with aridity and such effects were correlated with changes in chemical defenses. In contrast, sap-feeder's performance under drought was uncorrelated with aridity-associated traits. However, similar to monarchs, oleander aphid's drought response was correlated with changes in cardenolide concentrations in the same manner such that both herbivores performed worst in milkweed species that increased cardenolide concentrations under drought. Finally, in a common garden experiment, I investigated the chemical basis of bottom-up and top-down effects of drought on plant herbivory using the Heartleaf bittercress (*Cardamine cordifolia*) system. Results show that drought-stressed in plants decreased herbivory. However, in the presence of predators, herbivory was reduced more in well-watered than in droughted plants. The overall composition of chemical compounds associated with direct (glucosinolates), and indirect (volatile organic compounds [VOCs]) plant defenses were unaffected by plant drought stress. However, specific chemical functional groups in glucosinolates (indoles) and VOCs (alcohols) were affected by reduced water availability, and these may be responsible for the observed changes in herbivory patterns under reduced water conditions. In summary, my dissertation research revealed climate change effects on herbivores insects are mediated by both bottom-up and top-down effects and that accounting for plant variability and their response to climate change is crucial for understanding these mechanisms and predicting herbivore response to projected climate change

INTRODUCTION

Insect populations are declining at such alarming rates that some researchers have likened this phenomena to an insect apocalypse (Goulson 2019, Rhodes 2019). Herbivorous insects, in particular, play crucial ecological roles in both natural and agricultural systems (Basset & Lamarre 2019, Jankielsohn 2018), thus understanding the causes of this decline is imperative to mitigate further ecological and economic damage. Climate change is linked to insect biodiversity loss (Wilson et al. 2007) but the specific mechanisms behind species' responses to climate change are poorly understood. My dissertation work investigates these underlying mechanisms and argue that understanding the causes and consequences of such effects requires a system approach that accounts for species interactions as well as inter- and intra-specific variation among interacting species.

The *direct* effects of climate change on herbivores have been widely documented in the literature (reviewed in Rosenblatt & Schmitz 2016). As ectotherms, herbivorous insects are particularly sensitive to thermal changes (reviewed in Bale et al. 2002). For example, elevated temperatures have been shown to affect herbivore insects' life histories, increased their growth and voltinism in some species (Bale et al. 2002). Elevated temperatures and elevated CO_2 have also been shown to induced compensatory feeding behavior in insects by consuming more tissue of low-quality host plants to meet their nutritional needs (Facey et al. 2014, Lemoine et al. 2014). Similarly, there is ample evidence that climate warming is leading to shifts in the distribution of many herbivorous insects (Bellard et al. 2012, Grandpre et al. 2018, Pateman et al. 2012, Thomas et al. 2004), generally by prompting species to higher elevations and latitudes (Lemoine 2015, Parmesan et al. 1999, Parmesan & Yohe 2003).

The effects of climate change on herbivores may also occur via *indirect* effects mediated by interacting species. In nature, species are embedded in complex food webs composed by multiple interacting species occupying different trophic levels (Walther 2010). Indeed, all terrestrial communities are composed of at least three interacting levels: plants, herbivores, and natural enemies of herbivores (Price et al. 1980), where herbivores are the critical link between primary production and food webs (Burkepile & Parker 2017). These indirect effects on herbivores can be classified as bottom-up and top-down effects (Figure 1) depending upon the trophic level of origin (Rosenblatt & Schmitz 2016). Climate-change induced abiotic stress on plants may affect herbivores fitness from the bottom up through changes in plants physiological process that alter plant's water and nutritional content, secondary metabolites, and overall hostplant quality (reviewed in Jamieson et al. 2017, Stenseth & Mysterud 2002). Conversely, top-down effects result from any changes in natural enemies' pressure on herbivores. For example, predators exposed to higher temperatures can shift their dietary patterns to meet altered nutrient demands (Boersma et al. 2016, Rall et al. 2010). When these so-called top-down effects occur in response to changes in species occupying lower trophic levels, they are termed top-down cascading effects. For instance, top-down cascading effects have been observed in aphids raised under elevated CO_2 conditions are less sensitive to alarm pheromones from conspecifics than are aphids raised in ambient conditions and this in turn increase their risk for predation (Boullis et al., 2015). Similarly, multiple studies have shown that abiotic stress in plants may alter plant chemical cues used by natural enemies to locate their herbivore prey or host altering herbivore and predator population dynamics (Holopainen et al. 2013, Yuan et al. 2009).

The field of climate change ecology has made significant progress in the latest in – the *indirect* effect of climate change via trophic interactions – (DeLucia et al. 2012, Jamieson et al.

2012 and 2017, Stireman et al. 2005, Tylianakis et al. 2008, Yuan et al. 2009), especially in the subject of herbivore-plant interactions (reviewed in Bale et al. 2002, Bidart-Bouzat & Imeh-Nathaniel 2008, Coviella & Trumble 1999, Mundim & Bruna 2016) and we now have a better understanding of the role of the phytochemistry, phenology, as well as the main and interactive effects of the main axis of climate change (changes in temperature, precipitation, and CO_2). Despite these advances, our understanding of the effects of global climate change on herbivores is still not well developed. For instance, biotic interactions, including herbivore-plant interactions, are beginning to be recognized as important drivers of herbivores' distributional response to climate change (Araújo & Luoto 2007, Dilts et al. 2019, Kass et al. 2020, Lemoine 2015, Preston et al. 2008, Wharton & Kriticos 2004). However, both the magnitude and direction of these predictions can vary dramatically among species raging from range expansions (i.e. Pateman et al. 2012) to extinctions (i.e. Thomas et al. 2004). Moreover, one of the dominant axes of climate change is increased drought (Sheffield & Wood 2008), in particularly for the northern hemisphere (IPCC). Yet, its effects on herbivores are notoriously variable and difficult to predict (Gely et al. 2020, Huberty & Denno 2004). Because plants exhibit remarkably intra-and inter-specific variation, both in their traits and in their response to abiotic stresses (Henn et al. 2018, Jung et al. 2014, Lopez-Iglesias et al. 2014, Ouédraogo et al. 2013, Skelton et al. 2015), I hypothesize that variation in hostplant resources may mediate bottom-up and top-down effects on herbivores. To test this hypothesis, I addressed the following questions:

1. Does geographic variation in hostplant resources influence herbivore's distributional response to climate change?
2. Does intra-specific variation in hostplant traits associated with aridity gradients mediate herbivore drought response?

3. Are bottom-up and top- down drought effects on herbivores mediated by drought-induced changes in plant secondary chemicals?

To address these questions, I conducted three separate research projects using a range of tools spanning species distributions modeling, green house, and field experiments. In Chaper 1, I tested for the importance of hostplant availability and quality for the distribution and climate change response of the western monarch. To do so, I developed and compared species distribution models (SDM) incorporating hostplant distribution and quality to predict the contemporary and future distribution of western monarchs under climate change. For Chapter 2, again using the milkweed system, I conducted a drought manipulation in the greenhouse to investigate the role of intra-specific variation in plant traits associated with aridity in mediating herbivore drought response. In Chapter 3, I continued to explore the effects of drought on herbivores but focusing on how plant chemical defenses may mediate bottom-up and top-down effects. For this project, I conducted a field experiment where I manipulated water availability and herbivore/predator access to Bittercress plants (*Cardemone cordifolia*), to test whether drought affects herbivory pressure on plants via changes in plant secondary chemicals associated with direct (i.e., toxic chemicals to herbivores) and indirect (i.e., plant volatiles utilized by predators to locate prey) chemical defenses.

Figure 1. Conceptual framework

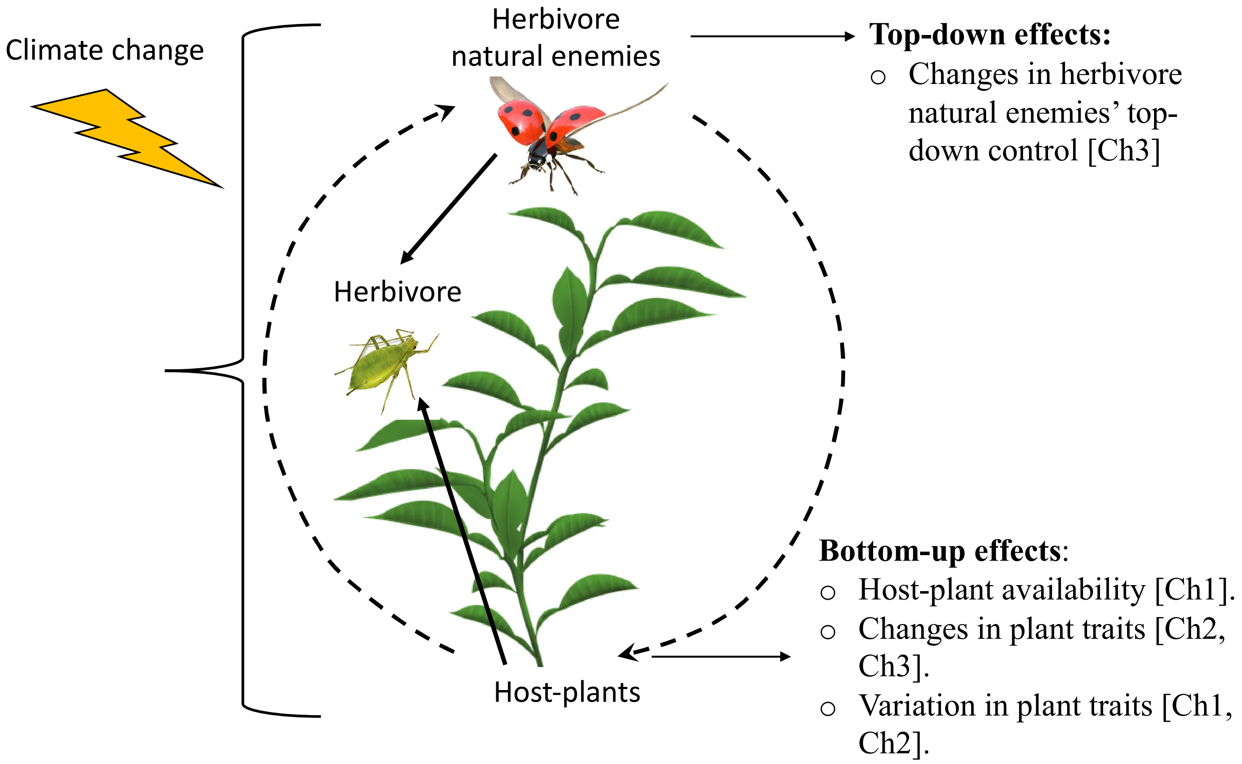


Fig. 1. Conceptual framework for investigating the effects of climate change effects on herbivorous insects. Solid arrows indicate direct effects and dashed arrows indirect cascading effects. Bottom-up effects originate from lower trophic levels (hostplants) whereas top-down effects originate from higher trophic levels (herbivore's natural enemies)

CHAPTER 1

Does geographic variation in hostplant resources affect herbivore's distributional response to climate change?

Abstract: *Species distributions are driven by abiotic and biotic factors, but the importance of variation in the availability and quality of critical resources is poorly understood. Disentangling the relative importance of these factors – abiotic environment, availability of critical resources, and resource quality– will be important to modeling species current distributions and responses to projected climate change. I address these questions using species distribution models (SDMs) for the western monarch butterfly population (Danaus plexippus), whose larvae feeds exclusively on Asclepias species known for their heterogeneously distribution and variation in host-quality. I modeled the distribution of 24 Asclepias species to compare three monarch distribution models with increasing levels of complexity: (i) a null model using only environmental factors (climate envelope model), (ii) a model using environmental factors and Asclepias availability estimated as species richness, (iii) and a model using environmental factors and Asclepias' availability weighted by hostplant quality as assessed through a greenhouse bioassay of larval performance. Asclepias models predicted that half of the Asclepias species will expand their ranges and shift towards higher latitudes, while half will contract. These patterns were uncorrelated with hostplant quality. Among the three monarch models, the climate envelope model was the poorest performing. Models accounting for hostplant availability performed best, while accounting for hostplant quality did not improve model performance. The climate envelope model estimated more restrictive contemporary and future monarch ranges compared to both* *hostplant models. Although all three models predicted future monarch range expansions, the projected future distributions varied among models. The climate envelope model predicted range expansions along the Pacific coast and contractions inland. In contrast, the hostplant availability and quality models predicted range expansions in both regions and, as a result, 14 and 19% increases in distribution (respectively) relative to the climate envelope model. These models do not include other factors affecting monarch persistence. Nevertheless, our findings suggest that accounting for information on hostplant availability and response to climate change is necessary to predict future species distributions, but that variation in the quality of those critical resources may be of secondary importance.*

1.1 Introduction

Climate change is expected to alter the distribution of most species (Bellard et al. 2012, Crozier 2004, Parmesan et al. 1999, Pauli et al. 2012) with many already experiencing range shifts, contractions, or facing extinctions (Bellard et al. 2012, La Sorte & Jetz 2010, Pauli et al., 2012, Sekercioglu et al. 2008). Understanding the underlying drivers is key to predicting such distributional response and also critical if we are to mitigate these impacts. Species distributions are presumed to be driven most strongly by abiotic factors, but biotic interactions have been increasingly recognized to also play a key role (Giannini et al. 2013, Guisan & Thuiller 2005, Van der Putten et al. 2010, Wisz et al. 2013). Because species often respond differently to abiotic stress (Schweiger et al. 2008, Van der Putten et al. 2010), producing accurate predictions necessitates that we also account for climate change effects on interacting species. This is especially true for species that engage in obligate interactions, as they depend on a few or even a single species to survive, and such species may not be available in all areas that are otherwise climatically suitable (Schweiger et al. 2008).

Herbivorous insects – the majority of multi-cellular species on earth (Lewinsohn et al., 2005) – are highly host-specific; thus, their response to climate change will likely be contingent on the responses of the plants upon which they depend. Indeed, most herbivorous insects feed on a single or a few plant families (Bernays 1989, Forister et al. 2015) with fewer than 10% feeding on plants belonging to more than three families (Price 1983). The strength of biotic interactions can vary substantially throughout the landscape (Thompson 1999) and spatial variation in competition (Boulangeat et al. 2012, Meier et al. 2011) and food resources (Kass et al. 2020, Koenig & Haydock 1999) have been shown to influence contemporary and future distributions of focal species. Thus, spatial variation in resource availability may play an important role in delimiting herbivore distributions. Furthermore, it has long been recognized that hostplants demonstrate considerable intra- and inter-specific variation in their resource quality to herbivores, and that resource quality is often heterogeneously distributed across landscapes (Denno & McClurc 1983, Hunter et al. 1992). Both intra- and inter-specific variation in host-quality can have large effects on herbivore performance (Singer et al. 2012) and may also play a significant role in determining the spatial distribution of host-specific herbivorous insects (Egan & Ott 2007, Mcmillin & Wagner 1998, Memmott et al. 1995). In addition, high- and low-quality host plants may respond differently to climate change due to evolutionary trade-offs constraining a plant's response to conflicting stresses (abiotic vs. biotic) (Agrawal et al. 2010). For example, plants adapted to resource-rich environments favor faster growth over herbivore resistance (Coley et al. 1985). Thus, fast-growing species are in turn predicted to be more sensitive to abiotic stress, including drought (Carvajal-Acosta et al. in review). This differential response to climate change of high- and low-quality hostplants may redistribute the hostplant quality across a landscape and thus alter herbivore distributions. Accordingly, understanding the role of hostplant quality as a driver of species distribution, and its implications for herbivores' distributional response to climate change, is critical but largely unknown.

In this study I investigated the importance of geographic variation in the availability and quality of host plant resources for herbivore distribution. More specifically, I characterized geographic variation in species richness and quality of herbivore food resources, and whether such variation drives distribution. Additionally, because high- and low-quality host plant species may not respond equally to climate change, I also investigated these dynamics with respect to projected future herbivore distributions. I use Species Distribution Models (SDMs), statistical tools that combine observations of species occurrences with environmental covariates to estimate species distributions. These models identify the factors driving contemporary species ranges and can infer species response to climate change based on projections for how these factors will change in the future (Elith & Leathwick 2009). SDMs have most often assumed that species distributions are defined by environmental factors alone. This so-called "climate envelope approach" is based on the Eltonian noise hypothesis, which posits that biotic interactions may be a major driver of abundance at smaller spatial resolutions, but at larger and coarser spatial resolutions the effects of biotic interactions may average out, leaving abiotic factors as the principal drivers (Elith & Leathwick 2009, Fraterrigo et al. 2014, Guisan & Thuiller 2005; Soberon & Nakamura 2009). Yet recent modelling studies have identified biotic factors as important drivers of species distributions (i.e. Araújo and Luoto 2007, Preston et al. 2008, Schweiger et al. 2008, de Araújo et al. 2014, Lemoine 2015, da Cunha et al. 2018, Dilts et al. 2019); and SDMs' predictions for species response to climate change have yielded contrasting results based upon the inclusions of biotic factors (Lemoine 2015, Preston et al. 2008, Schweiger et al. 2008). Accordingly, climate envelope modeling may accurately define the potential niche of a species, but the realized niche – defined in part by species interactions – may differ substantially.

Monarch caterpillars (*Danaus plexippus*, Lepidoptera: Nymphalidae) and their milkweed host plants (*Asclepias* spp., Apocynaceae) represent an ideal model system for this study's goals for many reasons: First, monarch larvae feed exclusively on plants in the *Asclepias* genus, thus providing the opportunity to model an herbivore and a tractably diverse set of obligate hostplants. Second, this system is exceptionally well-studied, and variation in milkweed quality for monarchs is clearly established to vary greatly among species (Agrawal and Fishbein 2006, Pocius et al. 2017). Third, at least some of this variation in host plant quality is driven by growth-defense trade-offs (Mooney et al. 2010). If plant growth strategy mediates a species' response to climate change, then climate change may have especially strong effects on redistributing these hostplant quality resources. Fourth, because monarch larvae are exceptionally toxic (Brower 1988), natural enemies are relatively less important in determining their performance compared to more palatable herbivores, thus providing a better opportunity to detect the direct effects of host plant quality on species distribution. Fifth, because of the charismatic nature of this herbivore, there are an exceptionally large number of observations, thus allowing development of robust SDMs. And finally, sixth, several past studies have established the role of milkweed species distribution, with some milkweeds being more important than others (Lemoine 2015, Svancara et al. 2019, Dilts et al. 2019, Kass et al. 2020), thus suggesting a potential role for variation in host plant quality. In summary, the biology of the monarch-milkweed system, as well as its past study, provides an ideal opportunity to test for the role of geographic variation in the availability and quality of hostplant resources for herbivore distribution.

I investigated the role of host plant resources in driving contemporary and projected future distributions of the monarch population west of the Rocky Mountains. To do so, we compared the performance of models including hostplant information against a traditional climate envelope model. In order of increasing complexity, these three models were: (i) a model using only climatic variables as predictors (climate envelope model); (ii) a model using climatic variables and *Asclepias'* availability represented by species richness as predictors (hereafter, hostplant-availability model); and (iii) a model that included climatic variables, hostplant availability and hostplant quality, which varied 10-fold among species as assessed through bioassays of larval performance (hereafter, hostplant quality model). I compared model's performance and identified the most important variables determining the distribution of the western monarch breeding range. These models were then used to project and estimate changes in their distribution. The role of milkweed distribution as a biotic factor driving monarch distribution is established for the contemporary distribution of both the eastern and western populations (Dilts et al. 2019, Kass et al. 2020, Lemoine 2015, Svancara et al. 2019) as well as for the future distribution of the eastern population (Lemoine 2015) and western population in Idaho (Svancara et al. 2019). These studies have been conducted with a conservation aim, with the primary intent of understanding the full set of biotic and abiotic factors driving current distributions (i.e., topographic, edaphic, hydrologic, land use and specific hostplant variables), and accurately predicting future distributions under climate change (Lemoine 2015, Svancara et al. 2019). In contrast, our aim was more narrowly focused on using this ideally suited system to explore the role of geographic variation in the quality of hostplant resources. Accordingly, we were exhaustive in the variables we include in our modeling efforts, although for heuristic purposes we nevertheless compare our findings with those of past studies. Our study is among the first to test for the role of geographic variation in resource availability and, to our knowledge, the first to explicitly test for the importance of geographic variation in the quality of resources for driving species contemporary and future distributions.

1.2 Methodology

Study system

Monarch butterflies occur world-wide and, in their larval stage, feed almost exclusively from plants in the milkweed family in the genus *Asclepias*, Apocyneceae: Asclepiadaceae) (Nail et al. 2019). In North America, there are two migratory populations that breed east and west of the Rocky Mountains, with each of these regions being populated by multiple and largely unique sets of hostplant species (USDA NRCS 2021). Western monarchs breed west of the Rocky Mountains and overwinter along the Pacific coast from Bodega Bay in northern California and as far south as Ensenada, Baja California, Mexico (Stevens and Frey 2010). During the spring, monarchs leave their overwintering sites and disperse throughout the western U.S. where they breed continuously during the summer. In the fall, adult monarchs return to their overwintering grounds (Pelton et al. 2019). Within North America, monarchs have been recorded feeding on over 50 plant species in the genus *Asclepias* (Agrawal et al. 2015); however, adult females may oviposit in any available *Asclepias* species. Thus, monarchs utilize multiple *Asclepias* species throughout their migratory paths (Agrawal et al. 2015)

The genus *Asclepias,* commonly known as milkweeds, consists of over 140 different species of which 130 are endemic to North America (Agrawal & Konno 2009). Milkweeds vary in their herbivore defensive strategies, which include various combinations of cardenolides, latex, and trichomes, among other traits (Agrawal & Fishbein, 2006). Inter-specific variation in plant defenses (Agrawal & Fishbein 2006) have been associated with monarch larval mass, developmental rate, and early instar survival (Zalucki et al. 2001; Pocius et al. 2017). Therefore, even though monarchs may feed on most *Asclepias* species, they vary greatly in terms of resource quality. This directly affects individual fitness, and may consequently affect monarch's population growth and ultimately, their distribution.

Previous studies have modelled the distribution of two of the largest monarch migratory populations in North America. Lemoine (2015) accounted for milkweeds' distribution in the eastern monarch population response to climate change, predicting a poleward range expansion facilitated by *Asclepias* range expansions. The contemporary distribution of western monarch was recently modeled at a regional (Dilts et al. 2019) and a local (Svancara et al 2019) scale. Both modeling efforts accounted for climatic and multiple abiotic factors (i.e*.,* land cover, edaphic factors) as well as milkweeds' distributions, again demonstrating the importance of hostplant presence. Svancara et al (2019) represents the first effort to include hostplant distributions for predicting the future distribution of the western monarch in Idaho. Although these studies accounted for hostplant presence, to our knowledge this is the first study to explicitly test for the role of hostplant availability and quality in driving the western monarch contemporary distribution and their distributional response to climate change.

Data collection

<u>Occurrence data</u>: I retrieved monarch and milkweed records for the United States using R Studio (R Studio Team, 2015) from multiple open source databases using the R packages SPOCC, Ecoengine, rbison (Chamberlain 2019, Chamberlain et al. 2014, Karthik 2014) and by accessing species occurrences directly from GBIF and iNaturalist databases (GBIF 2019,

iNaturalist 2019). For monarchs, we only selected eggs and larval records because they provide a direct index for the location of the monarch breeding grounds. Additional larval records were provided by the Monarch Larva Monitoring Program (MLMP) (Ries & Oberhauser 2015).

The occurrence data archived in open-source databases originates mainly from citizen scientist sightings and some from herbarium records. Because this type of data has some limitations such as sampling biases, potential misidentification and coordinate inaccuracies, and lack species absence records, I controlled for these limitations whenever possible. For example, I used filters that only retrieved records confirmed by experts and/or records classified as of research quality when permitted and spatial filtering to control for sampling biases. To focus on the western monarch population, I selected milkweeds and monarch larval records from states corresponding to this region: California, Nevada, Colorado, Washington, New Mexico, Arizona, Utah, Oregon, and Idaho. I compared milkweed records with milkweed distributions published in The Biota of North America Program (BONAP; [Kartesz 2015]) for record accuracy. After removing duplicate records, incorrect coordinates such as occurrences over oceans or inaccurate coordinates such as uncertainty over 1000 meters, and observations, the final databases were established. These included: 7,941 milkweed records for 51 species (Carvajal Acosta 2021a), and 904 monarch larval records (Carvajal Acosta 2021b). *A. fascicularis* and *A. speciosa* were the most common species with 22% (2,541) and 12% (1,404) of total milkweed records, respectively.

Environmental data and climate projections: Contemporary environmental bioclimatic variables and projections for the year 2070 were downloaded in R from the WorldClim website (Fick & Hijmans 2017) at 30-sec (approximately 1-km^2) grid cells, the finest spatial resolution available.

The current bioclimatic variables represent averages of a 50-year period from 1950 to 2000. Climate change projections for the year 2070 are based on average projections of a 30-year period from 2061 to 2080 based on the Hadley Centre Global Environmental Model, version 2, Earth System (HadGEM2-ES) model. The HadGEM2-ES model is recommended for ecological modeling as it accounts for ecologically-meaningful processes such as dynamic vegetation cover (The HadGEM2 Development Team: Martin et al., 2011). These projections are based on Representative Concentration Pathway (RCP) 8.5. The RCP 8.5 represents the worst-case scenario for greenhouse gas (GHG) concentrations, assuming that GHG emissions will continue to increase after the 21st century in contrast to other scenarios that assume GHG will remain stable or will decline after the 21st century (Collins et al. 2013). While a comparison of different projections for future climate would provide a more nuanced prediction for the future distributions of milkweeds and monarchs, using this single scenario met our primary purpose of evaluating the importance of host plant information in predicting specialist herbivore distributions.

Environmental layers were cropped to include the states corresponding to the range of the western monarch population. To reduce multicollinearity among variables, we removed highly correlated variables based on their Pearson correlation coefficients using a pairwise correlations approach following Dormann *et al.* (Dormann et al. 2013) but with a less restrictive threshold of 0.85 as in Elith *et al.* (Elith et al. 2006). First, I removed variables that were correlated with multiple other variables; when only two variables were correlated, I selected the variable that was less statistically derived. This process yielded 11 environmental predictors (Table 1.1).

Table 1.1 Selected environmental variables

Worldclim Code	Environmental Variable*
Bio1	Annual Mean Temperature
Bio5	Max Temperature of Warmest Month
Bio6	Minimum Temperature of Coldest Month
Bio7	Temperature Annual Range
Bio8	Mean Temperature of Wettest Quarter
Bio9	Mean Temperature of Driest Quarter
Bio12	Annual Precipitation
Bio15	Precipitation Seasonality
Bio17	Precipitation of Driest Quarter
Bio18	Precipitation of Warmest Quarter
Bio19	Precipitation of Coldest Quarter

*Selected environmental variables with Pearson correlation coefficient of 0.85 or lower.

Species distribution modeling

Because species occurrences in these datasets are available in the form of presence-only records, I used the maximum entropy method (hereafter MaxEnt) (Phillips et al. 2006) to model the current and future distribution of *Asclepias* and monarch breeding ranges (see Elith et al. 2006 for a list of SDM techniques using other data formats). The MaxEnt algorithm is a presence-background modeling tool based on Bayesian and maximum likelihood statistics (Elith et al. 2011). To estimate the probability of distribution of a species, MaxEnt uses species presence records and a set of environmental predictors (i.e., precipitation, temperature) across a pre-defined landscape that is divided into grid cells. From this landscape, background points are randomly selected to represent the species environmental domain or background environment. MaxEnt estimates the relative probability of occurrence for each grid cell by maximizing the similarity between the environmental conditions of presence records and that of the background environment, while constraining the prediction to have the same mean as the presence records. The relative probabilities (raw output) are transformed to probability of occurrence using post-logistic transformation (logistic output). Here I report the logistic output which assigns a probability of presence between 0 and 1 to each grid cell, assuming that typical presence localities have a probability of presence of 0.5. See Elith et al. (2011) for a comprehensive statistical explanation of MaxEnt.

Data collection, data processing, and modeling were performed in R studio (R Studio Team, 2015). Species distribution modeling was executed in MaxEnt using the ‘dismo’ package (Hijmans et al. 2011).

<u>Asclepias models:</u> I developed models for individual *Asclepias* species and estimated their distributions within an area restricted to the study region; therefore, our *Asclepias* ranges do not represent their full distributions but only represent areas where their range overlaps with that of the western monarch population. Due to the environmental heterogeneity of the region, *Asclepias* species were modelled separately because their distributions may be delimited by distinct environmental factors. I discarded records identified at the genus level and species with fewer than 40 records as this limited number of observations would not allow for an accurate estimation of their distributions. To correct for potential sampling biases, I used a spatial filtering approach which consists of randomly selecting one record per grid cell of a specified size (Kramer-Schadt et al. 2013). Spatial filtering was performed individually for each *Asclepias* species. This allowed us to retain records for multiple species co-occurring within a single grid cell as well as selecting the optimal spatial resolution that maximizes sample size while correcting for sampling biases. For example, species with a limited distribution (i.e., high-elevation species), were filtered at a finer spatial resolution of 1 km^2 and more widely distributed *Asclepias* species such as *A. speciosa,* were filtered at a 30-km^2 resolution. An additional two *Asclepias* species, *A. viridiflora* and *A. curassavica*, were discarded because their records were clearly subject to sample biases and spatial thinning decreased their number of records to less than 40. Furthermore, I excluded these

species because their records were restricted to urban areas, thus their presence is more likely to be influenced by anthropogenic factors (watering, fertilization) rather than climatic variation. The process of removing incorrect records and rare species, and spatial filtering, resulted in 24 *Asclepias* species databases each with a minimum of 40 records, totaling 3,549 *Asclepias* records (Table 1.2).

Table 1.2. Milkweeds models summary and estimated habitat suitability

***Asclepias* species**	**Spatial filtering (Km^2)**	**Total records**	**Filtered records**	**AUC for best model**	**Current estimated habitat (Km^2)**	**Projected estimated habitat (Km^2)**	**Host quality weight[1]**
A. albicans	1	434	209	0.977	37,937	28,444	**0.554**
*A. asperula**	30	586	244	0.815	614,502	678,420	0.078
*A. californica**	1	607	277	0.945	84,794	124,187	0.306
*A. cordifolia**	30	683	173	0.848	230,690	215,368	0.621
A. cryptoceras	30	210	128	0.839	416,872	246,545	0.218
*A. engelmanniana**	30	90	70	0.915	277,368	230,811	0.502
A. eriocarpa	30	878	145	0.888	147,561	235,229	0.541
*A. erosa**	30	524	145	0.885	285,506	466,099	0.671
*A. fascicularis**	30	2259	399	0.859	306,140	512,078	0.545
A. halli	1	48	48	0.915	197,755	2,896	0.898
*A. incarnata**	1	144	94	0.805	113,426	64,869	0.823
A. labriformis	1	68	57	0.945	22,126	4,863	**0.554**
*A. latifolia**	30	162	102	0.858	415,185	412,476	0.394
*A. linaria**	1	127	75	0.962	376,557	382,289	0.427
A. macrosperma	1	71	62	0.965	624,888	124,259	**0.554**
*A. macrotis**	1	46	44	0.925	196,251	151,159	**0.554**
*A. nycatginifolia**	1	154	100	0.958	112,228	233,621	**0.554**
*A. pumila**	1	89	73	0.985	69,502	5,581	0.660
*A. soloanoana**	1	132	68	0.995	17,704	65,588	1.00
A. speciosa	30	1359	478	0.746	958,479	937,320	0.768
*A. subulata**	30	627	114	0.927	114,507	379,000	0.606
*A. subverticillata**	30	545	286	0.809	594,885	674,988	0.505
*A. tuberosa**	30	184	88	0.831	305,144	470,641	0.681
*A. vestita**	1	159	70	0.983	34,449	80,731	0.286

*Species marked with an asterisk are projected to shift their distribution to higher latitudes (Appendix 1: Figure S1).

[1] Hostplant quality weight determined by the average weight of monarch larvae reared on 24 *Asclepias* species within a 5-day period. Bold numbers indicate the average weight assigned to species with missing hostplant quality information.

Spatially filtered data were randomly split into training and test data by withholding 25% of the occurrences and the remaining 75% was used for model training. This mimics the common approach to data partitioning used in most SDM studies (Hijmans 2012). To select background points, we first determined the *Asclepias* environmental domain, corresponding to an area of 50 km^2 surrounding *Asclepias* occurrences. The environmental domain was then divided into 1 km^2 grid cells, and background points were randomly selected from within the monarch environmental domain in a checkerboard fashion, following protocols described in Elith et al. (2006) and Hijmans and Elith (2019). Individual *Asclepias* species were modeled using background points from the environmental domain represented by all *Asclepias* species (Elith et al. 2011). This process yielded 9000 background points to model *Asclepias* species. The best-fitted models for *Asclepias* with the highest area-under-the curve (AUC) score, were used to estimate their current and projected distribution under climate change.

Asclepias quality: I used the average monarch larval weight supported by each *Asclepias* species grown under greenhouse conditions as proxy for hostplant quality (Table 1.2). I note that larval performance obtained from Anurag Agrawal and this data has been previously published (Agrawal and Fishbein 2006, Agrawal et al. 2015, Petschenka and Agrawal 2015) and other sources. This decision was based on three major premises. First, hostplant quality has been shown to strongly correlate with larval mass, an important component of herbivore fitness that influences survival and adult reproductive success (a.k.a. silver spoon effect; Grafen 1988; i.e. Coley et al. 2006, Travers-Martin and Müller 2008, Müller and Müller 2016). Second, this relationship has been demonstrated in the monarch-milkweed system (i.e. Zalucki et al. 2001, Pocious et al. 2017). And third, monarch larval mass has been associated with several measures of milkweeds quality including latex, cardenolides content, and trichomes densities (Zalucki et al. 2001, Robertson et al. 2015, Pocius et al. 2017), thus, providing an integrative index of hostplant quality. Rearing protocols are described in detail by Petschenka and Agrawal (2015).

Briefly, *Asclepias* plants were grown from seed in a greenhouse and after a period of 4-7 weeks neonates were placed individually upon the leaves of potted plants and weighed after 5 days. Assessing hostplant quality under controlled greenhouse conditions controls for extraneous factors such as natural predators, competition with other herbivores, induced plant defenses by other herbivores, and environmental variation that are necessarily associated with a field bioassay.

<u>Monarch models:</u> The monarch distribution was modeled using a similar approach to *Asclepias*. As described above, I used spatial filtering to correct for sampling biases. Larval records were first filtered at a range of resolutions (1 to 55 km^2) and the spatial resolution yielding the highest AUC was then selected. The final dataset used to model monarch breeding range was thinned using 30 km^2 grid cells (the best-fitted model) and included 110 observations. As with *Asclepias*, I withheld 25% of the data for model testing and the remaining 75% was used for model training. To determine the environmental domain of monarch larvae, I selected 4,000 background points following the same procedure described in the *Asclepias* modeling section (Elith et al. 2011), although the number of background points was lower due to the more restricted distribution of monarchs.

To test for the importance of hostplant availability, I combined *Asclepias* distribution layers to estimate *Asclepias* species richness (Ferrier & Guisan, 2006; Kass et al., 2020; Koenig & Haydock, 1999). In contrast, previous western monarch modeling studies (Dilts et al. 2019, and Svancara 2019), incorporated individual *Asclepias* distributions directly into the monarch distribution models as they sought to identify specific milkweed species relevant for monarch conservation and biology whereas I was interested in the role of hostplant availability as drivers of monarch distributions. Following Kass et al. (2019), I summarized the resulting individual

Asclepias distribution layers into a single predictor layer representing *Asclepias* species richness under current and projected environmental conditions (Figure 1.1 and 1.2). Because the ranges of many *Asclepias* species overlapped, forming the *Asclepias* distribution layer by summing probabilities captures not only the mean probability of distribution but also reflects species richness. The values assigned to grid cell in the hostplant-availability layer were determined by:

$$Pr(Asc)= p(Asc_1) + p(Asc_2) + \dots p(Asc_{24})$$

Where 'Pr' represents *Asclepias'* species richness and 'p' the probability of distribution of individual *Asclepias* species numbered from 1 to 24.

Although other variables such as milkweed abundance or total biomass may be more closely related to monarch biology, this type of data is often lacking (Hortal et al. 2015). Instead, an estimated richness may be a suitable proxy in systems such as the monarch–milkweed system where the focal species interact with multiple other species (Ferrier & Guisan, 2006).

To test for the importance of hostplant quality, I weighted each *Asclepias* species distribution layer according to its host quality estimated per larval mass (Figure 1.1). The milkweed with the greatest larval weight (*A. sololana*) was given a value of 1, and all other species were assigned values as proportions of this value, with the lowest quality weight being 0.078 (*A. asperula*) (Table 1.2). Five species with no information on larval weight were weighted by the average host quality weight of 0.55. Weighted layers were then summarized into a single predictive layer representing the hostplant probability of distribution and species richness weighted by hostplant quality (Figure 1.1 and 1.2). The values assigned to grid cells of the overall hostplant quality layer were calculated as follow:

$$Pq(Asc) = p[p(Asc_1) * (q_1)] + [p(Asc_2) * (q_2)] + \dots [p(Asc_{24}) * (q_{24}]$$

Where ‘Pq’ represents hostplant availability weighted by hostplant quality, ‘p’ the probability of distribution of individual *Asclepias* species numbered from 1 to 24, and ‘*q*’ the host-quality weight estimated for each *Asclepias* species. This approach is parallel to that used for the *Asclepias* distribution layer (above), capturing the effects of both the mean probability of distribution and species richness for all co-existing *Asclepias* species, but now weighting each species according to its relative hostplant quality.

Lastly, I used the summarized *Asclepias* layers as predictors to generate and compare three models: a null model (climate envelope model) using only environmental factors as predictors; a model using environmental factors and hostplant availability represented by *Asclepias* richness (hostplant-availability model); and a second model using environmental factors and *Asclepias* availability weighted by host quality (hostplant-quality model).

To identify the variables contributing more to each model, in addition to the “Analysis of Variable Contribution” reported by MaxEnt, I performed a jackknife test of variable importance. In a jackknife test, models are re-run using a single variable in isolation to identify the variables that yield the highest model gain when used in isolation. This test also identifies those variables that, when removed, decrease the model gain the most by re-running the models excluding one variable at a time (Shcheglovitova & Anderson 2013).

Since there is currently no consensus regarding a single most appropriate metric to evaluate SDMs performance (Peterson et al. 2011, Peterson et al. 2008, Warren & Seifert 2011), I evaluated model performance based on several criteria. The area-under-the-curve (AUC) statistic provides an estimate for the accuracy of predictions, with 0 indicating no predictive accuracy and 1 perfect predictive accuracy. An AUC score of 0.5 indicates that the model performs no better than random. I also estimated performance metrics based on the Akaike

Information Criterion corrected for small sample sizes (AICc). The AICc metric has the advantage of balancing both, model goodness-of-fit and model complexity. Furthermore, compared to AUC and BIC (Bayesian-Information-Criterion) based methods, AICc evaluation methods have been shown to favor models that more accurately estimate the relative importance of variables and habitat suitability, both in the training region and when models are extrapolated to a different time period (Warren & Seifert 2011). I calculated the AICc, delta AICc (ΔAICc), and Akaike weights (wAICc) for each model using the ENMeval package (Muscarella et al., 2014). The model with the lowest AICc value is considered the best model out of various candidate models. The ΔAICc is the difference between the best AICc and other candidate models. The best candidate model has a ΔAICc of 0 and models with ΔAICc lower than 2 are generally considered to have substantial support and should not be discarded (Muscarella et al., 2014). Akaike weights (wAICc) represent the likelihood of a model given the data. The weights are normalized to sum 1 and are interpreted as probabilities (Burnham & Anderson 2004).

Finally, I estimated suitable breeding area for monarchs and for *Asclepias* distribution from polygons drawn around areas with grid cell values higher than 0.5 from the output logistic layers projected from the final models.

1.3. Results

Asclepias models and estimated distribution

All *Asclepias* final models had AUC scores higher than 0.8, except for the *A. speciosa* model which yielded an AUC score of 0.74, indicating that these models are a good fit for the observations (Table 1.2). The current estimated distributional ranges (Appendix 1: Figure S1; left panel) were consistent with *Asclepias* species' distributions published by the Biota of North America Program (BONAP) (Kartesz, 2015).

Overall, within the study area, half of the *Asclepias* species are projected to expand their ranges by a mean of 88% (i.e., nearly doubling their distributions) whereas the other half will contract their ranges by a mean of 42% (i.e. more than halving their distributions) (Table 1.2 and Appendix 1: Figure S1). Of the 24 *Asclepias* species, 19 species are predicted to shift their distributions to higher latitudes (79%) both along the Pacific coast and inland, with 11 of these also expanding their distributions. Of the 4 species not shifting their distributions northward, 3 will contract their ranges: *A. albicans*, *A. cryptoceras* and, *A. hallii*. Specifically, five out of nine milkweed species with below-average hostplant quality are predicted to contract their ranges, two will expand, and two will remain the same. Out of the nine species with higher-than-average hostplant quality, five are predicted to expand and four contract their ranges (Table 1.2; Appendix 1: Figure S1).

Monarch models and estimated distribution

The AUC scores did not differ considerably among the three models, but AUC values were slightly higher for the hostplant-availability model (0.803) compared to both the hostplant-quality (0.800) and climate envelope model (0.799). However, the AIC-based metrics preferred the hostplant-availability model (ΔAICc=0, wAICc=1.00) over the hostplant-quality (ΔAICc=123.50, wAICc=1.515^{-27}) and climate envelope models (ΔAICc=168.52, wAICc=2.545^{-37}). The ΔAICc for the competing climate envelope and hostplant quality model was much larger than 2 indicating that these two models had limited support. Likewise, the wAICc of the hostplant model was nearly 1 suggesting that the likelihood of this model being the best-fitted model was high (Table 1.3).

Table. 1.3 Monarch model performance comparison and estimated habitat suitability

Model	*n*[1]	AUC	AICc	ΔAICc	wAICc	Current estimated habitat (Km^2)	Projected estimated habitat for 2070 (Km^2)
Climate envelope	60	0.799	25,694.99	168.52	2.545^{-37}	214,245	409,091
Hostplant-availability	61	0.803	25,526.46	0.000	1.00	252,464	466,306
Hostplant-quality	61	0.800	25,649.97	123.50	1.515^{-27}	252,465	486,200

*Estimated habitat was calculated by summarizing areas with probability of distribution higher than 0.5 from the logistic output layers produced by each model. [1] *n* gives the number of parameters of each model.

The environmental variables that contributed most to the climate envelope model were the "minimum temperature of the coldest month" (43.4% contribution) and "precipitation seasonality" (25.2% contribution, Figure 1.3). For both hostplant-availability and hostplant quality models, the hostplant variable was the second most important factor for predicting the western monarch breeding range. The hostplant variable contributed most to the hostplant distribution model (22.5%), after the "minimum temperature of the coldest month" (33.1%) (Figure 1.3). Although the hostplant-quality model did not produce the best-fit model, weighting the hostplant layer by host quality increased the contribution of the hostplant variable by 3% and decreased "minimum temperature of the coldest month" variable contribution by 7% compared to the hostplant distribution model (Figure 1.3). Both hostplant layers (weighted by host quality and unweighted) exhibited the highest gain (>0.40) in the jackknife test for variable importance in both hostplant models (Appendix 1: Figure S2). This indicates that the hostplant variable provided the most useful information for predicting where monarch breeding grounds occur. For all three models the "average precipitation of the warmest quarter" decreased model gain the most when omitted, suggesting that this environmental variable has the most information that is not present in other variables (Appendix 1: Figure S2).

The process of weighting the *Asclepias* distribution layer by quality did not dramatically alter the hostplant layer, and mainly rescaled the values of the layer (Figure 1.2, lower panel). This was probably due to large range overlaps among *Asclepias* species. The only area where weighting hostplants by quality appeared to change the grid cell values of the hostplant quality layer was the southwest region of Arizona and Utah which appeared to be occupied mostly by lower quality species, predominantly by *A. asperula*, our lowest quality hostplant (Fig 2, lower panel and Table 1.2).

The climate envelope model estimated more restricted ranges for the contemporary and future monarch distributions. Both hostplant models estimated nearly identical contemporary distributions for monarchs that were ~18% larger than that estimated by the climate envelope model (Figure 1.4, left panel and Table 1.2). Although all three models predicted future range expansions that nearly doubled their corresponding contemporary estimates, the hostplant-availability and hostplant-quality models projected an increase in habitat suitability 14 and 19% larger than that of the climate envelope model, respectively (Figure 1.4, right panel, and Table 1.2). Finally, I detected slight differences where hostplants models predicted that such range expansions will occur. For example, the hostplant quality model predicted a smaller range for monarchs in western New Mexico and a larger range in central Nevada, Utah and western Colorado. (Figure 1.4, right panel).

1.4. Discussion

In this study, I tested for the importance of geographic variation of hostplant resources in driving herbivores' contemporary and future distributions by comparing a climate envelope model with two models incorporating hostplant availability and quality. I used the western monarch butterfly to explore these questions, a model system especially suited for this purpose as it utilizes multiple hostplant species in the *Asclepias* genus varying in quality by an order of magnitude in this region. Although I did not exhaustively model all factors potentially influencing the western monarch future distribution, I showed that accounting for future hostplant availability is key for predicting herbivore response to climate change, but hostplant quality may play a secondary role. While climate envelope projected a more restrictive current monarch distributions than hostplant models, model comparisons suggested that hostplant information provided superior predictive power. Furthermore, the three models differed in their future monarch projections under climate change, with models including hostplant information predicting an increase in habitat suitability 14-19% larger than that of the climate envelope model. Despite the importance of hostplant information, models including hostplant quality did not perform better than a model based on hostplant presence. Our study suggests that information on critical resources is essential for predicting future species distributions under climate change.

The hostplant-availability model performed better than the traditional climate envelope model and the hostplant-quality model according to both AIC- and AUC-based metrics. Hostplant availability, together with the "minimum temperature of the coldest month", were the most importance predictors for monarch occurrence (Table 1.3 and Appendix 1: Figure S2), suggesting that the western monarch breeding range is co-limited by cold temperatures and hostplant availability. The finding that *Asclepias* are among the primary drivers of monarchs' distribution is consistent with past monarch modeling efforts regardless of model complexity, modeling scale, or how hostplant information was incorporated into the models. For example, Lemoine's (2015) models included individual *Asclepias* species distributions, topography, and climatic variables, and found that models accounting for both hostplants and environmental factors more accurately estimated the eastern monarch distribution. Similar regional (Dilts et al.

2019) and local (Idaho state; Svancara et al. 2019) modeling efforts for the western monarch accounted for multiple abiotic variables (i.e., topography, land cover, edaphic factors, and hydrology), and also incorporated individual *Asclepias* species distributions, identifying hostplants as key drivers for monarchs' distributions. The second result that monarch breeding range is delimited by winter cold temperatures may seem counterintuitive given monarchs' overwintering behavior. I speculate that this correlation is due to monarchs' larval occurrences being indirectly correlated with milkweed's habitat requirements which tend to be cold-sensitive (Lemoine 2015) as it is evident by their sharp decline beyond the Canadian border (Kartesz 2015). Taken together, our findings add to the increasing body of evidence suggesting that biotic interactions may govern species distributions as strongly as environmental conditions (i.e. Araújo and Luoto 2007, Preston et al. 2008, Schweiger et al. 2008, de Araújo et al. 2014, Lemoine 2015, da Cunha et al. 2018, Dilts et al. 2019, among others).

Hostplant quality, estimated by larval weight gain, varied ten-fold among milkweed species but did not have a large effect on the estimated breeding range of monarchs. There are several potential explanations for this surprising result. First, because monarchs are known to sequester cardenolides for predator defense (Malcolm & Brower 1989), it is possible that a direct negative effects of *Asclepias* on larval performed in turn has a counteracting, indirect positive effect by providing a stronger resistance against predators. Therefore, the expected negative relationship between low host-quality, and herbivore performance may change when the effect of a third trophic level is taken into consideration. Second, climate-change induced abiotic stress, including drought, may alter milkweed quality and therefore monarch larval performance (Hahn and Maron 2018, Carvajal-Acosta et al. in review). However, bioassays upon I based our hostplant quality measures, were performed in a controlled greenhouse environment. Thus, it is possible that host-plant quality in natural populations may vary under various abiotic conditions, thus influencing monarch performance. Third, it is also likely that, because *Asclepias* species exhibit substantial range overlaps in the American West and adult monarchs preferentially oviposit on higher quality milkweeds in areas with mixed quality resources (Pocius et al. 2018), the influence of low-quality milkweeds is diminished. Finally, because milkweed abundance data was unavailable to us, I used species richness as a proxy. It is possible that some high-quality species were also less abundant or had less biomass, thus reducing the probability of monarch encountering high-quality species. Hostplant models projected similar monarch distributions under a climate change scenario; however, their projections differed in some regions of the inland states of Utah, Nevada, New Mexico and Colorado. This implies that the importance of hostplant quality in determining herbivore distributions should not be discarded altogether as it may play a significant role in instances where herbivores rely on hostplants that are less speciose or have less geographic overlap, and therefore fewer food choices.

The importance of incorporating the climatic response of hostplants into models is underscored by the fact that only models including hostplant information predicted an inland range expansion while the climate-envelope model did not (Figure 1.4, right panel). The predicted inland range expansion of the western monarch breeding range appeared to be driven by higher hostplant availability in the regions of central Nevada, Utah and Colorado under future climatic conditions, identified by our models as one of the most important factors delimiting monarch distributions. Our results are congruent with previous findings by Lemoine (2015) whose study predicted northern range expansion of the eastern monarch population resulting from projected *Asclepias* range expansions under future climate change scenarios. In contrast, Svancara et al. (2019), studying only the Idaho range, found that suitable habitat for monarchs under projected climate change is likely to remain the same with range expansions nearly equal to range contractions. However, these distributional changes were largely driven by changes in the distribution of one milkweed species (*A. speciosa*) in combination with temperature increases during the wettest quarter, thus confirming the importance of hostplants and temperature for western monarch's habitat. Finally, it is worth noting that, although our milkweed models were based on climatic niches and may not accurately reflect the future distribution of milkweeds, past modeling studies (Lemoine 2015, and Svancara 2019) have accounted for other potentially important variables such as edaphic factors, topography, distance to water, and estimated future milkweed range expansions of similar magnitudes.

Our results demonstrate how climate envelope models that accurately represent current distributions may provide poor predictions for the future. This can occur when critical distributional drivers (i.e. hostplant distributions) correlate strongly with environmental factors under contemporary conditions (Wharton & Kriticos 2004) but not under climate change. These mechanistically-flawed models thus provide inaccurate predictions (Brewer & Gaston 2003; Soberon & Nakamura 2009). In our study, the climate envelope model – although more restricted – estimated very similar monarch contemporary ranges than hostplant models (Figure1.4, left panel) but differed in their future projections (Figure 1.4, right panel). Specifically, the two hostplant models predicted larger inland monarch range expansions than the climate envelope model. This suggests that the climate envelope model overpredicted monarch climatic limitations due to contemporary correlations between climatic factors and milkweed distributions, but that these correlations may not persist in the future. Accordingly, our results suggest that models based solely on climatic factors may be adequate for estimating contemporary species distributions, but nevertheless produce misleading projections under novel circumstances where abiotic conditions and biotic interactions do not respond in tandem to climate change. These findings are in agreement with past studies that have incorporated a wide range of biotic interactions – ranging from antagonistic to mutualism and from obligate to generalist – to predict future species' distributions under projected climate change. These studies have shown that incorporating these interactions not only improves model performance but also alters the outcome of future projections under climate change (i.e., Araujo and Luoto 2007, Preston et al. 2008, de Cunha et al. 2018, Gianini et al. 2013, Kass et al. 2019, Lemoine 2015).

Although my primary goal was not to accurately model the western monarch breeding range, our outcomes agree with past studies regarding the importance of the region along the California coast for monarchs' breeding. For instance, the "breeding model" from Dilts et al. (2019), also indicates that this area is highly suitable for monarch breeding despite having been developed using a different set of covariates (i.e., land use, topography, distance to water), distinct incorporation of hostplant information, sample bias correction techniques, and different number of records. This is also consistent with past isotopic (Yang et al. 2016) and spatial analysis (Stevens and Frey 2010) identifying this region as crucial natal grounds for overwintering western monarchs. In contrast to Dilts et al. (2019) and Yang et al. (2016), our models' projections estimated low habitat suitability in northern latitudes; however, this is consistent with Steven and Frey's (2010) study indicating that environmental conditions (cold temperatures and/or low precipitation) in the states of Oregon, Washington, Idaho, and northern Nevada are not ideal for larval development or milkweeds growth.

Finally, it is worth noting that due to the limited scope of this study and/or data limitations, our model's projections may not accurately represent the future western monarch distribution. Other than climate change, many other factors have been identified as drivers of monarch’s population declines. For example, insecticide use (Olaya-Arenas & Kaplan 2019; Pecenka & Lundgren 2015), land-use development (Crone et al. 2019), and milkweed abundance (Zalucki & Kitching 1982, Zalucki & Suzuki 1987) may all impact the distributions of the monarch’s breeding grounds. However, these data may be unavailable or difficult to incorporate into SDMs. Similarly, although most studies conducted so far (including our own) predict milkweed range expansions under climate change, these models did not account for milkweed’s seed dispersal ability or local adaptation which have been shown to influence plants’ distributional response to climate change (Chen et al. 2020, Dirnböck & Dullinger 2004). Lastly, our analysis focused on the larval stage when milkweed availability is critical. Nevertheless, various anthropogenic factors may significantly impact monarchs at other life stages and thus, the overall distribution of the western monarch under climate change. For example, overwintering habitat loss to housing development has been identified as a major factor driving western monarch populations declines (Pelton et al. 2019). Likewise, since autumn migrants often follow riparian corridors (Dingle et al. 2005), dams and human-facilitated invasions, may alter riparian areas (Poff et al. 2011) potentially disrupting monarch migration patterns. Future modeling studies aiming at accurately estimating the western monarch distribution under climate change should investigate whether inclusion of these additional factors improves the predictive performance of these models. Despite these limitations, SDMs can provide first-order predictions for evaluating factors driving current and future distributions of focal species and provide a means for assessing potential changes in habitat and distributions.

1.5. Conclusions

Our study is among the first attempt to assess the role of geographic variation in resource availability and the first to assess the role of resource quality in driving herbivore distribution and response to climate change. I show that accounting for obligate biotic interactions – and their distributional response to climate change – is required to predict the future distributions of specialist herbivores that depend on one or few plant hosts. A climate-envelope approach may be effective for estimating contemporary species distributions but may produce misleading future projections as climate change may uncouple suitable climate from essential biotic interactions. Hostplant quality did not play a significant role in delimiting monarch distribution in the American West where *Asclepias* ranges overlapped substantially. However, there were slight differences in some regions suggesting that host-quality may still be important for predicting distributions of species dependent on a fewer number of resources. These results are relevant, not only for most herbivorous insects which are highly host-specific, but for all organisms incurring in obligate biotic interactions (i.e., parasitic or mutualistic obligate interactions). Ultimately, accurate projections for the future will require incorporating inter-specific dynamics into our models.

Figures

Figure 1.1. Monarch species distributions models

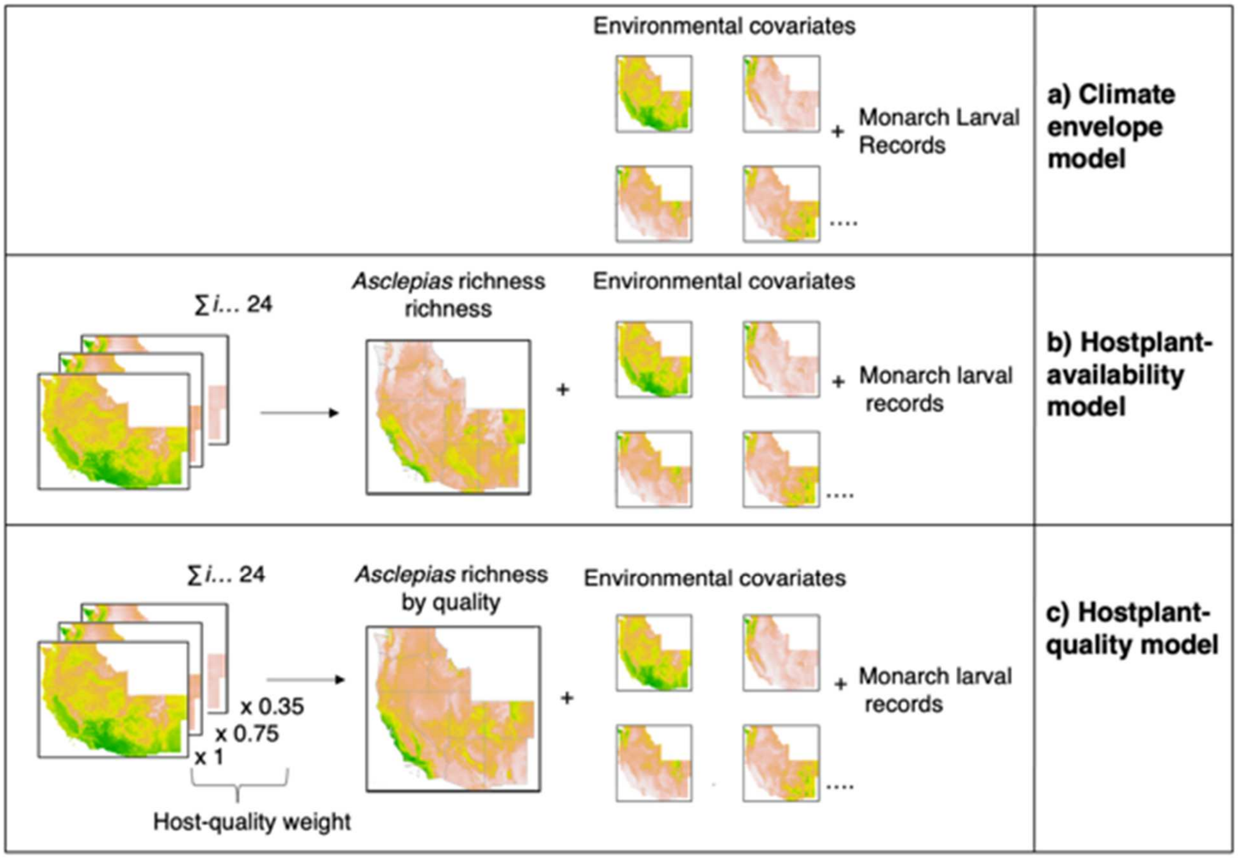


Fig. 1.1. Monarch species distribution models developed using environmental variables as predictors of monarch larval occurrences (a) climate envelope model; environmental variables and *Asclepias* richness as predictors of monarch larval occurrences (b) hostplant-availability model; and environmental predictors and *Asclepias* availability weighted by host-quality as predictors of monarch larval occurrences (c) hostplant-quality model.

Figure 1.2. Host plants predictors layers

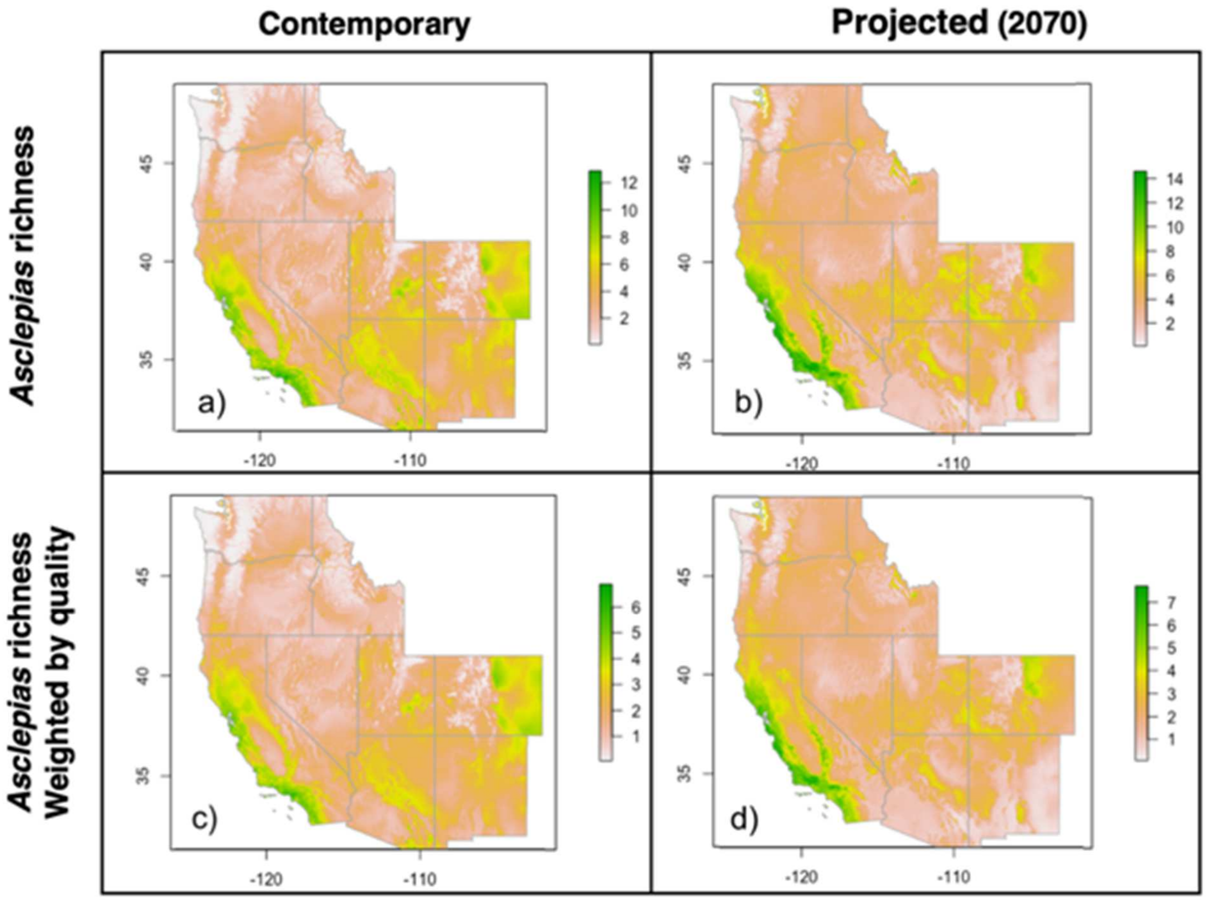
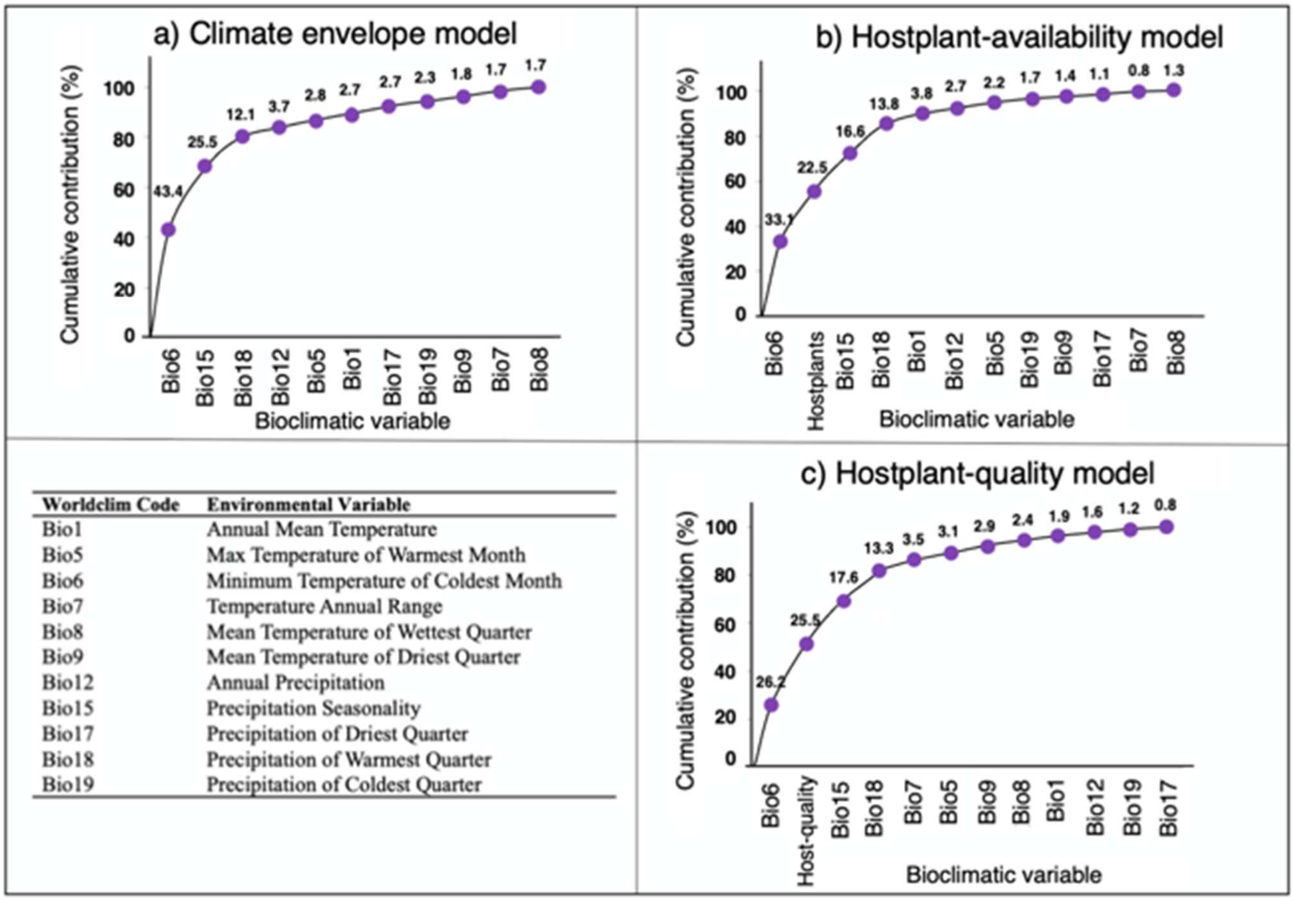


Fig. 1.2. *Asclepias* availability estimated by species richness (a) and *Asclepias* availability weighted by their host-quality (c) in the western U.S. Projected *Asclepias* availability estimated by species richness (b) and projected *Asclepias* availability weighted by their host-quality (d) in the western U.S. 'Green' indicate areas with high resource availability and/or host-quality, 'yellow' represent intermediate levels of resource availability and/or quality, and 'white' low probability of distribution, species richness and/or low quality.

Figure. 1.3. Analysis of variable importance



Worldclim Code	Environmental Variable
Bio1	Annual Mean Temperature
Bio5	Max Temperature of Warmest Month
Bio6	Minimum Temperature of Coldest Month
Bio7	Temperature Annual Range
Bio8	Mean Temperature of Wettest Quarter
Bio9	Mean Temperature of Driest Quarter
Bio12	Annual Precipitation
Bio15	Precipitation Seasonality
Bio17	Precipitation of Driest Quarter
Bio18	Precipitation of Warmest Quarter
Bio19	Precipitation of Coldest Quarter

Fig. 1.3. Percent contribution that each variable contributes to the models in decreasing order from left to right.

Figure. 1.4. Monarch contemporary and projected distributions

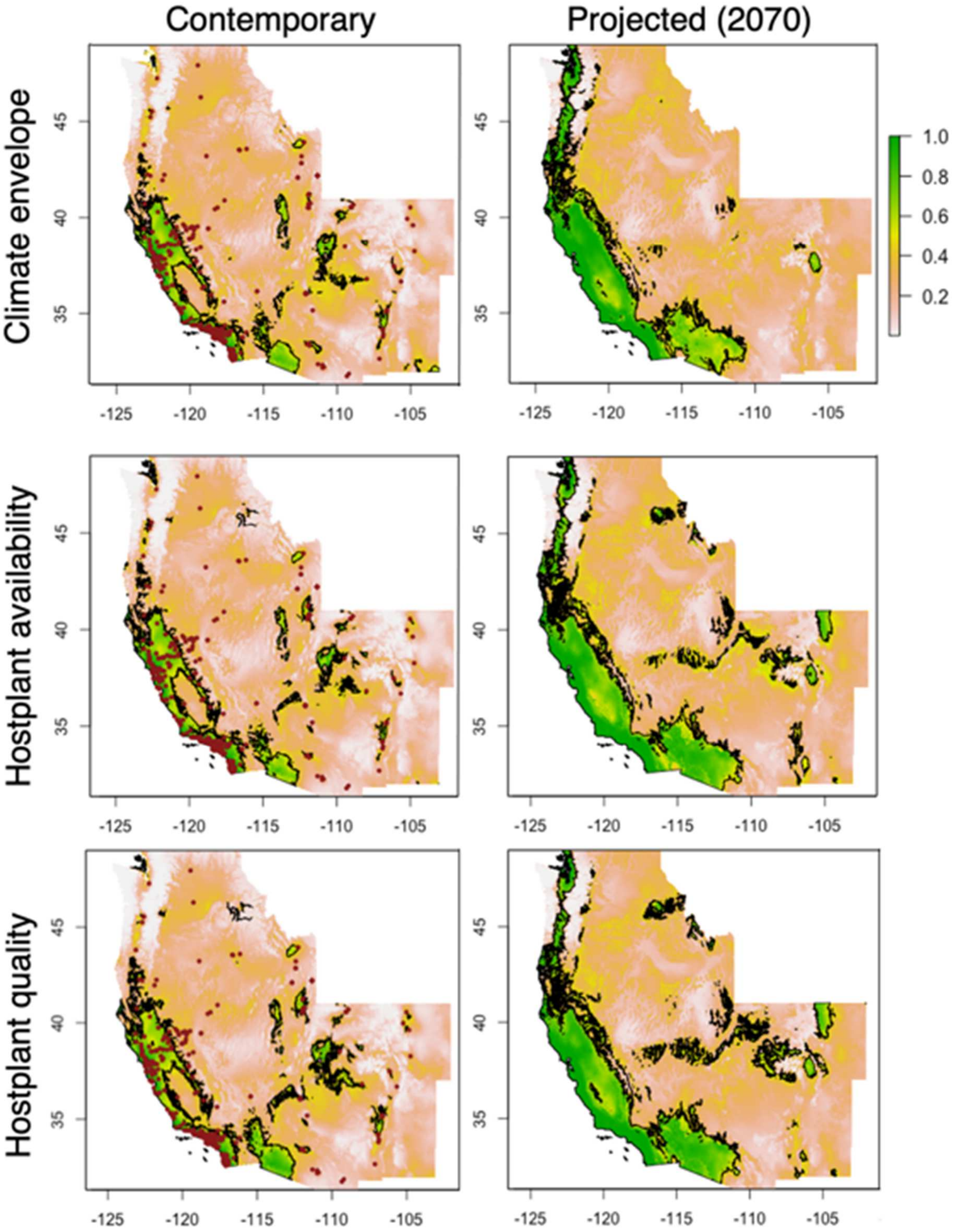


Fig. 1.4. Monarch contemporary and projected breeding ranges estimated by each model. Left panels indicate the current probability of distribution of the monarch breeding grounds estimated

by the three models, with ‘white’ representing low probability and ‘green’ high probability, from 0 to 1. Right panels indicate the projected probability of distribution of the monarch breeding grounds for the year 2070 estimated by the three models. Prime habitat for monarch breeding is delineated in black and represent areas with a probability of distribution greater than 0.5. Larval occurrences are indicated by red points.

CHAPTER 2

Does intra-specific variation in plant traits associated with aridity gradients mediate abiotic stress on herbivores?

Abstract: *The response of herbivorous insects to plant drought stress can range from positive to negative, and it has been challenging to understand the causes of this variation. I tested whether plant trait values associated with aridity gradients might underlie this variation and how effects vary among two insect feeding guilds. Here I propose that plants trait values associated with adaptation to arid environments would result in positive effects of experimental drought on herbivores, with such plant species adaptively shifting resources away from resistance to maintain performance under stress. In contrast, plant with trait values associated with adaptation to mesic environments would result in negative effects of drought because such species lose vigor and thus decline in their host-quality. I tested these predictions using experimental manipulations in 13 milkweed species (genus Asclepias) adapted to a wide range of environmental conditions, and herbivore performance of a specialist leaf-chewer (monarch butterfly; Danaus plexippus) and sap-feeder (oleander aphid; Aphis nerii). I exposed plants to species-specific watering regimes physiologically calibrated to maximize (100%) or reduce (50%) stomatal conductance and then monitored the performance of the herbivores.*

The effects of drought stress on herbivore survival ranged from strongly positive (50% increase) to strongly negative (80% decrease) among milkweed species, but these effects were inconsistent between the two herbivores. Plant trait values associated with adaptation to aridity were correlated with monarch survival in the predicted manner, such that milkweed species with high WUE and low relative water content increased monarch survival under drought. In contrast, aphid survival was unrelated to arid-adapted trait values. Drought-induced changes in plant *quality was negatively associated with both herbivore survival. Specifically, milkweed species that increased cardenolides concentration under drought conditions decreased monarch and aphid survival. In summary, I demonstrated that the indirect effects of drought on herbivores varied tremendously among closely related plant species and between co-occurring insect herbivores. I in turn present evidence that some of this variation is explained by plant traits associated with adaptation to arid vs. mesic environments for leaf-chewers but not sap-feeders and that such effects are mediated by drought-induced changes in chemical defenses.*

2.1 Introduction

Drought is common in many ecosystems (Kramer 1983) and can affect virtually every plant function (English-Loeb et al. 1997), but its effects on herbivores are notoriously variable and difficult to predict (Gely et al. 2020). Indeed, herbivore response to plant drought stress can range from instigating herbivore outbreaks (Mattson & Haack 1987) to population declines (*i.e.* Carnicer et al. 2019). Hundreds of empirical studies and many hypotheses have sought to characterize drought effects on herbivores, but the specific mechanisms driving herbivore response to drought in plants are still poorly understood. Climate change is predicted to intensify drought events in many parts of the world (Sheffield & Wood 2008) with significant consequences for ecosystem functioning and services, as well as economic activities such as forestry and agriculture. Thus, to better address the ecological and economic impacts of climate change, it is imperative that I develop a conceptual framework that allows us to predict herbivore response to plant drought stress.

The high variability of drought effects on herbivores have led to competing hypotheses: the plant stress hypothesis (PSH) and the plant vigor hypothesis (PVH). These hypotheses offer opposing predictions and have both received some empirical support (*i.e*. Bauerfeind and Fischer 2013, Grinnan et al. 2013). The PSH (White 1969) was proposed to explain patterns of insect outbreaks following drought events. This theory posits that herbivores benefit from drought-stressed plants due to 1) an increase in plant nutritional quality as plants flush nutrients (*i.e.* carbohydrates, nitrogen) to maintain osmotic potential under drought; and 2) a decrease in plant defenses as plants divert resources (*i.e.* carbon and nitrogen) from costly defense mechanisms to enhance survival under drought (Gutbrodt et al. 2011). By contrast, the PVH states that reduced plant performance from drought-stressed plants negatively affects herbivore fitness (Price, 1991). Low water availability reduces photosynthetic rate, above-ground dry mass, and leaf turgor (Eck et al. 2001, Gutbrodt et al. 2011, Ryan, 2011) and these effects are predicted to negatively affect insects that preferentially feed from fast-growing vigorous plants (Grinnan et al. 2013).

Multiple review papers and meta-analysis have tested and refined these predictions (Gely et al. 2020, Huberty & Denno 2004, Jactel et al. 2012, Koricheva & Haukioja 1997, Larsson 1989, Mattson & Haack 1987). In general, there is consensus that herbivore feeding guilds respond differently to drought-stressed plants since herbivores that feed from different plant tissues may differentially experience drought-induced changes in plant nutrition, chemistry, and growth. These studies have also revealed that drought effects on herbivores are often non-linear and depend on the mode and duration of the drought. Most feeding guilds are expected to initially benefit from a moderate drought as plant nutritional quality increases, but severe and prolonged drought typically have debilitating effects on both plants and herbivores (Matt & Hack 1987 and Gely et al. 2020). However, different drought types (*i.e.* short, long, moderate or severe) are often not well-defined by researchers, and experimental studies often fail to provide direct evidence that plants are indeed water stressed (Huberty & Denno 2004, Larsson 1989).

Huberty and Denno (2004) suggested that plants must experience intermittent periods of turgor recovery to allow nutrients to become available to sap-feeders (pulse stress hypothesis). Several other factors have also been considered to mediate herbivore response to drought, including plant phenological stage (White 2009) and herbivore diet breadth (Gely et al. 2020). Despite these advances, there is still considerable unexplained variation in the drought response of certain feeding guilds (*i.e.* leaf-chewers and leaf miners; Gely et al. 2020) and we have yet to unify these hypotheses under a single predictive framework.

Surprisingly, the role of plant traits values associated with water use strategies in mediating drought effects to herbivores is largely understudied. It is widely recognized that plant physiological responses to drought vary greatly within and among species dependent on their drought adaptation or acclimation capacity (Lopez-Iglesias et al. 2014, Mody et al. 2009, Ordoñez et al. 2009, Reich 2014, Turtola et al. 2005). Despite this variation, most drought experiments have been based on individual plant species and, while a diversity of plant-herbivore systems have been studied, I am aware of no work using a consistent methodology to explicitly test for how plant adaptation to aridity influence herbivore-plant dynamics under water deficit conditions.

I propose that plant traits values associated with aridity gradients can provide a means for integrating the PSH and PVH, and thus improve prediction of herbivore responses to plant drought stress. Plant traits that underlie adaptation to arid vs. mesic conditions include multiple morphological, physiological, and bio-chemical adaptations to avoid or tolerate drought (*i.e.* stomatal regulation, low specific leaf area (SLA), high water-use-efficiency (WUE), nitrogen and phosphorous content (Ordoñez et al. 2009, Passioura 1996, Reich 2014, Wright et al. 2001, 2005). I hypothesized that herbivores respond positively to drought-stressed plant species with aridity-associated traits values because these plants adaptively shift resources away from costly defensive compounds and increase nutrient content to maintain homeostasis under water limiting conditions (PSH). In contrast, herbivores respond negatively when feeding on drought-stressed plants with mesic-associated traits because these plants are unable to maintain homeostasis, lose vigor and decline in quality (PVH) (Figure 2.1). Our predictions are underlain by the more general observation that costly physiological investments lead to trade-offs in plant response to conflicting stresses (*i.e.* abiotic vs. abiotic) and that plants adapted to different environments evolve unique positions along such trade-off axes that are appropriate to their specific environments (Agrawal 2020, Blumenthal et al. 2020, Coley et al. 1985). Indeed, recent evidence suggests that drought-adapted species are genetically predisposed to prioritize investment in response to drought stress (*i.e*. proline and abscisic acid responsible for stomatal regulation) over their investment to face herbivore attack (*i.e.* jasmonic and salicylic acid) (Montesinos-Navarro et al. 2020).

To test our hypothesis, I imposed a physiologically calibrated water stress treatment on 13 milkweed species (*Asclepias* spp.) and tested for effects of aridity adaptation on the performance of two specialist herbivores from distinct feeding guilds, a leaf-chewer (monarch caterpillars; *Danaus plexippus*) and a sap-feeder (oleander aphids; *Aphis nerii*). The *Asclepias* genus has diversified into wet and arid habitats and herbivore defense traits are consistent among species across habitats (Agrawal et al. 2009), thus allowing us to mechanistically investigate how water stress indirectly affects herbivores. By taking a comparative approach and measuring nine functional traits, I sought to explicitly link traits values based on habitat affiliations with herbivore drought response. To our knowledge, this study represents the first formal test for how plant traits values associated with aridity gradients mediate drought effects on herbivores.

2.2. Methodology:

Study system

The milkweed (*Asclepias* genus, Apocynaceae) is a large plant genus consisting of over 140 known species widely spread in the American continent and the Caribbean (Woodson 1954). *Asclepias* species have diversified into a variety of habitats including tropical and temperate forests, deserts, wetlands, prairies and savannas (Agrawal, Fishbein, Jetter, et al., 2009). Plants in this genus have evolved a combination of anti-herbivore defense strategies (defense syndrome) with latex and cardenolides (cardiac glycosides) being the most characteristic defensive types (Agrawal & Fishbein 2006). Latex is exuded by plant tissues to deter further feeding damage by entangling herbivore's mouth parts (Agrawal et al. 2014). Cardenolides (cardiac glycosides) is a group of highly toxic steroidal compounds that impact the function of Na+/K+-ATPases (Agrawal et al. 2014). Both defenses types have been shown to decrease survival and slow down growth in monarch caterpillars (Agrawal et al. 2014, Agrawal & Fishbein 2006, Zalucki et al. 2001) and the development and fecundity of oleander aphids (Birnbaum & Abbot 2018). The monarch larvae and the oleander aphid are highly specialized to feed on all milkweed species despite its toxicity, they are broadly distributed and interact strongly in nature (Agrawal & Konno 2009, Dobler et al. 2011). Thus, the *Asclepias* genus allows to compare herbivore response to plant drought stress in species adapted to contrasting environments but with consistent defense types.

Study design

I selected 13 *Asclepias* species from a wide range of habitats, from arid (*i.e., A. californica*) to humid tropical environments (*i.e. A. curassavica*), that vary tremendously in traits associated with adaptation to mesic and dry habitats. The selected species were distributed across the *Asclepias* phylogeny (Appendix 2: Figure S3), increasing opportunities to effectively control for non-independence and test for evolutionary convergence between plant traits and indirect drought effects on herbivores. I grew plants from seeds for two months in a greenhouse and watered them to saturation twice a week. For each plant species, ten healthy plants were randomly divided into either the control or the drought treatment. Replication among the 26 species-by-treatment combinations (13 species, 2 water treatments) ranged from 3 to 5 plants, with a total sample size of 121 plants.

<u>Water treatment:</u> To impose a consistent physiological stress among species adapted to different moisture environments, I standardized water treatments based on stomatal response to water availability (Cowan 1978). I developed response curves for each *Asclepias* species by taking stomatal conductance measurements with a leaf porometer (Decagon Devices; model SC-1) over a gradient of soil moisture conditions (see Appendix 2: Protocols and Figure S4, for a detailed description of how response curves were constructed). Using these curves, I determined the amount of water needed to achieve the target pot weight at which a species reached its maximum stomatal conductance (100%; control) and a 50% reduction in stomatal conductance (drought) (Figure S4) (Skelton et al., 2015). Plants were watered twice a week, with each watering restoring the pot to its respective target weight. I imposed this treatment for two weeks. This duration was used because it imposes plants to drought stress while avoiding confounding effects of drought acclimation such as morphological adaptations in new leaves and roots (Touchette et al. 2007).

<u>Herbivore bioassay:</u> After two-weeks in their respective water regimes, I placed a single monarch larva and one aphid on each intact plant. Adult monarchs obtained from the UC Irvine campus and a captive colony housed at UC-Davis were caged and oviposited on *Asclepias*

curassavica as a source of neonate caterpillars. Oleander aphids were similarly collected from the UC Irvine campus and reared on *Asclepias fascicularis*. For aphids, I placed a gravid female on each plant which was then removed after reproducing, leaving one aphid nymph per plant. After seven days, I exhaustively searched each plant for the caterpillar and aphid, assuming that any missing herbivores had died. This assumption is supported by the fact that neonate movement among plants is extremely difficult (both in greenhouse and field conditions), and I found only a single instance of two herbivores on the same plant (two monarch larvae on *A. humistrata*). I maintained plants in their respective water regimes during the bioassay period for a total of three weeks (two weeks prior to biossays, and one during the bioassay) under the water manipulation treatments.

Plant trait selection and measurements

Plant trait selection: I selected a set of leaf traits each associated with water use strategies and host-quality to herbivores. For water-use associated traits, I selected specific leaf area (SLA), relative water content (RWC), intrinsic water-use efficiency (WUE), and maximum stomatal conductance (g_s *max*), all traits that have previously been associated with drought tolerance and avoidance strategies (Passioura 1996, Taiwo et al. 2020, Touchette et al. 2007, Volaire et al. 2014). Low SLA (or high leaf mass area [LMA], its inverse), act as a drought avoidance strategy to prevent water loss (Reich 2014, Wright et al. 2001, 2005). High g_s*max* values are associated with fast-growing acquisitive plant species that maximize photosynthesis and are less drought tolerance whereas species with low g_s*max* are typically slow-growing, water conservative, and thus, are more drought tolerant (Reich 2014, Wright et al. 2001). Plant species with drought tolerance strategies are also able to operate at low water content through resilience in plant metabolism (Taiwo et al. 2020). Species with high WUE, defined as the amount of water used (via transpiration) per unit dry matter produced (Touchette et al. 2007), are able to operate under water deficit conditions (Lopez-Iglesias et al. 2014, Touchette et al. 2007, Volaire et al. 2014). However, intrinsic WUE has also been associated with adaptation to aridity gradients and growth strategies (Moreno□Gutiérrez et al. 2012). I selected plant quality traits shown to affect herbivore performance under drought: nutrients content (protein, non-structural carbohydrates, and nitrogen) and defensive traits (latex and cardenolides) (Matts and Haack 1987, Lenhart et al. 2015, English et al., Couture et al. 2015, Gutbrodt et al. 2011, 2012).

Plant trait measurements: Trait measurements were obtained from 3-5 newly expanded and undamaged leaves collected from each experimental plant at the conclusion of the greenhouse experiment. Excised leaves were transferred to a paper envelope and immediately frozen at -20 °C for later characterization of plant traits. Because of the relatively short duration of the experiment, all leaves were formed prior to the initiation of the drought treatment. For water-use associated traits I measured leaves from plants in the control treatment as representative of their constitutive trait values because I were interested in testing whether plant adaptation to aridity gradients– not plant drought response– mediate drought herbivore response. To measure SLA, leaf area (cm^2)/dry mass (mg), frozen leaves were scanned, weighed, dried at 60 °C for 2 days, and reweighed to the nearest mg. Leaf water content was calculated as percentage estimated from the difference between leaf fresh weight and leaf dry weight. Dried leaves were pulverized using a Mixer Mill (Retsch MM 400) for stable isotope and macronutrient analyses. Foliar carbon isotopes ratio (^{13}C and ^{12}C) was estimated from pulverized foliar tissue as an indicator of WUE for a subset of samples (*n=46*) at the UC-Irvine Mass Spectrometry facility. Plants with high WUE, tend to be less fractionated in ^{13}C and therefore have less negative $\delta^{13}C$ values (Moreno□Gutiérrez et al. 2012). Maximum stomatal conductance ($g_s max$) was the raw stomatal conductance values achieved by each species when at its maximum level of stomatal conductance.

To quantify drought-induced changes in hostplant quality, I measured plant defenses and nutritional content from plants in the control and drought treatments. Latex exudation and cardenolides concentration were measured as the typical plant defensive traits in milkweeds (Agrawal & Fishbein 2006). Latex exudation was estimated by excising 2-3 mm off the tip of a new, undamaged, and fully extended leaf. The latex produced within 30 seconds was collected on pre-weighed 1 cm^2 filter paper discs which were then placed in pre-weighted 2 mL Eppendorf tubes. The tubes were immediately reweighed to estimate the mass of wet latex collected (Mooney & Agrawal 2008). This procedure was performed in the greenhouse after leaf collection to avoid inducing plant chemical defenses. Cardenolides concentrations were analyzed from a subsample of plants (n=47) from pulverized foliar tissue. I determined cardenolide concentration (mg/g dry tissue) from pulverized foliar tissue (n=47) by high-performance liquid chromatography following the methods of Züst et al. (2019) in the Ecology and Evolutionary Biology department at Cornell University (Appendix 2: Protocols).

Milkweeds nutritional profiles were characterized from pulverized leaf tissue by quantifying nitrogen, protein, and total non-structural carbohydrate concentrations for a subset of samples (n=46). An increased in nitrogen and carbohydrates as a result of osmotic adjustments under drought is the main proposed mechanisms for increased herbivore performance under drought (Bauerfeind and Fischer 2013, Lenhart et al. 2015, Mattson and Haack 1987). Nitrogen content (mg/g dry tissue) was estimated during stable isotope analysis at the UC-Irvine Mass Spectrometry facility. Soluble carbohydrates and carbohydrates from starch were extracted separately following protocols described by Chown and Landhässeur (2004) and quantified colourimetrically using the phenol-sulfuric acid assay (DuBois et al. 1956) optimized for microplate reading (Masuko et al. 2005). Protein was extracted by sonication following protocols described in Lenhart et al. (2015) and quantified colourimetrically using the Bio-Rad Bradford micro assay (Bradford, n.d.) in a microplate reader. Total carbohydrates and protein content were also estimated in a per mass basis (mg/g dry mass). Please refer to the supplemental material for a detailed description of carbohydrate and protein extraction and analyses (Appendix 2: Protocols).

Statistical analysis

Overview: Our general approach to analysis was to (i) test for milkweed species variation in herbivore response to our imposed drought stress, (ii) quantify that variation with an effect size metric (log response ratio of drought vs. control), (iii) explore the mechanisms underlying this variation separately for each herbivore through species correlations between drought effect size on herbivores and plant species traits related to adaptation to aridity (4 traits) and plant quality (4 traits) (Figure 2.1). This study thus addressed the same hypotheses with regards to two separate herbivore species and eight plant traits. Furthermore, these traits might reasonably be assessed based upon either variation in constitutive values (*i.e.,* under the control treatment) as well as the changes in those trait values induced by drought. I took several approaches to address the concern for inflated Type-1 error given the large number of test (*i.e.* 13 species, two treatments, nine traits) while not unnecessarily reducing statistical power (Garcia 2004).

I choose to assess the effects of constitutive values (only) for traits underlying adaption to arid vs. mesic environments, and I did not assess whether drought altered these traits, or whether such changes in traits explained variation in herbivore response. This approach is consistent with our hypothesis that variation in milkweed species drought adaptation drives variation in herbivore response to drought. While I explored reducing the dimensionality of the trait data through Principal Component Analysis (PCA), the resultant PCs did a poor job of explaining variation in drought adaptation traits (Appendix 2: PCA; Figure S5). Accordingly, I tested our hypotheses with trait-by-trait analyses.

In contrast to aridity associated traits, I choose to assess changes in trait values for plant quality traits. This approach is consistent with our hypothesis that variation in drought effects on herbivores is mediated by milkweed species changes in plant quality in response to drought. Here, to reduce Type-1 error, I performed a single multivariate test for whether there was species variation in the response of hostplant quality traits to drought, but then performed a trait-by-trait analysis to associate those changes with herbivore response.

I tested for phylogenetic signal for each set of traits in the "phylosig" package (Revell 2012), and corrected for potential effects of phylogenetic signal using a phylogenetically independent contrasts approach (PIC; Freckleton 2000).

Finally, I explored the overall pattern in our results by using the R function dbinom to quantify the probability of the observed number of significant ($\alpha = 0.05$) associations between plant traits and herbivore response based upon the total number of tests conducted. All statistical analyses were performed in R studio (R Studio Team, 2015; version 3.5).

Drought effects on herbivore survival: I used logistic regression models to test for the main drought effect and interactive effect of drought and *Asclepias* species on the survival of both herbivore guilds. To examine whether *Asclepias* species transmit similar drought effects to both herbivore guilds, I first calculated effect sizes (log response ratios, Ln R) using the formula Ln R= $\ln(\bar{x}_1/\bar{x}_2)$, where $\bar{x}_1$ represents the average herbivore survival for a given species in the drought treatment and $\bar{x}_2$ was the average herbivore survival of given species in the control or the well-watered treatment. A positive Ln R indicates that drought increased herbivore survival, 0 indicates no effects, and a negative Ln R indicates that drought decreased herbivore survival in a given species. I then conducted correlations of those effect size values to detect associations between the drought effect on monarchs and drought effect on oleander aphids.

Drought effect on herbivores as mediated by drought adaptation traits: I tested for correlations between each plant trait associated with aridity gradients (SLA, WUE, water content, and $g_s max$) and drought effects on the survival of the two herbivores (Ln R).

a) Drought effects on hostplant-quality traits: To test if plant defensive traits and plant nutritional content were affected by the water treatment, I first conducted a PerMANOVA. Then I identified which specific traits were affected by drought by conducting non-parametric ANOVAs based on permutations using cardenolides, latex, nitrogen, protein and carbohydrate content as the representative plant defenses and nutritional quality traits.

b) Drought effect on herbivores as mediated by changes in hostplant-quality: Once I identified which traits were affected by drought, I examined whether herbivore survival was associated with drought-induced changes in hostplant quality. I first estimated the effect sizes (Ln R) for each hostplant quality using the same formula, Ln R= ln ($\bar{x}_1$/ $\bar{x}_2$), but here $\bar{x}_1$ represented the average trait value under drought treatment and $\bar{x}_2$ was the average trait value under the control treatment. Then I tested for correlations between drought effects on each plant quality trait affected by the drought treatment (Ln R) and drought effects on the survival of the two herbivores (Ln R).

2.3 Results

Drought effect on herbivore survival

Monarch survival across all *Asclepias* species and water treatments was 71%. The effect of drought treatment on monarch survival differed among *Asclepias* species (plant species * treatment interaction, p=0.043; main effects: species p=0.09, and treatment p=0.28). Oleander aphid survival across all *Asclepias* species and water treatments was 56%. Similarly, the effect of drought on aphid survival differed among host-species (plant species * treatment interaction, p=0.09) and it was strongly influenced by *Asclepias* host-species (p=0.002) (Appendix 2: Figure S6).

I found no correlation between drought effects (Ln R) on monarch and oleander aphid survival among plant species (r^2=0.02, p=0.612) indicating that *Asclepias* species differentially transmitted drought effects to these herbivores in contrasting feeding guilds (Figure 2.2). Milkweed species occurred in all quadrants of the correlation plane, with five milkweed species transmitting concordant drought effects to the two herbivores (three positive, two negative), two species transmitted contrasting effects (*i.e.,* positive effects on one herbivore but negative effects on the other), and four species transmitted no effect to one herbivore and either positive or negative effect on the other (Figure 2.2).

Drought effect on herbivore survival as mediated by plant traits values associated with water-use strategies

Phylogenetic signal was weak for all plant traits associated with aridity gradients (Table 2.1); thus, I interpreted our results based on RAW correlations. For completeness I report both RAW and PIC results in Table 2.1 and PIC correlations are shown in Appendix 2: Figure S7. Drought effects on monarch survival (Ln R) varied from positive to negative and survival was positively correlated with two traits associated with drought tolerant strategies, WUE and RWC. Specifically, species with higher WUE (less negative δ^{13}C values) led to higher survival of monarchs under drought conditions (Ln R) ($p=0.015$, $r^2=0.42$). Drought effects on monarch survival (Ln R) were negatively correlated with RWC ($p=0.058$, $r^2=0.28$; Table 2.1) so that monarch performed better under drought conditions in those species with low RWC (Figure 2.3). Conversely, SLA and gs_{max} values were uncorrelated with monarch survival ($p=0.586$ and $p=782$, respectively).

Drought effects on oleander aphid survival (Ln R) also ranged from positive to negative but were not associated with *Asclepias* aridity-associated traits. I found no significant correlations between SLA, water content, WUE, or gs_{max} and drought effects on oleander aphid survival (Ln R) (Table 2.1, Figure 2.3; PIC correlations are shown in Appendix 2: Figure S7).

Table 2.1. Effect of drought on herbivore survival as mediated by plant trait values associated with aridity gradients

Species traits	**Phylogenetic signal**	**Monarch (p-values)**		**Aphid (p-values)**	
		Raw	PIC	Raw	PIC
SLA	$\lambda=7.34e\text{-}05^{+}$	0.586	0.884	0.436	0.131
Relative water content	$\lambda=7.34e\text{-}05^{+}$	0.019*	0.058·	0.362	0.569
gs_{max}	$\lambda=7.34e\text{-}05^{+}$	0.782	0.053·	0.568	0.394
$\delta^{13}C$	$\lambda=4.92e\text{-}05^{+}$	0.042*	0.015*	0.937	0.107

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

$^{+}$ $\lambda=0$ indicates no phylogenetic signal whereas $\lambda=1$ indicates phylogenetic signal.

Drought effect on hostplant-quality traits

Our PerMANOVA results revealed that differences among hostplant-quality traits values were driven by the water treatment ($p=0.001$) and that this treatment effect also varied among *Asclepias* species (species * treatment interaction: $p=0.001$). Our subsequent trait-by-trait analysis showed all plant quality traits were affected by drought ($p<0.05$) except for latex flow ($p=0.152$) (Table 2.2). However, the magnitude and direction of the effects differed among milkweed species. For each of the four plant traits, about half of the species increased and half decreased hostplant-quality trait values, with the exception of cardenolides, where most species increased concentrations under drought (eight increased vs. four decreased) (Appendix 2: Figure S8).

Table 2.2. Effect of drought on plant traits values associated with plant quality

Trait	**Species**	**Treatment**	**Species*Tmt**
Cardenolides	<0.001***	<0.001***	<0.001***
Latex	<0.001***	0.1528	0.958
Nitrogen	0.002***	0.0506·	0.002***
Protein	<0.001***	<0.001***	<0.001***
Total Carbs	<0.001***	<0.001***	<0.001***

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '·' 0.1 ' ' 1

Drought effect on herbivore survival as mediated by changes in host plant quality traits

Phylogenetic signal was weak for most drought-induced changes in plant-quality traits except for changes in total carbohydrate content (Table 2.3). Thus, I interpreted our results based on RAW correlations but report both PIC and RAW results. Drought effect on both herbivores' survival was uncorrelated with drought-induced changes in nutritional content (nitrogen, protein, and total carbohydrates). Latex flow was excluded from this analysis because it was not affected by drought (Table 2.2). I detected a significant correlation between drought effect on cardenolide concentrations and drought effects on both, monarch and oleander aphid survival (Ln R) (p= 0.037 and p=0.005, respectively; Table 2.3). Specifically, species where drought increased cardenolides concentrations were associated with negative drought effects on oleander aphid and monarch survival (Ln R) (Figure 2.4). PIC correlation shown in Appendix 2: Figure S9.

Table 2.3. Effect of drought on herbivore survival as mediated by drought-induced changes in plant quality traits

Species traits (Ln R)	**Phylogenetic signal**	**Monarch (p-values)**		**Aphid(p-values)**	
		Raw	PIC	Raw	PIC
Cardenolides	λ=7.34e-05^{+}	0.037*	0.584	0.058·	0.005**
Nitrogen	λ=7.34e-05^{+}	0.301	0.29	0.852	0.214
Protein	λ=7.34e-05^{+}	0.529	0.402	0.743	0.126
Total carbohydrates	λ=0.91879^{+}	0.819	0.571	0.390	0.983

Significance. Codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘·’ 0.1 ‘ ’

Note: latex was not found to be affected by drought and therefore was not included in this analysis.

$^{+}$Phylogenetic signal test performed on traits’ effect sizes. λ =0 indicates no phylogenetic signal whereas λ=1 indicates phylogenetic signal.

Inflated Type-1 Error

Overall, I tested for 8 associations between plant traits and the performance of each herbivore, for 16 total associations, and I detected five significant results. The probability of five significant (α = 0.05) associations out of 16 tests is low (p=0.0008). Inspecting the monarch and oleander aphid responses separately, the probability of detecting 4 significant associations in monarchs by chance alone is low (p=0.0004) while 1 significant association in aphids could have been detected by change (p=0.28), out of 8 respective set of tests. Thus, while we have evidence that the measured traits mediated drought effects on monarchs it is less clear for aphids.

2.4 Discussion

In this study, I analyzed the indirect effects of drought on *Asclepias* species and two herbivores with different feeding strategies. The species in the *Asclepias* plant genus have diversified into a variety of environments and employ similar herbivore defensive mechanisms. With a physiologically-customized treatment, I show that the effects of drought on herbivores varied dramatically among these closely-related plant species – in both magnitude and direction – ranging from strong decreases to increases in the survival of both herbivore species on drought stressed compared to control plants (Appendix 2: Figure S6). Past attempts at predicting drought stress effects on insects have struggled to unify varying empirical results without regard to plant traits, focusing on herbivore feeding guild or the characteristics of the drought. While such factors are undoubtedly important, our findings clearly establish that a plant's traits associated with aridity gradients can play a central role in mediating drought effects on herbivores.

Drought effects on herbivores varied tremendously among milkweed species but were uncorrelated between our two species in different feeding guilds (Figure 2.2), suggesting that drought effects may be driven by mechanisms that differ between herbivores. These results are consistent with past studies indicating that feeding guilds experience plant drought stress differently (Gely et al. 2020, Huberty & Denno 2004, Koricheva & Haukioja 1997).

For leaf-chewing monarchs I found evidence that the variation in drought effects was associated with plant traits values associated with drought-adaptation. These results are consistent with our proposed hypothesis that species with plant traits values associated with aridity transmit positive drought effects to herbivores, whereas plant traits values associated with mesic environments transmit negative drought effects. WUE, and RWC were each associated with drought effects on monarch survival in the predicted manner indicating that species with drought tolerant strategies transmit positive drought effects to monarchs (Table 2.1, Figure 2.3). In contrast, SLA and gs_{max} were uncorrelated with monarch survival. It is important to note that the selected aridity associated traits may not co-vary as a coordinated syndrome as they represent distinct drought adaptation strategies (avoidance, tolerance [Touchette et al. 2007]). Indeed, the lack of co-variation of the selected drought adaptation in our PCA (S.2) indicate that *Asclepias* species may have adapted unique water-use strategies when diversifying into different habitats.

I proposed that plant species with traits values associated with aridity maintain homeostasis and increase in quality whereas plants with mesic traits values are expected to decline in quality. Indeed, monarch survival was driven by drought-induced changes in chemical defenses. Monarch survival decreased on milkweed species that increased cardenolides concentrations under drought (Table 2.3; Figure 3). Drought-induced changes in these nutritional compounds have been correlated with increased performance in leaf chewers under drought conditions (i.e. Lenhart 2015). However, drought induced changes in milkweed nutritional quality (total carbohydrates, nitrogen, and protein content) appeared to have no effect on monarch survival in response to plant drought (Table 2.2; Figure 5). Similarly, Hahn et al. (2018) found no evidence that monarch mortality in drought-stressed *A. syriaca* was associated with changes in nutritional content (nitrogen) and speculate that monarch's drought response was driven by changes in cardenolides but this was not measured in this study. In contrast, Couter et al. (2014) found that monarch larvae performed better in *A. syriaca* subject to intermittent water stress, and it was associated with an increased in nitrogen foliar content. Because of the relative short duration of the experiment, one possible explanation for the lack of association between monarch survival and plant nutritional traits these did not change by the magnitude necessary to be detected by our statistical analysis. But monarchs may have responded prophylactically to a signal of plant quality decline and these quality changes may have eventually been detected in a longer drought period. A more plausible explanation is that our assessment of fitness was based on survival, and changes in nutritional content may be more evident in other components of fitness such as growth or reproduction.

In contrast to the monarch's findings, our hypothesis was not supported for the oleander aphid, whose performance under drought was uncorrelated with plant drought traits (Table 2.1; Figure 4). Nevertheless, oleander aphid performance was also associated with drought-induced changes in cardenolides concentrations. Sap-feeders have been traditionally predicted to benefit from higher nutrient content in drought-stressed plants (Larsson & Björkman 1993), but drought-induced changes in plant nutritional quality (carbohydrates, protein, and nitrogen) did not influence oleander aphids. One potential explanation for the lack of response to changes in nutritional content is that our drought treatment was not pulsed, but rather maintained throughout the duration of the experiment; thus, nutrient effects might have become apparent only with a return of plant turgor (pulse stress hypothesis; Huberty & Denno 2004). As mentioned previously, it is also likely that other components of fitness not measured here were impacted.

Unstudied here, but potentially important, is how variation in drought characteristics (*i.e.,* duration or intensity) mediate herbivore responses. Our manipulation was short enough that herbivores were responding to relatively rapid plant physiological responses, and not acclimation or compensation (*i.e.,* through the production of new leaves with altered traits). It is worth noting that, although relatively short, our drought treatment did elicit a physiological response in all milkweed species, as drought affected nearly all plant traits associated with hostplant quality except for latex flow. I also noted other signs of plant physiological stress such as leaf shedding. However, milkweed species exhibited a wide range of response to drought stress with some *Asclepias* species either increasing or decreasing in hostplant-quality (*i.e.,* toxicity and nutrients) under drought conditions (Appendix S2: Figure S8).

2.5. Conclusions

To my knowledge, this represents the first study to systematically test for the role of plant traits in mediating drought response to herbivores by comparing herbivore drought response on multiple plant species adapted to contrasting environments and by ensuring that all plant species experienced the same level of drought stress. I show that plant trait variation associated with aridity gradients could be key to predicting drought effects on herbivores. For two distinct feeding guilds, I observed the dynamics predicted by both the PSH and PVH (drought increasing and decreasing herbivore performance, respectively) depending on the hostplant species; thus, plant traits may be important for resolving the apparent conflict between these hypotheses regardless of the herbivore feeding guild. It is worth noting that I tested our hypothesis with respect to recently evolved trait variation among a genus of herbaceous plants and it will be important to test whether these dynamics hold in a community context with coexisting plant species and multiple leaf-feeding and sap-feeding taxa. However, this may be challenging as more distantly related plant species may vary not just in the magnitude of trait expression but also in the type of traits. In conclusion, resolving some of the variation in how drought stress impacts herbivores can be gained by linking plant drought adaptation strategies to functional traits that span abiotic and biotic interactions. Progress in this field is important given projections for the increasing frequency and severity of drought with climate change.

Figures

Figure 2.1. Plant Trait Hypothesis

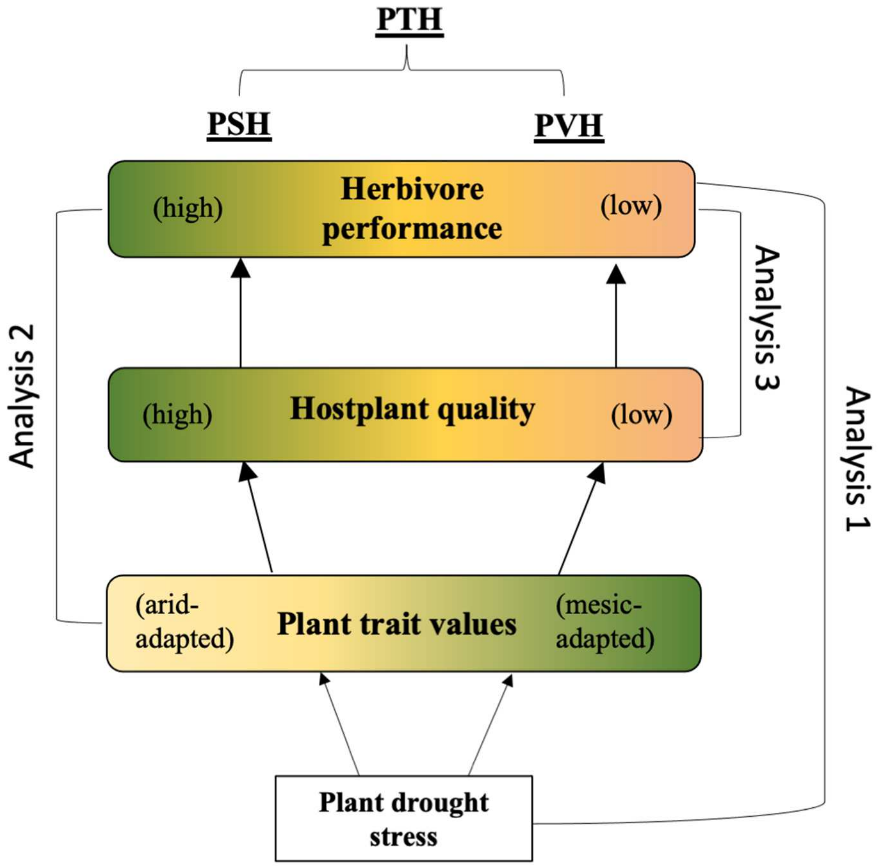


Fig. 2.1. Schematic showing mechanism and hypotheses linking drought adaptation traits and herbivores response to drought in arid and mesic adapted species. Plant trait values are represented by a gradient from arid (light yellow) to mesic (green). Plant quality and herbivore performance shown from low (light orange) to high (green). Analysis 1 tests for drought effects on herbivore survival. Analysis 2 tests for associations between plant traits underlying adaptation to arid vs. mesic environments and drought effects on herbivores. Analysis 3 tests for associations between drought effects on hostplant quality and drought effects on herbivores.

Figure. 2.2. Correlation between drought effects on monarch larvae and aphid survival

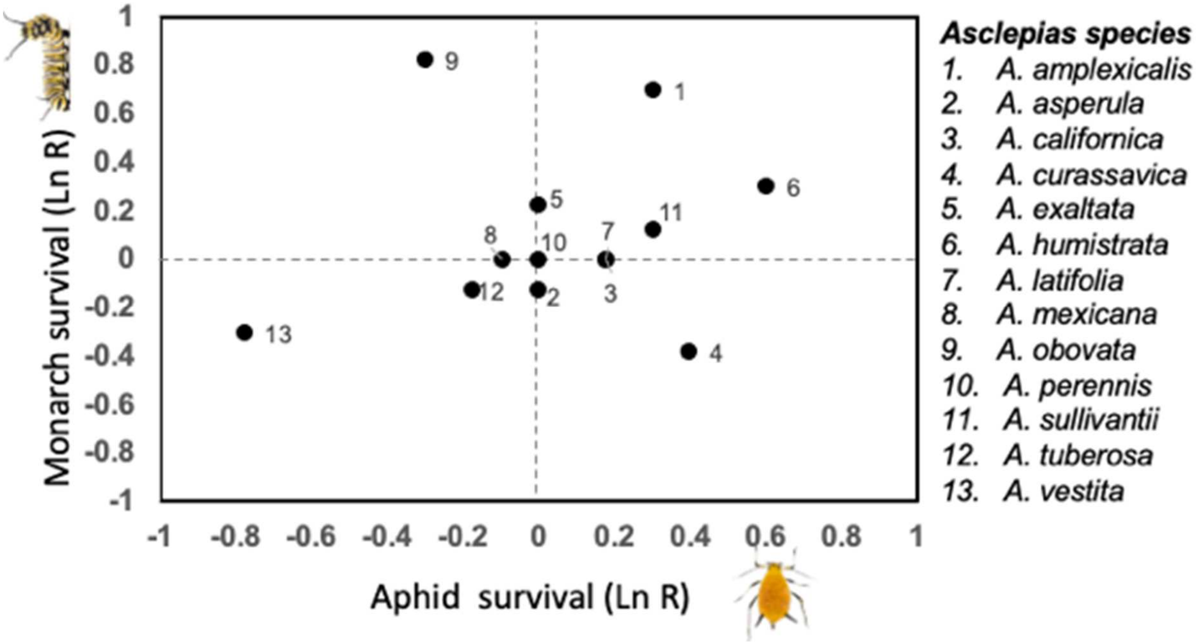


Fig 2.2. Each point represents drought effects on herbivore survival per *Asclepias* species. Positive values indicate an increased survival under drought while negative values indicate a decreased in survival under drought. Horizontal grey dotted line indicates no drought effect on monarch survival and vertical line no effect on oleander aphid survival.

Figure 2.3. Drought effects on herbivores as mediated by water use strategy traits

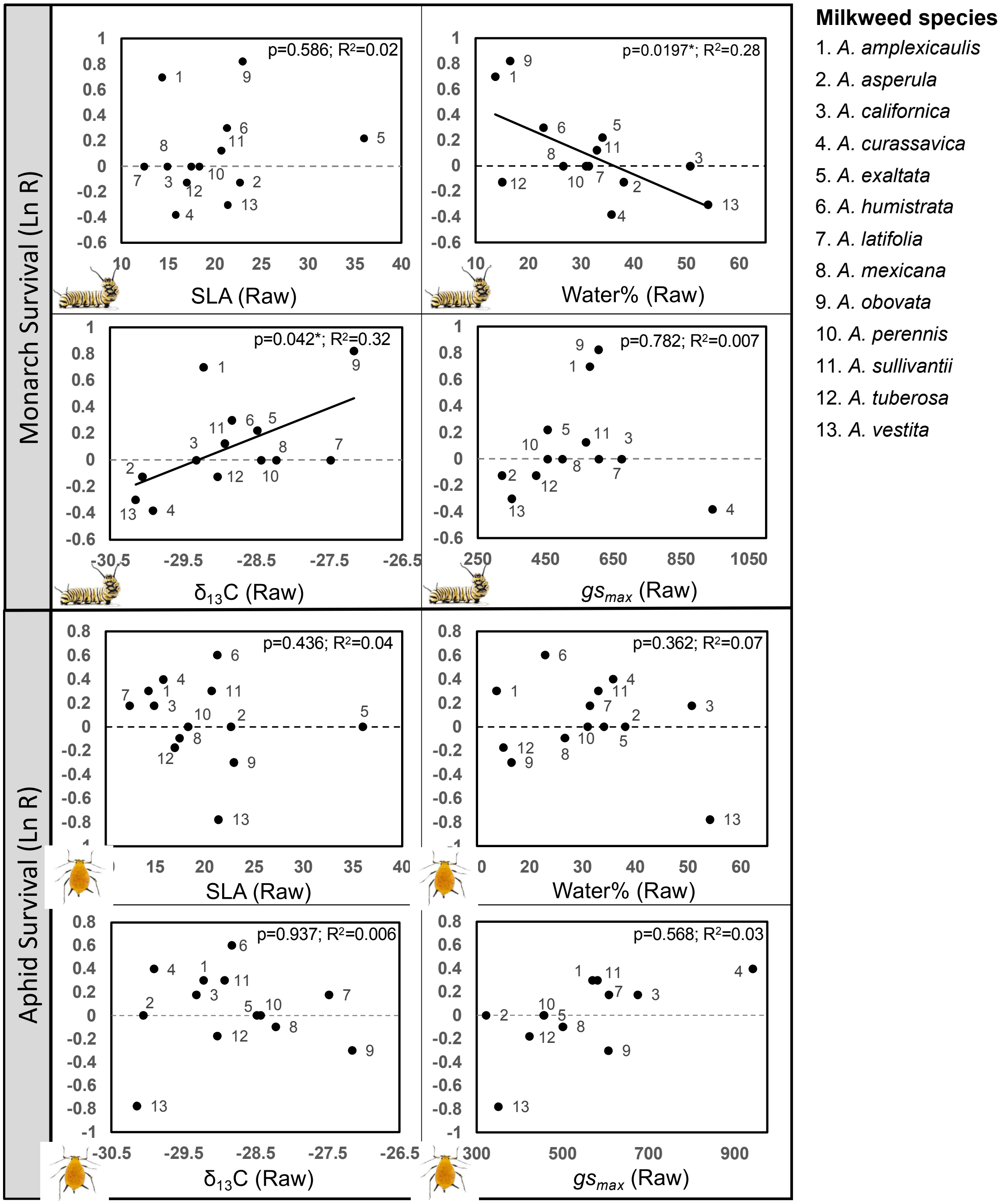


Fig. 2.3. Raw correlations between plant trait values associated with aridity gradients and drought effects on monarch larvae (top panel) and aphid (lower panel) survival. Each point represents the mean constitute trait values of each milkweed species. Grey dotted line indicates no drought effect on monarch survival. PIC correlations are reported in Appendix 2: Fig. S7

Figure. 2.4. Drought effects on herbivores as mediated by changes in host plant quality traits

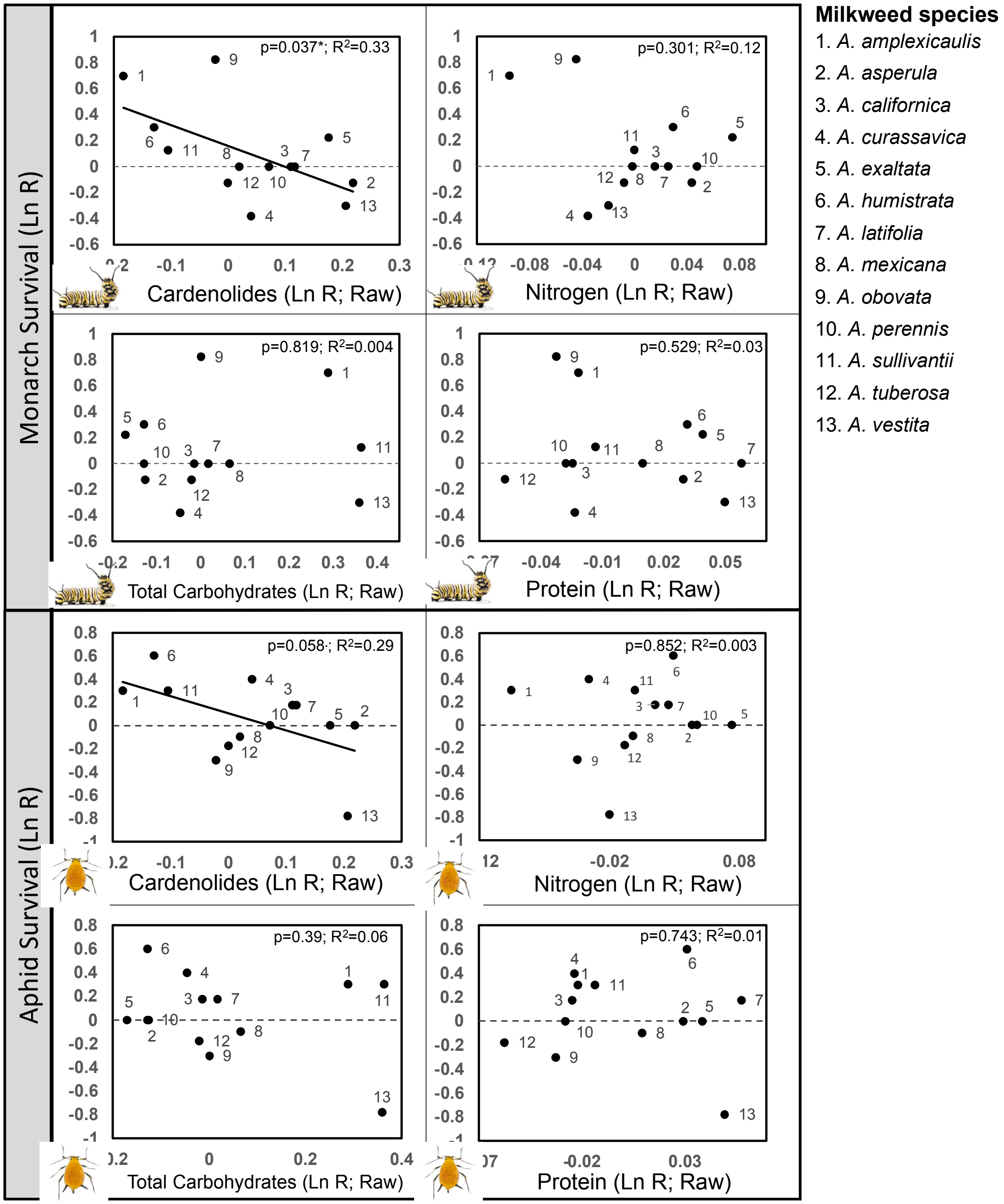


Fig. 2.4. Raw correlations between drought effects on plant quality traits and drought effects on monarch (top panel) and aphid (lower panel) survival. Each point represents the effect size of drought on plant quality trait values of each milkweed species. Grey dotted line indicates no drought effect on oleander aphid survival. PIC correlations are reported in Appendix 2: Fig. S9.

CHAPTER 3

Are bottom-up and top- down drought effects on plant herbivory mediated by plant secondary chemicals associated with direct and indirect plant defenses?

Abstract: *Drought events are predicted to increase due to climate change, yet consequences for plant–insect interactions are only partially understood. Drought stress can alter plant's ability to produce defensive chemicals (direct defenses) as well the emission of plant volatiles associated with the attraction of herbivore's natural enemies (indirect defenses) with potential consequences for plant-herbivore interactions. Moreover, plant habitat adaptations may also influence how drought stress alters plant, herbivore, and predator dynamics. Disentangling the relative importance of these factors is important for predicting how herbivore-plant interactions will be affected by increasingly common drought events. To investigate drought effects on plant herbivory, I conducted a common garden experiment by manipulating water availability and herbivore/predator access to Heartleaf bittercress (Cardamine cordifolia) plants from distinct habitat types. The experiment consisted of experimental blocks with two levels of water (drought and ambient) and a trophic treatment with three levels (control [no herbivores], herbivores, and herbivores plus predators) achieved with plants enclosures. After two weeks in the respective treatment combinations, we measured herbivory, and measured leaf herbivory and leaf chemicals associated with direct defenses (glucosinolates) and plant volatiles using HPLC-MS and GC-MS, respectively. In the absence of predators, herbivory increased with increasing soil water content; but in the presence of predators, herbivory was decreased with increasing soil water content. With respect to habitat type, herbivory did not vary as a function of soil water content among plants from different habitat types. We detected 14 glucosinolates and 21* *volatile compounds in Cardamine cordifolia plants but neither glucosinolates nor plant volatile emissions were affected by the water treatments based on a distance-based Redundancy Analysis (db-RDA). Glucosinolates profiles primarily varied by habitat source while volatiles were primarily affected by herbivory. Taken together, these results suggest that drought effect are mediated by both, bottom-up effects from plants to predators, as well as top-down effects from predators but that neither plant habitat adaptation nor plant secondary chemistry play a major role in mediating these effects.*

3.1. Introduction

Global climate change may affect species directly and indirectly by resetting complex coevolutionary relationship with cascading effects at population, community, and ecosystem levels (Ode et al. 2014, Tylianakis et al. 2008, Van der Putten et al. 2010). The composite effect of climate change on species reflects some combination of the direct effect of abiotic stress on organisms as well as the indirect effects mediated by species interactions. Thus, to understand the ecological impacts of global climate change requires a multi-trophic framework.

Herbivory is an interaction between plants and their consumers which has important ecological and evolutionary consequences (Coley & Barone 1996, Marquis 1992). Despite this importance, our understanding of how climate variability and changes affects these interactions is limited. In nature, herbivore-plant interactions are part of complex food webs composed by multiple species occupying different positions within the food web known as trophic levels. Particularly, herbivore natural enemies (i.e., predators, parasitoids, and pathogens) can have a strong effect on herbivores and plants by altering the ways herbivore impact plants (Facey et al. 2014). Thus, to understand the impacts of climate change on these critical interactions we must investigate them from a pairwise and tri-trophic perspective to disentangle the relative importance of bottom-up effects mediated by plants and top-down effects mediated by predators. Nonetheless, to date, research into the impacts of global climate change on more than two trophic levels is scarce focusing on the direct effects of climate change in a pairwise fashion either herbivore-host plants (reviewed in Facey et al., 2014; Jamieson et al., 2012) or herbivore-natural enemies (Holopainen et al., 2013; Stireman et al. 2005; Facey et al. 2014).

One mechanism by which climate change may affect herbivore-plant interactions is by altering plant defenses. Plants have evolved multiple ways to defend against herbivore attack which can be classified as direct and indirect defenses. Direct defenses are those that directly affect the attacker and can be physical (i.e., spines, thorns, waxes) or chemical (i.e., toxic compounds). Plants may also defend against herbivore attack through so-called “indirect defenses” by recruiting herbivore natural enemies such as predators, parasitoids or pathogens. These may include the provision of shelter (i.e., domatia), food rewards (e.g. extrafloral nectar) in exchange of protection from herbivores or through the emission of volatile organic compounds (VOCs) that herbivore enemies use as cues to locate their prey or host (see reviews by Dicke 2009, and Gols 2014). Direct defenses have long been known to be affected by environmental stress such as elevated temperatures, elevated CO2, and drought by up or downregulating the production of toxic chemicals (Coviella & Trumble 1999, English-Loeb et al. 1997). Indirect defenses such as herbivore-induced plant volatiles (HIPVs) can be particularly sensitive to abiotic stresses. This is because any climatic factors that alter plant growth, maturation, can affect plant VOC emission and composition (Yuan et al. 2009).

One of the dominant axes of climate change is increased drought (Sheffield & Wood 2008), in particular, alpine ecosystems are at high risk of drought because earlier snowmelt is predicted to decrease summer water availability in snowmelt-dominated regions such as the U.S.

Intermountain Region (National Research Council 2010). However, the drought effects on herbivores are notoriously variable and difficult to predict (Gely et al. 2020). Most of the research on this topic has focused on the drought effects on plant-herbivore interactions from a bottom-up perspective (i.e. via drought-induced changes in hostplant quality) (reviewed in Gely et al. 2020, Huberty & Denno 2004). Predators and parasitoids rely heavily on chemical cues from plants for foraging and are capable of differentiating VOC blends based on plant species, plant growth stages (Ballhorn et al. 2008), and the attacking insect species (Schettino et al. 2017). Therefore, minor changes in VOC blends could result in miscues by parasitoid/predator affecting their ability to locate their host or prey. Nonetheless, the role of herbivore natural enemies in mediating drought effects on plant-herbivore interactions is rarely studied. Another factor that has received little attention in the literature, is the role of plant habitat adaptations in mediating drought on plant-herbivore interactions. It is widely recognized that plants often evolved trade-offs in resource allocation that are unique to their specific environments (Agrawal 2020, Blumenthal et al. 2020, Coley et al. 1985). There is also evidence that these habitat adaptation strategies can also influence how plants respond to dual stresses of drought and herbivory (Montesinos-Navarro et al. 2020). Thus, plant adaptations to different habitats may be important for mediating drought effects on leaf herbivory.

In this research project, I investigated bottom-up and top-down effects of drought on leaf herbivory and whether such effects differ among Bittercress (Brassicaceae: *Cardamine cordifolia*) from distinct habitat types. *C. cordifolia* grows across a microgeographic mosaic of contrasting herbivory and light regimes ranging from high herbivory in meadows (sun habitats) and low herbivory in deeply shaded forest understories (shade habitats) and, as result, this species has differentiated into distinct morphological and defensive genotypes (Humphrey et al.

2018). To investigate drought effects on plant herbivory, I conducted a common garden experiment by manipulating water availability and herbivore/predator access to *C. cordifolia* plants from distinct habitat types. I further investigated the mechanisms driving these effects by exploring how the secondary plant chemistry driving direct and indirect defenses is affected by water availability, and whether these responses also varied among plants sourced from across distinct habitat types. Specifically, I sought to answer the following questions: 1) Does drought stress affect leaf herbivory and if so, are these mediated by bottom-up and/or top-down effects? 2) Does plant drought stress affect plant secondary chemistry associated with direct and indirect plant defenses? 3) Do the drought effects on leaf herbivory and chemical defenses vary among plants from distinct habitat types?

Water availability plays a prominent role in mediating plant-insect interactions (Grinnan et al. 2013, Mattson & Haack 1987) and it may be particular importance for this plant species distributed in wet microhabitats. Thus, I expect that water availability would strongly influence plant herbivory as well as plant chemistry. Based on past evidence showing that environmental stress decreased concentrations of defensive compounds in *C. cordifolia* leaves (Louda and Collinge 1992), I hypothesize a decrease in direct defenses in response to drought stress and an increase in plant herbivory. Although limited research has been conducted on the effects of environmental stress on indirect defenses in this system, I hypothesize that *Cardamine cordifolia*'s VOCs profiles will be affected by drought stress and consequently altering indirect resistance from predators.

3.2. Methodology

Study system

This experiment was conducted at the Rocky Mountain Biological Laboratory (RMBL) located near Crested Butte, Colorado, from June to August 2019. The habitat in this area is typically montane and the vegetation types has been described in detail by Langenheim (1962).

Bittercress (Brassicaceae: *Cardamine cordifolia*) is a clonal perennial forb native to montane regions of western North America (Harrington 1954). Growth begins immediately after snowmelt (Louda & Collinge 1992) in stands found on moist ground, primarily in the shade of willows and in riparian areas (Louda & Rodman 1983). This plant occurs in three distinct light habitat types. In full shade habitat, *C. cordififolia* occurs sparsely and reproduces sexually growing underneath tree stands, thus it receives limited sun light throughout the growing season. In partial shade habitat, *C. cordifolia* is more common and grows in large clonal patches underneath willow stands. In this habitat type, plants receive full light early in the season, but as the season progresses, sunlight availability diminishes as the willow vegetation becomes denser. In full light habitats, plants also reproduce clonally in open areas exposed to sunlight throughout the growing season.

Glucosinolates are a diverse group of compounds that are repellent or toxic to non-adapted insects (Halkier & Gershenzon 2006, Louda & Rodman 1983) and they represent the main secondary compounds present in plants in the Brassicaceae family. Glucosinolates profiles have been shown to vary among populations from different habitat types (Humphrey et al., 2018). Plants from sun populations tend to be more defended because they are subject to higher herbivore pressures (Humphrey et al. 2018, Louda & Rodman 1983). These compounds have also been shown to be induced by herbivore and pathogen damage (Halkier and Gershezon 2006). The VOC profile of *Cardamine cordifolia* has not yet been characterized. In this study region, over 25 insect herbivores utilize the leaves of bittercress. The dominant insects at this site are the leaf miner *Scaptomyza nigrita*, *Phaedon sp. oviformis* (Chrysomelidae), and *Aphalara sp. nov*. (Psyllidae) (Collinge & Louda 1989). The chrysomelid beetle (*Phaedon sp. oviformis*) can be the most common and damaging herbivore at some sites. Indeed, Louda (1984) found that herbivore damage by *P. spp. oviformis* larvae had strong negative fitness effects on *C. cordifolia*. In this site, *P. spp. oviformis* was the dominant herbivore and it was primarily distributed in shady habitats under dense willow vegetation.

Common garden design

To investigate how drought effects on plant herbivory are mediated by bottom-up (plant drought stress) and top-down (predators) effects and if these differ by habitat types, I conducted a common garden experiment by manipulating water availability as well as herbivore (*Phaedon* spp. *oviformis*) and/or predator access to *C. cordifolia* plants originating from distinct habitat types, hereafter termed habitat source. The common garden location was in an abandoned beaver pond (38°57′31″N 106°59′16″W) with dense, mixed vegetation cover, including willows (*Salix* sp.) and Bittercress (*C. cordifolia*), and *P.* spp. *oviformis*.

I collected *Cardamine cordifolia* clonal matts and individual plants from four different sites in early June 2019, soon after snowmelt and before most insects become active. Within each site, I collected genets from three different light habitat types (shade, sun, partial shade). The roots of clonal mats were severed to form individual clones which were potted in 10 cm diameter pots filled with soil collected from each site. Plants from shade habitats reproduced sexually, thus genets from these sites were collected as individual plants and were also potted in soil from their respective sites. This resulted in 23 non-clonal plants from shaded habitat, each a unique genet, and 83 plants from 14 clonal mats for a total of 106 plants from 36 genets. Plants were grown in a weather port for three weeks and were watered to saturation 2-3 times per week.

The experiment consisted of blocks of plants exposed to a manipulation of water (drought and ambient; see below for details) and trophic levels (control, herbivores alone, and herbivores plus predators; see below for details) for a total of 6 treatment combinations within each block. Potted plants were transported to the common garden site and distributed among 16 blocks, each with 6-8 plants per block. 11 blocks were stocked with plants sourced from a single genet from a clonal mat from either sun or partial shade habitat distributed among the 6 treatment combinations. 5 blocks had a mix of genets sourced from clonal mats from sun and partial shade habitat and non-clonal replicates from full shade, with plants randomly distributed among 6 treatment combinations within each block.

The water treatment was designed to impose two levels of watering, hereafter referred to as "ambient" and "drought" with the intent of achieving 100% and 60% stomatal conductance, respectively. A stomatal response curve constructed as a function of soil volumetric water content (VWC) using plants representative of each site and habitat types. Based on this curve, maximum stomatal conductance was achieved at ~35% VWC and a 60% stomatal conductance was observed at a VWC of ~22%. During the experiment, plants were then watered with the intent of achieving the target levels of stomatal conductance based upon measuring soil moisture 2-3 times per week with a soil moisture meter. However, soil moisture varied significantly both within treatment levels and over time, ranging from 13-24 VWC % in plants assigned to the drought group, and 25-56 VWC % in plants assigned to the ambient group. Accordingly, I also estimated the average soil water content per plant over the duration of the experiment as a continuous variable.

The trophic treatment consisted of three levels achieved via insect enclosures/ex-closures: ‘control’ (no herbivores), ‘herbivores alone’ (herbivores without predators), and herbivore with predators (hereafter the "predator" level). Plants in the control level had no herbivores and were covered with protective mesh to prevent any insect access to the plant throughout the experiment. The herbivore alone level consisted of adding five beetle larvae (*Phaedon* spp. *oviformis*) to each plant within the same protective mesh to exclude predators. Plants in the herbivore plus predator level also received five beetle larvae but I cut four large horizontal slits in the protective mesh to allow predator access.

Data collection and chemical analysis

After a period of 2 week in their respective treatment combinations, I measured leaf herbivory, volatile emissions, and glucosinolates content. This duration was used because it imposes plants to drought stress while avoiding confounding effects of drought acclimation such as morphological adaptations in new leaves and roots (Touchette et al. 2007).

Herbivory and leaf volatiles area were measured from the leaves of 2-3 stems collected from a subset of plants (n=60) within 11 of the 16 experimental blocks. I chose blocks that included plant replicates representatives of all habitat sources. To measure herbivory, individual leaves were scanned and for total leaf area and leaf area consumed using the app Leaf Byte application (Getman-Pickering et al. 2019) in order to calculate total herbivory (leaf area consumed) for the plant stems assayed. Leaf volatiles were collected from 60 plants from August 5-8, 2019. Samples were collected between 11 AM and 2 PM during which time temperatures ranged from 23-45°C. Procedures for scent sampling were modified from Cambell et al. (2019). Scent traps, consisting of a glass capillary tube filled with 5 mg of Tenax TA and held with plugs of silianised quartz wool, were cleaned before initial use by heating in an oven for 30 min at 250 °C. Dynamic headspace samples of leaf volatiles were taken by enclosing 2-3 stems per plant in 20 x 10 cm nylon-6 oven bags (Reynolds, USA) unsealed on both sides. This design allows to cover the entire vegetative part of the stems while excluding the flowerhead. The bags were tied on both sides with twisters and volatiles were allowed to equilibrate for 30 min., after which, samples were pumped for 30 min through a scent trap using a pump (Supelco PAS-500, Spectrex, Redwood City, California, USA) set to a pre-trap flow rate of 200 mL/min. Ambient controls (n=9) were taken from the area adjacent to each of the experimental blocks from where leaf volatiles were collected using an empty oven bag sampled for the same duration as the experimental samples. These ambient samples were used to identify contaminants or background compounds from surrounding vegetation. Samples were stored in capped glass vials until analysis.

Volatiles and ambient samples were analyzed using thermal desorption - gas Chromatography- mass Spectrometry (GC-MS), together with blank/unused scent traps (n=12). Settings for thermal desorption and GC temperature ramp followed those in Mullins et al. (2020). Peak deconvolution, integration, and tentative compound identification were performed in the Automated Mass Spectral Deconvolution and Identification System (AMDIS) using the NIST 2017 mass spectral library. Peaks were included if they had mass spectral match scores greater than 75%. Compounds present at higher concentrations in the blank and ambient samples than in plant samples were removed from our final dataset. Compounds with high retention times (i.e., compounds with molecular weights of > 300 Daltons) were also excluded as contaminants. Stems used for volatile collection were saved for measuring leaf area and amount of herbivory as described in the previous section. Volatile emissions were quantified based on peak values and standardized by total leaf area.

Glucosinolates content was analyzed at the University of Chicago using High-Performance Liquid Chromatography (HPLC-MS). Glucosinolates were extracted from leaf tissue collected from all experimental plants (n=106) following protocols described in (Humphrey et al. 2018). Per each plant, we collected ~10 discs (total weight ~90 mg) from fully expanded leaves of similar size and located near the mid-position of the stem. Each disc was immediately placed in a 2ml Eppendorf tube containing an 80% methanol solution to prevent glucosinolates hydrolyzation. Samples were preserved at -20 °C for later characterization of glucosinolates. Peaks values were corrected by sinigrin content.

Statistical analysis

Effects on leaf herbivory: To investigate the effects of soil water availability on leaf herbivory, I used linear mixed models to test for the main and interactive effects of soil water content (continuous, see common garden design description), trophic treatments (control, herbivore, predator) and habitat source (sun, partial shade, shade), and the interactive effect of soil water content with trophic treatment and habitat source on leaf herbivory (n=60), with genet as a random factor implemented in R package lme4 (Bates et al. 2014). The main purpose of these analysis was to test whether the effect of water availability varied with trophic level (soil water content by trophic treatment interaction) and by habitat type (soil water content by habitat source). However, because there is ample evidence showing that herbivores differentially feed from plants from distinct habitat sources, I additionally tested for the interactive effect of trophic treatment and habitat source.

Effects on plants secondary chemistry: I employed two distinct approaches to analyze effects on GSL and VOCs: a multivariate analysis with all compounds, and a univariate analysis with chemical functional groups. Assessing functional groups provides a more direct test of the ecological significance of changes in plant secondary chemistry. The goal of these analyses was to assess how drought stress affected the chemical traits mediating plant direct defense (GLSs) and VOCs, which can also be involved in indirect defense (VOCs), and whether such effects depended on the light environments from which plants were sourced. Accordingly, in all analyses plant chemistry was modeled as dependent upon soil water availability, habitat source, and their interactive effects. However, because both classes of chemical traits (GSLs and VOCs) are expected to be impacted (i.e., induced) by herbivore damage, we controlled for herbivory in both analyses accordingly to isolate the effects of drought and plant source habitat.

a) Multivariate analysis: To analyze differences in GSL and VOC profiles, I conducted a constrained distance-based Redundancy Analysis (db-RDA) with bray distances after Hellinger transformation as implemented in the function capscale from the R package vegan (R Core Team, v.4.0; Oksanen et al. 2019). In contrast to unconstrained methods, this approach is appropriate for assessing multi-variate responses among predefined explanatory variables (i.e., soil water content, and habitat source). To control for the effect of herbivory, this term was included first in the model to examined whether soil water content and habitat type have additional effects beyond that of herbivory (Oksanen et al. 2019). To do so, I classified plants in two categories: "herbivore", which included plants in the herbivore and predators levels, and "no herbivores" which included plants in the control group. I refer to this treatment as the herbivory treatment. I used this approach as opposed to including herbivory as a continuous variable because these measurements were only available for a subset of samples (n=60), and this would significantly reduce the sample size. Using an insufficient sample size in multivariate analyses, may yield unstable coefficient estimates with inflated standard errors (Bühlmann & Van der Geer 2011) leading to reduced statistical power and erroneous conclusions regarding relationships between independent and dependent variables. I tested for the main effect of herbivory (herbivores, no herbivores), soil water content (as continuous), and habitat source (sun, shade, partial shade) on GSLs and VOCs, and determined whether there was a significant interaction after accounting for the main effects. Analyses were performed on compound relative abundances (% of total emissions), although analyses of emission rates were qualitatively identical (not shown). For visualization and to improve interpretation of the results, the analysis was repeated with the terms that were found in the final models to drive variation in chemical composition (Figure 3.5).

b) Univariate analysis: For the univariate analysis by functional groups, I used linear mixed models in the lme4 R package (Bates et al. 2015), with genet as a random effect, and tested for the main and interactive effects of soil water content and habitat source on volatiles and glucosinolates major functional groups. To control for herbivory, my approach was to first test for the effect of herbivory on each functional group and if it was found significant, I controlled for this term by adding it as a covariate. I also tested for these effects on total volatile emissions and total glucosinolates content. When model residuals were not normally distributed, the dependent variable was log-transformed.

3.3. Results

Effects on leaf herbivory:

Herbivory ranged from 0 to 17 % across treatment combinations. While there were no main effects of trophic treatment, soil water content, or habitat source on leaf herbivory, there were significant interactive effects of trophic treatment-by-soil water content and trophic treatment-by-habitat source. Results are summarized in Table 3.1. With respect to trophic treatment, in the absence of predators, herbivory increased with increasing soil water content; but in the presence of predators, herbivory was decreased with increasing soil water content (Figure

3.1). With respect to habitat type, herbivory did not vary as a function of soil water content among plants from different habitat types (Figure 3.2.). Finally, trophic treatment differentially affected plants from distinct habitat types (Figure 3.3).

Table 3.1: Summary results reporting significance values of explanatory variables on leaf herbivory.

Explanatory variables	**Herbivory**
Trophic	0.27
Soil water content	0.35
Habitat source	0.27
Soil water content source:trophic	0.006**
Soil water content:habitat source	0.44
Trophic:habitat source	0.05*

Signif. codes: 0 '****', < 0.001 '***', < 0.01 '**', < 0.05 '*', < 0.6 '.', < 0.1 '', < 1

Effects on plant secondary chemistry

Glucosinolates

I detected 14 glucosinolates including 8 aliphatic, 3 indoles, and 3 aromatic compounds (Appendix 3: Table S1). Multivariate analysis of glucosinolates composition (n=106) shows that these were not affected by either soil water content (db-RDA, p=0.104) or herbivory treatment (db-RDA, p=0.302) but varied significantly by habitat source. I detected no significant interactive effect of soil water content and habitat source (Table 3.2). The model explained 13% of the total variation, with CAP 1 (40%) and CAP 2 (33%) together capturing 73% of this explained variation. Two compounds, 1-methylpropyl and indol-3-ylmethyl, contributed more strongly to the explained variance captured by CAP1 and were negatively correlated with each other. 2-methylpropyl and an unidentified aliphatic compound contributed more to the variance explained by CAP 2 and were also negative correlated. Ordination plot of simplified model including "habitat source" as explanatory variable is shown in Figure 3.4.

Table 3.2: Db-RDA summary results reporting significance values of explanatory variables for GSL and VOCs profiles.

Explanatory variables	**GSL**	**VOCs**
Soil water content	0.104	0.007**
Herbivory	0.302	0.927
Habitat source	0.001**	0.816
Soil water content:habitat source	0.928	0.519

Signif. codes: 0 '****', < 0.001 '***', < 0.01 '**', < 0.05'*', < 0.6 '.', <0.1 '',< 1

Dividing compounds by functional group also revealed no impacts of herbivory (Table 3.3). Aromatic compounds were unaffected by soil water content and habitat source (Table 3.3). Indoles was the only group that appeared to be affected by soil water content, but this response did not vary with habitat source. There was a negative trend of indoles with increasing water suggesting they may be downregulated with water stress. Aliphatic compounds were not affected by soil water content or habitat source, although the interactive effect of soil water content and habitat source was significant; plants from partial shade had higher aliphatic concentrations under water limited conditions, while plants from sunny habitats increased these concentrations under well-water conditions, and plants from shade habitats had equal concentrations regardless of soil water content. Total glucosinolates content was not affected by soil water content, although the interactive effect of soil water content and habitat source was significant suggesting that water availability appeared to differentially affect total glucosinolates content among plants from different habitat types.

Table 3.3: Glucosinolates functional groups summary reporting p-values of linear mixed models

Chemical functional group	**Herbivory (cm2)$^{+}$**	**Soil water content**	**Habitat type**	**Soil water content*habitat type**
Aromatics(log)	0.44	0.71	0.97	0.82
Indoles	0.11	0.02*	0.61	0.25
Aliphatics	0.37	0.46	0.17	0.03*
Totals	0.58	0.11	0.21	0.02*

Signif. codes: 0 '****', 0.001 '***', 0.01 '**', 0.05 '*', 0.6 '.', 0.1 '', 1

$^{+}$Term was tested separately and included as a co-variate in the final model if found significant.

Volatile's emissions:

We detected 21 volatile compounds including 3 alcohols, 5 ketones, 3 isothiocyanates, 7 alkanes, 1 monoterpene, and 1ether (Appendix 3: Table S2).

Volatile's compositions (n=60) primarily varied by the herbivory treatments (db-RDA, p=0.007**) but not by soil water content (db-RDA, p=0.927) nor habitat source (p=0.816) (Table 3.2). I detected no interactive effect of soil water content and trophic treatments on volatile composition (db-RDA, p=519). The model explained 11% of the total variance with CAP 1 (47%) and CAP 2 (33%) accounting for 80% of this explained variation. Nonane.5.butyl and Isopropyl isothiocyanate contributed more to the variation explained by CAP 1 and were negatively correlated with each other. Nonane.5.butyl and 2.Ethyl.hexanol contributed more to the variance explained by CAP 2 and were also negatively correlated. These results are summarized in Table 3.4. Ordination plot for simplified model including "herbivory" treatment as explanatory variable is shown in Figure 3.5.

Volatile functional groups were affected by the various treatment combinations in different ways (Table 3.4). Herbivory had a strong effect on isothiocyanates and total volatiles emissions. Total volatiles and isothiocyanates were positively associated with herbivory suggesting that these compounds were induced by herbivory. Isothiocyanates were not affected by soil water content or habitat source after accounting for herbivory as a covariate. Effect of herbivory on alcohol emissions was marginally significant so it was not included as a covariate in the model. Alcohols was the only group that appeared to be affected by soil water content but, that effect did not vary by habitat source. Alkanes were unaffected by herbivory and soil water content. Alkanes' emissions differed significantly by habitat source; plants from sunny habitats had higher concentrations, partial shade intermediate levels, and shade the lowest. Ketones' emissions appeared to be unaffected by either treatment or treatment combination and did not differ by habitat source. Total volatile emissions were not affected by either by soil water content or habitat source.

Table 3.4: Volatiles functional groups result summary reporting p-values of linear mixed models

Chemical functional group	**Herbivory (cm^2)$^+$**	**Soil water content**	**Habitat type**	**Soil water content*habitat type**
Alcohols(log)	0.09.	0.04*	0.61	0.10
Ketones(log)	0.10	0.62	0.31	0.12
Isothiocyanates(log)	0.007**	0.57	0.34	0.83
Alkanes(log)	0.54	0.12	0.02*	0.82
Totals(log)	0.008**	0.10	0.31	0.38

Signif. codes: 0 ‘****’, <0.001 ‘***’,<0.01 ‘**’, <0.05 ‘*’, <0.6 ‘.’, <0.1 ‘’, <1

$^+$Term was tested separately and included as a co-variate in the final model if found significant.

3.4. Discussion

This study is among the first to investigate drought effects on plant-herbivore interactions from a pairwise and tri-trophic perspective, and in doing so, disentangle the relative importance of bottom-up effects of plants from the top-down effects of predators. We further investigated potential mechanisms underlying these dynamics by exploring the role of plant chemistry and plant habitat adaptations in mediating drought effects on plant-herbivore interactions. These results revealed that drought effects on herbivory are mediated by bottom-up forces as herbivory decreased significantly in plants under low water availability conditions, as well as top-down forces as plant drought stress appear to suppress predators’ top-down control on herbivores (Figure 3.1). Conversely, although plant direct defenses varied significantly among habitat types, habitat source did not appear to play a major role in mediating drought effects on herbivore-plant interactions. Plant secondary chemistry (direct and indirect defensive compounds) did not appear to be significantly altered by water stress in plants. Thus, the observed patterns of herbivory under limited water availability conditions cannot be explained by changes in plant chemical composition. However, specific functional groups, associated with both direct and indirect defenses, responded to stresses imposed by herbivory and water limitation, thus, it is possible that these compounds are responsible for mediating bottom-up and top-down effects of drought on leaf herbivory. Overall, our finding suggests that drought effects on plant-herbivore interactions are mediated by bottom-up effects and top-down effects and that these effects may be associated with drought-induced changes in direct chemical defenses to drought stress that are dependent upon their unique habitat adaptations.

Contrary to my prediction that water stress would increase herbivory performance and therefore herbivory, plant drought stress suppressed herbivory. This prediction was based on work by Louda and Rodman (1996) concluding that water stress in *C. cordifolia*, led to decreased glucosinolates concentrations and thus increased herbivory in water stress plants. However, the authors did not directly manipulate water availability. These conclusions were based on correlational patterns among herbivory, glucosinolates concentrations, and plant water deficits in plants exposed to sun vs. shaded plants. It was also observed that herbivores abundance was higher in the sun; therefore, it is likely that the differentially higher levels of insect herbivory in the sun were due solely to higher herbivore pressures in sunny habitats. More broadly, these results support the Plant Vigor Hypothesis (Price 1991) which predicts that more vigorous plants will experience more attack by herbivores than drought-stressed, lower quality plants.

With respect to predators, results were in line with my predictions that plant drought stress will strongly influence predators' top-down control on plant herbivory. Under well-watered conditions predator's top-down control was stronger than in water limited conditions, although the specific mechanisms by which this occurred are unclear. One potential explanation is that herbivore mortality was higher in drought-stressed plants than non-stressed plants.

Therefore, predators had a stronger effect on plants under well-water conditions where herbivores were more abundant. Another explanation is that predator's ability to locate herbivores was impaired due to drought-induced changes in VOCs. There is ample evidence that herbivore's natural enemies are particularly sensitive to changes in chemical cues resulting from abiotic stress (see reviews by Dicke 2009, and Gols 2014). However, as discussed later, our multivariate analysis did not support these predictions since overall volatile composition was not affected by plant water stress. Nonetheless, volatiles blends are quite complex and minor changes in particular compounds or even the ratio of emissions may result in miscues in plant signaling (Ballhorn et al. 2008, Schettino et al. 2017). Thus, it is likely that changes in specific volatile compounds may be responsible for mediating top-down effects of drought on leaf herbivory.

Plant habitat adaptation played no significant role in mediating drought stress on leaf herbivory. Among habitat types, heartleaf bittercress displays clear differentiation in their morphology, defensive chemicals, and their mode of reproduction. In general, plants from sunny habitats are more defended because they are subject to higher herbivory pressures. Thus, based on resource allocation theory, I anticipated trade-offs among habitat types in allocation to conflicting stresses (biotic and abiotic) (Agrawal 2020, Coley et al. 1985) which may in turn lead to differences in herbivory pressures. I found no evidence that these habitat adaptation influence tri-trophic dynamics in this system. I speculate this is because these plant adaptations are not directly associated with water stress but light availability and herbivore pressures. Furthermore, the effect of herbivores and predators on plant herbivory, differed among habitat types (Figure 3.3), with shaded plants experiencing higher herbivory than plants from sun and partial shade. This is consisting with past studies in this system showing that herbivores differentially feed from shade habitat plants than more defended sun habitat plants (Humphrey et al. 2018, Louda & Rodman 1983). Predators, on the other hand, seemed to reduced herbivory equally across habitat types. Thus, top-down control of predators is more important for plants from the shade.

Heartleaf bittercress occurs in moist grounds, commonly along riparian areas. Thus, I expected that drought stress would strongly affect plant chemistry. Multivariate analysis of both GSL and VOCs suggest drought did not significantly altered their composition. With respect to glucosinolates, these compounds were primarily separated by habitat source (Figure 3.4), indicating that plants adapted to different light environments and herbivore pressures had distinct glucosinolates profiles. This not surprising as habitat differentiation in glucosinolates composition in Heartleaf bittercress has been previously documented in past studies (Louda & Rodman 1983). Therefore, these results suggest that direct chemical defenses do not mediate drought stress in this plant species. However, a closer examination of functional groups, revealed that some glucosinolates groups are affected by water stress, and also interacted with habitat source. Thus, these complex responses may in turn influence how herbivores respond to drought stress. Herbivory often induced plant direct chemical defenses, therefore, it was surprising that neither glucosinolates overall composition nor specific groups were unaffected by herbivory. It is possible that glucosinolates are produced constitutively by plants in this system and that these do not change with short-term environmental or abiotic stress. Conversely, VOCs composition did not vary with habitat source, but its composition was primarily affected by herbivory (Figure 3.5), suggesting that these VOCs are induced upon herbivore damage. Thus, although these compounds may be associated with plant indirect defenses, they did not appear to play a role in mediating drought stress on plant herbivory. Nonetheless, as mentioned earlier, due to the complexity of these chemical signals minor changes in VOCs blends may lead to communication break down between plants and herbivore natural enemies. Of course, because we did not directly assess predator attraction to plants or specific volatile compounds, we can speculate about the underlying mechanism leading to changes in predator pressures. These questions can be addressed in future studies using insect choice bioassays or field observation studies.

Figures

Figure 3.1. Soil water content effects on leaf herbivory by trophic treatment.

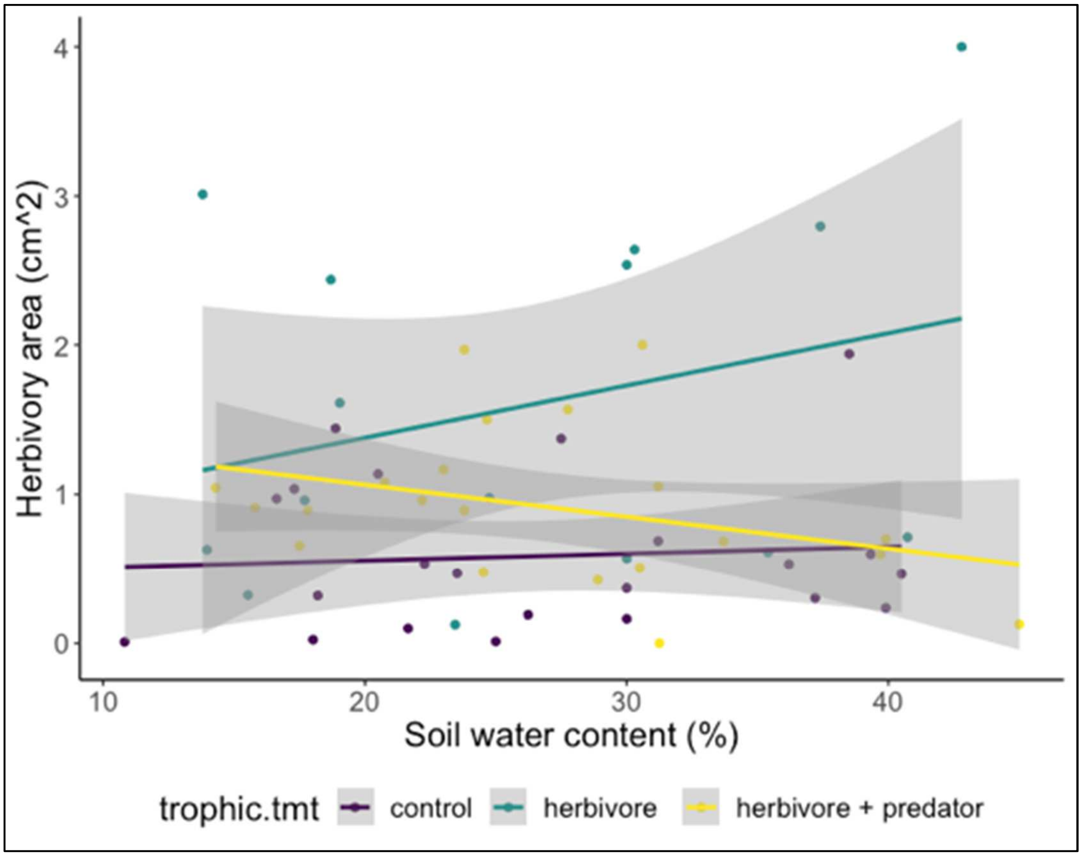


Fig. 3.1. Plant in the herbivore group had herbivores excluded from predators, and plants in the predator group had herbivores exposed to predators. Standard deviation shown in gray.

Figure 3.2. Effects of soil water content on leaf herbivory by habitat source

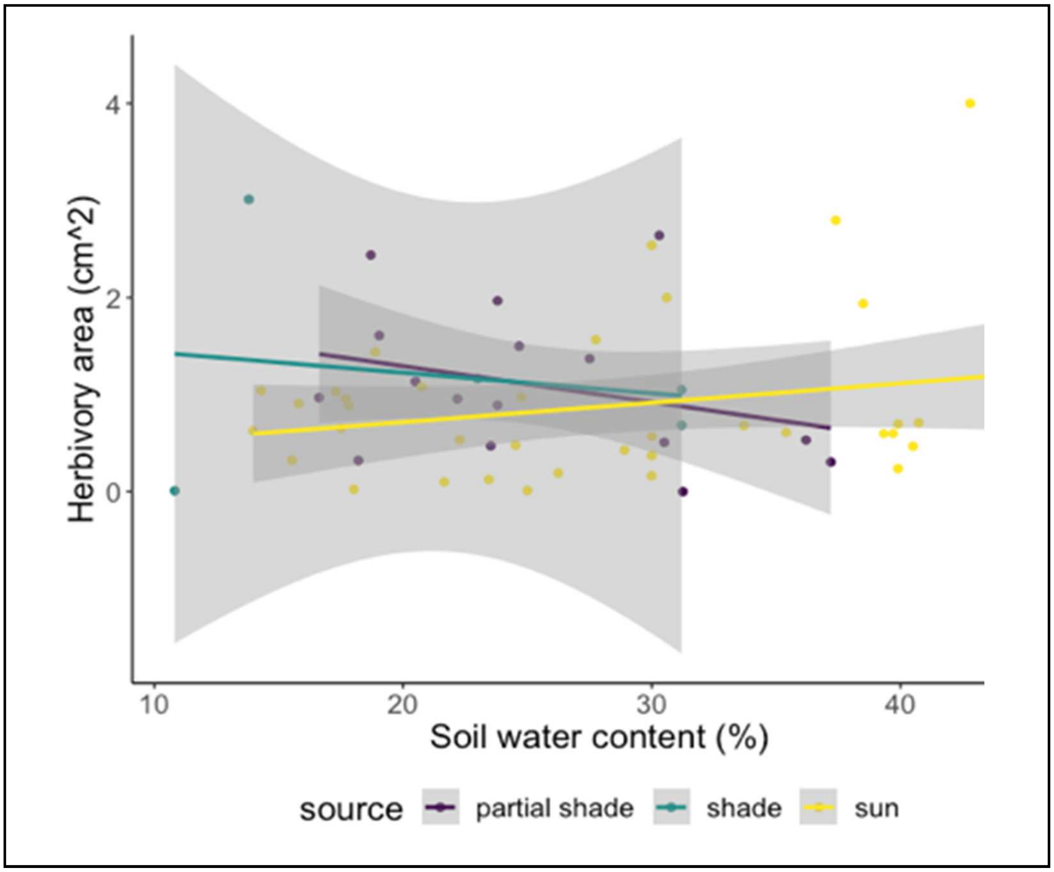


Fig. 3.2. Linear regressions showing leaf herbivory as a function of soil water content by habitat source. Standard deviation shown in gray.

Figure 3.3. Leaf herbivory by trophic treatment and habitat source

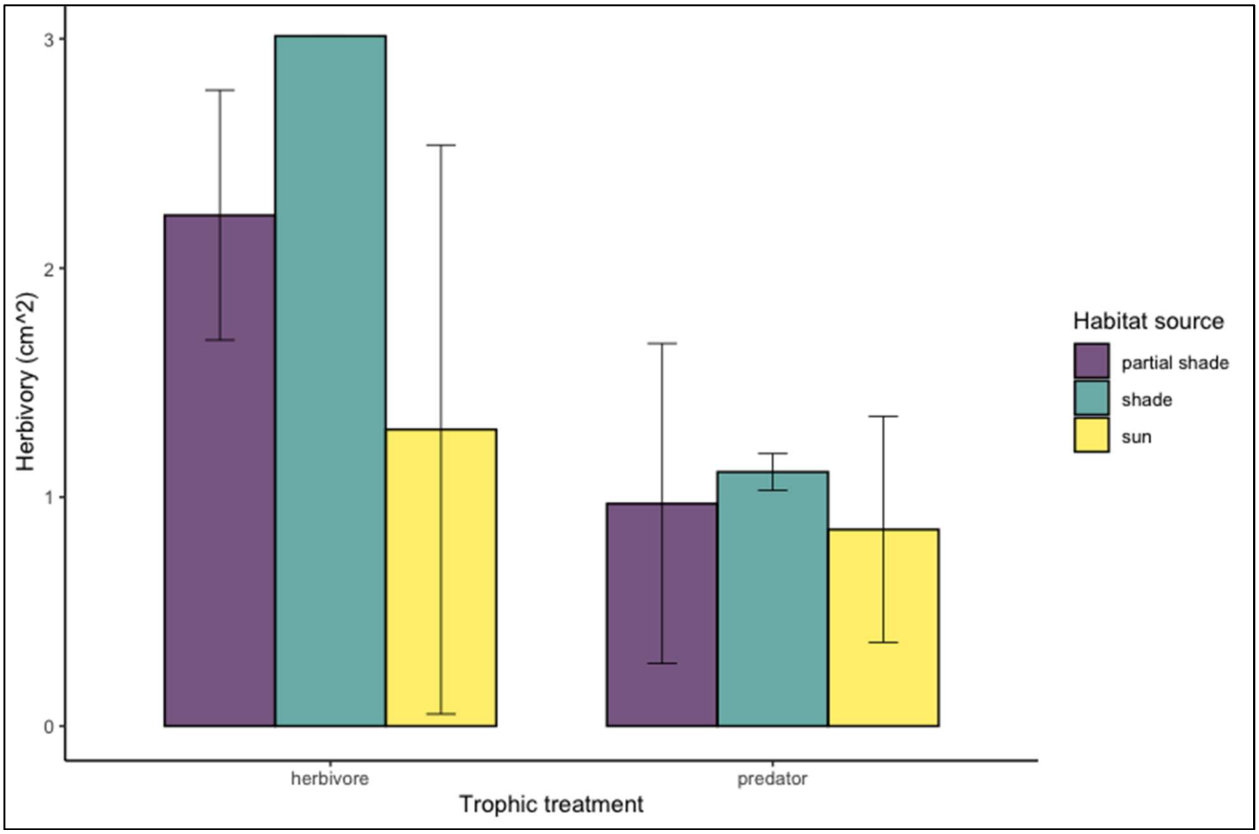


Fig. 3.3. Average leaf herbivory (cm^2) by "herbivore" and "predator" trophic treatments. Plants in the herbivore treatment received five beetle larvae, and plants in the predator treatment also receive herbivore larvae but were exposed to predators. Error bars represent standard deviations.

Figure 3.4. Glucosinolates ordination plot

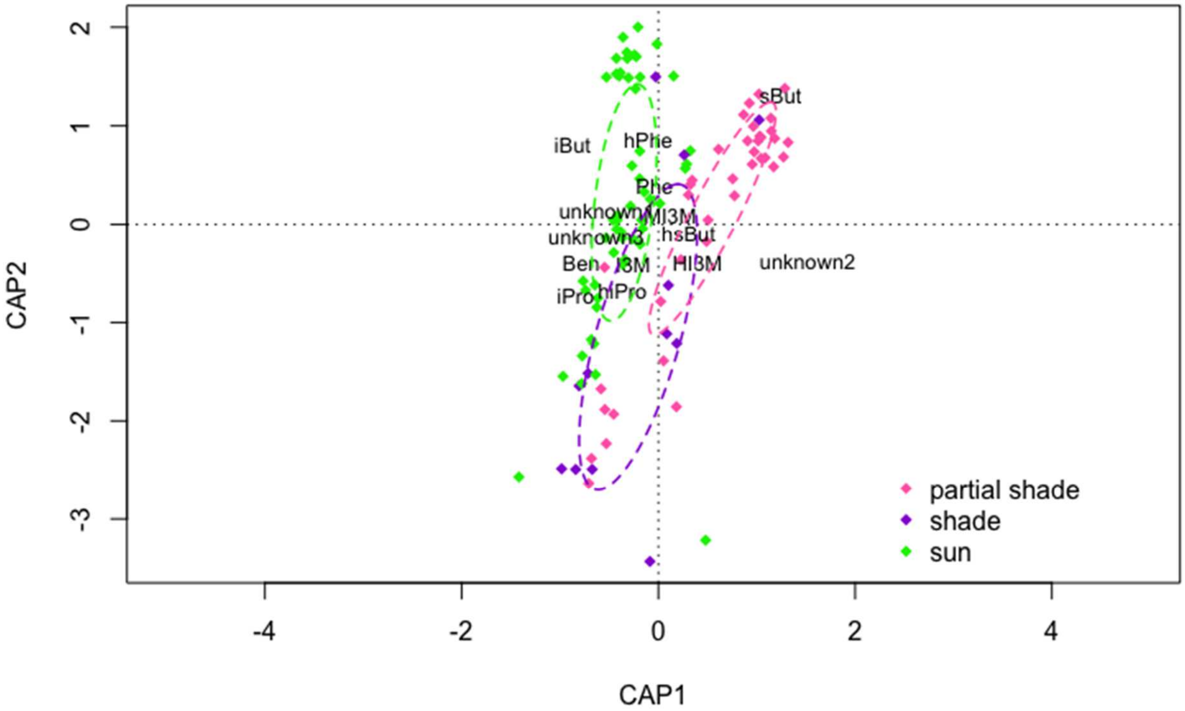


Fig. 3.4. Db-RDA ordination plot showing variation in glucosinolates composition (relative content) among habitat types 'sun', 'partial shade', and 'shade'. Glucosinolates compounds are shown in black. Plants in the "no.herbviore" group represented plants in the "control" while plants in the "herbivore" group represented plants in the "herbivore" and "predator" from the trophic treatment manipulation. Codes represent hiPro=1-hydroxymethyl ethyl, iPro=1-methylethyl, hsBut=1-hydroxymethyl propyl, iBut=2-methylpropyl, sBut=1-methylpropyl, HI3M=4-hydroxyindol-3-ylmethyl, I3M=indol-3-ylmethyl, MI3M=?-methoxyindol-3-ylmethyl, Ben=benzyl, Phe=2-phenethyl, hPhe=2-hydroxy-2-phenethyl, and unkown 1-3= unidentified aliphalic compounds.

Figure 3.5. Volatile organic compounds (VOCs) ordination plot

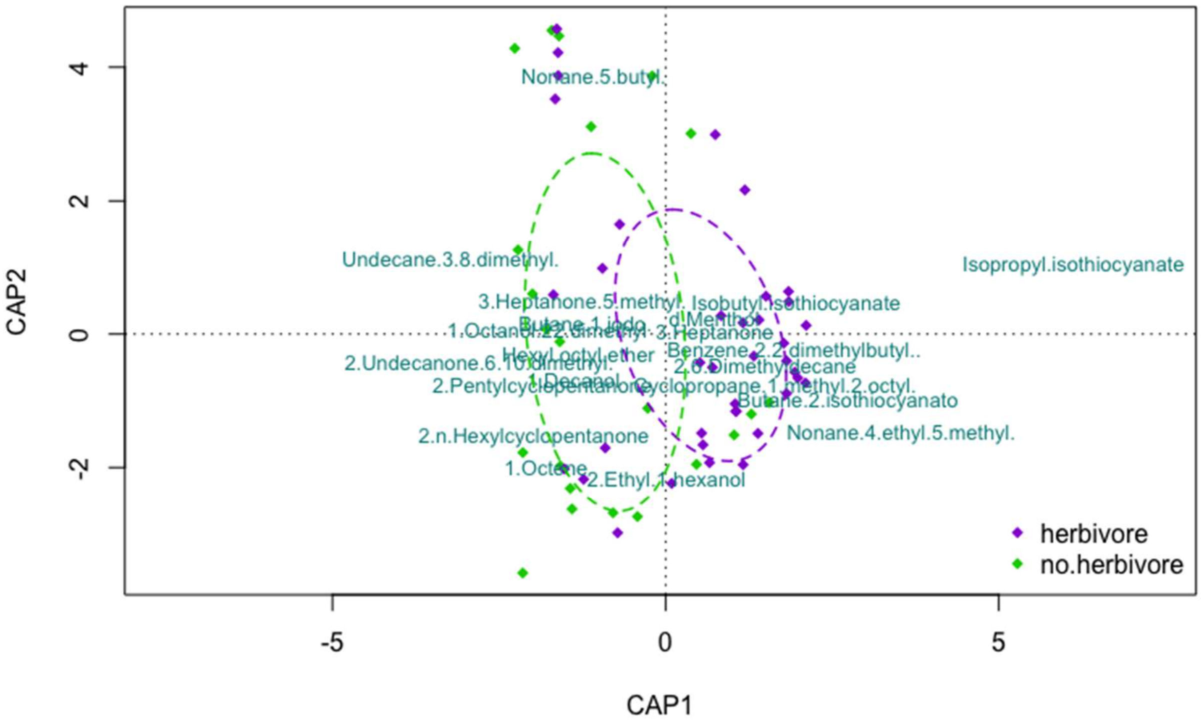


Fig. 3.5. Db-RDA ordination plot and CAP 1 and CAP 2 showing how volatile composition (relative emissions) differ between plants with herbivores and plants without herbivores. Plants in the "no.herbviore" group represented plants in the "control" while plants in the "herbivore" group represented plants in the "herbivore" and "predator" from the trophic treatment manipulation. Chemical compounds scores displayed in 'light blue'.

REFERENCES

Agrawal, A. A. (2020). A scale-dependent framework for trade-offs, syndromes, and specialization in organismal biology. *Ecology*, *101*(2). https://doi.org/10.1002/ecy.2924

Agrawal, A. A., Ali, J., Rasmann, S., & Fishbein, M. (2015). Macroevolutionary trends in the defense of milkweeds against monarchs: Latex, cardenolides, and tolerance of herbivory. In *Monarchs in a changing world: Biology and conservation of an iconic butterfly* (pp. 47–59). Cornell University Press. http://www.jstor.org/stable/10.7591/j.ctt20fw696.9

Agrawal, A. A., & Fishbein, M. (2006). Plant defense syndromes. *Ecology*, *87*(sp7), S132–S149. https://doi.org/10.1890/0012-9658(2006)87[132:PDS]2.0.CO;2

Agrawal, A. A., Fishbein, M., Halitschke, R., Hastings, A. P., Rabosky, D. L., & Rasmann, S. (2009). Evidence for adaptive radiation from a phylogenetic study of plant defenses. *Proceedings of the National Academy of Sciences*, *106*(43), 18067–18072. https://doi.org/10.1073/pnas.0904862106

Agrawal, A. A., Fishbein, M., Jetter, R., Salminen, J.-P., Goldstein, J. B., Freitag, A. E., & Sparks, J. P. (2009). Phylogenetic ecology of leaf surface traits in the milkweeds (*Asclepias* spp.): Chemistry, ecophysiology, and insect behavior. *New Phytologist*, *183*(3), 848–867. https://doi.org/10.1111/j.1469-8137.2009.02897.x

Agrawal, A. A., & Konno, K. (2009). Latex: A model for understanding mechanisms, ecology, and evolution of plant defense against herbivory. *Annual Review of Ecology, Evolution, and Systematics*, *40*(1), 311–331. https://doi.org/10.1146/annurev.ecolsys.110308.120307

Agrawal, A. A., Patrick, E. T., & Hastings, A. P. (2014). Tests of the coupled expression of latex and cardenolide plant defense in common milkweed (*Asclepias syriaca*). *Ecosphere*, *5*(10), art126. https://doi.org/10.1890/ES14-00161.1

Araújo, M. B., & Luoto, M. (2007). The importance of biotic interactions for modelling species distributions under climate change. *Global Ecology and Biogeography*, *16*(6), 743–753. https://doi.org/10.1111/j.1466-8238.2007.00359.x

Bale, J. S., Masters, G. J., Hodkinson, I. D., Awmack, C., Bezemer, T. M., Brown, V. K., Butterfield, J., Buse, A., Coulson, J. C., Farrar, J., Good, J. E. G., Harrington, R., Hartley, S., Jones, T. H., Lindroth, R. L., Press, M. C., Symrnioudis, I., Watt, A. D., & Whittaker, J. B. (2002). Herbivory in global climate change research: Direct effects of rising temperature on insect herbivores: rising temperatures and insect herbivores. *Global Change Biology*, *8*(1), 1–16. https://doi.org/10.1046/j.1365-2486.2002.00451.x

Ballhorn, D. J., Kautz, S., Lion, U., & Heil, M. (2008). Trade-offs between direct and indirect defences of lima bean *(Phaseolus lunatus*): Trade-off between cyanogenesis and VOC-emission. *Journal of Ecology*, *96*(5), 971–980. https://doi.org/10.1111/j.1365-2745.2008.01404.x

Basset, Y., & Lamarre, G. P. A. (2019). Toward a world that values insects. *Science*, *364*(6447), 1230–1231. https://doi.org/10.1126/science.aaw7071

Bauerfeind, S. S., & Fischer, K. (2013). Testing the plant stress hypothesis: Stressed plants offer better food to an insect herbivore. *Entomologia Experimentalis et Applicata*, *149*(2), 148–158. https://doi.org/10.1111/eea.12118

Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., & Courchamp, F. (2012). Impacts of climate change on the future of biodiversity: Biodiversity and climate change. *Ecology Letters*, *15*(4), 365–377. https://doi.org/10.1111/j.1461-0248.2011.01736.x

Bernays, E. A. (1989). Host range in phytophagous insects: The potential role of generalist predators. *Evolutionary Ecology*, *3*(4), 299–311. https://doi.org/10.1007/BF02285261

Bidart-Bouzat, M. G., & Imeh-Nathaniel, A. (2008). Global Change Effects on Plant Chemical Defenses against Insect Herbivores. *Journal of Integrative Plant Biology*, *50*(11), 1339–1354. https://doi.org/10.1111/j.1744-7909.2008.00751.x

Birnbaum, S. S. L., & Abbot, P. (2018). Insect adaptations toward plant toxins in milkweed-herbivores systems—A review. *Entomologia Experimentalis et Applicata*, *166*(5), 357–366. https://doi.org/10.1111/eea.12659

Blumenthal, D. M., Mueller, K. E., Kray, J. A., Ocheltree, T. W., Augustine, D. J., & Wilcox, K. R. (2020). Traits link drought resistance with herbivore defence and plant economics in semi-arid grasslands: The central roles of phenology and leaf dry matter content. *Journal of Ecology*, *108*(6), 2336–2351. https://doi.org/10.1111/1365-2745.13454

Boersma, M., Mathew, K. A., Niehoff, B., Schoo, K. L., Franco-Santos, R. M., & Meunier, C. L. (2016). Temperature driven changes in the diet preference of omnivorous copepods: No more meat when it's hot? *Ecology Letters, 19*, 45–53.

Boulangeat, I., Gravel, D., & Thuiller, W. (2012). Accounting for dispersal and biotic interactions to disentangle the drivers of species distributions and their abundances: The role of dispersal and biotic interactions in explaining species distributions and abundances. *Ecology Letters*, *15*(6), 584–593. https://doi.org/10.1111/j.1461-0248.2012.01772.x

Boullis, A., Francis, F., & Verheggen, F. J. (2015). Climate change and tritrophic interactions: Will modifications to greenhouse gas emissions increase the vulnerability of herbivorous insects to natural enemies? *Environmental Entomology, 44*(2), 277–286.

Bradford, M. M. (n.d.). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. 7.

Brewer, A. M., & Gaston, K. J. (2003). The geographical range structure of the holly leaf-miner. II. Demographic rates. *Journal of Animal Ecology*, *72*(1), 82–93. https://doi.org/10.1046/j.1365-2656.2003.00682.x

Brower, L. P. (1988). Avian Predation on the Monarch Butterfly and Its Implications for Mimicry Theory. *The American Naturalist*, *131*, S4–S6. https://doi.org/10.1086/284763

Bühlmann, P., & Van der Geer, S. (2011). *Statistics for high-dimensional data: Methods, theory and applications*. Springer Science & Business Media.

Burkepile, D. E., & Parker, J. D. (2017). Recent advances in plant-herbivore interactions. *F1000Research*, *6*, 119. https://doi.org/10.12688/f1000research.10313.1

Burnham, K. P., & Anderson, D. R. (2004). Multimodel inference: Understanding AIC and BIC in model selection. *Sociological methods & research, 33*, 261–304.

Cambell, D. R., Sosenski, P., & Raguso, R. A. (2019). *Phenotypic plasticity of floral volatiles in response to increasing drought stress*. *123*, 601–610. https://doi.org/doi: 10.1093/aob/mcy193

Carnicer, J., Stefanescu, C., Vives-Ingla, M., López, C., Cortizas, S., Wheat, C., Vila, R., Llusià, J., & Peñuelas, J. (2019). Phenotypic biomarkers of climatic impacts on declining insect populations: A key role for decadal drought, thermal buffering and amplification effects and host plant dynamics. *Journal of Animal Ecology*, *88*(3), 376–391. https://doi.org/10.1111/1365-2656.12933

Carvajal-Acosta, A. N., Mooney, K. A., & Agrawal, A. A. (*In Review)*. Plant traits as mediators of herbivore drought response: Phylogeny, physiology, and functional traits. *Functional Ecology*

Chamberlain, S. (2019). *Rbison:Interface to the "USGS" "BISON" API* (0.8.0) [Computer software]. https://CRAN.R-project.org/package=rbison

Chamberlain, S., Ram, K., & Hart, T. (2014). *spocc: R interface to many species occurrence data sources* (0.1.0) [Computer software]. https://github.com/ropensci/spocc

Chen, Q., Yin, Y., Zhao, R., Yang, Y., Teixeira da Silva, J. A., & Yu, X. (2020). Incorporating Local Adaptation Into Species Distribution Modeling of Paeonia mairei, an Endemic Plant to China. *Frontiers in Plant Science*, *10*, 1717. https://doi.org/10.3389/fpls.2019.01717

Chow, P. S., & Landhausser, S. M. (2004). A method for routine measurements of total sugar and starch content in woody plant tissues. *Tree Physiology*, *24*(10), 1129–1136. https://doi.org/10.1093/treephys/24.10.1129

Coley, P., Bryant, J., & Chapin III, F. S. (1985). Resource availability and plant antiherbivore defense. *Science, 230*(4728), 895–899. https://doi.org/10.1126/science.230.4728.895

Coley, P. D., & Barone, J. A. (1996). Herbivory and plant defenses in tropical forests. *Annual review of ecology and systematics, 27*, 305–335.

Coley, P. D., Bateman, M. L., & Kursar, T. A. (2006). The effects of plant quality on caterpillar growth and defense against natural enemies. *Oikos*, *115*(2), 219–228. https://doi.org/10.1111/j.2006.0030-1299.14928.x

Collinge, S. K., & Louda, S. M. (1989). Influence of plant phenology on the insect herbivore/bittercress interaction. *Oecologia*, *79*(1), 111–116. https://doi.org/10.1007/BF00378247

Collins, M., & et al. (2013). *Long-term climate change: Projections, commitments and irreversibility. Climate Change 2013-The Physical Science Basis: Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* 1029–1136.

Coviella, C. E., & Trumble, J. T. (1999). Review: Effects of Elevated Atmospheric Carbon Dioxide on Insect-Plant Interactions. *Conservation Biology*, *13*(4), 700–712.

Cowan, I. R. (1978). Stomatal behaviour and environment. In *Advances in botanical research* (Vol. 4, pp. 117–228). Academic Press.

Crone, E. E., Pelton, E. M., Brown, L. M., Thomas, C. C., & Schultz, C. B. (2019). Why are monarch butterflies declining in the West? Understanding the importance of multiple correlated drivers. *Ecological Applications*, *29*(7). https://doi.org/10.1002/eap.1975

Crozier, L. (2004). Warmer winters drive butterfly range expansion by increasing survivorship. *Ecology*, *85*(1), 231–241. https://doi.org/10.1890/02-0607

da Cunha, H. F., Ferreira, É. D., Tessarolo, G., & Nabout, J. C. (2018). Host plant distributions and climate interact to affect the predicted geographic distribution of a Neotropical termite. *Biotropica*, *50*(4), 625–632. https://doi.org/10.1111/btp.12555

de Araújo, C. B., Marcondes-Machado, L. O., & Costa, G. C. (2014). The importance of biotic interactions in species distribution models: A test of the Eltonian noise hypothesis using parrots. *Journal of Biogeography*, *41*(3), 513–523. https://doi.org/10.1111/jbi.12234

DeLucia, E. H., Nabity, P. D., Zavala, J. A., & Berenbaum, M. R. (2012). Climate change: Resetting plant-insect interactions. *Plant Physiology*, *160*(4), 1677–1685. https://doi.org/10.1104/pp.112.204750

Denno, R. F., & McClurc, M. S. (1983). Individual, population, and geographic patterns in plant defense. *Variable plants and herbivores in natural and managed systems*, 463-512. Academic Press.

Dicke, M. (2009). Behavioural and community ecology of plants that cry for help. *Plant, Cell & Environment*, *32*(6), 654–665. https://doi.org/10.1111/j.1365-3040.2008.01913.x

Dilts, T. E., Steele, M. O., Engler, J. D., Pelton, E. M., Jepsen, S. J., McKnight, S. J., Taylor, A. R., Fallon, C. E., Black, S. H., Cruz, E. E., Craver, D. R., & Forister, M. L. (2019). Host Plants and Climate Structure Habitat Associations of the Western Monarch Butterfly. *Frontiers in Ecology and Evolution*, *7*, 188. https://doi.org/10.3389/fevo.2019.00188

Dingle, H., Zalucki, M. P., Rochester, W. A., & Armijo-Prewitt, T. (2005). Distribution of the monarch butterfly, Danaus plexippus (L.) (Lepidoptera: Nymphalidae), in western North America: Monarch Butterflies in Western North America. *Biological Journal of the Linnean Society*, *85*(4), 491–500. https://doi.org/10.1111/j.1095-8312.2005.00512.x

Dirnböck, T., & Dullinger, S. (2004). Habitat distribution models, spatial autocorrelation, functional traits and dispersal capacity of alpine plant species. *Journal of Vegetation Science*, *15*(1), 77–84. https://doi.org/10.1111/j.1654-1103.2004.tb02239.x

Dobler, S., Petschenka, G., & Pankoke, H. (2011). Coping with toxic plant compounds – The insect’s perspective on iridoid glycosides and cardenolides. *Phytochemistry*, *72*(13), 1593–1604. https://doi.org/10.1016/j.phytochem.2011.04.015

Dormann, C. F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., Marquéz, J. R. G., Gruber, B., Lafourcade, B., Leitão, P. J., Münkemüller, T., McClean, C., Osborne, P. E., Reineking, B., Schröder, B., Skidmore, A. K., Zurell, D., & Lautenbach, S. (2013). Collinearity: A review of methods to deal with it and a simulation study evaluating their performance. *Ecography*, *36*(1), 27–46. https://doi.org/10.1111/j.1600-0587.2012.07348.x

DuBois, Michel., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, Fred. (1956). Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry*, *28*(3), 350–356. https://doi.org/10.1021/ac60111a017

Eck, G., Fiala, B., Linsenmair, K. E., Hashim, R. B., & Proksch, P. (2001). Trade-Off Between Chemical and Biotic Antiherbivore Defense in the South East Asian Plant Genus Macaranga. *Journal of Chemical Ecology*, 18.

Egan, S. P., & Ott, J. R. (2007). Host plant quality and local adaptation determine the distribution of gall-forming herbivore. *Ecology*, *88*(11), 2868–2879. https://doi.org/10.1890/06-1303.1

Elith, J., H. Graham, C., P. Anderson, R., Dudík, M., Ferrier, S., Guisan, A., J. Hijmans, R., Huettmann, F., R. Leathwick, J., Lehmann, A., Li, J., G. Lohmann, L., A. Loiselle, B., Manion, G., Moritz, C., Nakamura, M., Nakazawa, Y., McC. M. Overton, J., Townsend Peterson, A., … E. Zimmermann, N. (2006). Novel methods improve prediction of species' distributions from occurrence data. *Ecography*, *29*(2), 129–151. https://doi.org/10.1111/j.2006.0906-7590.04596.x

Elith, J., & Leathwick, J. R. (2009). Species Distribution Models: Ecological Explanation and Prediction Across Space and Time. *Annual Review of Ecology, Evolution, and Systematics*, *40*(1), 677–697. https://doi.org/10.1146/annurev.ecolsys.110308.120159

Elith, J., Phillips, S. J., Hastie, T., Dudík, M., Chee, Y. E., & Yates, C. J. (2011). A statistical explanation of MaxEnt for ecologists: Statistical explanation of MaxEnt. *Diversity and Distributions*, *17*(1), 43–57. https://doi.org/10.1111/j.1472-4642.2010.00725.x

English-Loeb, G., Stout, M. J., & Duffey, S. S. (1997). Drought Stress in Tomatoes: Changes in Plant Chemistry and Potential Nonlinear Consequences for Insect Herbivores. *Oikos*, *79*(3), 456. https://doi.org/10.2307/3546888

Facey, S. L., Ellsworth, D. S., Staley, J. T., Wright, D. J., & Johnson, S. N. (2014). Upsetting the order: How climate and atmospheric change affects herbivore–enemy interactions. *Current Opinion in Insect Science*, *5*, 66–74. https://doi.org/10.1016/j.cois.2014.09.015

Ferrier, S., & Guisan, A. (2006). Spatial modelling of biodiversity at the community level. *Journal of Applied Ecology*, *43*(3), 393–404. https://doi.org/10.1111/j.1365-2664.2006.01149.x

Fick, E. C., & Hijmans, R. J. (2017). *WorldClim-Global Climate Data*. WorldClim Version2| New 1-Km Spatial Resolution Climate Surfaces for Global Land Areas. http://www.worldclim.org

Forister, M. L., Novotny, V., Panorska, A. K., Baje, L., Basset, Y., Butterill, P. T., Cizek, L., Coley, P. D., Dem, F., Diniz, I. R., Drozd, P., Fox, M., Glassmire, A. E., Hazen, R., Hrcek, J., Jahner, J. P., Kaman, O., Kozubowski, T. J., Kursar, T. A., … Dyer, L. A. (2015). The global distribution of diet breadth in insect herbivores. *Proceedings of the National Academy of Sciences*, *112*(2), 442–447. https://doi.org/10.1073/pnas.1423042112

Fraterrigo, J. M., Wagner, S., & Warren, R. J. (2014). Local-scale biotic interactions embedded in macroscale climate drivers suggest Eltonian noise hypothesis distribution patterns for an invasive grass. *Ecology Letters*, *17*(11), 1447–1454. https://doi.org/10.1111/ele.12352

Freckleton, R. P. (2000). Phylogenetic BlackwellScience,Ltd tests of ecological and evolutionary hypotheses: Checking for phylogenetic independence. *Functional Ecology*, 6.

Garcia, L. V. (2004). *Escaping the Bonferroni iron claw in ecological studies*. *105*(3), 657–663.

GBIF.org. (2019). GBIF Home Page. https://doi.org/10.15468/dl.sjg2d2

Gely, C., Laurance, S. G. W., & Stork, N. E. (2020). How do herbivorous insects respond to drought stress in trees? *Biological Reviews*, 15.

Getman-Pickering, Z. L., Campbell, A., Aflitto, N., Grele, A., Davis, J. K., & Ugine, T. A. (2019). *LeafByte: A mobile application that measures leaf area and herbivory quickly and accurately*. Methods in Ecology and Evolution.

Giannini, T. C., Chapman, D. S., Saraiva, A. M., Alves-dos-Santos, I., & Biesmeijer, J. C. (2013). Improving species distribution models using biotic interactions: A case study of parasites, pollinators and plants. *Ecography*, *36*(6), 649–656. https://doi.org/10.1111/j.1600-0587.2012.07191.x

Gols, R. (2014). Direct and indirect chemical defences against insects in a multitrophic framework: Plant chemical defences against insects. *Plant, Cell & Environment*, *37*(8), 1741–1752. https://doi.org/10.1111/pce.12318

Goulson, D. (2019). *The insect apocalypse, and why it matters*. *29*(19), R967–R971.

Grafen, A. (1988). *On the uses of data on lifetime reproductive success*. *363*, 1635–1645.

Grandpre, L., Pureswaran, D., Bouchard, M., & Kneeshaw, D. (2018). Climate-induced range shifts in boreal forest pests: Ecological, economic, and social consequences. *Canadian Journal of Forest Research, 48*(3), v–vi.

Grinnan, R., Carter, T. E., & Johnson, M. T. J. (2013). The effects of drought and herbivory on plant-herbivore interactions across 16 soybean genotypes in a field experiment: Drought and plant-herbivore interactions. *Ecological Entomology*, *38*(3), 290–302. https://doi.org/10.1111/een.12017

Guisan, A., & Thuiller, W. (2005). Predicting species distribution: Offering more than simple habitat models. *Ecology Letters*, *8*(9), 993–1009. https://doi.org/10.1111/j.1461-0248.2005.00792.x

Gutbrodt, B., Mody, K., & Dorn, S. (2011). Drought changes plant chemistry and causes contrasting responses in lepidopteran herbivores. *Oikos*, *120*(11), 1732–1740. https://doi.org/10.1111/j.1600-0706.2011.19558.x

Hahn, P. G., & Maron, J. L. (2018). Plant water stress and previous herbivore damage affect insect performance: Abiotic and biotic effects on insect performance. *Ecological Entomology*, *43*(1), 47–54. https://doi.org/10.1111/een.12468

Halkier, B. A., & Gershenzon, J. (2006). Biology and biochemistry of glucosinolates. *Annual Review of Plant Biology*, *57*(1), 303–333. https://doi.org/10.1146/annurev.arplant.57.032905.105228

Harrington, H.D. (1954). Manual of the Plants of Colorado. In *Manual of the Plants of Colorado* (p. 666). Sage Books.

Henn, J. J., Buzzard, V., Enquist, B. J., Halbritter, A. H., Klanderud, K., Maitner, B. S., Michaletz, S. T., Pötsch, C., Seltzer, L., Telford, R. J., Yang, Y., Zhang, L., & Vandvik, V. (2018). Intraspecific Trait Variation and Phenotypic Plasticity Mediate Alpine Plant Species Response to Climate Change. *Frontiers in Plant Science*, *9*, 1548. https://doi.org/10.3389/fpls.2018.01548

Hijmans, R. J. (2012). Cross-validation of species distribution models: Removing spatial sorting bias and calibration with a null model. *Ecology*, *93*(3), 679–688. https://doi.org/10.1890/11-0826.1

Hijmans, R. J., & Elith, J. (2019). *Spatial Distribution Models*. 73.

Hijmans, R. J., Phillips, S., Leathwick, J. R., & Elith, J. (2011). *Package 'dismo.'* http://cran.r-project.org/web/packages/dismo/index.html.

Holopainen, J. K., Himanen, S. J., & Poppy, G. M. (2013). Climate change and its effects on the chemical ecology of insect parasitoids. In E. Wajnberg & S. Colazza (Eds.), *Chemical Ecology of Insect Parasitoids* (pp. 168–190). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118409589.ch8

Hortal, J., de Bello, F., Diniz-Filho, J. A. F., Lewinsohn, T. M., Lobo, J. M., & Ladle, R. J. (2015). Seven Shortfalls that Beset Large-Scale Knowledge of Biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, *46*(1), 523–549. https://doi.org/10.1146/annurev-ecolsys-112414-054400

Huberty, A. F., & Denno, R. F. (2004). Plant water stress and its consequences for herbivorous insects: A new synthesis. *Ecology*, 85(5), 1383–1398. https://doi.org/10.1890/03-0352

Humphrey, P. T., Gloss, A. D., Frazier, J., Nelson–Dittrich, A. C., Faries, S., & Whiteman, N. K. (2018). Heritable plant phenotypes track light and herbivory levels at fine spatial scales. *Oecología, 18*(2), 427–445. https://doi.org/doi: 10.1007/s00442-018-4116-4

Hunter, M. D., Ohgushi, T., & Price, P. W. (1992). Effects of resource distribution on animal-plant interactions. Academic Press.

Jactel, H., Petit, J., Desprez-Loustau, M.-L., Delzon, S., Piou, D., Battisti, A., & Koricheva, J. (2012). Drought effects on damage by forest insects and pathogens: A meta-analysis. *Global Change Biology*, *18*(1), 267–276. https://doi.org/10.1111/j.1365-2486.2011.02512.x

Jamieson, M. A., Burkle, L. A., Manson, J. S., Runyon, J. B., Trowbridge, A. M., & Zientek, J. (2017). Global change effects on plant–insect interactions: The role of phytochemistry. *Current Opinion in Insect Science*, *23*, 70–80. https://doi.org/10.1016/j.cois.2017.07.009

Jamieson, M. A., Trowbridge, A. M., Raffa, K. F., & Lindroth, R. L. (2012). Consequences of Climate Warming and Altered Precipitation Patterns for Plant-Insect and Multitrophic Interactions. *Plant Physiology*, 160(4), 1719–1727. https://doi.org/10.1104/pp.112.206524

Jankielsohn, A. (2018). The Importance of Insects in Agricultural Ecosystems. *Advances in Entomology*, 06(02), 62–73. https://doi.org/10.4236/ae.2018.62006

Jung, V., Albert, C. H., Violle, C., Kunstler, G., Loucougaray, G., & Spiegelberger, T. (2014). Intraspecific trait variability mediates the response of subalpine grassland communities to extreme drought events. *Journal of Ecology*, 102(1), 45–53. https://doi.org/10.1111/1365-2745.12177

Kartesz, J. T. (2015). *Floristic Synthesis of North America* (Version 1.0.) [Map]. Taxonomic Data Center. http://www.bonap.net/tdc

Karthik, R. (2014). Ecoengine: Programmatic interface to the API serving UC Berkeley's Natural History Data (1.9) [Computer software]. https://github.com/ropensci/ecoengine

Kass, J. M., Anderson, R. P., Espinosa-Lucas, A., Juárez-Jaimes, V., Martínez-Salas, E., Botello, F., Tavera, G., Flores-Martínez, J. J., & Sánchez-Cordero, V. (2020). Biotic predictors with phenological information improve range estimates for migrating monarch butterflies in Mexico. *Ecography*, *43*(3), 341–352. https://doi.org/10.1111/ecog.04886

Koenig, W. D., & Haydock, J. (1999). Oaks, acorns, and the geographical ecology of acorn woodpeckers. *Journal of Biogeography*, *26*(1), 159–165. https://doi.org/10.1046/j.1365-2699.1999.00256.x

Koricheva, J., & Haukioja, S. L. E. (1997). Insect Performance on Experimentally Stressed Woody Plants: A Meta-Analysis. *Annual review of entomology,* 43(1), 195-216.

Kramer, P. J. (1983). Water relations of plants. Academic Press. Inc. New York.

Kramer-Schadt, S., Niedballa, J., Pilgrim, J. D., Schröder, B., Lindenborn, J., Reinfelder, V., Stillfried, M., Heckmann, I., Scharf, A. K., Augeri, D. M., Cheyne, S. M., Hearn, A. J., Ross, J., Macdonald, D. W., Mathai, J., Eaton, J., Marshall, A. J., Semiadi, G., Rustam, R., … Wilting, A. (2013). The importance of correcting for sampling bias in MaxEnt species distribution models. *Diversity and Distributions*, *19*(11), 1366–1379. https://doi.org/10.1111/ddi.12096

La Sorte, F. A., & Jetz, W. (2010). Projected range contractions of montane biodiversity under global warming. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1699), 3401–3410. https://doi.org/10.1098/rspb.2010.0612

Langenheim, J. H. (1962). Vegetation and environmental patterns in the Crested Butte area, Gunnison County, Colorado*. Ecological Monographs,* 3(32), 249–285.

Larsson, S. (1989). Stressful Times for the Plant Stress: Insect Performance Hypothesis. *Oikos*, *56*(2), 277. https://doi.org/10.2307/3565348

Larsson, S., & Björkman, C. (1993). Performance of chewing and phloem-feeding insects on stressed trees. *Scandinavian Journal of Forest Research*, 8(1–4), 550–559. https://doi.org/10.1080/02827589309382801

Lemoine, N. P. (2015). Climate change may alter breeding ground distributions of eastern migratory monarchs (Danaus plexippus) via range expansion of *Asclepias* host plants. *PLOS ONE*, *10*(2), e0118614. https://doi.org/10.1371/journal.pone.0118614

Lemoine, N. P., Buckerpile, D. E., & Parker, J. D. (2014). Variable effects of temperature on insect herbivory. *PeerJ 2,* e376.

Lenhart, P. A., Eubanks, M. D., & Behmer, S. T. (2015). Water stress in grasslands: Dynamic responses of plants and insect herbivores. *Oikos*, 124(3), 381–390. https://doi.org/10.1111/oik.01370

Lewinsohn, T., Novotny, V., & Basset, Y. (2005). *Insects on plants: Diversity of herbivore assemblages revisited*. *36*, 597–620.

Lopez-Iglesias, B., Villar, R., & Poorter, L. (2014). Functional traits predict drought performance and distribution of Mediterranean woody species. *Acta Oecologica*, *56*, 10–18. https://doi.org/10.1016/j.actao.2014.01.003

Louda, S. M. (1984). Herbivore Effect on Stature, Fruiting, and Leaf Dynamics of a Native Crucifer. *Ecology*, *65*(5), 1379–1386. https://doi.org/10.2307/1939118

Louda, S. M., & Collinge, S. K. (1992). Plant Resistance to Insect Herbivores: A Field Test of the Environmental Stress Hypothesis. *Ecology*, *73*(1), 153–169. https://doi.org/10.2307/1938728

Louda, S. M., & Rodman, J. E. (1983a). Concentration of glucosinolates in relation to habitat and insect herbivory for the native crucifer Cardamine cordifolia. *Biochemical Systematics and Ecology*, *11*(3), 199–207. https://doi.org/10.1016/0305-1978(83)90054-6

Malcolm, S. B., & Brower, L. P. (1989). Evolutionary and ecological implications of cardenolide sequestration in the monarch butterfly. *Experientia*, *45*(3), 284–295. https://doi.org/10.1007/BF01951814

Marquis, R. J. (1992). Selective impact of herbivores. In *In: Frtiz RS, Simms EL, editors, Plant resistance to herbivores and pathogens: Ecology, evolution, and genetics* (pp. 301–324). University of Chicago Press.

Masuko, T., Minami, A., Iwasaki, N., Majima, T., Nishimura, S.-I., & Lee, Y. C. (2005). Carbohydrate analysis by a phenol–sulfuric acid method in microplate format. *Analytical Biochemistry*, 339(1), 69–72. https://doi.org/10.1016/j.ab.2004.12.001

Mattson, W. J., & Haack, R. A. (1987). The Role of Drought in Outbreaks of Plant-Eating Insects. *Bioscience,* 37(2), 10.

Mcmillin, Joel. D., & Wagner, M. R. (1998). Influence of host plant vs. Natural enemies on the spatial distribution of a pine sawfly, Neodiprion autumnalis. *Ecological Entomology*, *23*(4), 397–408. https://doi.org/10.1046/j.1365-2311.1998.00146.x

Meier, E. S., Edwards Jr, T. C., Kienast, F., Dobbertin, M., & Zimmermann, N. E. (2011). Co-occurrence patterns of trees along macro-climatic gradients and their potential influence on the present and future distribution of Fagus sylvatica L.: Influence of co-occurrence patterns on Fagus sylvatica. *Journal of Biogeography*, *38*(2), 371–382. https://doi.org/10.1111/j.1365-2699.2010.02405.x

Memmott, J., Day, R. K., & Godfray, H. C. J. (1995). Intraspecific variation in host plant quality: The aphid Cinara cupressi on the Mexican cypress, Cupressus lusitanica. *Ecological Entomology*, *20*(2), 153–158. https://doi.org/10.1111/j.1365-2311.1995.tb00441.x

Mody, K., Eichenberger, D., & Dorn, S. (2009). Stress magnitude matters: Different intensities of pulsed water stress produce non-monotonic resistance responses of host plants to insect herbivores. *Ecological Entomology*, *34*(1), 133–143. https://doi.org/10.1111/j.1365-2311.2008.01053.x

Montesinos-Navarro, A., Pérez-Clemente, R. M., Sánchez-Martín, R., Gómez-Cadenas, A., & Verdú, M. (2020). Phylogenetic analysis of secondary metabolites in a plant community provides evidence for trade-offs between biotic and abiotic stress tolerance. *Evolutionary Ecology*, *34*(3), 439–451. https://doi.org/10.1007/s10682-020-10044-2

Mooney, K. A., & Agrawal, A. A. (2008). Plant Genotype Shapes Ant-Aphid Interactions: Implications for Community Structure and Indirect Plant Defense. *The American Naturalist*, *171*(6), E195–E205. https://doi.org/10.1086/587758

Mooney, K. A., Halitschke, R., Kessler, A., & Agrawal, A. A. (2010). Evolutionary Trade-Offs in Plants Mediate the Strength of Trophic Cascades. *Science*, *327*(5973), 1642–1644. https://doi.org/10.1126/science.1184814

Moreno-Gutiérrez, C., Dawson, T. E., Nicolás, E., & Querejeta, J. I. (2012). Isotopes reveal contrasting water use strategies among coexisting plant species in a Mediterranean ecosystem. *New Phytologist*, *196*(2), 489–496. https://doi.org/10.1111/j.1469-8137.2012.04276.x

Müller, T., & Müller, C. (2016). Adult beetles compensate for poor larval food conditions. *Journal of Insect Physiology*, *88*, 24–32. https://doi.org/10.1016/j.jinsphys.2016.02.009

Mullins, M., J. D. Uyl, E. Cruz, S. Trail, B. Davidson, D. Campbell, and E. Mooney. (2020). Advanced phenology of higher trophic levels shifts aphid host plant preferences and performance. *Ecological Entomology* 45:1004-1014.

Mundim, F. M., & Bruna, E. M. (2016). Is There a Temperate Bias in Our Understanding of How Climate Change Will Alter Plant-Herbivore Interactions? A Meta-analysis of Experimental Studies. *The American Naturalist*, *188*(S1), S74–S89. https://doi.org/10.1086/687530

Muscarella, R., Galante, P. J., Soley-Guardia, M., Boria, R. A., Kass, J. M., Uriarte, M., & Anderson, R. P. (2014). ENMeval: An R package for conducting spatially independent evaluations and estimating optimal model complexity for MAXENT ecological niche models. *Methods in Ecology and Evolution*, *5*(11), 1198–1205. https://doi.org/10.1111/2041-210X.12261

Nail, K. R., Drizd, L., & Voorhies, K. J. (2019). Butterflies Across the Globe: A Synthesis of the Current Status and Characteristics of Monarch (Danaus plexippus) Populations

Worldwide. *Frontiers in Ecology and Evolution*, *7*, 362. https://doi.org/10.3389/fevo.2019.00362

National Reseach Council. (2010). *Advancing the Science of Climate Change*. The National Academies Press. https://doi.org/10.17226/12782.

Naturalist *[online]. Website*. (2019, January). INaturalist. https://www.inaturalist.org

Ode, P. J., Johnson, S. N., & Moore, B. D. (2014). Atmospheric change and induced plant secondary metabolites—Are we reshaping the building blocks of multi-trophic interactions? *Current Opinion in Insect Science*, 5, 57–65. https://doi.org/10.1016/j.cois.2014.09.006

Olaya-Arenas, P., & Kaplan, I. (2019). Quantifying Pesticide Exposure Risk for Monarch Caterpillars on Milkweeds Bordering Agricultural Land. *Frontiers in Ecology and Evolution*, *7*, 223. https://doi.org/10.3389/fevo.2019.00223

Ordoñez, J. C., van Bodegom, P. M., Witte, J.-P. M., Wright, I. J., Reich, P. B., & Aerts, R. (2009). A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. *Global Ecology and Biogeography*, 18(2), 137–149. https://doi.org/10.1111/j.1466-8238.2008.00441.x

Ouédraogo, D.-Y., Mortier, F., Gourlet-Fleury, S., Freycon, V., & Picard, N. (2013). Slow-growing species cope best with drought: Evidence from long-term measurements in a tropical semi-deciduous moist forest of Central Africa. *Journal of Ecology*, 101(6), 1459–1470. https://doi.org/10.1111/1365-2745.12165

Parmesan, C., Ryrholm, N., Stefanescu, C., Hill, J. K., Thomas, C. D., Descimon, H., Huntley, B., Kaila, L., Kullberg, J., Tammaru, T., Tennent, W. J., Thomas, J. A., & Warren, M. (1999).

Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature*, 399(6736), 579–583. https://doi.org/10.1038/21181

Parmesan, C., & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, *421*(6918), 37–42. https://doi.org/10.1038/nature01286

Passioura, J. B. (1996). *Drought and drought tolerance. Plant growth regulation*. 20(2), 79–83.

Pateman, R. M., Hill, J. K., Roy, D. B., Fox, R., & Thomas, C. D. (2012a). Temperature-Dependent Alterations in Host Use Drive Rapid Range Expansion in a Butterfly. *Science*, *336*(6084), 1028–1030. https://doi.org/10.1126/science.1216980

Pateman, R. M., Hill, J. K., Roy, D. B., Fox, R., & Thomas, C. D. (2012). Temperature-Dependent Alterations in Host Use Drive Rapid Range Expansion in a Butterfly. *Science*, *336*(6084), 1028–1030. https://doi.org/10.1126/science.1216980

Pauli, H., Gottfried, M., Dullinger, S., Abdaladze, O., Akhalkatsi, M., Alonso, J. L. B., Coldea, G., Dick, J., Erschbamer, B., Calzado, R. F., Ghosn, D., Holten, J. I., Kanka, R., Kazakis, G., Kollar, J., Larsson, P., Moiseev, P., Moiseev, D., Molau, U., … Grabherr, G. (2012). Recent plant diversity changes on Europe's mountain summits. *Science*, 336(6079), 353–355. https://doi.org/10.1126/science.1219033

Pecenka, J. R., & Lundgren, J. G. (2015). Non-target effects of clothianidin on monarch butterflies. *The Science of Nature*, 102(3–4), 19. https://doi.org/10.1007/s00114-015-1270-y

Pelton, E. M., Schultz, C. B., Jepsen, S. J., Black, S. H., & Crone, E. E. (2019). Western monarch population plummets: Status, probable causes, and recommended conservation actions. *Frontiers in Ecology and Evolution*, 7, 258. https://doi.org/10.3389/fevo.2019.00258

Peterson, A., Soberon, J., Pearson, R. G., Anderson, R. P., Martinez-Meyer, E., Nakamura, M., & Araújo, M. (2011). *Ecological niches and geographic distributions.* Princeton University, Press.

Peterson, A. T., Papeş, M., & Soberón, J. (2008). Rethinking receiver operating characteristic analysis applications in ecological niche modeling. *Ecological Modelling*, *213*(1), 63–72. https://doi.org/10.1016/j.ecolmodel.2007.11.008

Petschenka, G., & Agrawal, A. A. (2015). Milkweed butterfly resistance to plant toxins is linked to sequestration, not coping with a toxic diet. *Proceedings of the Royal Society B: Biological Sciences*, 282(1818), 20151865. https://doi.org/10.1098/rspb.2015.1865

Phillips, S. J., Anderson, R. P., & Schapire, R. E. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, *190*(3–4), 231–259. https://doi.org/10.1016/j.ecolmodel.2005.03.026

Pocius, V. M., Debinski, D. M., Pleasants, J. M., Bidne, K. G., & Hellmich, R. L. (2018). Monarch butterflies do not place all of their eggs in one basket: Oviposition on nine midwestern milkweed species. *Ecosphere*, 9(1). https://doi.org/10.1002/ecs2.2064

Pocius, V. M., Debinski, D. M., Pleasants, J. M., Bidne, K. G., Hellmich, R. L., & Brower, L. P. (2017). Milkweed matters: Monarch butterfly (Lepidoptera: Nymphalidae) survival and development on nine midwestern milkweed species. *Environmental Entomology*, 46(5), 1098–1105. https://doi.org/10.1093/ee/nvx137

Poff, B., Koestner, K. A., Neary, D. G., & Henderson, V. (2011). Threats to Riparian Ecosystems in Western North America: An Analysis of Existing Literature1: Threats to Riparian Ecosystems in Western North America: An Analysis of Existing Literature. *Journal of the*

American Water Resources Association, *47*(6), 1241–1254. https://doi.org/10.1111/j.1752-1688.2011.00571.x

Preston, K. L., Rotenberry, J. T., Redak, R. A., & Allen, M. F. (2008). Habitat shifts of endangered species under altered climate conditions: Importance of biotic interactions. *Global Change Biology*, *14*(11), 2501–2515. https://doi.org/10.1111/j.1365-2486.2008.01671.x

Price, P. W. (1983). Hypotheses on organization and evolution in herbivorous insect communities. In *Variable Plants and Herbivores in Natural and Managed Systems* (pp. 559–598). Academic Press.

Price, P. W. (1991). The Plant Vigor Hypothesis and Herbivore Attack. *Oikos*, *62*(2), 244. https://doi.org/10.2307/3545270

Price, W. P., Bouton, C. E., Gross, P., McPheron, B. A., Thompson, J. N., & Weis, A. E. (1980). Interactions among three trophic levels: Influence of plants on interactions between insect herbivores and natural enemies. *Annual review of Ecology and Systematics, 11*(1), 41–65.

R Studio Team. (2015). *R Studio*. Integrated Development for R. RStudio, Inc. http://www.rstudio.com

Rall, B. C., Vucic-Pestic, O., Ehnes, R. B., Emmerson, M., & Brose, U. (2010). Temperature, predator–prey interaction strength and population stability. *Global Change Biology* 16(8), 2145–2157.

Reich, P. B. (2014). The world-wide ‘fast-slow’ plant economics spectrum: A traits manifesto. *Journal of Ecology*, *102*(2), 275–301. https://doi.org/10.1111/1365-2745.12211

Revell, L. J. (2012) phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology & Evolution*, 3, 217-223.

Rhodes, C. J. (2019). Are insect species imperilled? Critical factors and prevailing evidence for a potential global loss of the entomofauna: A current commentary. *Science Progress*, *102*(2), 181–196. https://doi.org/10.1177/0036850419854291

Ries, L., & Oberhauser, K. S. (2015). *A citizen army for science: Quantifying the contributions of citizen scientists to our understanding of monarch butterfly biology*. *65*(4), 419–430.

Robertson, G. F., Zalucki, M. P., & Paine, T. D. (2015). Larval host choice of the monarch butterfly (*Danaus plexippus* L.) on four native California desert milkweed species. *Journal of Insect Behavior*, *28*(5), 582–592. https://doi.org/10.1007/s10905-015-9524-2

Rosenblatt, A. E., & Schmitz, O. J. (2016). Climate change, nutrition, and bottom-up and top-down food web processes. *Trends in Ecology & Evolution*, *31*(12), 965–975. https://doi.org/10.1016/j.tree.2016.09.009

Ryan, M. G. (2011). Tree responses to drought. *Tree Physiology*, *31*(3), 237–239. https://doi.org/10.1093/treephys/tpr022

Schettino, M., Grasso, D. A., Weldegergis, B. T., Castracani, C., Mori, A., Dicke, M., Van Lenteren, J. C., & Van Loon, J. J. A. (2017). Response of a Predatory ant to Volatiles Emitted by Aphid- and Caterpillar-Infested Cucumber and Potato Plants. *Journal of Chemical Ecology*, *43*(10), 1007–1022. https://doi.org/10.1007/s10886-017-0887-z

Schweiger, O., Settele, J., Kudrna, O., Klotz, S., & Kühn, I. (2008). Climate change can cause spatial mismatch of trophically interacting species. *Ecology*, *89*(12), 3472–3479. https://doi.org/10.1890/07-1748.1

Sekercioglu, C. H., Schneider, S. H., Fay, J. P., & Loarie, S. R. (2008). Climate change, elevational range shifts, and bird extinctions: *Elevation, Climate Change, and Bird Extinctions*. *Conservation Biology*, *22*(1), 140–150. https://doi.org/10.1111/j.1523-1739.2007.00852.x

Shcheglovitova, M., & Anderson, R. P. (2013). Estimating optimal complexity for ecological niche models: A jackknife approach for species with small sample sizes. *Ecological Modelling*, *269*, 9–17. https://doi.org/10.1016/j.ecolmodel.2013.08.011

Sheffield, J., & Wood, E. F. (2008). Projected changes in drought occurrence under future global warming from multi-model, multi-scenario, *IPCC AR4 simulations*. 27.

Singer, M. S., Farkas, T. E., Skorik, C. M., & Mooney, K. A. (2012). Tritrophic interactions at a community level: Effects of host plant species quality on bird predation of caterpillars. *The American Naturalist, 179*(3), 363–374.

Skelton, R. P., West, A. G., & Dawson, T. E. (2015). Predicting plant vulnerability to drought in biodiverse regions using functional traits. *Proceedings of the National Academy of Sciences*, *112*(18), 5744–5749. https://doi.org/10.1073/pnas.1503376112

Soberon, J., & Nakamura, M. (2009). Niches and distributional areas: Concepts, methods, and assumptions. *Proceedings of the National Academy of Sciences*, *106*(Supplement_2), 19644–19650. https://doi.org/10.1073/pnas.0901637106

Stenseth, N. C., & Mysterud, A. (2002). Climate, changing phenology, and other life history traits: Nonlinearity and match-mismatch to the environment. *Proceedings of the National Academy of Sciences*, *99*(21), 13379–13381. https://doi.org/10.1073/pnas.212519399

Stevens, S. R., & Frey, D. F. (2010). Host plant pattern and variation in climate predict the location of natal grounds for migratory monarch butterflies in western North America. *Journal of Insect Conservation*, *14*(6), 731–744. https://doi.org/10.1007/s10841-010-9303-5

Stireman, J. O., Dyer, L. A., Janzen, D. H., Singer, M. S., Lill, J. T., Marquis, R. J., Ricklefs, R. E., Gentry, G. L., Hallwachs, W., Coley, P. D., Barone, J. A., Greeney, H. F., Connahs, H., Barbosa, P., Morais, H. C., & Diniz, I. R. (2005). Climatic unpredictability and parasitism of caterpillars: Implications of global warming. *Proceedings of the National Academy of Sciences*, *102*(48), 17384–17387. https://doi.org/10.1073/pnas.0508839102

Svancara, L. K., Abatzoglou, J. T., & Waterbury, B. (2019). Modeling current and future potential distributions of milkweeds and the monarch butterfly in Idaho. *Frontiers in Ecology and Evolution*, *7*, 168. https://doi.org/10.3389/fevo.2019.00168

Taiwo, A. F., Daramola, O., Sow, M., & Semwal, V. K. (2020). *Ecophysiology and responses of plants under drought. In: Hasanuzzaman M. (eds) Plant ecophysiology and adaptation under climate change: Mechanisms and perspectives I.* (pp. 231–268). Springer Singapore. https://doi.org/10.1007/978-981-15-2156-0_8

The HadGEM2 Development Team: G. M. Martin, Bellouin, N., Collins, W. J., Culverwell, I. D., Halloran, P. R., Hardiman, S. C., Hinton, T. J., Jones, C. D., McDonald, R. E., McLaren, A. J., O&apos;Connor, F. M., Roberts, M. J., Rodriguez, J. M., Woodward, S., Best, M. J., Brooks, M. E., Brown, A. R., Butchart, N., Dearden, C., … Wiltshire, A. (2011). The HadGEM2 family of Met Office Unified Model climate configurations. *Geoscientific Model Development*, *4*(3), 723–757. https://doi.org/10.5194/gmd-4-723-2011

Thoen, M. P. M., Davila Olivas, N. H., Kloth, K. J., Coolen, S., Huang, P.-P., Aarts, M. G. M., Bac-Molenaar, J. A., Bakker, J., Bouwmeester, H. J., Broekgaarden, C., Bucher, J., Busscher-Lange, J., Cheng, X., Fradin, E. F., Jongsma, M. A., Julkowska, M. M., Keurentjes, J. J. B., Ligterink, W., Pieterse, C. M. J., … Dicke, M. (2017). Genetic architecture of plant stress resistance: Multi-trait genome-wide association mapping. *New Phytologist*, *213*(3), 1346–1362. https://doi.org/10.1111/nph.14220

Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., Erasmus, B. F. N., de Siqueira, M. F., Grainger, A., Hannah, L., Hughes, L., Huntley, B., van Jaarsveld, A. S., Midgley, G. F., Miles, L., Ortega-Huerta, M. A., Peterson, A. T., Phillips, O. L., & Williams, S. E. (2004). *Extinction risk from climate change*. *427*, 5.

Thompson, J. N. (1999). The evolution of species interactions. *Science*, *284*(5423), 2116–2118. https://doi.org/10.1126/science.284.5423.2116

Touchette, B. W., Iannacone, L. R., Turner, G. E., & Frank, A. R. (2007). Drought tolerance versus drought avoidance: A comparison of plant-water relations in herbaceous wetland plants subjected to water withdrawal and repletion. *Wetlands*, *27*(3), 656–667. https://doi.org/10.1672/0277-5212(2007)27[656:DTVDAA]2.0.CO;2

Travers-Martin, N., & Müller, C. (2008). Matching plant defence syndromes with performance and preference of a specialist herbivore. *Functional Ecology*, *22*(6), 1033–1043. https://doi.org/10.1111/j.1365-2435.2008.01487.x

Turtola, S., Rousi, M., Pusenius, J., Yamaji, K., Heiska, S., Tirkkonen, V., Meier, B., & Julkunen-Tiitto, R. (2005). Clone-specific responses in leaf phenolics of willows exposed to enhanced UVB radiation and drought stress. *Global Change Biology*, *11*(10), 1655–1663. https://doi.org/10.1111/j.1365-2486.2005.01013.x

Tylianakis, J. M., Didham, R. K., Bascompte, J., & Wardle, D. A. (2008). Global change and species interactions in terrestrial ecosystems. *Ecology Letters*, *11*(12), 1351–1363. https://doi.org/10.1111/j.1461-0248.2008.01250.x

USDA NRCS. (2021, April 22). *National Plant Data Center*. The PLANTS Database. http://plants.usda.gov

Van der Putten, W. H., Macel, M., & Visser, M. E. (2010). Predicting species distribution and abundance responses to climate change: Why it is essential to include biotic interactions across trophic levels. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1549), 2025–2034. https://doi.org/10.1098/rstb.2010.0037

Volaire, F., Barkaoui, K., & Norton, M. (2014). Designing resilient and sustainable grasslands for a drier future: Adaptive strategies, functional traits and biotic interactions. *European Journal of Agronomy*, *52*, 81–89. https://doi.org/10.1016/j.eja.2013.10.002

Walther, G.-R. (2010). Community and ecosystem responses to recent climate change. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1549), 2019–2024. https://doi.org/10.1098/rstb.2010.0021

Warren, D. L., & Seifert, S. N. (2011). Ecological niche modeling in Maxent: The importance of model complexity and the performance of model selection criteria. *Ecological Applications*, *21*(2), 335–342. https://doi.org/10.1890/10-1171.1

Wharton, T. N., & Kriticos, D. J. (2004). Biodiversity: The fundamental and realized niche of the Monterey Pine aphid, Essigella. *Diversity and Distributions*, 10.

White, T. C. R. (1969). *An index to measure weather-induced stress of trees associated with outbreaks of psyllids in Australia*. *50*(5), 905–909.

Wilson, R. J., Davies, Z. G., & Thomas, C. D. (2007). Insects and climate change: Processes, patterns and implications for conservation. In A. J. A. Stewart, T. R. New, & O. T. Lewis (Eds.), *Insect conservation biology* (pp. 245–279). https://doi.org/10.1079/9781845932541.0245

Wisz, M. S., Pottier, J., Kissling, W. D., Pellissier, L., Lenoir, J., Damgaard, C. F., Dormann, C. F., Forchhammer, M. C., Grytnes, J., Guisan, A., Heikkinen, R. K., Høye, T. T., Kühn, I., Luoto, M., Maiorano, L., Nilsson, M., Normand, S., Öckinger, E., Schmidt, N. M., … Svenning, J. (2013). The role of biotic interactions in shaping distributions and realised assemblages of species: Implications for species distribution modelling. *Biological Reviews*, *88*(1), 15–30. https://doi.org/10.1111/j.1469-185X.2012.00235.x

Woodson, R. E. (1954). The North American species of Asclepias L. *Annals of the Missouri Botanical Garden*, *41*(1), 1. https://doi.org/10.2307/2394652

Wright, I. J., Reich, P. B., Cornelissen, J. H. C., Falster, D. S., Groom, P. K., Hikosaka, K., Lee, W., Lusk, C. H., Niinemets, Ü., Oleksyn, J., Osada, N., Poorter, H., Warton, D. I., & Westoby, M. (2005). Modulation of leaf economic traits and trait relationships by climate: Modulation of leaf traits by climate. *Global Ecology and Biogeography*, *14*(5), 411–421. https://doi.org/10.1111/j.1466-822x.2005.00172.x

Wright, I. J., Reich, P. B., & Westoby, M. (2001). Strategy shifts in leaf physiology, structure and nutrient content between species of high- and low-rainfall and high- and low-nutrient habitats: Leaf physiology, structure and nutrients. *Functional Ecology*, *15*(4), 423–434. https://doi.org/10.1046/j.0269-8463.2001.00542.x

Yang, L. H., Ostrovsky, D., Rogers, M. C., & Welker, J. M. (2016). Intra-population variation in the natal origins and wing morphology of overwintering western monarch butterflies *Danaus plexippus*. *Ecography*, *39*(10), 998–1007. https://doi.org/10.1111/ecog.01994

Yuan, J. S., Himanen, S. J., Holopainen, J. K., Chen, F., & Stewart, C. N. (2009). Smelling global climate change: Mitigation of function for plant volatile organic compounds. *Trends in Ecology & Evolution*, *24*(6), 323–331. https://doi.org/10.1016/j.tree.2009.01.012

Zalucki, M., Brower, L., & Alonso, A. (2001). Detrimental effects of latex and cardiac glycosides on survival and growth of first-instar monarch butterfly larvae Danaus plexippus feeding on the sandhill milkweed Asclepias humistrata. *Ecological Entomology*, *26*, 212–224.

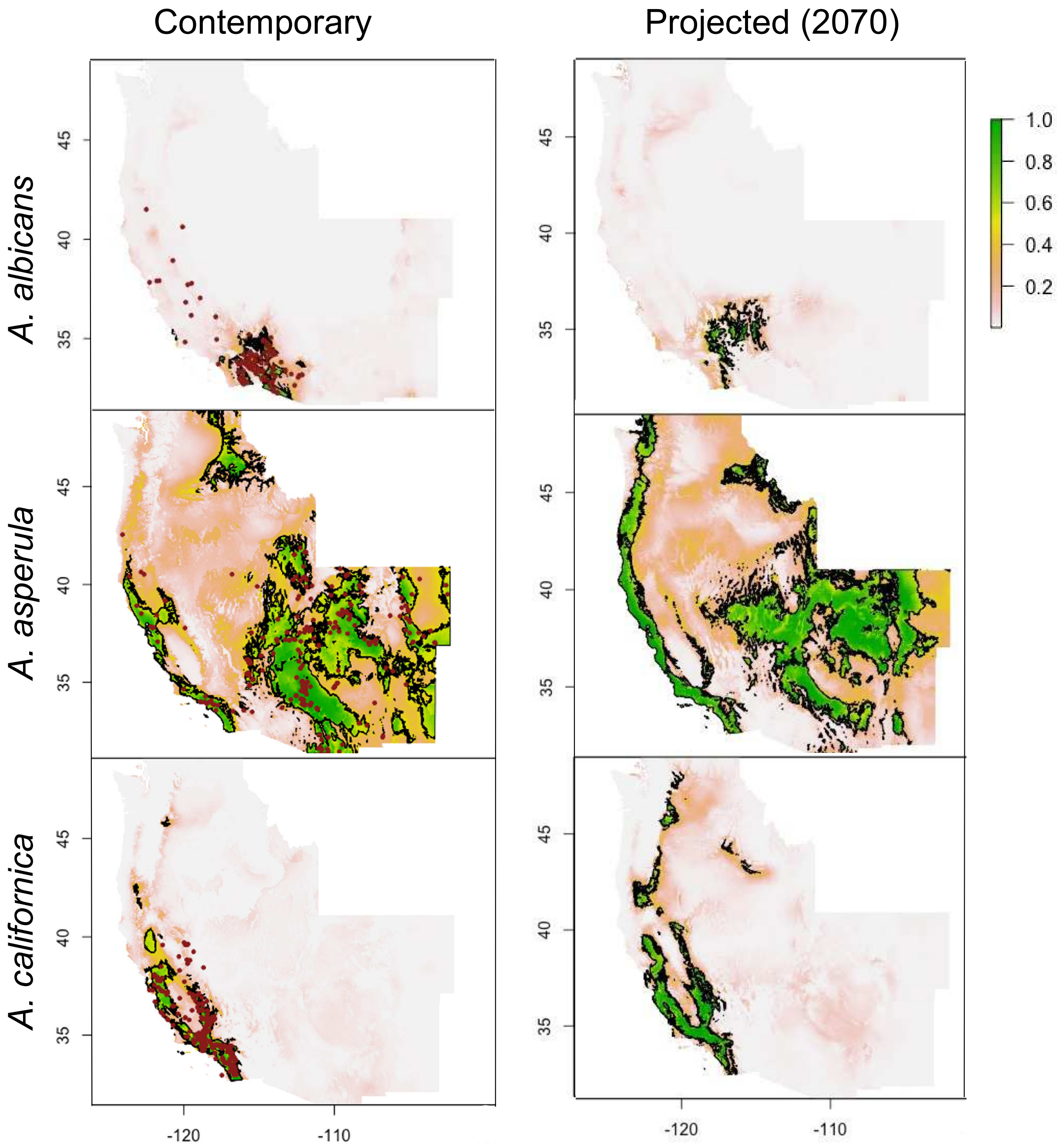
Zalucki, M. P., & Kitching, R. L. (1982). Temporal and spatial variation of mortality in field populations of *Danaus plexippus* L. and *D. chrysippus* L. Larvae (Lepidoptera: Nymphalidae). *Oecologia*, *53*(2), 201–207. https://doi.org/10.1007/BF00545664

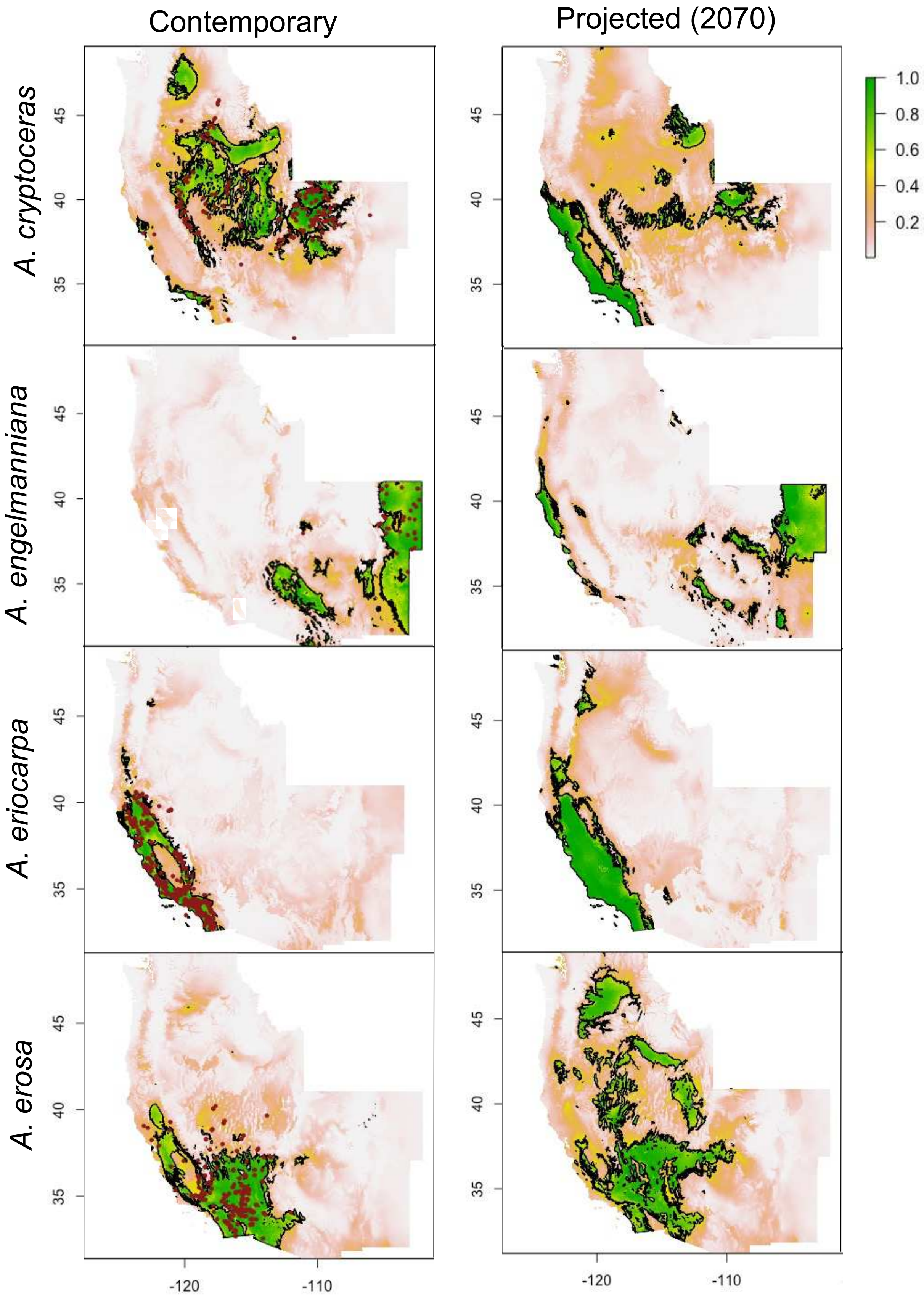
Zalucki, M. P., & Suzuki, Y. (1987). Milkweed patch quality, adult population structure, and egg laying in the monarch butterfly. *Journal of the Lepidopterists Society*, *41*(1), 13–22.

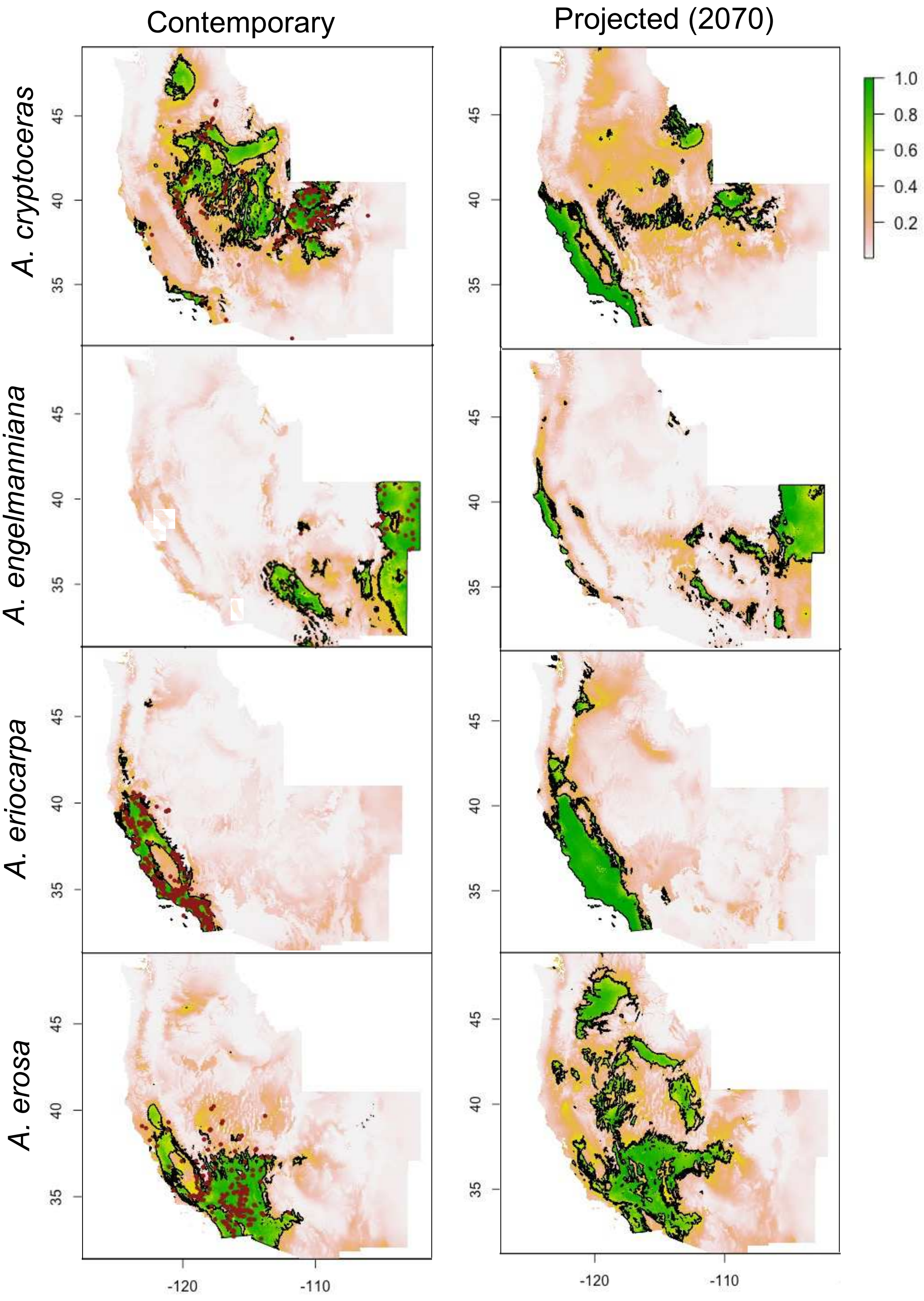
Züst, T., Petschenka, G., Hastings, A. P., & Agrawal, A. A. (2019). Toxicity of milkweed leaves and latex: chromatographic quantification versus biological activity of cardenolides in 16 *Asclepias* Species. *Journal of Chemical Ecology*, *45*(1), 50–60. https://doi.org/10.1007/s10886-018-1040-3

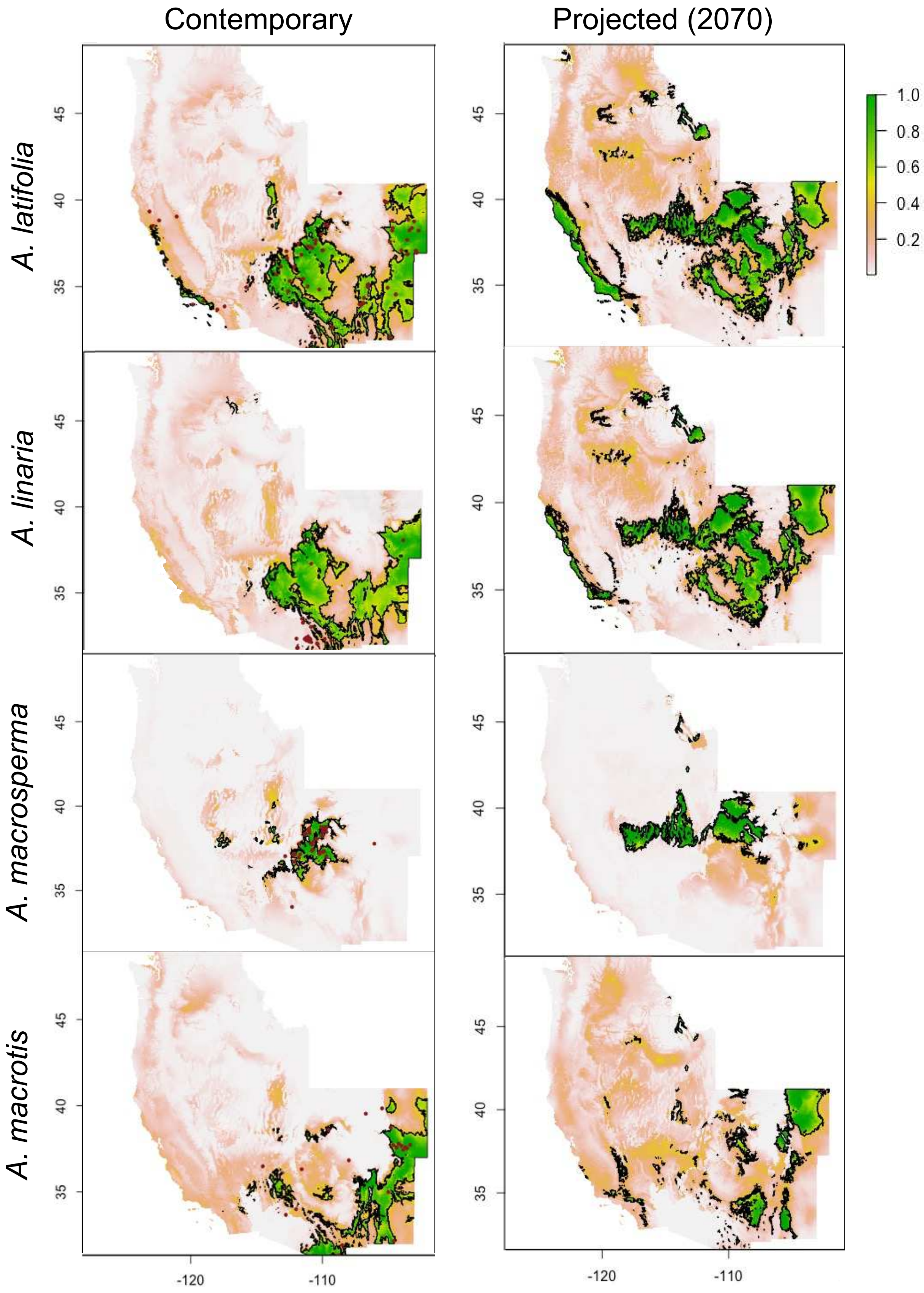
APPENDIX 1

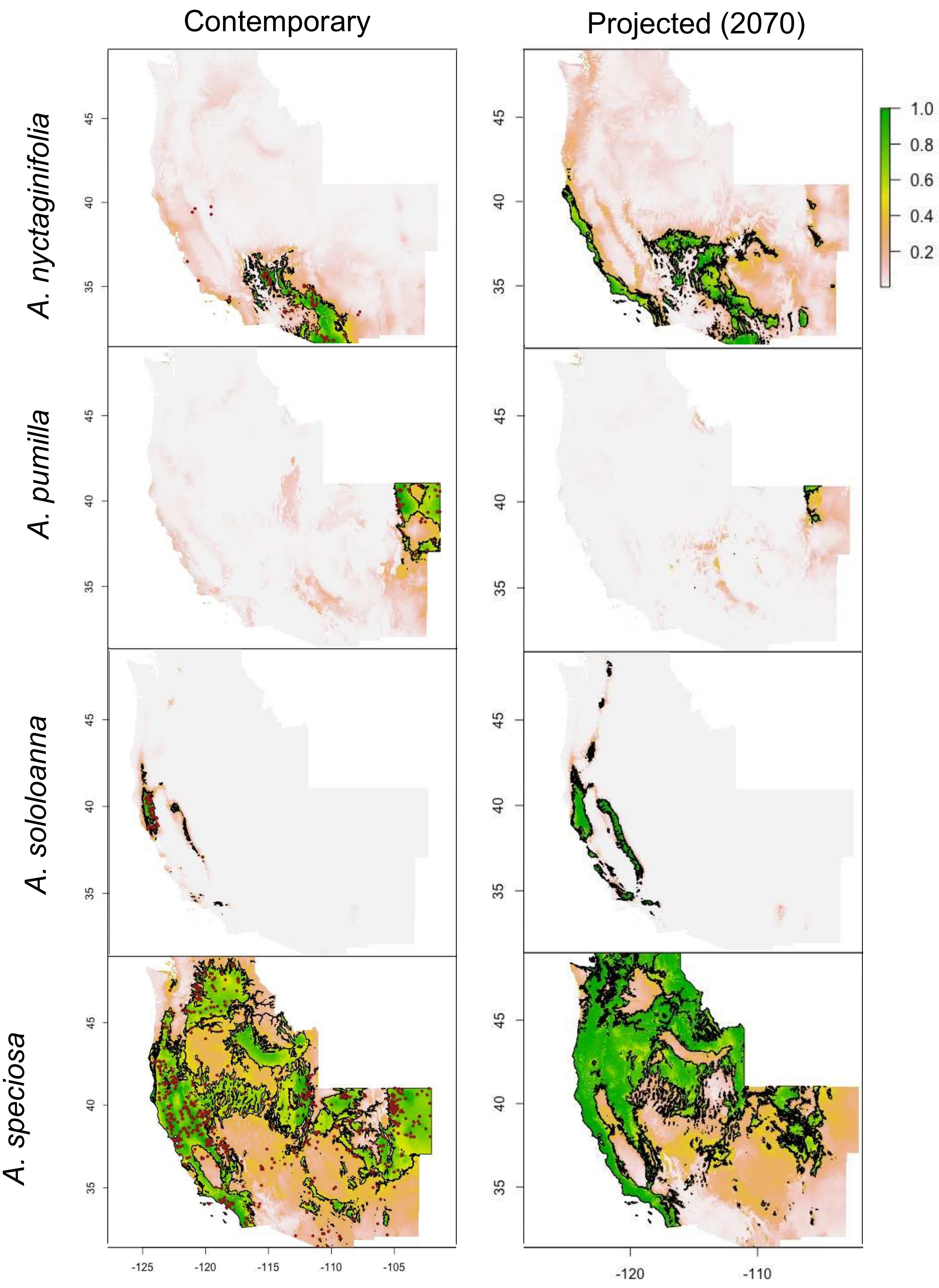
Figure S1. Estimated contemporary and future distribution for 24 *Asclepias* species

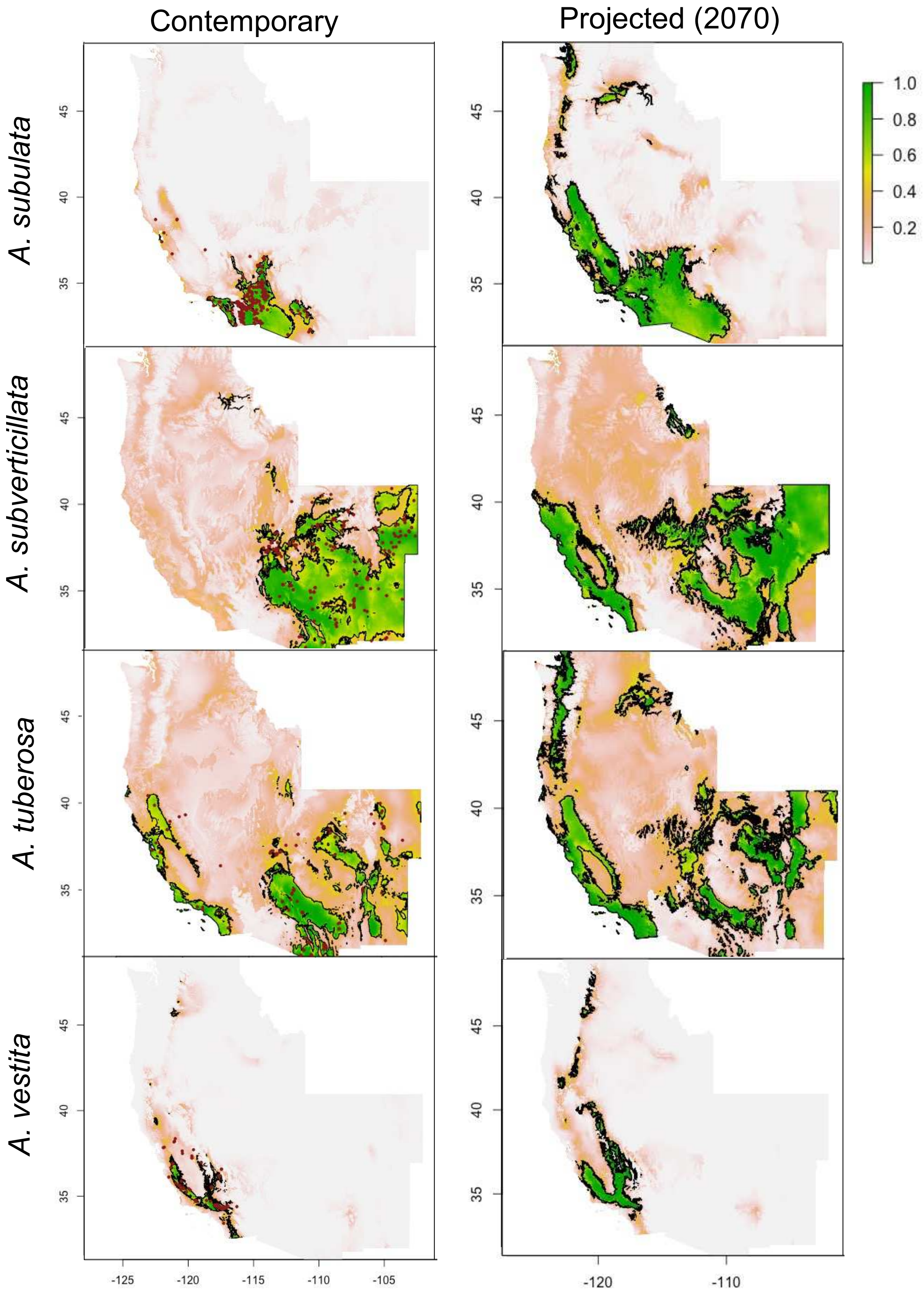



Contemporary
Projected (2070)
A. cryptoceras
A. engelmanniana
A. eriocarpa
A. erosa
45
40
35
-120
-110
1.0
0.8
0.6
0.4
0.2


Contemporary
Projected (2070)
A. cryptoceras
A. engelmanniana
A. eriocarpa
A. erosa
1.0
0.8
0.6
0.4
0.2
45
40
35
-120
-110


Contemporary
Projected (2070)
A. latifolia
A. linaria
A. macrosperma
A. macrotis
45
40
35
-120
-110
1.0
0.8
0.6
0.4
0.2


Contemporary
Projected (2070)
A. nyctaginifolia
A. pumilla
A. sololoanna
A. speciosa
45
40
35
-125
-120
-115
-110
-105
-120
-110
1.0
0.8
0.6
0.4
0.2


Contemporary
Projected (2070)
A. subulata
A. subverticillata
A. tuberosa
A. vestita
45
40
35
-125
-120
-115
-110
-105
-120
-110
1.0
0.8
0.6
0.4
0.2

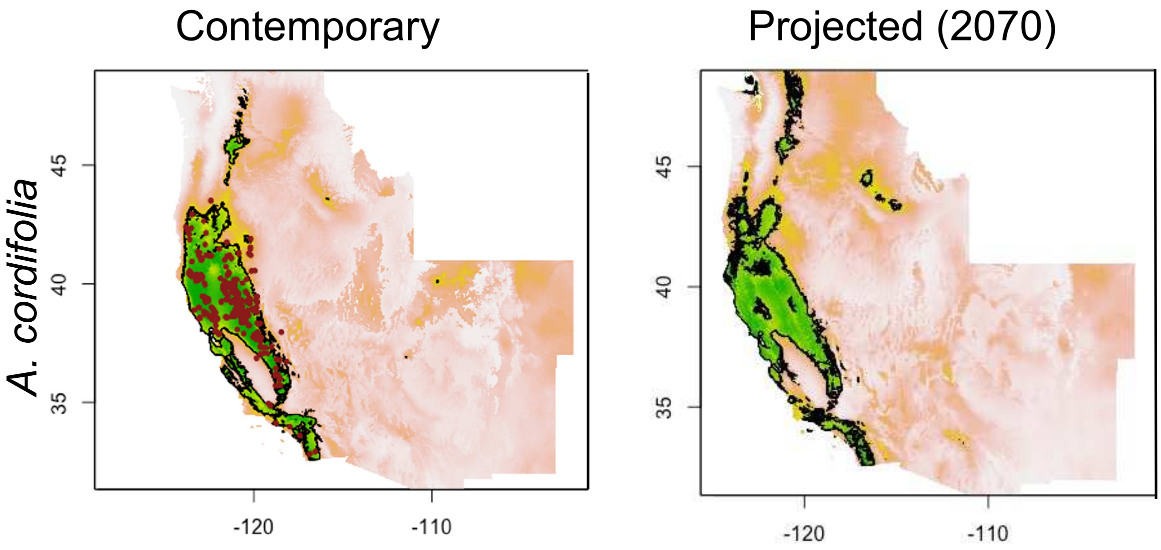
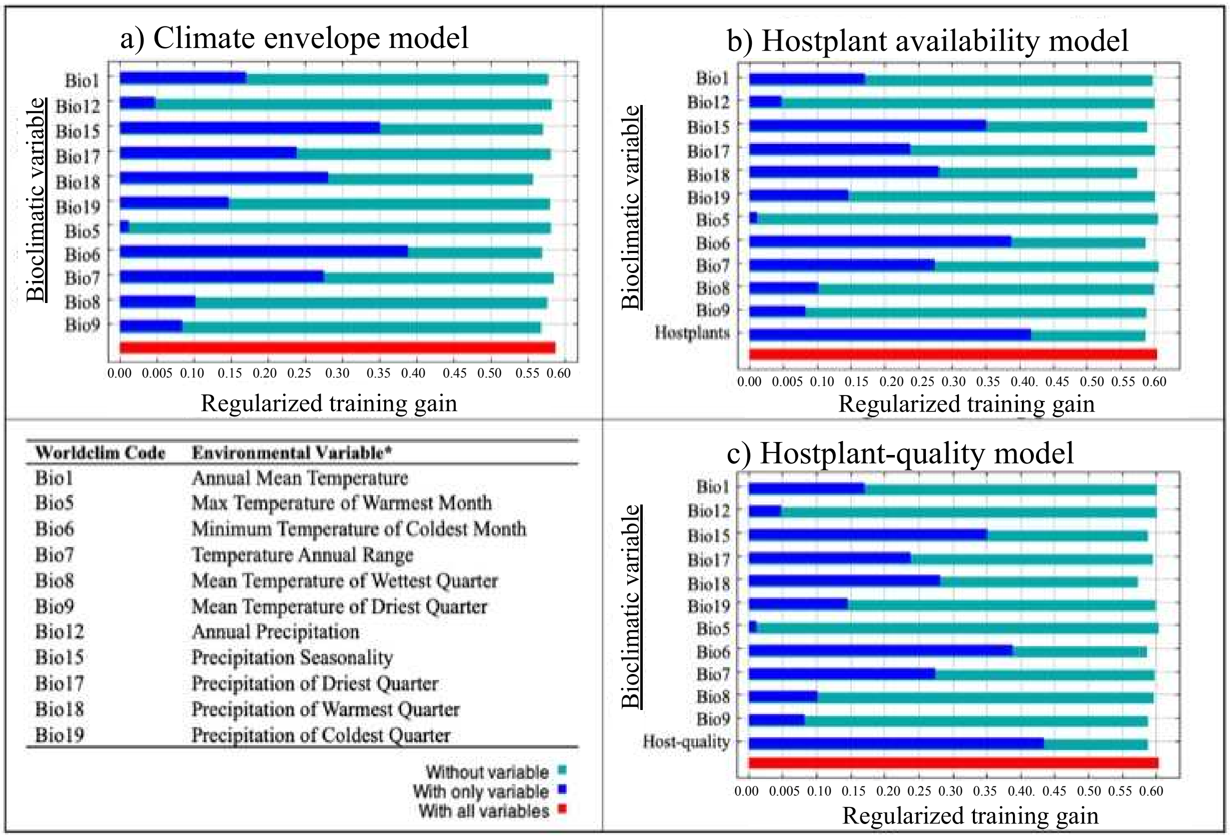


Fig. S1. Left panels indicate the current probability of distribution with 'white' representing low probability and 'green' high probability, from 0 to 1. Right panels indicate the projected probability of distribution of the monarch breeding grounds for the year 2070 estimated by the three models. Prime habitat is delineated in black and represent areas with a probability of distribution greater than 0.5. Occurrence records are indicated by red points.

Figure S2. Jackknife test of regularized training grain



Worldclim Code	Environmental Variable*
Bio1	Annual Mean Temperature
Bio5	Max Temperature of Warmest Month
Bio6	Minimum Temperature of Coldest Month
Bio7	Temperature Annual Range
Bio8	Mean Temperature of Wettest Quarter
Bio9	Mean Temperature of Driest Quarter
Bio12	Annual Precipitation
Bio15	Precipitation Seasonality
Bio17	Precipitation of Driest Quarter
Bio18	Precipitation of Warmest Quarter
Bio19	Precipitation of Coldest Quarter

Fig. S2. Jackknife test for each model: a) Climate envelope, b) Hostplant availability, and c) Hostplant-quality. Blue bars represent model gain when each variable is used in isolation, turquoise bars represent model gain when a single variable is excluded, and red bars represent model gain when all variables are included.

APPENDIX 2

Figure S3. ***Asclepias*** **phylogeny**

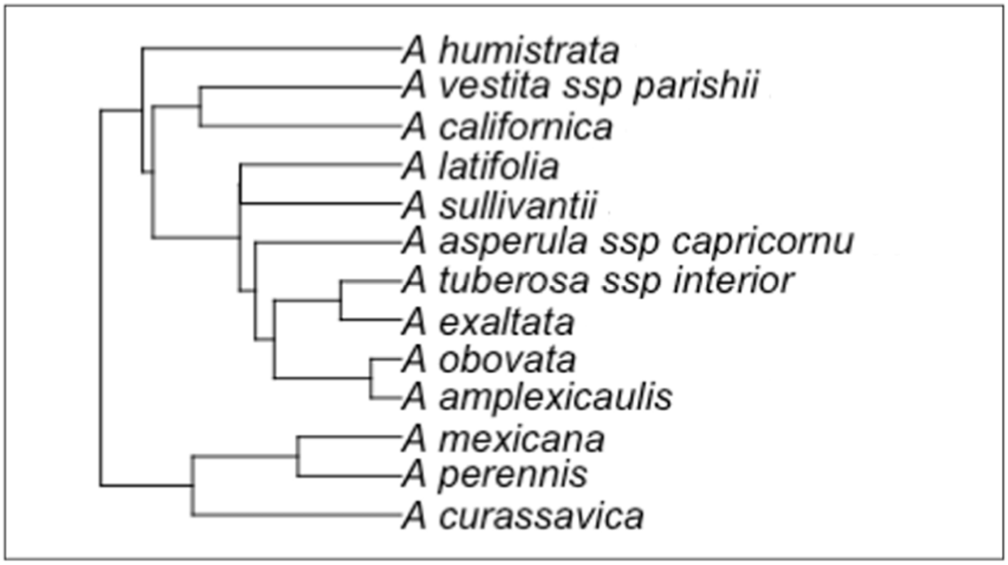


Fig. S3. Phylogenetic relationships of 13 selected Asclepias species.

Protocols

1. Stomatal conductance curves

Response curves were developed for each milkweed species based on their stomatal response to reduced soil moisture conditions. To do so, we selected three replicates per *Asclepias* species, watered pot to saturation (100% soil moisture) and took stomatal measurements daily between 10 am and 1 pm, during times of maximum stomatal opening, until plants nearly close its stomata (< 200 mmol m−2 s−1). We transformed the average stomatal conductance values per species to percentages, with 100% being the maximum stomatal conductance and 0% the lowest stomatal conductance value achieved by a species. We estimated relative soil moisture indirectly using pot weight as a proxy for soil moisture content so that a pot was at 100% saturation at its maximum weight soon after being watered, and at 0% saturation at its minimum weight when completely dried. We then correlated stomatal conductance (%) to relative soil moisture content (%) to construct stomatal response curves for each *Asclepias* species.

Figure S4. Stomatal conductance curves for each Asclepias species

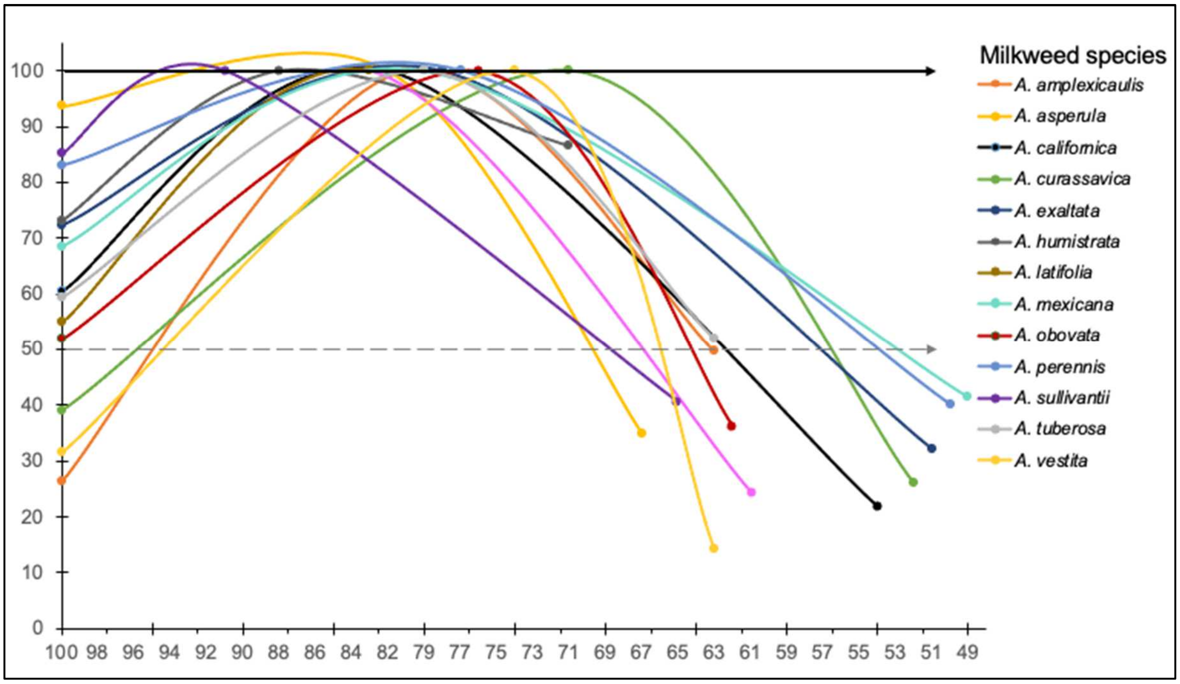


Fig. S4. Average stomatal conductance response for each *Asclepias* species as a function to soil water content.

2. Carbohydrate Analysis

We quantified total non-structural carbohydrate concentrations for a subset of samples (*n=46*). Soluble carbohydrates and carbohydrates from starch were extracted separately from 20 mg pulverized leaf tissue. To extract soluble carbohydrates, we used the MCW method described in Chow & Landhausser (2004) which uses fractionation to separate soluble carbohydrates from other solutes in a methanol:chloroform:water (MCW) solution. Plant samples were mixed with the 2mL MCW solution in 2 mL Eppendorf tubes. Tubes were placed in a sonic bath for 5 seconds and then centrifuged for 10 minutes at 2,500 rmp to separate the chloroform phase from the methanol:water phase where sugars get dissolved. This process was repeated three times until the supernatant (the methanol:water phase) was clear. I then combined supernatants resulting from each iteration and removed starch for further carbohydrate analysis. A 1 mL sample from the combined supernatant solution was mixed with 600 mL of pure distilled water and again separated into two phases by centrifuging at 2500 rpm for 5 min. The chloroform phase was discarded and the methanol:water phase was analyzed for carbohydrate content.

Carbohydrates from starches resulting from the previous process were extracted via hydrolyzation by dissolving the plant material in a 0.005 sulfuric acid solution and re-flux for 1 hour in a 95°C water bath (Chow & Landhausser, 2004). Extracted soluble and starch carbohydrates were quantified colourimetrically in triplicates against a glucose standard (0-800 µg concentrations) using the phenol-sulfuric acid assay (DuBois et al., 1956) optimized for microplate reading (Masuko et al., 2005).

3. Protein analysis

Protein was extracted from 20 mg samples dissolved in a 500 µl 0.1 M sodium hydroxide (NaOH) by sonication for 30 minutes and heating at 90°C for 15 min. Samples were centrifuged at 13,000 rpms for 10 min, supernatants containing proteins were removed. The remaining pellet was washed with 300 µl of 0.1 M NaOH, centrifuged again, and supernatant removed. After combining supernatants, the pH was neutralized using 11 µl of 5.8 M hydrochloric acid (HCl). Protein was then precipitated with 90 µl of 100% trichloroacetic acid. The samples were centrifuged to form a pellet of protein that was quickly washed with 100 µl of –20°C acetone after the supernatant was removed. The acetone was allowed to evaporate, and proteins were resuspended in 1 ml of 0.1 M NaOH and then diluted to ensure the concentration of NaOH were less than 0.01 M so that it did not interfere with Coomassie blue solution used by the Bradford assay (Bradford, 1976). To quantify protein, I used the Bio-Rad micro assay based on the Bradford assay (Bradford, 1976) with 0-8 µg of IgG (bovine gamma globulin) as the standard with samples read in triplicate.

4. Cardenolides analysis

We determined cardenolide concentration (mg/g dry tissue) by high-performance liquid chromotography following the methods of Züst et al. (2019). We used a Gemini C18 reversed-phase, 3 um, 150 mm x 4.6 mm column and an Agilent 1100 series instrument with a diode array detector. Briefly, 50 mg of dried and pulverized leaf tissue was analyzed by a methanolic extract. 1.5 ml of 100% methanol (including a 20 ug digitoxin spike as an internal standard) was added to each sample with 20 FastPrep beads (MP Biomedicals, CA, USA) and agitated twice on a FastPrep-24 homogenizer for 45 s at 6.5 m/s each time, followed by centrifugation at 20,800 g for 12 min. Supernatants were dried down in a vacuum concentrator, resuspended in 250 µl methanol, and filtered using 0.45 um hydrophilic PTFE membranes. Cardenolides were eluted at a constant flow of 0.7 ml/min with a gradient of acetonitrile and water as follows: 0–2 min at 16% acetonitrile; 2–25 min from 16% to 70%; 25–30 min from 70% to 95%; 30–35 min at 95%; followed by 10 min reconditioning at 16% acetonitrile. Peaks were recorded at 218 nm and absorbance spectra were recorded between 200 nm to 300 nm. Peaks showing a characteristic single absorption maximum between 214 and 222 nm, corresponding to an unsaturated lactone functional group, were considered cardenolides. Concentrations of cardenolide compounds were calculated by relating peak areas to the area of the internal standard.

Principal component analysis (PCA)

We conducted a principal component analysis on constitutive values of traits associated with drought adaptation: specific leaf area (SLA), water content (%), water use efficiency (WUE), and maximum stomatal conductance (gs_{max}). The purpose of this analysis was to reduce the number of variables as we suspected drought adaptation traits would co-variate among arid adapted and among mesic adapted milkweed plants. Constitutive drought adaptation trait values were represented by plants in the control treatment (n=61), with 3-5 replicates per *Asclepias* species. Water use efficiency (WUE) was estimated by measuring δ_{13}C from a subset of plants (n=31), thus we did not have δ_{13}C values for all replicates. We approached missing data by assigning the average trait values per species to the missing values (imputing the means). PC analysis was conducted in R (V.3.5) using the built-in function "prcomp" with center and scaling arguments set as "TRUE" for data normalization.

Results

Our PC analysis shows that the drought adaptation traits measured in this study (SLA, WUE, water content, and gs_{max}) did not load into a single principal component suggesting that these traits do not co-variate within the *Asclepias* genus. SLA and gs_{max} loaded more strongly on the first principal component (0.57 and -0.67, respectively) and were negatively correlated indicating that, plants with higher SLA have generally lower gs_{max} or more stomatal regulation. Two traits, δ_{13}C and water content loaded more strongly on component 2 with loadings of -0.74 and 0.50, respectively. These traits were also negatively correlated such that, the higher a plant's water content the lower its WUE (Fig. S3). The first two principal components contributed to 58% to the variation in the data.

Importance of components:

	PC1	PC2	PC3	PC4
Standard deviation	1.135	1.0269	0.9679	0.8488
Proportion of Variance	0.322	0.2636	0.2342	0.1801
Cumulative Proportion	0.322	0.5857	0.8199	1.0000

PC Loadings

	PC1	PC2	PC3	PC4
SLA	0.5725387	-0.3961292	0.4354255	-0.57068884
RWC %	-0.3104444	0.5025199	0.8056175	-0.04559029
WUE	-0.3421149	-0.7448910	0.3581055	0.44705162
gs_{max}	-0.6773338	-0.1889253	-0.1820591	-0.68729951

Figure S5. PCA ordination plot

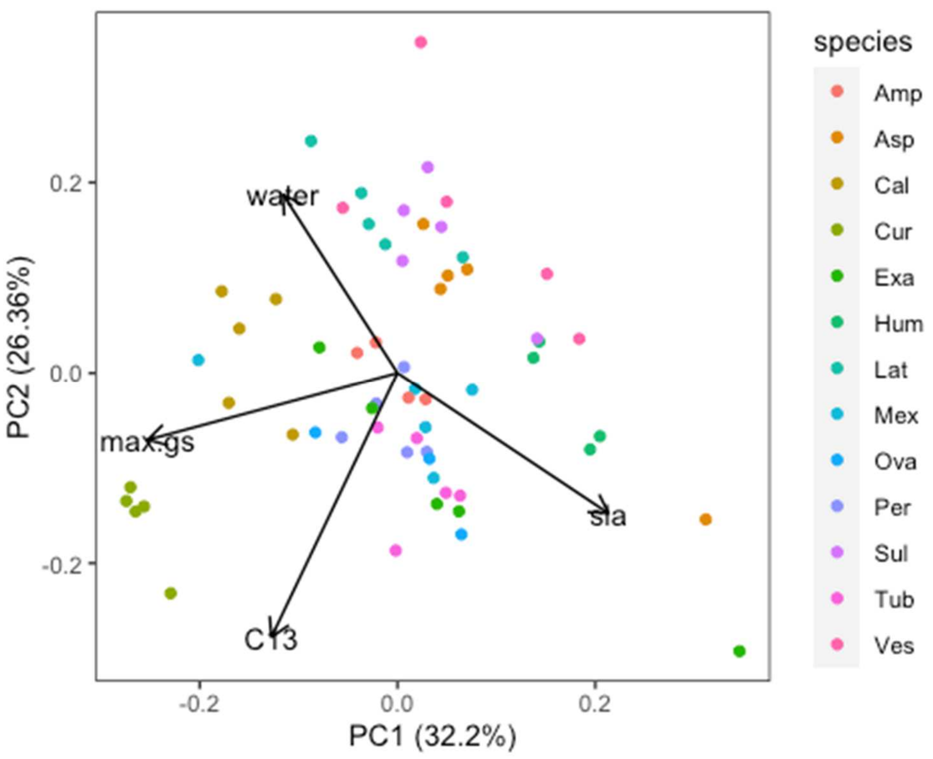


Figure S6. Herbivore survival by species in the control and drought treatment

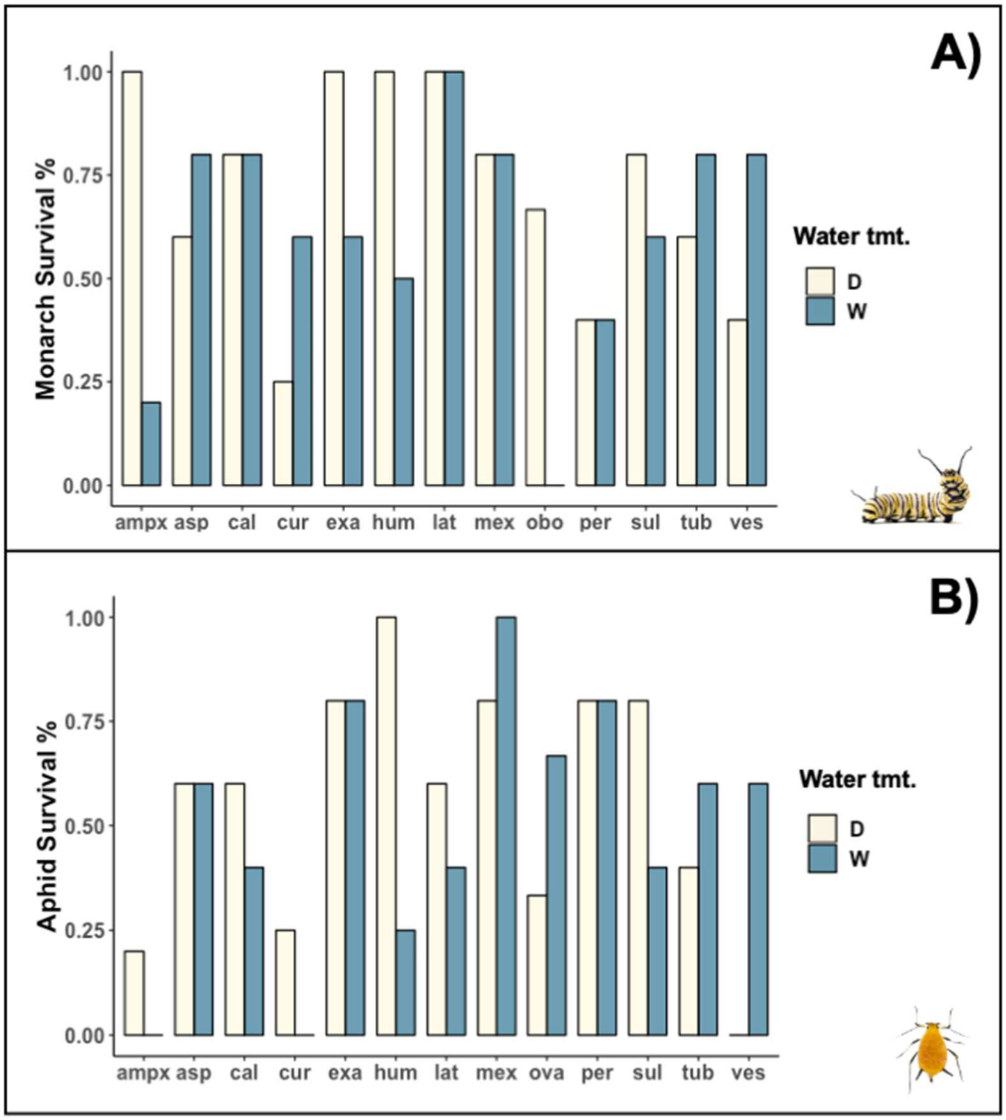


Fig. S6. Proportion of monarchs (A) and oleander aphids (B) that survive in each Asclepias species by treatment. Light blue bars represent herbivore survival in the wet (W) treatment and light yellow in the drought treatment (D). Species codes: ampx= A. amplexicaulis, asp= A. asperula, cal= A. californica, cur= A. curassavica, exa= A. exaltata, hum= A. humistrata, lat= A. latifolia, mex= A. mexicana, ova= A. obovata, per= A. perennis, sul= A. sullivantii, tub= A. tuberosa, ves= A. vestita.

Figure S7. PIC correlations between drought effects on herbivore survival and water-use strategy traits

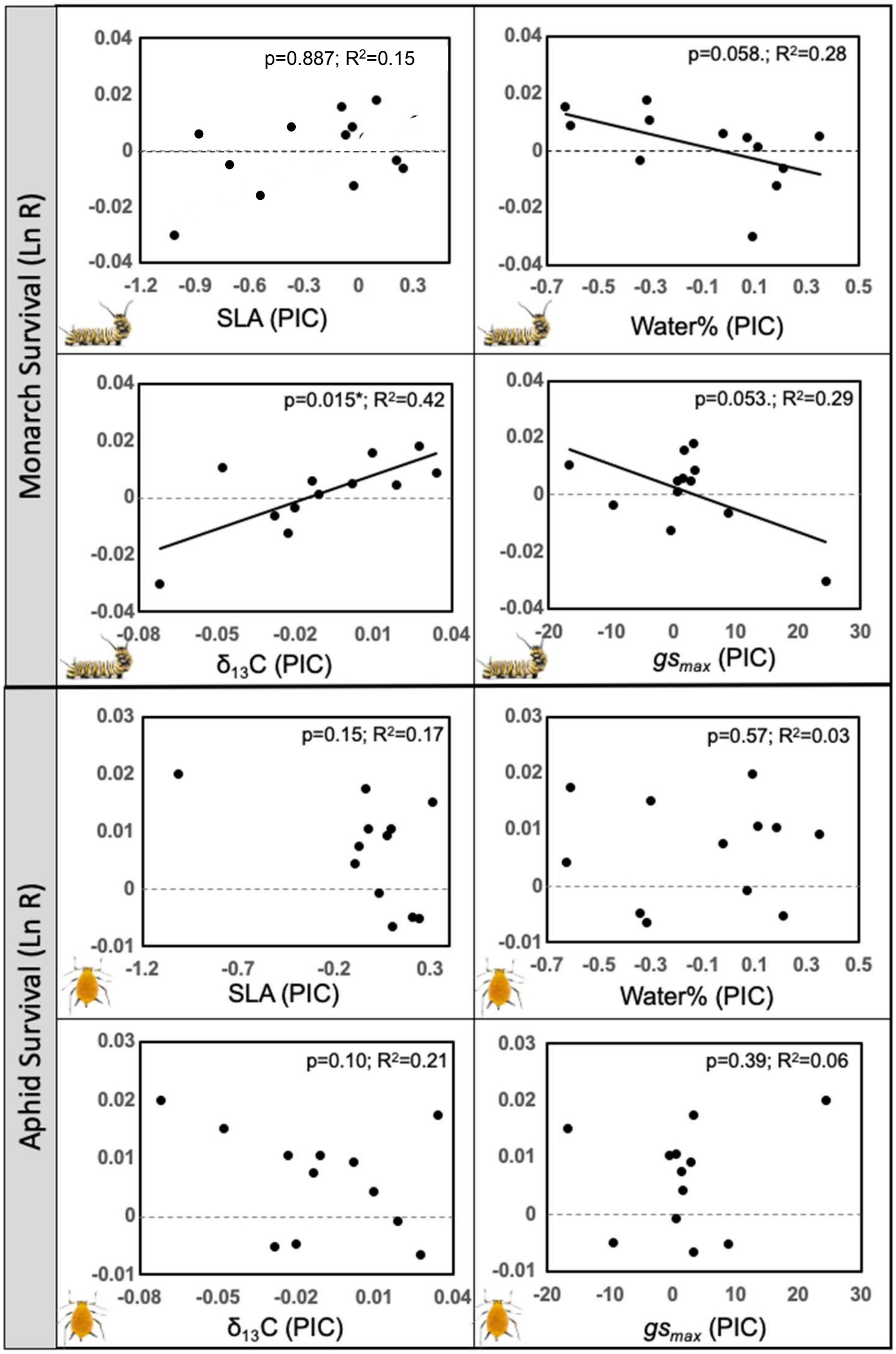


Fig. S7. Phylogenetically corrected correlations between drought effects on herbivore survival (monarch [top panels], and aphid survival [lower panels]) and plant traits associated with water use strategies. Each dot represents a phylogenetic contrast.

Figure S8. Plant traits associated with host plant quality in the control and drought treatment

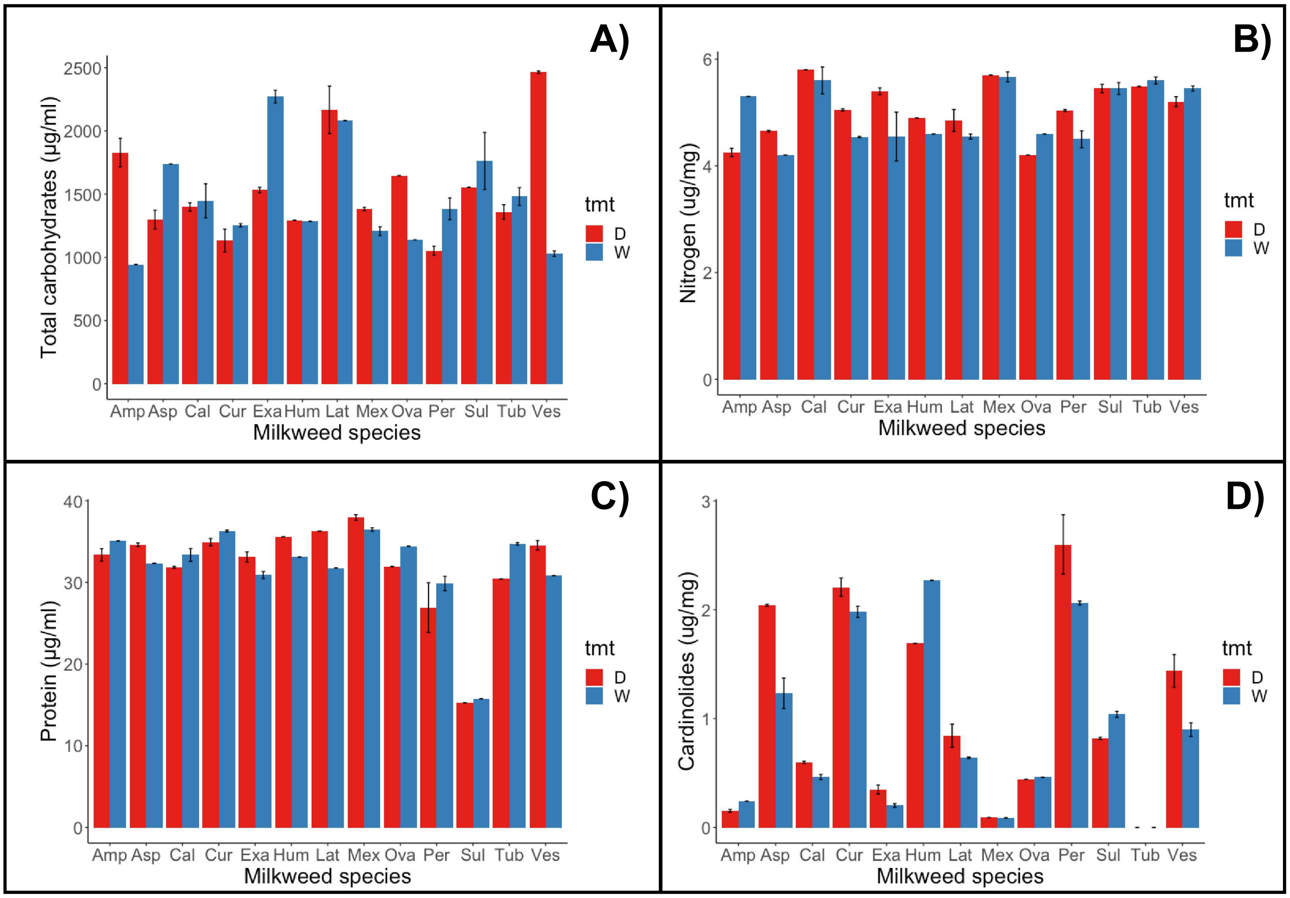


Fig. S8. Plant quality traits measured per milkweed species by treatment represented by nutrient content including total non-structural carbohydrates (A), nitrogen (B), and protein (C) and cardenolides concentrations (D). Red bars represent the milkweed species in the drought treatment (D) and blue the wet/control treatment (D). Error bars represent one standard deviation. Species codes: ampx= *A. amplexicaulis*, asp= *A. asperula,* cal= *A. californica*, cur= *A. curassavica*, exa= *A. exaltata*, hum= *A. humistrata*, lat= *A. latifolia*, mex= *A. mexicana*, ova= *A. obovata*, per= *A. perennis*, sul= A. *sullivantii*, tub= *A. tuberosa*, ves= *A. vestita*.

Fig. S9. PIC correlations between drought effects on herbivore survival and drought effects on hostplant quality traits

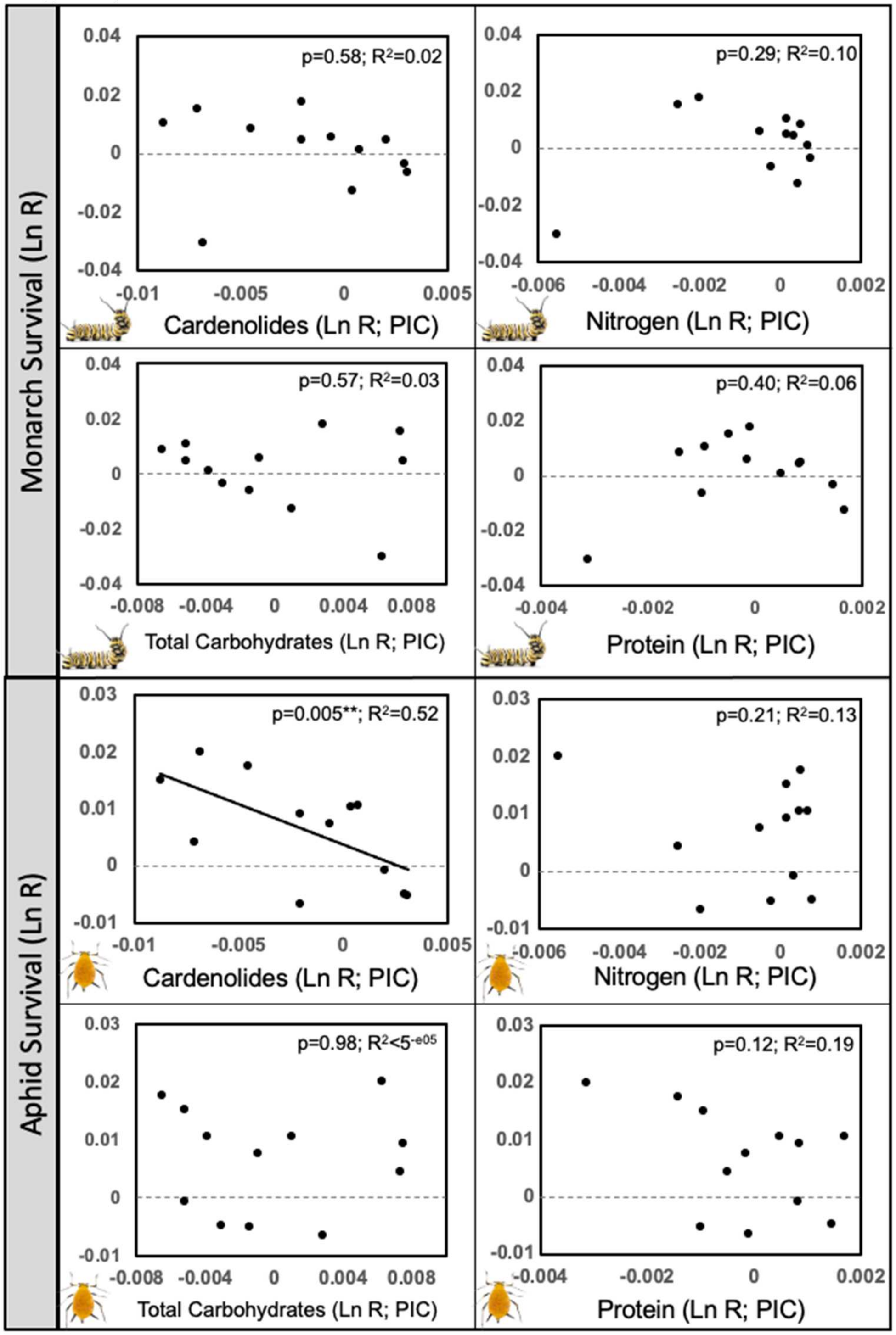


Fig. S9. Phylogenetically corrected correlations between drought effects on herbivore survival (monarch [top panels], and aphid survival [lower panels]) and drought effects on plant traits associated with hostplant quality. Each dot represents a phylogenetic contrast.

APPENDIX 3

Table S1. Plant volatile organic compounds

Chemical compound	Chemical functional group
1-Decanol	alcohol
1-Octanol, 2,2-dimethyl-	alcohol
1-Octene	alkane
2-Ethyl-1-hexanol	alcohol
2-n-Hexylcyclopentanone	ketone
2-Pentylcyclopentanone	ketone
2-Undecanone, 6,10-dimethyl-	ketone
2,6-Dimethyldecane	alkane
3-Heptanone	ketone
3-Heptanone, 5-methyl-	ketone
Benzene, (2,2-dimethylbutyl)-	benzenoid
Butane, 1-iodo-	alkane
Butane, 2-isothiocyanato-	isothiocyanate
Cyclopropane, 1-methyl-2-octyl-	alkane
d-Menthol	monoterpene
Hexyl octyl ether	ether
Isobutyl isothiocyanate	isothiocyanate
Isopropyl isothiocyanate	isothiocyanate
Nonane, 4-ethyl-5-methyl-	alkane
Nonane, 5-butyl-	alkane
Undecane, 3,8-dimethyl-	alkane

Table S2. Glucosinolates compounds

Compound	**Chemical functional group**
1-(hydroxymethyl)ethyl	aliphatic
1-methylethyl	aliphatic
1-(hydroxymethyl)propyl	aliphatic
2-methylpropyl	aliphatic
1-methylpropyl	aliphatic
4-hydroxyindol-3-ylmethyl	indole
indol-3-ylmethyl	indole
-methoxyindol-3-ylmethyl	indole
benzyl	aromatic
2-phenethyl	aromatic
2-hydroxyl-2-phenethyl	aromatic
Unknown 1	aliphatic
Unknown 2	aliphatic
Unknown 3	aliphatic