THESIS

# IMPLICATIONS OF GLOBAL CLIMATE CHANGE ON COW PARSNIP IN COLORADO: FROM FLOWERING PHENOLOGY TO MULTITROPHIC INTERACTIONS

Submitted by

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# ABSTRACT

# IMPLICATIONS OF GLOBAL CLIMATE CHANGE ON COW PARSNIP IN COLORADO: FROM FLOWERING PHENOLOGY TO MULTITROPHIC INTERACTIONS

Plants play a central role in structuring nearly all terrestrial communities. As ectothermic organisms, the synchronization of plant and insect life history events (phenology) are strongly dependent on temperature and precipitation, two of the major components of climate change. Plants can serve as indicator species for how climate change might alter community composition that contributes to ecosystem and landscape stability. I investigated elevation as a proxy for climate change from ten spatially separate Colorado populations of cow parsnip *Heracleum maximum* Bartram (Apiaceae) in both 2017 and 2018. I studied, plant flowering phenology and plant trait measurements that attributed to plant fitness measures (seed production and weight). Additionally, I investigated floral visitors in association with flowering phenology and how the presence or absence of parsnip webworm *Depressaria radiella* Goeze (Lepidoptera: Depressariidae) impacted pollinators visiting plants.

I found that elevation does not clearly explain phenological differences amongst *H. maximum* plants in high elevations (>2600m), yet insect diversity decreases as elevation increases. Additionally, *D. radiella* presence increases plant seed production, and secondary plant umbels compensate for consumed primary umbel flowers and fruit. Additionally, male flowers with pollen or female flowers with nectar do not significantly affect the insect families that visit them. Lastly, both abiotic conditions and biotic plant-insect interactions may contribute to multitrophic community assemblages under warming temperatures.

# ACKNOWLEDGEMENTS

I would like to thank my advisor Paul Ode for helping throughout the process of this master’s thesis, without which my pursuit for ecological questions would not have been as exciting nor nearly as successful. I would also like to thank my committee members Arathi Seshadri and Lou Bjostad, as well as the Ode lab for their encouragement, comments, and feedback. I would also like to thank my family, friends, and peers for their commitment to keeping me sane in times of chaos and for their kindness and support. Lastly, to Sarah who has supported me through sleepness nights, mid-thesis crises, and the stresses of being me….I can’t thank you enough.

# DEDICATION

This research is especially dedicated to my mother and father Marcie and Jeffrey Smith whom helped raise me to ask questions and look for answers. To my brothers Josh, Andrew, and Shlowmo who accept and humble me. To my grandparents, Natalie VanBueren, Arnold and Shirley Enkelis who always encouraged me to learn as much as I can. To Mary Alice Neal and Stanley Rappaport who keep me sharp and challenge how I look at the world. To Jim Bird Smith who taught me to make fudge and to listen, really listen. To my uncle, Mark VanBueren and the VanBueren family that taught me to be prepared and occasionally ‘relax’. To the Smiths, especially Matt, that showed me how to be one with nature and sometimes find yourself getting lost in it. To the DePerrys, Youngs, Buffs, Enkelis’, Vivians, Dennisons, Jirkils, and Hays that remind me that family means everything, and that I probably wouldn’t have even survived without them to keep me out of trouble. Lastly, to my teachers over the years for which none of this would have been possible.

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## Introduction

Plants play a central role in structuring nearly all terrestrial communities and providing ecosystem services. As ectothermic organisms, the timings of plant and insect life history events (phenology) are strongly dependent on temperature and precipitation, two of the major components of climate change. As such, plants can serve as indicator species for how climate change might alter community composition and trophic level structuring that in turn contribute to ecosystem and landscape stability. Yet, less than a quarter of all ecological studies published in over the last three decades focus on multitrophic interactions between plants and insects and the ecosystem services they provide (Carmel *et al.* 2013). A multi-species perspective will result in greater ecological insights by addressing direct and indirect responses to global climate change.

Global climate change has created drastic alterations in the mean and variance of temperature and precipitation that affects species occurrence and abundance. Global land temperatures are predicted to increase 1.2-4.8˚C above current conditions between the years 2081-2100 (IPCC 2013 Table 12.2). By altering the development and geographic ranges of species, climate change influences how and when species interact (Memmott *et al.* 2007, Gilman *et al.* 2010, Potts *et al.* 2010, Schweiger *et al.* 2010). With decreasing levels of precipitation and increasing temperatures resulting from increased $CO_2$ concentrations flowering time for several herbaceous plants are occurring earlier and lasting longer (IPCC 2013). Consequently, ecological Interactions between plants and their insect herbivores and pollinator associates may be modified due to asynchronous responses to climatic conditions resulting in phenological mismatches. Altered plant-insect interactions due to climate change can affect community assemblages specifically plant-pollinator mutualisms (Aizen *et al.* 2008).

Climate change can directly impact species through inducing phenological shifts resulting in possible trophic mismatches as well as promoting range expansion and local adaptation (Bradley *et al*. 2010, Dale *et al.* 2001, Van der Putten *et al.* 2010, Wolkovich *et al.* 2013). Phenological mismatch is defined as the asynchrony of species life histories that results in disrupted trophic associations (Durant *et al.* 2007). For example, the timing of pollinator flights must coincide with the availability of floral resources if pollinators are to access pollen or nectar and subsequently spread pollen to receptive flowers resulting in increased plant fitness. If flower bud-burst is early and seen in warm years as with several sub-alpine plants in the Colorado Rockies, plant fitness rates may decrease due to missed pollination opportunities from pollinator species whose flight phenologies lag-behind or accelerate past floral phenology (Inouye 2008). As temperatures increase, species may expand their ranges to occupy newly habitable conditions (Atwater *et al.* 2018). Species that require cool conditions to survive are predicted to either perform more poorly or compensate physiologically under warming climatic conditions (Śniegula *et al.* 2011).

Plant-insect interactions are also impacted by species competition and selection pressures such as herbivory (Bokhari *et al*. 2007). For example, plants respond to herbivore tissue damage with defensive chemical strategies (Joshi and Vrieling 2005). As temperatures warm the production of secondary metabolites by plants at higher elevations/latitudes may increase in response to increased herbivore pressure moving upward in elevation and latitude (Moreira *et al.,* 2018). If novel species are able to enter a community due to changed conditions created by global warming, they may also indirectly re-arrange niche roles that structured previous communities. Invasive and/or non-indigenous species thus add to the harmful effects of global climate change, which today is one of the greatest drivers of species extinction to date (Ceballos *et al.* 2017).

Invasive species throughout this literature review are characterized by their ability to rapidly reproduce and spread causing ecological and economic harm (Pyšek *et al.* 2004, Beck *et al.* 2008). Multiple hypotheses have been developed to explain the complex mechanisms by which both plants and phytophagous insects become invasive (Table 1). Invasions seldom affect only one species. Rather, multiple species and their communities are often affected. As such, there is currently neither a completely accurate nor single way to interpret the full effects of global climate change on all species. One suggestion to improve ecological insight is to first acknowledge that multiple species are impacted simultaneously and in complex ways by global climate change. Further, predicting impacts on global landscapes and ecosystems starts with considering habitats today-with their communities of various species.

This introductory chapter aims to provide a brief background on how the various facets of climate change (phenological shifts and possible mismatches, range expansion, and successful invasive species establishment) alter plant-pollinator community assemblages (Figure 1.). By focusing on plant-pollinator interactions as a basis for these mechanisms, my aim in this introduction is to provide support for the argument that multitrophic species studies are important for investigating global climate change. The goal for my thesis is to provide a foundation for understanding the ramifications of global climate change on community structure and function with a specific focus on the added complexities and impacts that go beyond single species. The scope of this introductory chapter will discuss 1) climatic effects on plants and their pollinators, and 2) shifts in insect assemblages and pollinator suites under warming conditions.

Additionally, this introduction portrays the complex ecological concepts that surround plant-insect interactions involving cow parsnip *Heracleum maximum* Bartram (Apiaceae). The following two chapters investigate the role of elevation as a proxy for climate change and its effect on plant phenology and multitrophic interactions involving *H. maximum*, its pollinators, and herbivore parsnip webworm *Depressaria radiella* Goeze (Lepidoptera: Depressariidae). This introduction ultimately aims to provide a foundation for how we think about climate change when looking into multitrophic interactions and community structure and assemblages.

## Climatic effects on plants and their pollinators

Plant traits are highly variable amongst species and are frequently studied through measures of fitness, fecundity, life span, photosynthetic rate, stomatal conductance, leaf area ratio, specific leaf area, leaf N content, underground (root) biomass and aboveground (shoot) biomass (Van Kleunen *et al.* 2010). These traits are related to patterns of reproduction and growth and are both affected by and affecting interspecific relationships and interactions. Plant chemical defenses (e.g., toxins, volatiles, etc.) for example, mediate relationships of the plant with other species including other plants, fungi, and insect herbivores and pollinators. Chemical cues (volatile organic compounds; VOCs) or toxic compounds reduce herbivory and fitness losses by attracting natural enemies of herbivores or directly providing resistance to herbivory (Figure 2) (Kessler and Baldwin 2001). VOCs are also used by the plant to attract pollinators (Farré-Armengol *et al.* 2013), where common attractant and defensive VOCs produced by some plants in the Apiaceae is included in Table 2. Production of VOCs is sensitive to temperature and can vary spatiotemporally (i.e., hourly, daily, seasonally, and annually) (Krischik and Denno

1983, Zobel and Brown 1990, Schweiger *et al.* 2010). VOCs in addition to both morphological and physiological flower signals (shape and color) and floral rewards (pollen and nectar), aid in pollinator attraction (Schiestl *et al.* 2014) and are specific to insect or pollinator functional types (*see* Gilman *et al.* 2010).

In addition to how plants mediate insect interactions, climate change can result in the asynchronization or phenological mismatch with mutualist pollinators that help spread pollen (Durant *et al.* 2007, Hegland *et al.* 2009). For example, Alaskan sockeye salmon spawn and kneeling angelica (*Angelica genuflexa*) flowering phenology were found to match-up with that of the blowfly (Calliphoridae) pollinator development (Lisi and Schindler 2011). Blowfly adults require *A. genuflexa* nectar to produce eggs, while larvae required remnants of salmon protein to mature. *Angelica genuflexa* plants required several species of calliphorids for pollination. Similar to the example above, plant species exhibiting obligate outcrossing are expected to see the greatest impacts of climate change and phenological shifts between plants and their mutualist pollinators (Potts *et al.* 2010).

Warming conditions affect plants' ability to offer pollinators sufficient floral rewards as nectar volume may decrease and pollinator-attractive VOC compositions can dissipate more quickly (Farré-Armengol *et al.* 2013). Other evidence of shifting life history phenology involves decreased snowpack and earlier flowering time in sub-alpine flowers in Gothic, CO (Inouye 2008). A meta-analysis of 429 plant species and over 1,400 pollinator species, showed that plant-pollinator phenological mismatches could account for up to 50% reduced floral resources available to pollinators (Memmott *et al.* 2007). In support of this work, increased warming has been shown to be associated with increased rates of insect herbivory and expanding insect ranges that coincide with warmer habitats (Dukes *et al.* 2009: Currano *et al.* 2008 and Parmesan 2006).

The following section discusses how insect herbivory complicates plant-pollinator interactions under the scope of global climate change.

### *i. Herbivore effects on plant-pollinator interactions*

Variable host plant quality, environmental conditions, and herbivore pressure affect pollinator choices. Herbivory can mediate changes in defensive plant traits as well as impact energetic resources needed to produce vegetative and reproductive plant tissues (Joshi and Vrieling 2005, Handley *et al.* 2008). Specialist herbivores while better than generalists at metabolizing plant chemical defenses induced by their host plants (Berenbaum *et al.* 1991) are also further threatened by declining plant populations under global climate change (Memmott *et al.* 2007).

Chemical defenses and herbivory can decrease the size of floral displays negatively affecting pollinator attraction and detection to host plants, ultimately lowering plant fitness (Strauss 1997, Takabayashi and Dicke 1996, Jogesh *et al*. 2013). Parsnip webworm (*D. radiella)* herbivory for example, host plant quality by consuming flowers and fruits in cow parsnip (*H. maximum*) (Lohman *et al.* 1996). Plant defense production and diversity are affected by herbivore pressure and frequency as well as changes in elevation gradients and temperature (Moles *et al.* 2011, Rasmann *et al.* 2014). Relationships of floral volatiles and floral displays with pollinators are crucial for plant fitness and are intensified by phenological lags and pollinator asynchronization when plants depend on out-crossing pollination. In sticky polemonium (*Polemonium foliosissimum*), the majority of plants that flower in synchrony (higher floral density) experience lower seed-set and fewer pollinators (Zimmerman 1980). However, staggered flowering time within a population can also increase fitness advantages for individuals that receive more outcrossing events or pollination (Rathcke and Lacey 1985).

Volatile secondary metabolites serving as chemical communication might help indicate how plant-pollinator-herbivore relationships are formed and/or change over time. Attractive floral olfactory cues are positively associated with plant fitness, but pollinators also depend on visual cues such as: flower shape, color and morphology in host selection (Schiestl 2014). Although, there is debate surrounding the mechanisms of how insects choose their host plants and what strategies plants use for pollination attraction and herbivory defense (Bruce *et al.* 2005, Fraenkel 1959), plant chemistry forms a foundation for guiding plant-insect interactions. These chemical cues help determine how a plant navigates its interactions with herbivores and pollinators.

## Shifts in insect assemblages in response to climate change

Plant community composition can drastically change in association with the abundance and richness of its insect herbivores and pollinators (Strauss 1997, Fontaine *et al.* 2015). Changes in plant-pollinator community assemblages can reflect preferences for flower functional types (e.g., tube-shaped flowers over open flowers) attracting pollinators with distinct morphologies (e.g., bees with long tongues, moths with proboscis, and short tongued flies). For example, in a study conducted in field cage experiments in France, Fontaine *et al.* (2015) found that a mixture of plant functional types performed better (higher fitness, greater seed-set) when communities included diverse functional types of pollinators (i.e., syrphid fly and bumblebee species) as opposed to communities associated with one functional pollinator type. Because insect functional types are associated with both generalist and specialist pollinators, it is suggested that distinct pollinator species maintain similar effectiveness across plant-pollinator networks (Waser *et al.* 1996).

In plants in the family Apiaceae, such as hogweed (*Heracleum sphondylium*) and wild parsnip (*Pastinaca sativa*), male-stage flowers that bloom prior to female flowers receive the most pollinator visits (Zych 2007, Hendrix and Trapp 1981, Lohman *et al.* 1996). If pollinators respond similarly to their plant host(s) with respect to environmental gradients, they should coincide with the availability of resources and floral rewards across the elevational gradient (Burkle and Alarcón 2011). This retention of resources and floral rewards that plants and their insect associates share may be conserved overtime within a particular niche (i.e., niche conservatism) (Wiens *et al.* 2010). Niche conservatism theory explains that small environmental changes have less evolutionary impact on species expansion and community structure due to phylogenetic conservation of species within their specified niche (Wiens *et al.* 2010). Species experiencing climate change have been found to shift their geographic ranges within their historic 'ancestral environments' versus adapting locally (Wiens *et al.* 2010: Eldredge *et al.* 2005). Plant evolution responding to pollinator selection pressure or pollinators adapting to plant evolutionary convergence and/or divergence is reciprocal, and the strength of conservatism may strengthen future directions in plant-pollinator assemblages.

Plant-pollinator community assemblages may be affected by shifts in both pollinator functional type (Gratton and Denno 2005) and environmental and biotic selection pressure. While warming temperatures are forcing species upward in elevation, species in cool climates have constricting ranges, where distributional changes or range change occurs at various rates (Menéndez 2007). Plants and pollinators may tolerate both warming temperatures and fluctuating herbivore populations under global climate change in different ways (Lortie *et al.* 2004). Pollinator guilds may be generalized for example in their interactions with plant species, but they may also be plastic in their ability to utilize changing resources (Burkle and Alarcón 2011).

The abundance and richness of plants also help structure plant-pollinator community assemblages. For example, the California endemic gunsight clarkia (*Clarkia xantiana*) in populations with congener plant species were more efficiently and frequently pollinated by specialist pollinators in comparison to *C. xantiana* populations alone (Moeller 2005). As several generalist pollinator species have redundant pollination functions in plant communities, it is thought that generalists may shift easily with plants under warming conditions (Memmott *et al.* 2004), while specialist pollinators will be less successful in adapting. Although, it is still unknown how much of a role the differentiation of pollinator functional type really plays in the success and stability of a community. Global climate change directly and indirectly affects plant-pollinator communities. Indirect species effects, such as pollinators increasing body size and rates of herbivory and plants shifting phenology and growth have more strongly been associated with changes in plant-insect community structure and assemblages than direct climate effects alone (Gilman *et al.* 2010).

Community-level pollination structures shift with spatiotemporal time-frames (i.e., daily, weekly, seasonal, or annual) creating fluctuations in pollinator frequencies (Burkle and Alarcón 2011). In studies looking at pollinators across several years Dupont *et al.* (2009) found that less than 25% of insect interactions were re-observed in the consecutive year, while Lazaro *et al.* (2010) found that insects depend on flowering time, duration and synchronization with their host plants. Phenological matching of plants and their associated pollinators changes temporally (Burkle and Alarcón 2011) and can shift with elevation and climatic conditions. In a review focusing on plant-insect phenological mismatch, great tits (*Parus major*) exhibit early egg laying that precedes the peak time of winter moth (*Opheroptera brumata*) abundance (Visser and Both 2005). *Opheroptera brumata* larval development also depends on the availability of new vegetative growth by oak trees (*Quercus robur*) which relies on synchronized bud-burst and flowering time. Larvae emerging on leaves prior to or after new leaf growth exhibit reduced fitness due to a mismatch in leaf chemical defenses which are more potent in older leaf tissues (Visser and Both 2005). Climate change results in phenological mismatch between some plants and their insect associates.

Alpine sky pilots (*Polemonium viscosum*) pollinator suites shift with changes in floral chemistry, where beetles and flies are attracted to plants at lower elevation and bees and bumblebees are attracted to plants at higher elevations (Galen 1989). In experimental studies of wild radish (*Raphanus raphanistrum*), pollinator visitation was investigated between plants experiencing herbivory by cabbage white butterflies (*Pieris rapae*) and plants not experiencing herbivory (Strauss 1997). Pollinators are less likely to visit herbivore-damaged plants, and as a result male *R. raphanistrum* flowers from damaged plants produced less pollen than undamaged plants (Strauss 1997). Yet, plants that produce defensive metabolites in large concentrations may exhibit ecological costs in terms decreasing attractiveness to pollinators, thus decreasing plant reproductive opportunities and fitness (Züst and Agrawal 2017). There remains a complex balance in plant-pollinator community assemblages where plants attract and present themselves to pollinators and pollinators experience choices that match flower availability and resources provided to them. Shifts in insect assemblages depend on the type of insects involved and their tolerances to climate change, resource fluctuation, and the conserved links with their plant host(s) (Menéndez 2007).

### *i. Pollinator species across elevation gradient*

With changes in global climate insect pollinators may: 1- locally adapt to their environmental conditions, 2-shift from native to invasive plants (host-shift), 3-compete between invasive and native plant communities, or 4-migrate or go extinct (Memmott *et al.,* 2007, Schweiger *et al.,* 2010, Vanbergen *et al.* 2018). With the potential for altered effectiveness in outcrossing pollen, increased stigma clogging and hybridization, many plants depend on their pollinators for survival (Schierenbeck and Ellstrand 2009). However, plant-herbivore-pollinator interactions often change across elevation. For example, plant floral characteristics associated with more generalist pollinators may have greater phenotypic variation than plants with more specialized pollinators (Fenster *et al.* 2004). Additionally, plants at lower, warmer elevations or sites near the equator are thought to have increased herbivore pressure and thus produce more defensive traits (i.e., latitudinal herbivory-defense hypothesis) (Anstett *et al.* 2018, Moreira *et al.* 2018). However, with increasing elevation, plant strategies may be more variable than traditionally thought, as climatic conditions are not always consistent moving towards higher latitudes or elevations. For example, chemical defenses were not found to increase with latitude in a study investigating latitudinal herbivory-defense hypothesis (LHDH) in 80 *Oenothera* species (Onagraceae) in North and South America (Anstett *et al.* 2018).

Community networks of plant-pollinator interactions rely on the frequency of connections between species (i.e., links) (Waser *et al.* 1996). In general pollinators are nested within plant communities and are associated by connectedness, whereby generalist pollinators have the most links and specialist pollinators have the least links (Waser *et al.* 1996, Memmott *et al.* 2004). While there are no significant trends across latitude for plant-pollinator relationships it is thought that temperate habitats have more generalist pollinators than tropical habitats (Ollerton and Cranmer 2002). Considering diversity, tropical habitats are more species rich than temperate habitats, but specialist pollination syndromes in the tropics may not be more ecologically specialized than temperate habitats (Ollerton and Cranmer 2002, *see* Van Kleunen *et al.* 2010 for alternative evidence). This is to say that ecologically and functionally, plant-community assemblages may not fully rely on species richness and diversity, but strengths and connectedness of species involved in the community network. In relation to the latitudinal herbivory-defense hypothesis mentioned previously, this hypothesis may not be as strongly supported when fluctuations in environmental conditions, herbivore selection pressure, and the ability for plants to adapt to their changing landscapes is based on community network linkages, and not simply latitude (Kergunteuil *et al.* 2018).

It is clear that plant-pollinator assemblages do rapidly change. Insects influence plant fitness rates and plant diversity influences the types of pollinators present in a community. Following the removal of non-native common reed (*Phragmites australis)* and the re-introduction of regional smooth cordgrass (*Spartina alterniflora*) to New Jersey, salt marsh native arthropod community assemblages returned to surrounding plant species, thus rearranging higher arthropod trophic levels (Gratton and Denno 2005). Meanwhile, looking toward multitrophic arthropod interactions, congener hybridization between yellow toadflax (*Linaria vulgaris*) and dalmatian toadflax (*Linaria dalmatica*) in non-native ranges has altered the efficacy of species-specific weevil biocontrol agents (Ward *et al.* 2009). Although, hybridization and phenotypic plasticity can explain rapid adaptation to environmental and climatic changes, plant-pollinator community assemblages are impacted by biotic pressures in addition to environmental conditions.

Lastly, it is obvious that there remain several gaps in the literature in our understanding of climate change on plant-pollinator community assemblages. While environmental conditions fluctuate with climate change and types of pollinators respond differently to plant communities and resources. It may be best to focus more on the mechanisms of how and why communities change prior to creating future plant-pollinator community predictions. Lortie *et al.* (2004) suggest that the concept of integrated communities (IC) may best fit unpredictable and complex questions related to the ecological processes behind plant community assemblage variability. Yet, I do not believe that there is, nor ever will be, any one clear answer.

## Discussion

It is common to look for responses to multiple environmental stressors. Abiotic changes in temperature, $CO_2$, salinity, soil moisture, and nutrients create changes in the environments that species live in. Plants are studied for their responses to these direct changes by metabolic functions and partitioning of the remaining resources. Insects responded to plant traits creating ties that structure communities and scaffold ecosystem dynamics. Arguably, climate change is altering both resource availability as well as species relationships.

Plant-pollinator community assemblages are typically strengthened with increasing species biodiversity. Species richness and diversity may be impacted by both direct and indirect effects of climate change. For example, resource levels fluctuate, phenologies shift, and pollinators may miss opportunities for pollination. Invasive species that enter native communities that are slower to respond to climate change may better match these habitat conditions. More so, insect functional types generally fit into categories of plant functional needs, where links of community connectedness can be repetitive or conserved to niches. Higher trophic level interactions are difficult to predict when there is still much to research and study at the level of the plant in its community. Between trophic levels, resources are directed by herbivore selection pressure, elevational gradient, and environmental conditions.

Lastly, plant communities have various insect species that may not be successful in altered habitats under warming conditions. It should be noted that spatial and temporal scale are important factors when addressing multitrophic interactions (Moreira *et al.* 2018). As scale can be arbitrary in the measurement of natural populations of moving species in a community, this too can affect future results and patterns. Agrawal (2011) points out that short-term common garden studies cannot possibly reflect the dynamics, changes, and conditions related to community-level interactions. Work by Davies *et al.* (2007) similarly voiced these concerns in that productivity of Californian serpentine sites was statistically significant across three separate spatial scales, but species diversity for native and exotic species relationships were positively correlated at each individual spatial scale. For example, the smallest spatial ‘high productivity’ sites ($1m^2$) species diversity decreased, but at ‘low productivity’ sites of the same size species diversity increased.

In conclusion, how plant-pollinator assemblages change and react under global climate change is still unknown. Ecological concepts are complex, and several aspects of abiotic and biotic conditions are difficult to predict in conjunction with natural systems. Maybe something can be said instead toward the types of information currently being used to address these and similar ecological questions. Where global climate change threatens to impact more than just individual species but communities and ecosystems. Under warming conditions ecological models currently predict greater changes to ecosystem stability, and as such it is imperative that we currently investigate community multitrophic interactions now prior to shifts in plant-herbivore-pollinator assemblages.

# FIGURES

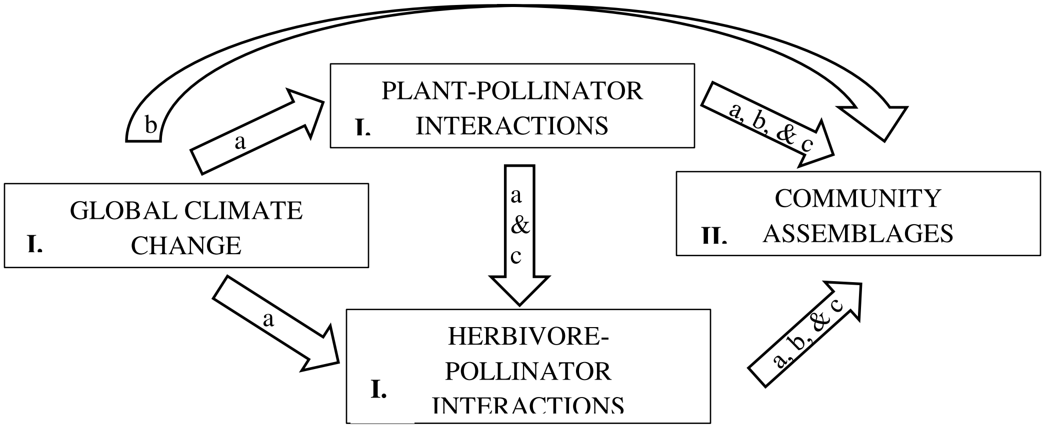

FIG. 1. Conceptual map of key topics in the introduction (squares) and the subheadings discussed within those topics (arrows). The subheadings (arrows) include **a**-phenological shifts or mismatch, **b**-successful species colonization/ invasion hypotheses, **c**- range expansion. Arrow direction denotes the directed impact on that subject area, ex. global climate change directly impacts insect assemblages via phenological shifts or mismatch. The future directions section is not included in the above diagram (no square shown) but incorporates concepts from each section to suggest strengths for multi-species studies with global climate change.

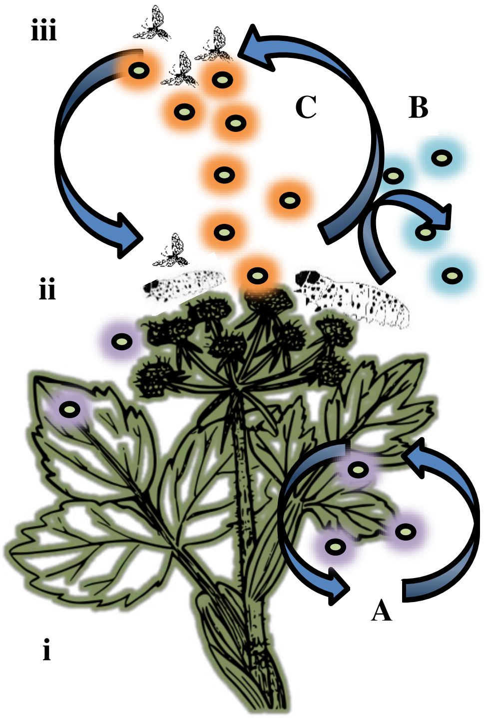

FIG. 2. Representation of volatile organic compound production in *H. maximum*; A) constitutive chemicals produced regardless of herbivory, B) herbivore-induced direct resistance to quell tissue feeding, and C) herbivore-induced indirect resistance that attracts predators/parasitoids to the herbivore. Trophic levels: i-plant (*H. maximum*), ii-herbivore (*D. pastinacella*), iii-predator/parasitoid

# TABLES

TABLE. 1 Theories and hypotheses that may address how invasive species colonize and establish in non-indigenous habitats. Examples are given from primary literature, and if not cited separately are referenced by the source in the right column.

| **Invasion theory/ hypothesis** | **Definition/Explanation** | **Example** | **Reference** |
|---|---|---|---|
| Evolution of increased competitive ability hypothesis (EICA) | Invasive species increase growth and require less energetically expensive chemical defenses due to enemy release in non-indigenous regions. | Purple loose strife plants (*Lythrum salicaria*) are larger, less defended in Germany as compared to native range plants in New York. | (Blossey and Nötzold 1995) |
| Enemy release hypothesis (ERH) | Release from specialist enemies enables plants to re-allocate resources to primary metabolism functions versus defenses. | Liu and Stiling's (2006) meta-analysis found liberation from natural enemies lowers herbivore insect richness on invasive plants versus native plants. | (Keane and Crawley 2002) |
| Advantages from functional traits | Functional traits hold a genetic basis that respond to both abiotic and biotic factors. There are innate differences in functional traits between species that can lead to greater invasive success. | Common mullein *(Verbascum thapsus)* has a seed-set of 100,000 to 223,000 seeds per plant when experiencing low to no competition or herbivory (Gucker 2008). | (Drenovsky *et al.* 2012) |
| Recruitment of other invasive species into non-indigenous regions | Invasive species may form complexes of invasive species that together take advantage of non-indigenous habitats. | *Myrica faya* recruits Japanese white-eyes (*Zosterops japonica*) to disperse seeds, both species outcompete native Hawaiian species. | (Vitousek and Walker 1989) |
| Fluctuating resource hypothesis | Environmental drivers increase invasion rates when there are more unused resources available. | Cheat grass (*Bromus tectorum*) invasion in the Great Basin has increased grass-fire regime cycles, making it difficult for native species to re-establish. | (Chambers *et al.* 2007), |
| Rapid adaptation and hybridization | Invasive hybrids are larger and more fecund compared to non-hybrid species. | Schierenbeck and Ellstrand's (2009) meta-analysis found hybridization can trigger plant invasion by mechanisms such as clonal growth, allopolyploidy, recombination, and agamospermy. | (Hovick and Whitney 2014) |
| Increased phenotypic plasticity in changing landscapes | Phenotypic plasticity may hasten invasion by allowing introduced species to adapt to and tolerate greater environmental variability than their native neighbors. | Polyploidy in spotted knapweed (*Centaurea stoebe*) increased phenotypic plasticity traits associated with native European tetraploids and diploids versus N. American tetraploid plants (Hahn et al. 2012). | (Davidson *et al.* 2011) |
| Lag-phase establishment and Propagule Pressure | Colonization and establishment may 'lag' when initial invasive species numbers (propagule size) are low or undetected. | Grevstad (1999) found that larger beetle (*Galerucella calmariensis)* propagule size increased the probability of species establishment and growth rate. | (Simberloff 2009) |

TABLE 2. Known secondary metabolites found within Apiaceae plant tissues. Where + represents defensive plant compounds and ++ are known floral volatiles.

| Secondary metabolite (+, ++) | Associated Plant | References |
|---|---|---|
| Imperatorin+ | *Pastinaca sativa 1,3*<br>*Heracleum lanatum* (*H. maximum*) 2<br>*Angelica archangelica 4*<br>*Peucedanum spp. 5* | 1-Berenbaum & Zangerl 1986<br>2-Camm et al. 1976<br>3-Nitao & Zangerl 1987<br>4-Steck & Bailey 1969<br>5-Hadaček et al. 1994 |
| Psoralen+ | *Pastinaca sativa 3,6*<br>*Heracleum lanatum* (*H. maximum*) 2<br>*Angelica archangelica 4*<br>*Apium graveolens 6* | 3-Nitao & Zangerl 1987<br>4-Steck & Bailey 1969<br>6-Peroutka et al. 2007 |
| Angelicin+ | *Pastinaca sativa 3,6*<br>*Apium graveolens 6* | 3-Nitao & Zangerl 1987<br>6-Peroutka et al. 2007 |
| Xanthotoxin+ | *Pastinaca sativa 1,6*<br>*Heracleum lanatum* (*H. maximum*) 2<br>*Petroselinum spp. 2,6*<br>*Angelica archangelica 4*<br>*Peucedanum spp. 5*<br>*Apium graveolens 6* | 1-Berenbaum & Zangerl 1986<br>2-Camm et al. 1976<br>4-Steck & Bailey 1969<br>5-Hadaček et al. 1994<br>6-Peroutka et al. 2007 |
| Isopimpinellin+ | *Pastinaca sativa 1,6*<br>*Peucedanum spp. 5*<br>*Apium graveolens 6*<br>*Petroselinum sativum 6* | 1-Berenbaum & Zangerl 1986<br>5-Hadaček et al. 1994<br>6-Peroutka et al. 2007 |
| Bergapten+ | *Pastinaca sativa 1,6*<br>*Heracleum lanatum* (*H. maximum*) 2<br>*Angelica archangelica 4*<br>*Peucedanum spp. 5*<br>*Apium graveolens 6*<br>*Petroselinum sativum 6* | 1-Berenbaum & Zangerl 1986<br>2-Camm et al. 1976<br>4-Steck & Bailey 1969<br>5-Hadaček et al. 1994<br>6-Peroutka et al. 2007 |
| Sphondin+ | *Pastinaca sativa 1,6* | 1-Berenbaum & Zangerl 1986<br>6-Peroutka et al. 2007 |
| Methyl-anthranilate++ | *Pastinaca sativa 7,8* | 7-Berenbaum & Zangerl 2006<br>8-Borg-Karlson et al. 1994 |
| 3-carene++ | *Anthriscus sylvestris 8* | 8-Borg-Karlson et al. 1994 |
| Limonene++ | *Aegopodium podagraria 8*<br>*Carum carvi 8*<br>*Thapsia villosa 9* | 8-Borg-Karlson et al. 1994<br>9- Drew et al. 2010 |
| Myrcene++ | *Anthriscus sylvestris 8,10*<br>*Laserpithum latifolium 8*<br>*Thapsia villosa 9* | 8-Borg-Karlson et al. 1994<br>9- Drew et al. 2010<br>10-Tollsten et al. 1994 |
| Cis-ocimene++ | *Anthriscus sylvestris 8*<br>*Heracleum sibiricum 8*<br>*Pastinaca sativa 8*<br>*Angelica archangelica 10* | 8-Borg-Karlson et al. 1994<br>10-Tollsten et al. 1994 |
| Trans-ocimene++ | *Anthriscus sylvestris 8*<br>*Heracleum sibiricum 8*<br>*Pastinaca sativa 8*<br>*Angelica archangelica 10*<br>*Ferula communis 11* | 8-Borg-Karlson et al. 1994<br>10-Tollsten et al. 1994<br>11- Rubiolo et al. 2006 |
| Linalool++ | *Heracleum sibiricum 8* | 8-Borg-Karlson et al. 1994 |
| α-farnesene++ | *Heracleum sibiricum 8* | 8-Borg-Karlson et al. 1994 |

| | | |
|---|---|---|
| trans-β-farnesene++ | *Carum carvi 8*<br>*Heracleum sibiricum 8*<br>*Pastinaca sativa 8* | 8-Borg-Karlson et al. 1994<br>9- Drew et al. 2010 |
| γ-cadinene++ | *Anthriscus sylvestris 8*<br>*Heracleum sibiricum 8*<br>*Pastinaca sativa 8*<br>*Thapsia villosa 9* | 8-Borg-Karlson et al. 1994<br>9- Drew et al. 2010 |
| γ -terpinene++ | *Aegopodium podagraria 8* | 8-Borg-Karlson et al. 1994 |
| α-pinene++ | *Laserpithum latifolium 8*<br>*Thapsia villosa 9* | 8-Borg-Karlson et al. 1994<br>9- Drew et al. 2010 |
| β-pinene++ | *Aegopodium podagraria 8*<br>*Laserpithum latifolium 8* | 8-Borg-Karlson et al. 1994 |
| β -caryophyllene++ | *Carum carvi 8*<br>*Thapsia villosa 9* | 8-Borg-Karlson et al. 1994<br>9- Drew et al. 2010 |

# CHAPTER 2: FLOWERING PHENOLOGY AND LIFE HISTORY OF COW PARSNIP *HERACLEUM MAXIMUM* BARTRAM (APIACEAE)

## Introduction

There are over 3,000 species of Apiaceae plants worldwide, including approximately 60 species in the genus *Heracleum* L. The majority of *Heracleum* species are native to Asia and Europe (Gültekin 2005, Pimenov and Lenov 2004). Introduced umbelliferous plants of Eurasian origin in North America include carrot, celery, parsley, dill, fennel, caraway, and anise which have been used commercially for root crops and spices. Additionally, North American native apiaceous plants such as oshá (*Ligusticum porteri*) have been used medicinally by indigenous peoples to treat sore throats, influenza, and gastrointestinal illnesses (Myhal 2017). Ecologically numerous species of herbivores and pollinators use plants in the family Apiaceae (Hilty 2017). Wild parsnip, angelica, poison hemlock, giant hogweed, sweet cicely, biscuitroot, rattlesnake master, and cow parsnip are involved in trophic interactions with numerous species (Zych 2007, Hilty 2017). As such, community interactions involving Apiaceae plants require more study. Threatened by global climate change where host plants can experience shifts in flowering time resulting in changes such as premature bud-burst or delayed seed-set, plant-insect communities involving both herbivores and pollinators are predicted to change (Memmott *et al.* 2007).

Given that plants and insects are ectothermic, global climate change may interfere with plant and insect phenological development, resource partitioning, and the ability to interact with other species (Menéndez 2007, Schweiger *et al.* 2010). In Colorado subalpine habitats, for example, insect pollinators rely on the early emergence of cow parsnip (*Heracleum maximum* Bartram) flowers for pollen and nectar resources (Robertson 1929). Under warming conditions plant-pollinator interactions in subalpine elevations (3000-3500m) may change with fluctuations in the availability of floral resources (e.g., nectar and pollen) (Schiestl *et al.,* 2014, Theobald *et al.* 2017). If cow parsnip and pollinator phenologies do not respond similarly to changes in temperature, they may become mismatched resulting in a lack of floral resources for pollinators, as well as, a lack of pollinators, and reduced seed-set for cow parsnip plants. Few studies investigate impacts of global climate change on multitrophic interactions and plant-insect communities, even though mismatches and phenological shifts are expected to impact plant-pollinator interactions and community assemblages.

Several life history and phenology studies have been conducted on Apiaceae plants: wild parsnip *Pastinaca sativa* (Berenbaum 1983, Hendrix 1984), common hogweed *Heracleum sphondylium* (Sheppard 1991, Zych 2007), poison hemlock *Conium maculatum* (Baskin and Baskin 1990), and kneeling angelica *Angelica genuflexa* (Lisi and Schindler 2011). It is necessary that time is spent to detail the life history and phenological development of *H. maximum* throughout Colorado, USA because under warming conditions multitrophic interactions may change, destabilizing ecosystem services. *Heracleum maximum* has a broad distribution across elevation (1500-3300m) and likely structures insect community assemblages ranging across several habitats (e.g., from subalpine meadows to riparian stream beds). Several plant communities and species in multiple taxa depend on these long-lived Apiaceae plants, such as *H. maximum* for ecosystem stability (Campbell 1991, Sheppard 1991).

Changes in the developmental time or phenology of a species can lead to phenological mismatch, defined as the asynchronization of life history events between trophic levels (Durant *et al.* 2007). Under warming climatic conditions, several insects may become phenologically mismatched with their host plants. Common patterns of plant-insect phenological mismatch include moths with their host plants and butterflies with nectar source plants (Visser and Both 2005, Menéndez 2007), where lepidopteran larvae emerge prior to plant bud-burst and flowering. There is a high degree of synchronization between flowering time, herbivory, and pollination in communities involving *H. maximum.* Parsnip webworm *Depressaria radiella* Goeze (Lepidoptera: Depressariidae) relies on emerging floral tissue to complete its life cycle, and parsnip webworm's parasitoid *Copidosoma sosares* Walker (Hymenoptera: Encrytidae) attacks *D. radiella* eggs laid prior to *H. maximum* flower emergence (a one-two week window) (Ode *et al.* 2004). In instances of warming conditions *D. radiella* development may occur earlier than *H. maximum* bud-burst impacting *D. radiella* and *C. sosares* trophic interactions and survival (Figure 5).

This investigation explores whether *H. maximum* phenology and life history traits vary within and between study sites in Colorado across elevation gradient (2200-3000m). Elevation may be used as a proxy for climate change when indirect changes to species interactions are elicited by phenological mismatches. Moreover, we can make predictions about how plant, herbivore, and pollinator trophic levels change across elevation in response to warming temperatures attributed to global climate change. In this investigation elevation is utilized as a proxy for climate change, where we investigate *H. maximum* phenology and its interaction with *D. radiella* and pollinators across an elevation gradient in Colorado.

### *Objectives and Hypotheses*

### *To determine if flowering phenology and associated plant life history traits in H. maximum differ across populations experiencing varied elevation in Colorado.*

As several Apiaceae species overwinter as vegetative leaves prior to flowering, plants in the genus *Heracleum* can be relatively long-lived (up to 20-25 years old) and thus depend on climatic cues to determine if they will flower or remain vegetative in unfavorable seasons (Sheppard 1991). Plants growing at high elevations are restricted by cool temperatures, snow melt, and available sunlight. Similar to some insect species, plants at high elevations may experience elevational or latitudinal compensation where floral development and phenology is hastened to achieve optimal growth in a condensed timeframe (Śniegula *et al.* 2011). However, with increased warming conditions and less precipitation plants grown in cool climates may experience range constriction from plant species moving upward in elevation, where plants experience less time to develop and mature (Menéndez 2007).

I predict that *H. maximum* plants at high elevation (>2600m) will experience delayed flowering phenology (June-July) and additionally will compensate with shorter plants and reduced seed production in comparison to plants at lower elevations. Plant reproductive trade-off patterns associated with seed-set and seed weight (Smith and Fretwell 1974, Venable 1992) would predict that plants that experience large seed-sets will have reduced individual seed weights. Total seed production may be highly variable as seen with *H. sphondylium* where 0-5000 seeds are produced per plant (Sheppard 1991). In *H. maximum,* seed-set may be more representative of seasonal climatic conditions, herbivore pressure, or resource limitation.

## Methods

### *Study Species*

*Heracleum maximum* is a monocarpic perennial plant that produce flowering stalks, first produced is a single primary umbel and then two or three secondary umbels develop subsequently. Occasionally, tertiary umbels are formed. Basal leaves are ternately compound and alternate around a hollow herbaceous stem (Hilty 2017). Umbels at primary, secondary, and tertiary positions consist of small, white disk-shaped flowers that develop centripetally in umbellets (peripheral flowers develop more quickly than central flowers) (Cruden and Hermann-Parker 1977, Nitao and Zangerl 1987). Monecious flowers consist of five petals and are protandrous whereupon male stage (staminate) flowers develop prior to hermaphroditic ‘functionally-female’ (pistillate) flowers (Hendrix 1984). Plants flower between late June and early July and are hypothesized to be self-compatible with some degree of out-crossing (Schlessman 1978, Lindsey 1982). Fleshy schizocarps (fruit) develop in late-July before drying into two-seeded mericarps which ripen on the plant in August and September (Hendrix *et al.* 1991). Seed oil ducts contain defensive furanocoumarins (e.g., xanthotoxin, isopimpinellin, bergapten, psoralen, and sphondin) (Berenbaum and Zangerl 1986, Nitao and Zangerl 1987). Additionally, plant juices are photooxidative causing severe ‘parsnip burns’ following skin exposure to UV light (Carroll and Berenbaum 2006). Typically found in moist deciduous forests, open meadows, disturbed areas, man-made stream drainages, and ditches (Esser 1995) *H. maximum* is likely sensitive to temperature fluctuation and long-term warming.

Seed germination of *H. maximum* may be similar to conditions required to germinate poison hemlock (*Conium maculatum*), which requires after-ripening and natural freeze-thaw cycles that replicate temporal and seasonal thermoperiods (Baskin and Baskin 1989, 1990). Rates of germination increase after passage of seeds through the digestive tract of grizzly bears (*Ursus arctos horribilis*) but seeds may also be consumed by black bears, sheep, cows, deer, and elk (Applegate *et al.* 1979, Campbell 1991). Seeds may vary in size and weight, in wild parsnip (*Pastinaca sativa)* secondary and tertiary umbels produce seeds with lower seed weights that germinate more effectively than heavier seeds produced in the primary umbel (Hendrix 1984, Hendrix and Sun 1989, Sheppard 1991). Production of seed-set may be dependent on photosynthetically active radiation (PAR) and canopy cover. Shaded hogweed (*H. sphondylium*) plants were found to produce fewer umbels and lower seed-sets in comparison to plants in partial and full sun (Sheppard 1991).

### *Study Site Descriptions and Map*

Ten study sites were selected across a range of elevation (2230-3050m) spanning five counties in Colorado in 2017 (Table 3). Each site included a minimum of 30 H. *maximum* plants on publicly accessible lands. *Heracleum maximum* habitat ranged from open subalpine meadows to shaded aspen understories. In 2018, six study sites (Joe Chamber's Campsite, Crooked Creek Pass, Cebolla Creek, Shambhala/69E, Cherokee Park Rd., and McClure Pass) were chosen from the original ten 2017 study sites for continuous study. A map of the 2018 study sites is indicated in Figure 3. The six sites in 2018 were chosen on the presence or absence of *D. radiella* and proximity of sites to Colorado State University, Fort Collins (CSU). Site elevation and proximity of sites from each other were also considered. These same sites are also the subject of investigation in Chapter 3.

### *Flowering phenology*

Twenty plants from each study site were randomly selected and assigned a flowering phenology rank (0-7) representative of the stage of floral development (e.g., unopened flowers, immature staminate) during each study visit from 2017 and 2018 (Figure 4). Flowering phenology at each site was tracked throughout the field season from mid-June (June 20th) to mid-August (August 18th). For each plant, flowering phenology rank (0-7) was recorded separately for the primary umbel and a single marked secondary umbel. In instances of mixed flower phenology within the umbel the more advanced flowering phenology rank was recorded when 50% or more of the flowers within an umbel exhibited that ranking. For example, if half of the flowers were rank 3 (old staminate/immature pistillate) and half were rank 4 (mature pistillate), the rank would be classified as 4, the more advanced ranking. When less than 50% of flowers were in a more advanced flowering phenology rank, the more conservative, less advanced rank was recorded (see Figure 4 rank "1" for an example).

### *Plant trait measurements*

Plant traits: number of secondary umbels, number of umbellets in both the primary and marked secondary umbel, average number of flowers per two randomly selected umbellets in the primary and marked secondary umbel, plant height (cm), *umbel diameter (cm), and *average umbellet diameter (cm) from two randomly selected umbellets were recorded for each of the sites' twenty plants throughout the 2017 (* not measured in 2017) and 2018 flowering seasons. Measurements were recorded from the primary umbel and a single marked secondary umbel in each plant. Plants were marked with an aluminum tag fastened around the stem so that they could be tracked throughout each field season, a single secondary umbel in each plant was marked with a piece of yarn (=marked secondary umbel). Plant trait measurements and observations were recorded from single site visits in 2017 but repeated for study site visits in 2018. Total visits varied per study site in 2018 due to large driving distances from Fort Collins: Joe Chamber's Campsite (n=4), Crooked Creek Pass (n=4), Cebolla Creek (n=3), Shambhala/69E (n=9), Cherokee Park Rd. (n=10), and McClure Pass (n=3).

### *Fruit and Seed-Set Count*

In addition to tracking *H. maximum* plant trait measurements, fruit and seed-set counts were monitored in July and August from sites in 2018, in 2017 fruit counts were not recorded. Initial fruit counts (flowering phenology ranks 5 and 6) were recorded for the primary and all secondary umbels from each study site plant. Initial fruit counts were occasionally repeated to measure differences in counting performed by students and myself. To account for possible fruit abortion, final fruit counts were recorded later in the season on mature (rank 6) fruits prior to the plant setting seed (a couple days to ≥2 weeks following initial fruit counts). Mature fruits and seeds (rank 6 and 7) were collected from the primary and all secondary umbels mid-August (August 14th-18th) provided there were at least 25 fruits or seeds to collect.

Fruits and seeds were air dried immediately following collection and subsequently freeze-dried. Mericarps were separated during the drying process allowing me to sort seeds and to determine if they were viable or inviable by placing them on a lightbox (Baskin and Baskin 1990). Viable seeds contained a visible, solid endosperm whereas inviable seeds remained papery and empty. To verify that viable seeds were healthy and not aborted, approximately 10-20% of seeds were cut in half to examine the endosperm and check for desiccation (Jongejans and Telenius, 2001). In 2017, in addition to sorting seeds, 20 viable seeds were randomly chosen per plant seed-set and weighed individually using a microbalance (±0.0001*mg*, ME5 Sartorius AG, Germany).

### *D. radiella presence*

Parsnip webworm *Depressaria radiella* Goeze (Lepidoptera: Depressariidae) larvae develop in *P. sativa* and *H. maximum* floral and fruit tissue (Berenbaum 1983). Prior to *H. maximum* floral development (late June) *D. radiella* moths lay eggs on leaves of plants with unopened flower buds and developing umbels. Herbivory damage consists of floral and fruit consumption where *D. radiella* larvae feed within silken masses that encompass several *H. maximum* flowers and fruits. Late instar larvae consuming heavily defended fruit tissue can metabolize defensive plant furanocoumarins (Carroll *et al.* 2006) prior to pupation inside hollow *H. maximum* stems.

Depressariidae from the field were identified using Triplehorn and Johnson (2005) in both 2017 and 2018 study sites. As multiple Depressariidae species were identified from *H. maximum* plants (*D. radiella, D. discipunctella, and Agonopterix spp.*), presence of *D. radiella* was determined at the end of the field season when it could be more easily determined. Late instar *D. radiella* larvae, pupae, exuviae, (un)emerged moths, or parasitized mummies were observed from opened *H. maximum* stems and peduncles as it is uncharacteristic of the other Depressariidae species to pupate within *H. maximum* stems.

## Data and Analysis

### *Data Description*

An observational study was conducted in Colorado across ten spatially separated *H. maximum* study sites in 2017 and six study sites in 2018 to determine if viable seed weight (mg) (2017 only) and viable seed counts differ between sites experiencing varied elevation and herbivore pressure. Twenty plants were observed in each of the ten sites (n=320 observed plants total). Linear models were fit to the data with viable seed weight (mg) and viable seed counts as the response variables. A 1-way ANOVA was conducted to test if viable seed weights and counts differed across study sites. The following categorical predictor variables were analyzed in multiple linear regressions: study site, parsnip webworm (*D. radiella*) presence (yes) or absence (no), and umbel position (1˚ or 2˚ umbels). With the following continuous predictor variables: number of viable seeds, viable seed weight (mg), number of secondary umbels, average flowers per two randomly selected umbellets, average number of umbellets from two randomly selected umbellets, and plant height (cm). In addition to 2017 continuous variables, umbel diameter (cm) and umbellet diameter (cm) were measured in 2018 to determine floral area and possible correlation to seed-set and plant fitness. A generalized linear model for the binary predictor variable of *D. radiella* presence (yes or no) was investigated with the following three predictor coefficients: plant height (cm), elevation (m), and the year plants were sampled. For viable seed production impacted by *D. radiella* a linear fixed-effects model was used where lo-transformed viable seed count was treated as the predictor variable, and the following two coefficients were treated as fixed effects: *D. radiella* presence (yes or no) and plants nested within elevation. A Kenward-Roger's approximation of degrees of freedom was used in the linear mixed-effects model.

### *Statistical Analyses*

Data analysis was conducted using R Version 3.4.1 (R Development Core team, 2017) where the 'Anova()' function was used to calculate *p-values* from the 'car' package (Fox and Weisber, 2011). Additionally, single and multiple linear regressions were conducted using the 'lm' function. Estimated marginal means and Tukey adjusted pairwise comparisons were found using the 'emmeans' and 'cld' function from the 'emmeans' package (Lenth, 2018). Logistic regressions were used to investigate binary response variables from the glm() using the "logit" function. From the 'MASS' package 'dose.p()' was used to find the "dose" or in this case probable elevation (m) that would lead to the event of *D. radiella* presence in 50% or 90% of infested study site plants (Venables and Ripley, 2002). Linear mixed-effects models were fit by REML t-tests using the Welch-Satterthwaite's method from the 'lme4' package (Bates *et al.* 2015). Mean viable seed counts were reported on the log transformed scale, but data represented throughout figures in this chapter were presented in the original scale. Traditional backwards model selection in the 'MuMIn' package (Barton, 2018) was used to determine the best fit models for viable seed counts and viable seed weights (in 2017) amongst plant trait measurements.

## Results

### *Flowering phenology*

In the 2017 and 2018 *H. maximum* study sites, elevation had an observable effect on flowering phenology. Between study sites there was an approximately 2.5-week delay (18 days) from when primary umbel male staminate flowers emerged (rank 2, 3). Yet, there was no clear pattern for flowering phenology across elevation, high elevation plants were not always found to lag behind lower elevation sites. The earliest site to flower from primary umbels began at Cherokee Park Rd. on June 20th and the last study sites to flower were on July 6th and 7th from Coalmont, Clark, McClure Pass, and NF550. Male staminate flowers in secondary umbels were present across all sites for approximately two weeks (13 days) from June 26th to July 8th (first to flower Cherokee Park Rd., last to flower Shambhala/69E). In female pistillate flowers (rank 4) flowers emerged for about 9 days between June 28th to July 6th in primary umbels (first to flower Cherokee Park Rd., last to flower Clark). Similarly, secondary umbels flowered for 9 days from July 1st to July 10th (first to flower Cherokee Park Rd., last to flower Shambhala/69E). Plants on average spent approximately 19 days flowering, from the emergence of male staminate flowers to the end of the female pistillate stage, where the lowest elevation site (Cherokee Park Rd.) was consistently the earliest to develop phenologically.

### *Plant trait measurements*

The values presented in this section are reported as averages across all plants and sites. Plant characteristics were measured in 2017 and 2018, Colorado *H. maximum* plants produced, on average, three secondary umbels with maximum production of six secondary umbels. Plant height averaged 108 cm, the smallest plants around 54 cm and the tallest plants between 165-200 cm from the basal stem to the tip of the tallest umbel. Primary umbels contained on average 23 umbellets where secondary umbels had 28 umbellets, each umbellet consisted of approximately 25 flowers. From 2018 plants, umbel diameters averaged 14.5 cm across, where primary umbels were larger (18.6 cm) than secondary umbels (12.9 cm). Umbellet diameter in primary umbels averaged 3.4cm and secondary umbellets were 2.1 cm. Plants in 2018 produced an average of approximately 288 fruits initially, 180 mature fruits prior to setting seed (two seeds per fruit), and 343 total seeds (195 viable seeds) in primary umbels. Secondary umbels contained initially 254 fruits, 109 fruits prior to seed-set, and 104 total seeds of which 54 were viable on average.

Plant height (cm) in 2018 was positively correlated with (log-transformed) viable seed count (r>0.6) from Pearson's correlation and a single linear regression (80.87±SE 3.67, F=91.37, df=1 and 171, $R^2$=0.4081, $p<0.0001$) (Figure 8). Plant height was also found to be collinear with umbel diameter (r>0.4, $p<0.0001$) where umbel diameter and umbellet diameter were also collinear (r>0.7, $p<0.0001$). Umbel diameter alone did positively correlate (r>0.3) with viable seed count (14.51±SE 0.62, F=25.16, df=1 and 159, $R^2$ =0.1431, $p<0.0001$) but plant height was a stronger predictor for potential plant fitness. Plant height was significantly different between study sites (F=70.09, df=7 and 219, $p<0.0001$, $R^2$ =0.6776), where umbel diameter (cm) and (0.99±SE 0.4, F=6.24, df=7 and 219, $p<0.05$) umbellet diameter increased plant height (cm) (3.71±SE 1.78, F=4.37, df=7 and 219, $p<0.05$). In a single linear regression with elevation (m) and plant height (cm), plant height was significant across elevation (F=65.09, df= 1 and 240, $p<0.0001$) where a 1000m increase in elevation decreased plant height by (54.62±SE 6.8 cm). Plants at the lowest elevation site (Cherokee Park Rd.) had the tallest plants on average estimated at (134.0± SE=5.0 cm, F=69.36, df=7 and 219, $p<0.0001$), but high elevation sites Crooked Creek Pass, Joe Chamber's campsite did not consistently have the shortest plants (98.0±SE 5.15 cm and 115.0± SE 8.89 cm) (F=69.36, df=7 and 219, $p<0.01$ and $p<0.0001$). Both Crooked

Creek Pass (3058m) and Shambhala/69E (2696m) decreased plant height (cm) (-23.53±SE 3.92 to -34.55±4.19 cm, F=69.36, df=7 and 219, $p<0.0001$).

### *Fruit and Seed-Set Count*

#### i. *Viable Seed Weight*

Study site location (F=21.371, df=15 and 127, $p<0.0001$) and umbel position (1° or 2° umbels) (F=9.096, df=15 and 127, $p<0.001$) were significant predictors for *H. maximum* viable seed weight (mg) in 2017 (F=116.965, df=15 ad 127, $R^2=0.627$, $p<0.001$). Secondary umbels decreased viable seed weight (mg) by (-1.62±SE 0.61, F=16.616, df=15 and 127, $p<0.05$). Neither *D. radiella* presence (F=1.83, df=15 and 127, $p=0.18$), plant height (cm) (F=0.00, $p=0.99$), average flowers per umbellet (F=0.18, df=15 and 127, $p=0.70$), average number of umbellets (F=0.937, df=15 and 127, $p=0.33$), nor numbers of secondary umbels (F=1.46, df=15 and 127, $p=0.23$) were significant measures for viable seed weight (mg). Mean viable seed weights ranged from (4.41 to 11.32mg) and were significantly different across study sites (F=12.021, df=15 and 127, $p<0.0001$). Differences between study sites was unclear and was not exclusively based on study site location, where the greatest difference between viable seed weight (mg) was between the study sites NF550 and McClure Pass (-4.95±SE 0.86, F=12.021, df=15 and 127, $p<0.001$) (Figure 6).

Mean viable seed weights were not however, significantly different across elevation (m) using the same model as above (F=0.838, df=7 and 135, $p=0.36$). Instead, for every 1cm increase in plant height (cm) average viable seed weight (mg) increased by (0.039±SE 0.0089 mg, F19.31, df=7 and 135, $p<0.0001$) and decreased by (2.67±0.66 mg, F=16.44, df=7 and 135, $p<0.0001$) for producing one secondary umbel. However, when the model above includes only elevation and umbel position as predictor variables, mean viable seed weight (mg) significantly decreased across elevation. Where a 1000m increase in elevation decreased viable seed weight (mg) by (2.69±0.82 mg, F=10.83, df=2 and 154, $p<0.05$). Additionally, secondary umbels also decreased viable seed weight (mg) (-2.36±SE 0.63 mg, F=14.063, df=2 and 154, $p<0.0001$). Summary statistics from mean viable seed weights and viable seed counts from both study years are listed in (Table 4). Mean viable seed weights were statistically significant between study sites and are presented as estimated marginal means averaged over the levels of umbel position and *D. radiella* presence in (Table 5, Figure 6).

### *ii. Viable Seed Count*

Viable seed counts in both 2017 and 2018 were log transformed. Study sites in both high and low elevations increased viable seed counts in 2017 (1.195±SE 0.41 to 2.199±SE 0.77, F=8.164, df=11 and 134, $p<0.01$). Where plant height (0.0098±SE 0.0058, F=2.796, df=11 and 134, $p=0.097$) and average number of umbellets (0.036±SE 0.019, F=3.695, df=11 and 134, $p=0.057$) increased viable seed count, but were marginally or non-significant coefficients. McClure Pass was significantly different from all other study sites and had the largest estimated viable seed counts (464.44±SE 29.77 seeds, F=7.341, df=11 and 134, $p<0.001$). Mean viable seed count was significantly different between study sites but marginally significant across elevation in 2017 (F=2.75, df=4 and 141, $p=0.099$) (Figure 6).

Primary umbel viable seed counts in 2018 were statistically significant across study sites (F=12.909, df=10 and 98, $R^2=0.5195$, $p<0.0001$). Flower and fruit herbivory by *D. radiella* larvae increased viable seed counts by (3.87±SE 0.90, F=21.525, df=10 and 98, $p<0.001$) in logscale. Plant height (cm) (0.04±SE 0.01, F=26.871, df=10 and 98, $p<0.001$) and average number of umbellets (0.062±SE 0.035, F=3.01, df=10 and 98, $p=0.0859$) also increased primary umbel viable seed counts (Figure 7), though number of umbellets was marginally significant.

Viable seed counts per umbel were summarized across elevation in (Figure 7). Neither umbel diameter (cm) (F=1.75, df=10 and 98, $p=0.19$), umbellet diameter (cm) (F=0.043, df=10 and 108, $p=0.84$), nor average number of flowers per umbellet (F=1.11, df=10 and 108, $p=0.29$) significantly affected viable seed counts.

Umbellet diameter and plant height (*cm*) significantly increased secondary umbel viable seed production (0.76±SE 0.30, F=6.56, df=10 and 108, $p<0.05$), where a 10cm increase in plant height increases viable seed production by (0.34±SE 0.082 seeds, F=13.71, df=10 and 108, $p<0.0001$). Umbel diameter (*cm*) (F=0.0091, df=10 and 108, $p=0.924$), average flowers per umbellet (F=0.002, df=10 and 108, $p=0.97$), number of umbellets (F=0.30, df=10 and 108, $p=0.59$), study site (F=1.57, df=10 and 108, $p=0.19$), and *D. radiella* presence (F=1.04, df=10 and 108, $p=0.31$) were not significant predictors for secondary umbel mean viable seed count (F=6.99, df=10 and 108, $R^2=0.436$, $p<0.001$).

### *iii. Elevation effects on fruit and seed production*

In the following analyses elevation (m) was treated as a continuous variable. Single linear regressions and Pearson's correlations were conducted to look at the effect of elevation (m) on the following predictor variables: initial fruit counts, final fruit counts, total seed count, and viable seed count from primary and the marked secondary umbels. Initial fruit counts were statistically significant across elevation for both primary (r>-0.3, F=31.96, df=1 and 178, $R^2=0.1522$, $p<0.0001$) and secondary umbels (r>-0.6, F=151, df=1 and 198, $R^2=0.4326$, $p<0.0001$). Final fruit counts were also statistically significant for primary (r>-0.3, F=17.97, df= 1 and 104, $R^2=0.1473$, $p<0.0001$) and secondary umbels (r>-0.5, F=22.01, df=1 and 66, $R^2=0.2501$, $p<0.0001$) across elevation. For log-transformed total seed counts (including counts from viable and inviable seeds) the primary umbel (r>-0.1, F=4.657, df=1 and 174, $R^2=0.0261$,

$p<0.05$) and secondary umbels (r>-0.2, F=11.34, df=1 and 198, $R^2=0.0542$, $p<0.001$) were statistically significant across elevation, but linear models were not fit well. Elevation was a significant predictor of log-transformed viable seed counts in both the primary (r>-0.3, F=22.2, df= 1 and 174, $R^2=0.1132$, $p<0.0001$) and the marked secondary umbels (r>-0.2, F=13.03, df=1 and 198, $R^2=0.0618$, $p<0.001$). Lastly, initial fruit count, final fruit count, and seed production all decreased significantly as elevation increased for both primary and secondary umbels.

*D. radiella presence*

From 2017 and 2018, between 20-85% of plants were attacked by *D. radiella* (n=56) while 42 plants were unaffected by *D. radiella* from McClure Pass, Cebolla Creek, and Spring Creek. Between all 2018 study sites, plants produced greater seed-sets than un-infested plants in the same study sites (3.87±SE 0.90, F=6.83, df=12 and 163, $p<0.0001$). However, the flowering stage of plants during *D. radiella* attack did not significantly affect viable seed count (F=1.16, df=12 and 163, $p=0.33$). Meaning that *D. radiella* herbivory in un-emerged flowers in buds (rank 0) had similar effects on seed count on flowers attacked post bud-burst (rank 1 and beyond).

From sites with *D. radiella* presence only (McClure Pass, Cebolla Creek, and Spring Creek) log-transformed primary umbel viable seed counts were analyzed from both 2017 and 2018. I excluded secondary umbel data as these counts were not collected in 2017. It was found that (log-transformed) primary umbel viable seed count itself is not significant (F=0.1601, df=5 and 85, $p=0.69$) across study sites, but study sites are significantly different (F=11.853, df=5 and 85, $p<0.0001$), where McClure Pass plants increase viable seed counts (2.28±SE 0.62, F=11.853, df=5 and 85, $p<0.001$). When elevation (m) is treated as a continuous predictor variable log-transformed mean viable seed count became significant (F=22.857, df= 4 and 85, $p<0.0001$).

Within this model elevation significantly increased viable seed count in primary umbels, where a 1000m increase in elevation increased viable seed count by (26.98±SE 5.55 seeds, F=23.596, df=4 and 85, *p<0.0001*). *Depressaria. radiella* presence and herbivory was found to increase viable seed count (3.46±SE 1.74 seeds, F=2.049, df= 4 and 85, *p<0.05*) but the year plants were sampled (2017 or 2018) was not a significant predictor of viable seed count (F=3.567, df= 4 and 85, *p=0.0623*) (Figure 9). Although, *D. radiella* presence increased viable seed production *D. radiella* presence or absence was not significantly different from each other.

From 2017, viable seed weight (mg) was significantly different between all three study sites (F=249.15, df=3 and 47, *p<0.0001*) where Spring Creek and McClure Pass increased viable seed weight by (8.46±SE 0.54 and 1.74±SE 0.56 mg, F=30.987, df=3 and 47, *p<0.0001*), and Cebolla Creek decreased viable seed weight by (2.73±SE 0.54 mg, F=30.987, df=3 and 47, *p<0.0001*). Further *D. radiella* presence was marginally significant in increasing viable seed weight (0.98±SE 0.51 mg, F=3.77, df= 3 and 47, *p=0.058*). With elevation in the model, viable seed weight was significantly different across elevation (m), but *D. radiella* presence was no longer marginally significant (F=0.542, df=2 and 48, *p=0.47*). For a 1000m increase in elevation (m) viable seed weight increased by (32.1±SE 7.37 mg, F=18.94, df=2 and 48, *p<0.0001*).

A logistic regression was used to test the odds of plants containing *D. radiella* (yes), with three predictor variables: elevation (m), plant height (cm), and year sampled. For a 1000m decrease in elevation, the odds of a plant containing *D. radiella* larvae (versus not containing *D. radiella*) increased by a factor of (965, df=1 and 197, *p<0.0001*). Neither plant height (cm) (df=1 and 197, *p=0.13*) nor the year plants were sampled (df=1 and 197, *p=0.13*) were significant indicators of *D. radiella* in *H. maximum* plants. However, in order for half of *H. maximum* plants to contain *D. radiella* (from the infested sites only) the elevation was estimated to be 2587±SE

30.53m. Where 90% of plants containing *D. radiella* corresponds to 2525±SE 29.12m in elevation, and 10% of plants with *D. radiella* is estimated to occur at 2649±SE 34.06m in elevation. As elevation increases it is less likely that *D. radiella* will attack or be found in *H. maximum* plants in Colorado ($p<0.0001$).

Within plant populations with *D. radiella* presence, the average of all plants within an elevation had an average intercept of (-0.1401±SE 1.767, F=5.974, df=2 and 79, $p=0.937$) a non-significant predictor of log-transformed viable seed count when plants nested within elevation is hypothetically excluded from the model. Yet, *D. radiella* presence or absence was significant in explaining log-transformed viable seed count, however plants with or without *D. radiella* were not significantly different from each other (F=5.974, df= 2 and 79, $p<0.005$).

## Discussion

My results show that there are differences in *H. maximum* plants between Colorado study sites for flowering phenology, associated plant traits, and viable seed count and weight. Flowering phenology varies in *H. maximum* plants throughout Colorado to some degree, ranging from a few days to more than a week between study site plant development, but phenology does not exclusively lag as elevation increases. Due to high variability between *H. maximum* plants across study sites, the role of elevation is less clear, but our results suggest that elevation does not drive associated plant traits (e.g. plant height) or seed production. Varied conditions within sites (e.g., soil water moisture, sunlight), herbivore pressure, and seasonal temperatures may effectively explain some of our results between study site plants.

Plant trait patterns for plant height, number of secondary umbels, and flowers per umbellet may be both genetically and environmentally constrained. Our results showed that plant height (cm) and umbellet diameter (cm) could increase secondary umbel viable seed counts, and that height and *D. radiella* increased primary umbel seed-set. Though plant height may positively correlate with seed production, this pattern was not consistent for pants across elevation. Decreased seed-set and secondary umbels within Apiaceae plants (*H. sphodylium*) for example, may be attributed to reduced photoactive radiation and shaded understories (Sheppard 1991). In Shambhala/69E, plants experienced more shade and less partial sunlight than any other study site and produced fewer secondary umbels and smaller seed-sets. Additionally, there is evidence that fruit 'carpel' abortion in lower order umbels (Sheppard 1991) occurs to some degree in *H. maximum* plants, where this will be further investigated in Chapter 3. Secondary and tertiary umbels frequently did not set fruit or seeds, but plants may have compensated for lost seed production by producing larger primary umbel seed-sets.

Parsnip webworm herbivory has been found to reduce up to 75% of seed-set in wild parsnip (*Pastinaca sativa*) (Lohman *et al.* 1996). In our study *D. radiella* herbivory had varied results on *H. maximum* viable seed production and seed weight. Although, *D. radiella* effects on seed production and weight was not attributed to flowering phenology stage during attack in the model, in the field there was stronger relationship with *D. radiella* timing. For example, in Cebolla Creek, flower buds (rank 0) infested with *D. radiella* produced few if any seeds as most floral tissue was consumed prior to bud-burst, but plants attacked post bud-burst (rank 1) were able to produce flowers, fruits, and seeds. As such the degree of *D. radiella* attack and infestation and the timing of female moths needs further investigation. For example, studies with *H. sphondylium* have found that seed production is unaffected if less than half of foliar tissue is manually removed by hand (Sheppard 1991). Such that *D. radiella* may need to remove at least half of *H. maximum* floral tissue to significantly affect seed production.

Between sites with *D. radiella* presence, elevation is a predictor for viable seed count, where increase in elevation gradient typically lowers the probability of plants being attacked by *D. radiella*. Additionally, data show that *D. radiella* presence significantly increases plant viable seed production and may increase viable seed weight (mg). Further, it is known that *D. radiella* larvae reduce floral area and attractive qualities in both *P. sativa* and *H. maximum* (Hendrix 1979, Lohman *et al*. 1996). Yet the full effect of *D. radiella* on *H. maximum* fitness, either with directly limiting seed-set or indirectly altering floral area and/or floral scent associated with attracting pollinators has yet to been known and needs further investigation. Especially, as our results found that the probability of *D. radiella* attack on plants increased with lower elevation (2587 to 2525m) yet the actual elevations of our study sites attacked by *D. radiella* were (2580, 2588, and 2670m).

Elevation may play some role to *D. radiella* survival where lower elevations are warmer and may have the earliest flowering phenologies. However, our results also suggest that *D. radiella* can survive at higher elevations, which may indicate individual differences in plant populations in addition to abiotic conditions as a driver for *D. radiella* colonization. For example, plant viable seed production was not exclusively addressed by directionality of elevation or plant trait measures such as plant height or umbel diameter (cm). Rather it is appropriate that *H. maximum* fitness depends on biotic interactions with pollinators in addition to abiotic conditions. Lastly, in cases where *D. radiella* larvae developed within unopened buds it was not possible to record the following plant traits: number of secondary umbels, umbel diameter, umbellet diameter, number of umbellets and flowers, and seed-set, creating instances of missing data values.

## Future directions

In relation to the next chapter, this chapter provides an outline and a foundation for plant traits and phenology that may impact plant-insect community assemblages. Flowering phenology is expected to be an important indicator for pollinator attraction, where male and female stage *H. maximum* flowers offer separate floral awards, pollen and nectar (Zych 2007). Literature suggests that larger floral display will attract more pollinators (Danderson and Molano-Flores 2010, Brody and Mitchell 1997). Such that *H. maximum* umbel diameter may play a role in insect, pollination visitation. *Depressaria radiella* herbivory, however, may reduce pollinator visitation by decreasing floral area or eliciting defensive plant cues that pollinators elude. Chapter 3 will investigate insect and pollinator communities as attributed to elevational gradient, *H. maximum* umbel position, flowering rank, and *D. radiella* presence.

FIGURES

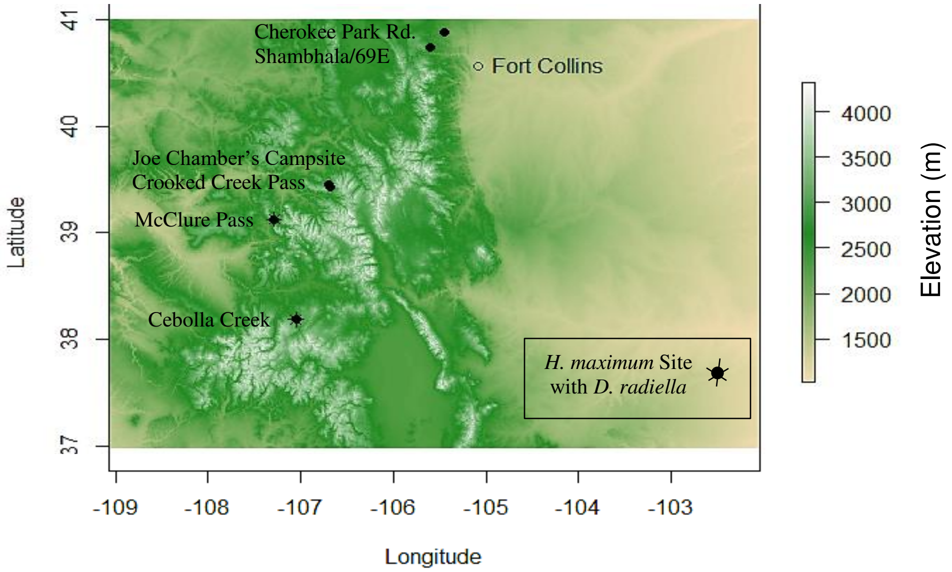

FIG. 3. The location of populations observed for *H. maximum* phenology throughout the state of Colorado in 2018.

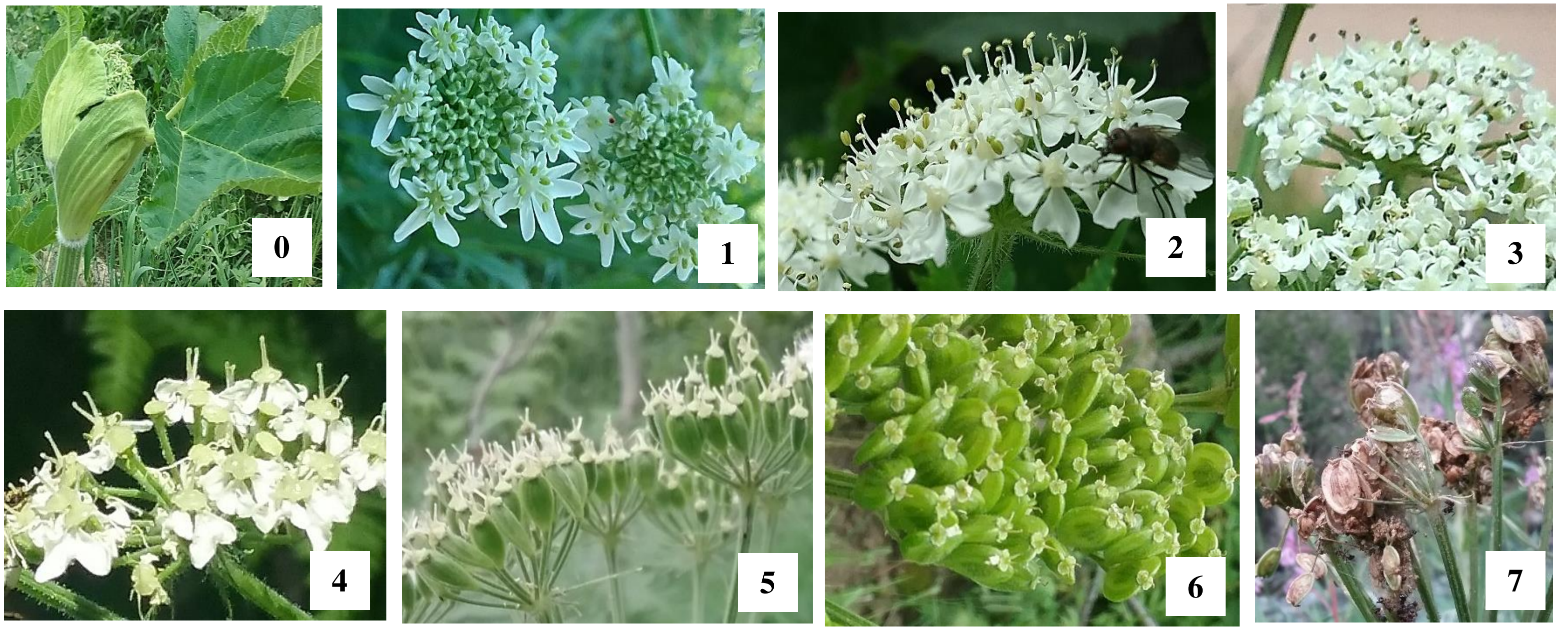

FIG. 4. Pictures of representative *H. maximum* flowering phenology ranks. Note that in rank 3 (old staminate/ immature pistillate) anthers dehisce and appear dry and 'green.' (Photo credit: B. Smith)

Floral development and flowering phenology rankings for *Heracleum maximum*. Descriptions have been adapted from Lindsey (1982) and Devlin and Stephenson (1985) that investigated similar protandrous apiaceous species for flowering phenology. **Rank 0**-Both staminate and pistillate flowers in a tight bud, **Rank 1**-Immature staminate= flowers recently open but not yet yielding pollen, **Rank 2**-Mature staminate=pollen present, **Rank 3**-Old staminate/ immature pistillate= old/no pollen; stigma exerted but receptive surface not exposed, **Rank 4**-Mature pistillate= stigma receptive surface open (stigmas bifurcated), **Rank 5**-Old pistillate/ fruiting= flowers wilting; fruit developing but immature, **Rank 6-**Past anthesis=mature fruit developing, **Rank 7**-Seeds dried on plant; mericarps splitting

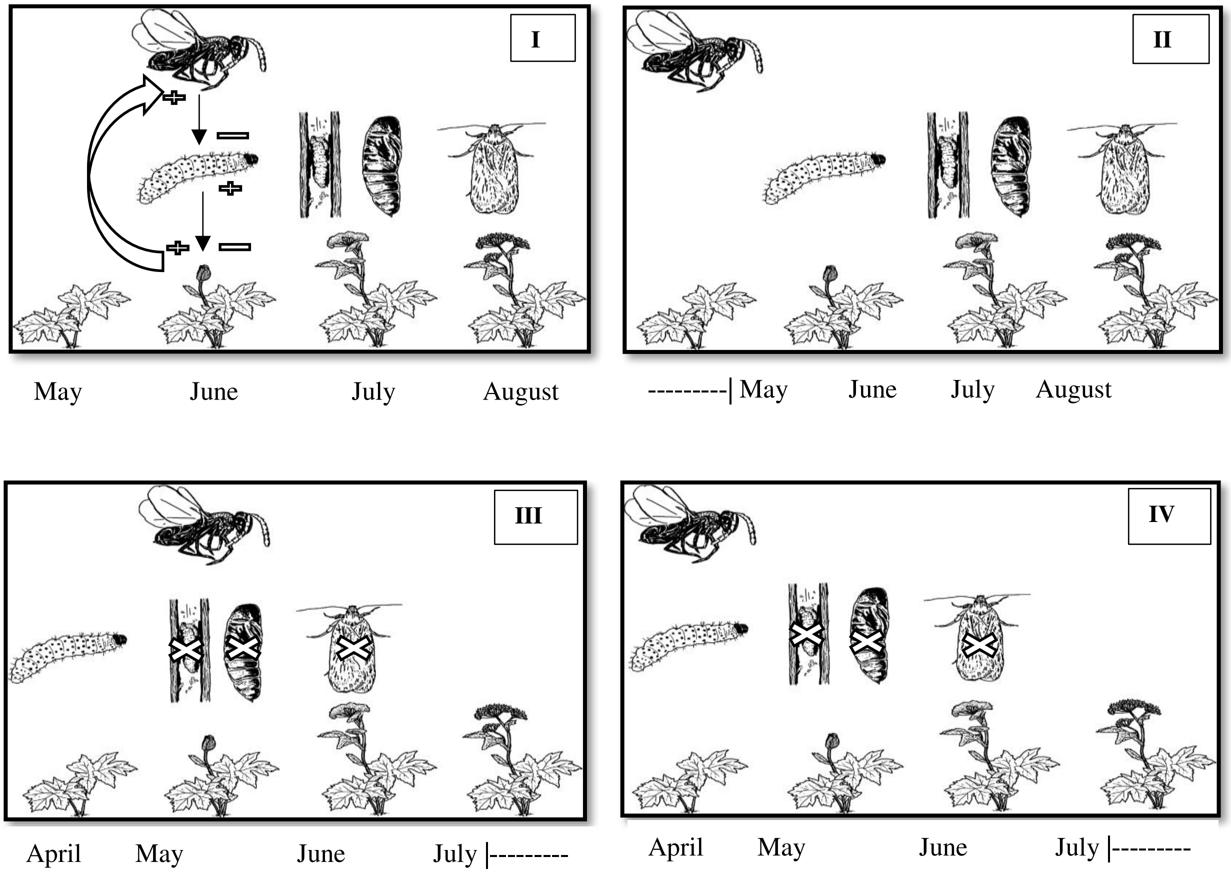

FIG. 5. Representative phenological match (I) and mismatch (II, III, IV) of *H. maximum*, its herbivore *D. radiella*, and parasitoid wasp *C. sosares* experiencing changing climatic conditions. Ilust. T. Barosh.. Plants past the vegetative stage starting in June represent flowering phenology ranks (2-4) with plants setting seed in August (rank 6, 7).

Panel (**I**) represents normal climate conditions and synchronous life histories, or phenological matching between all three species. Panel (**II**) details cooling temperatures where, plant phenology lags, though matched with *D. radiella*, *C. sosares* emerges prior to its plant host thus the parasitoid will not survive. Panels (**III**) and (**IV**) represent warming conditions where plant phenology shifts earlier. Panel (**III**)- warming temperatures accelerate *D. radiella* phenology, moths arrive prior to plant floral resources and are unable to develop without floral tissue. *C. sosares* matches *H. maximum* phenology but no longer has its host to survive. Panel (**IV**)- tri-trophic phenologies are matched, but *D. radiella* females lay eggs earlier than floral tissue is available. While *C.sosares* can parasitize first instar *D. radiella*, neither larvae nor parasitoid broods survive with lack of *H. maximum* flower and fruit that are required for larval growth.

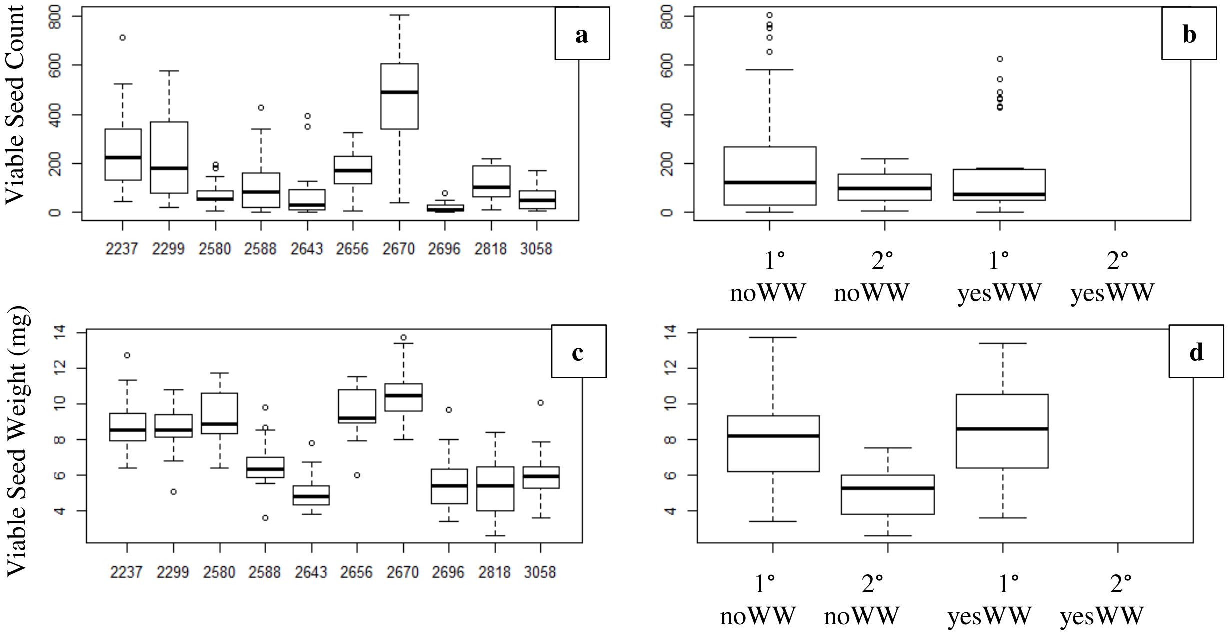

FIG. 6. Viable Seed Count and Weight (mg) boxplots from 2017. **Top row**: **a**-viable seed counts across elevation, and **b**- viable seed counts for primary (1°) and secondary (2°) umbels either with (yes) or without (no) *D. radiella* presence. **Bottom row**: **c**- viable seed weights across elevation, and **d**- viable seed weights for primary (1°) and secondary (2°) umbels either with (yes) or without (no) *D. radiella* presence.

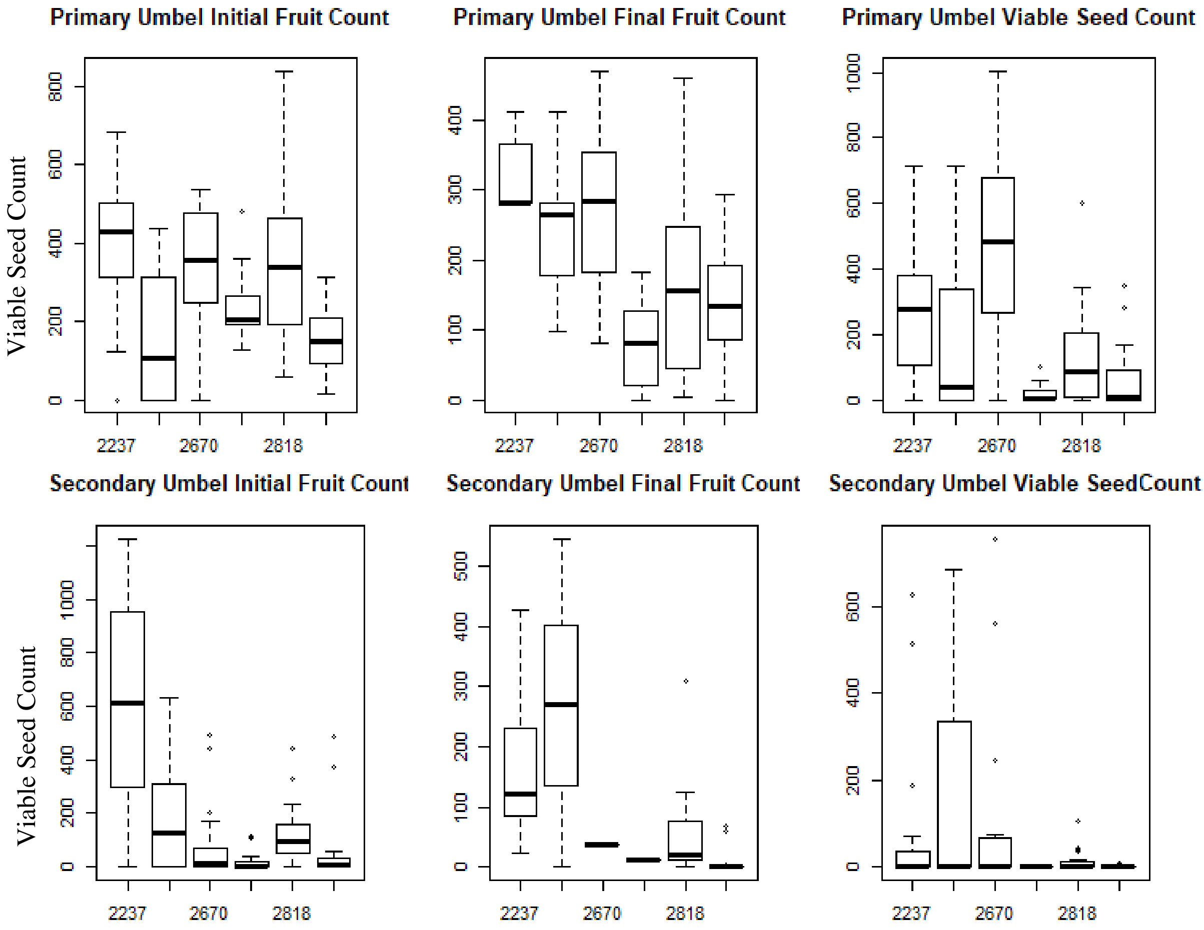

FIG. 7. Fruit and seed counts from primary and secondary umbels in 2018. Initial fruit counts are for immature (rank 5) fruits while final fruit counts are mature (rank 6) fruits. Two seeds are produced per fruit.

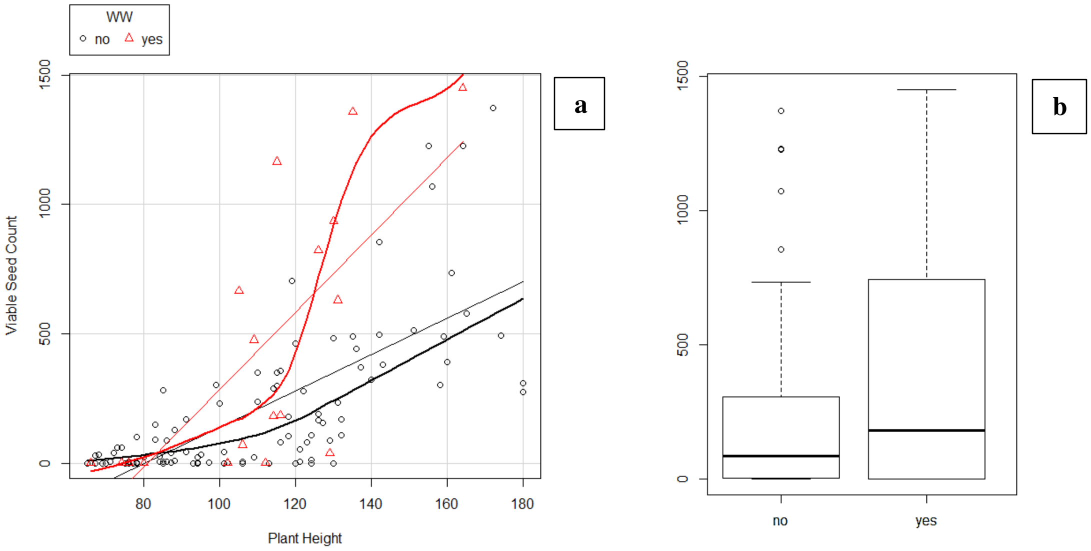

FIG. 8. **a**- The 2018 Primary umbel viable seed counts, with *D. radiella* presence (red/yes) or absence (black/ no). Straight lines represent line of best fit. **b**- Viable Seed count based on *D. radiella* presence or absence.

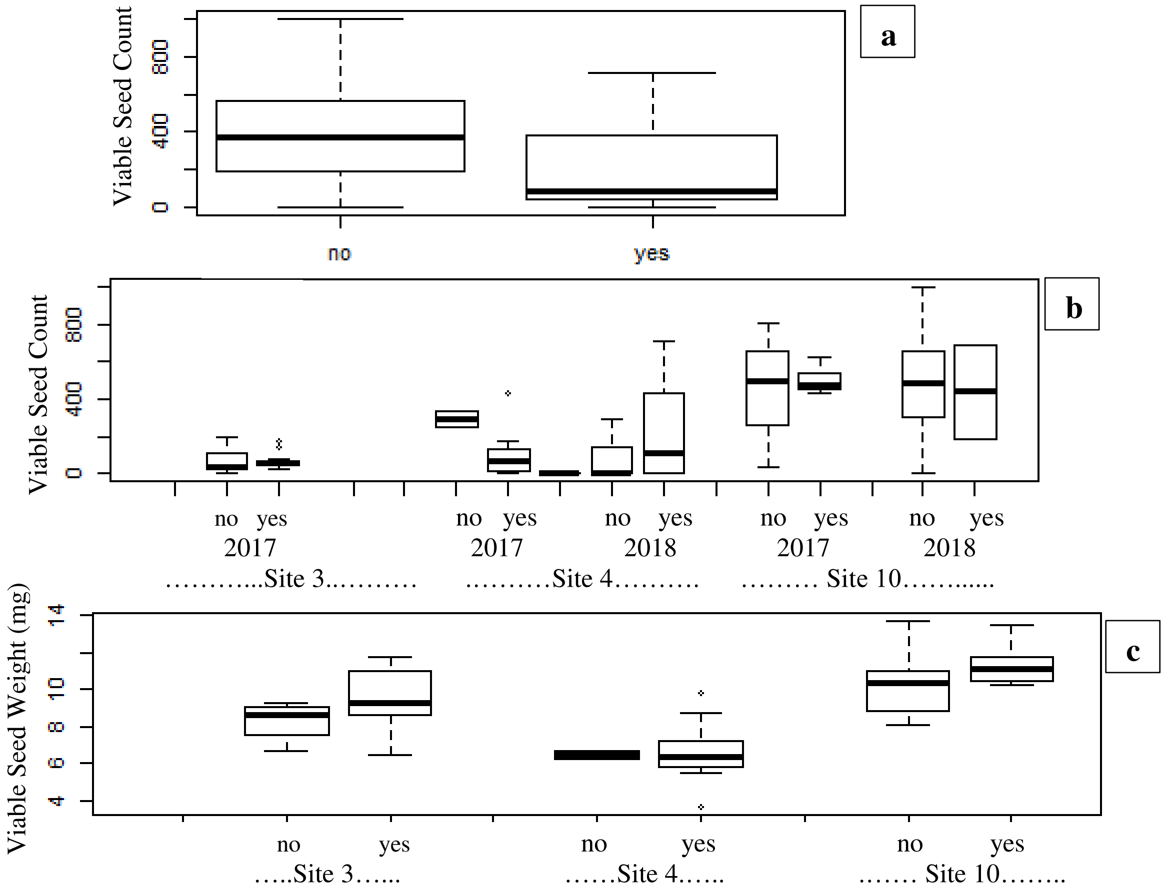

FIG. 9. Viable seed count and weight for study sites attacked by *D. radiella*. **a-** Viable seed count grouped by *D. radiella* presence (no) or (yes). **b-** Viable seed count for study sites with and without *D. radiella*, split between study years 2017 and 2018. Site 3(Spring Creek), Site 4 (Cebolla Creek), and Site 10 (McClure Pass). **c-** Viable seed weight (mg) from 2017 study sites with or without *D. radiella* presence.

# TABLES

TABLE 3. Site information including GPS location, elevation, habitat and *D. radiella* presence in ten populations of *H. maximum* observed throughout Colorado in 2017 and 2018.

| Study Site Name | Year Observed | County | Location | Elevation (m) | Habitat | *D. radiella*? |
|---|---|---|---|---|---|---|
| Joe Chamber's Campsite | 2017, 2018 | Eagle | 39.4564, -106.7039 | 2818 | Aspen understory | No |
| Crooked Creek Pass | 2017, 2018 | Eagle | 39.4254, -106.6856 | 3058 | Subalpine meadow | No |
| Spring Creek | 2017 | Eagle | 39.3694, -106.6810 | 2580 | Conifer understory, Creek below | Yes |
| Cebolla Creek | 2017, 2018 | Gunnison | 38.1834, -107.0539 | 2588 | Conifer understory, Creekside | Yes |
| Shambhala/69E | 2017, 2018 | Larimer | 40.7442, -105.6012 | 2696 | Aspen understory | No |
| Coalmont | 2017 | Routt | 40.38002, -106.57981 | 2656 | Aspen understory | No |
| Clark | 2017 | Routt | 40.7363, -106.90939 | 2300 | Aspen understory | No |
| NF 550 | 2017 | Routt | 40.98767, -106.90897 | 2643 | Conifer understory, Creek below | No |
| Cherokee Park Rd. | 2017, 2018 | Larimer | 40.88176, -106.90897 | 2237 | Aspen understory, Creekside | No |
| McClure Pass | 2017, 2018 | Gunnison | 39.1286, -107.2870 | 2670 | Aspen understory | Yes |

TABLE 4. Summary statistic data from the ten *H. maximum* study sites in 2017 (top) and 2018 (bottom). WW denotes *D. radiella* presence and no WW denotes no *D. radiella* presence.

| **Site ID** | **Study Site Name (County, State)** | **Elevation (*m*)** | **Umbel Position** | **Mean Viable Seed Count ± SD** | **Mean Viable Seed Weight (mg) ± SD** |
|---|---|---|---|---|---|
| 17CO01 | Joe Chamber's (Eagle, CO) | 2818 | 1°(n=3),<br>2°(n=10) | 101.333 ±96.111<br>119.4 ±75.98 | 6.899 ± 2.039,<br>4.906 ± 1.615 |
| 17CO02 | Crooked Creek Pass (Eagle, CO) | 3058 | 1°(n=13),<br>2°(n=3) | 56.769 ±46.693<br>71.667 ±63.532 | 6.415 ± 1.400,<br>4.406 ± 0.820 |
| 17CO03 | Spring Creek (Eagle, CO) | 2580 | 1° (n=14: WW=11, noWW=3)<br>2°(n=1) | 75.545 ±45.888,<br>79.667 ±100.381<br>99.000 ± Na | 8.837± 0.458,<br>9.494 ± 1.779<br>6.691 ± Na |
| 17CO04 | Cebolla Creek (Gunnison, CO) | 2588 | 1°(n=17: WW=15, noWW=2)<br>2°(n=0) | 97.867 ±106.685,<br>296.000 ±65.054<br>Na | 6.622 ± 1.521,<br>6.461± 0.299<br>Na |
| 17CO05 | Shambhala/Rd 69E (Larimer, CO) | 2696 | 1°(n=16)<br>2°(n=1) | 17.125 ± 21.112<br>29.000± Na | Na, Na<br>5.810 ± Na |
| 17CO06 | Coalmont (Jackson, CO) | 2656 | 1°(n=17),<br>2°(n=3) | 177.529 ± 97.403<br>Na, Na | 9.513 ± 1.491<br>Na, Na |
| 17CO07 | Clark (Routt, CO) | 2300 | 1°(n=14),<br>2°(n=6) | 231.357 ± 181.235<br>Na | 8.514 ± 1.493<br>Na |
| 17CO08 | NF 550 (Routt, CO) | 2643 | 1°(n=13),<br>2°(n=7) | 89.833± 135.724<br>Na | 5.087± 1.169<br>Na |
| 17CO09 | Cherokee Park Rd. (Larimer, CO) | 2237 | 1°(n=19),<br>2°(n=1) | 245.263 ±171.479<br>Na | 8.887 ± 1.685<br>Na |
| 17CO10 | McClure Pass (Gunnison, CO) | 2670 | 1°(n=19: WW=6, noWW=13)<br>2°(n=1) | 502.833 ±71.575,<br>460.385 ±250.667<br>Na | 11.315± 1.193,<br>10.133± 1.55<br>Na |
| 18CO01 | Joe Chamber's (Eagle, CO) | 2818 | 1°(n=20),<br>2°(n=20) | 128.800 ±150.887<br>12.950 ± 25.893 | Na<br>Na |
| 18CO02 | Crooked Creek Pass (Eagle, CO) | 3058 | 1°(n=20),<br>2°(n=20) | 62.900 ±100.000<br>0.5500 ±1.877 | Na<br>Na |
| 18CO04 | Cebolla Creek (Gunnison, CO) | 2588 | 1°(n=20: WW=16, noWW=4)<br>2°(n=20: WW=16, noWW=4) | 209.563 ±240.021,<br>87.75 ±137.866<br>192.688 ± 247.957<br>0.000 ±0.000 | Na<br>Na<br>Na<br>Na |
| 18CO05 | Shambhala/Rd 69E (Larimer, CO) | 2696 | 1°(n=20)<br>2°(n=20) | 22.100 ± 27.371<br>Na | Na<br>Na |
| 18CO09 | Cherokee Park Rd. (Larimer, CO) | 2237 | 1°(n=19),<br>2°(n=20) | 272.632 ± na<br>72.300 ± 176.467 | Na<br>Na |
| 18CO10 | McClure Pass (Gunnison, CO) | 2670 | 1°(n=20: WW=2, noWW=18)<br>2°(n=20: WW=2, noWW=18) | 439.500 ±359.917,<br>481.778 ± 295.869<br>378.000 ± 534.573<br>58.167 ± 138.904 | Na<br>Na<br>Na<br>Na |

TABLE 5. Estimated marginal means of Viable seed count and Viable seed weights from study sites in 2017 and 2018 (no seed weights were collected for 2018). Matched group numbers (last column) signify non-significant differences.

2017 Viable Seed Weight (*mg*)

```
Site  emmean     SE         df   lower.CL   upper.CL   group
8     4.897101   0.8277814  127  3.259071   6.535131   1
 4    5.172920   0.5715748  127  4.041876   6.303963   1
 5    5.300637   0.6302125  127  4.053560   6.547714   1
 2    5.922824   0.5525774  127  4.829374   7.016275   1
 1    6.273888   0.5729365  127  5.140150   7.407626   12
 7    8.117786   0.6055941  127  6.919424   9.316147    23
 9    8.245105   0.5937614  127  7.070158   9.420051    23
 3    8.308255   0.5084760  127  7.302072   9.314437    23
 6    9.197344   0.5778057  127  8.053971   10.340718    3
 10   9.847754   0.5135526  127  8.831526   10.863982    3
Results are averaged over the levels of: Umbel Position and D. radiella presence.
Confidence level used: 0.95
```

2017 Viable Seed Count:

```
Site  emmean     SE        df    lower.CL     upper.CL   group
 3    36.10399   32.97194  133   -29.113236   101.3212   1
 1    47.32820   37.46791  133   -26.781885   121.4383   1
 2    64.05525   34.53125  133    -4.246214   132.3567   12
 5    99.61668   38.78748  133    22.896550   176.3368   123
 4    133.96924  35.87720  133    63.005535   204.9330   123
 6    161.78038  29.53203  133   103.367167   220.1936   123
 8    196.89376  56.04288  133    86.043110   307.7444   123
 7    209.68747  32.19930  133   145.998498   273.3764    23
 9    261.75714  29.86841  133   202.678586   320.8357     3
 10   464.44202  29.76557  133   405.566877   523.3172      4
Results are averaged over the levels of: Site.
Confidence level used: 0.95
```

2018 Viable Seed Count:

```
Site  emmean    SE        df  lower.CL  upper.CL  group
 1    123.3223  72.58577  44  -22.96474  269.6093  1
 9    171.2077  64.42658  44   41.36446  301.0509  1
 10   221.9989  62.75196  44   95.53066  348.4672  1
 2    252.2982  69.99577  44  111.23104  393.3654  12
 5    326.7095  73.64207  44  178.29364  475.1253  12
 4    528.6425  58.79766  44  410.14358  647.1414   2
Results are averaged over the levels of: Site.
Confidence level used: 0.95
```

# CHAPTER 3: MULTITROPHIC COMMUNITY INTERACTIONS: HERBIVORY EFFECTS ON POLLINATOR DIVERSITY AND PLANT PERFORMANCE IN COW PARSNIP

## Introduction

Multitrophic interactions are less frequently studied than single species ecological investigations (Carmel *et al.,* 2013). Even fewer studies have addressed how global climate change and elevation gradients influence multitrophic interactions (Memmott *et al.,* 2007, Moreira *et al.,* 2018). Yet, communities of plants and their insect associates are dependent on synchronous phenologies (Visser and Both 2005). Increases in global temperatures are speeding up insect development and plant hosts may be subject to intensified herbivory or experience visits by fewer pollinators (Menéndez 2007). Intensity of biotic interactions in plant-herbivore communities and the level of investment in plant defenses has not been clearly linked to latitude (Anstett *et al.,* 2018), which is often used as a proxy to understand the effects of climate change. Instead, links (the relationship of plant-pollinator trophic interactions), between plants and their insect associates may rely on matching between species whose phenologies may differ in their responses to changes in temperature (Waser *et al.,* 1996).

In cow parsnip (*Heracleum maximum* Bartram) (Apiaceae), flowering and development are synchronized with their herbivore, the parsnip webworm *Depressaria radiella* Goeze (Lepidoptera: Depressariidae), and pollinators flight times. *Heracleum maximum* plants flower in late-June at lower elevations (e.g., 1500-2200m) and mid-July at higher elevations (≥3000m). Under warming conditions, plants in montane regions have been documented to flower and set-seed earlier than historically cooler conditions (Inouye 2008). Lepidopteran species have also been recorded to emerge before plant hosts in warmer conditions associated with climate change

(Menéndez 2007). In the *H. maximum* system, the effect of *D. radiella* herbivory on plant seed production is attributed to floral development (see *Chapter 2*). As *D. radiella* feeds on flowers and fruits, it may reduce pollination visitation rates, and hence *H. maximum* fitness.

*Heracleum maximum* is known to provide both pollen and nectar floral rewards to pollinators. I am interested in identifying whether multitrophic interactions are affected by elevation and *D. radiella* herbivory.

### *Objectives and Hypotheses*

*To determine if insect families visiting H. maximum change with flowering stage and/or increased elevation gradient.*

*To investigate how the presence or absence of parsnip webworm Depressaria radiella Goeze (Lepidoptera: Depressariidae) affects H. maximum floral visitors.*

Most pollinator visits to Apiaceae plants have been associated with insects in the orders Coleoptera, Hymenoptera, and Diptera, where male-stage flowers receive more pollinator visits than female-stage flowers in hogweed (*H. sphondylium*) (Sheppard 1991, Zych 2002 and 2007). Additionally, herbivory by *Coleotechnites eryngiiella* (Lepidoptera: Gelechiidae) in rattlesnake master (*Eryngium yuccifolium*) has been shown to reduce Coleoptera, Hymenoptera, and Diptera pollinator visits as compared to undamaged plants (Danderson and Molano-Flores 2010). Similarly, I predict that *H. maximum* pollinators will consist mainly of insects in coleopteran, dipteran, or hymenopteran orders and that elevation will affect pollinator diversity. Across elevation gradient, cooler temperatures are typically associated with reduced pollinator and insect diversity as compared to lower elevations with warmer climates (Moreira *et al*. 2018).

In relation to the second objective, parsnip webworm (*D. radiella*) may alter *H. maximum* floral attraction to insects. Reduced floral area (umbel diameter) via webbing, increased plant defensive cues (e.g., xanthotoxin, isopimpinellin, sphondin, psoralen, and bergapten), or volatiles that warn of damage have been suspected to alter insect associate interactions (Camm *et al.* 1976, Berenbaum 1983, Berenbaum *et al.,* 1986). Alternatively, pollinator choice may be attributed more to flower morphology (e.g., flower size, shape, and color) and floral awards (nectar and pollen) regardless of *D. radiella* presence (Maloof and Inouye 2000, Irwin *et al.* 2004). Lastly, several apiaceous species have been found to utilize out-crossing pollination due to temporal flowering synchronization among umbels (Ponomarev 1960). Previous literature suggests that out-crossing occurs in apiaceous plants due to varied floral timing among hermaphroditic, pistillate, and/or staminate flowers between umbels and umbellets (i.e., floral dichogamy) (Schlessman 1978). However, self-compatibility in Apiaceae plants is also common (Lindsey 1982). Therefore *H. maximum* seed production at unconsumed umbels may not be as affected by *D. radiella* herbivory or lack of pollinator visitation. Instead abiotic and environmental conditions such as soil moisture and resource availability may be more responsible for seed production when plants self-fertilize.

## Methods

This chapter uses results and data from the previous chapter. The same study sites, plants, herbivore, and plant trait measurements were discussed in Chapter 2.

### *Insect Visitors and Pollinators*

Insects were collected and identified from *H. maximum* inflorescences throughout ten study sites in 2017 and a subset of six study sites in 2018. Insects were collected manually or with use of aspirators in the field, and identified to family, and occasionally genus and species by Dr. Boris Kondratieff. Insects were recorded with respect to the marked plant and umbel each was collected from. Additionally, a list of common insects observed and collected in 2017 was compiled and used to identify insects by sight in 2018 (Table 6). Insect visitation rates were conducted in 2018, whereupon each plant in a study site (n=20) was observed for insects and pollinators from the primary and marked secondary umbel.

Insects were observed on the umbel(s) in 3-4 min increments from the following time intervals: morning (8:00 am-12:00 pm), early afternoon (12:00-2:30 pm), and late afternoon (2:30-5:30 pm). In a single study site visit, I typically conducted several insect observations usually from two different time intervals (e.g., morning and early afternoon). Weather, cloud cover, and approximate temperature were also recorded during insect observations at each study site visit but were not further analyzed. Sites were visited at different time intervals to try to collect visitation rates from all time periods and plant phenology stages in the 2018 field season (June 20th-July 14th). Occasionally, flowering stages were missed in the field such that obtaining insect observations across all time periods was not possible. However, it was later found that the time insect visitation and counts were collected did not affect insect diversity or visits.

Insect floral visitors were counted separately on the primary and marked secondary umbel at the start of the observation and as they contacted *H. maximum* flowers during the 3-4 min time interval. Insects that visited after an observation period, on unmarked secondary umbels, or on tertiary umbels in the same plant were not recorded. Insects that left the umbel and returned within the time interval were occasionally counted multiple times, especially if several insects were present and moving at the same time. To account for unequal study site visits, a single observation of all six study sites was compared for insect counts. The single observation dates were chosen to coincide with similar flowering stages or ranks across all six sites in which dates varied with individual study site plant development (June 26th, July 5th, July 6th, July 8th, July 13th, and July 14th). Additionally, individual insect counts were analyzed as insect family count, defined as the number of distinct insect families that visited an umbel within the time interval. Individual insect species were not investigated across sites because several reported families consist of a single species, such that insect family diversity is also a sufficient indicator of species diversity (Table 6).

## Data and Analysis

### *Data Description*

Insect family count and viable seed-set (log transformed) were treated as response variables in linear multiple regressions. Continuous predictor variables included: elevation (m), number of secondary umbels, average flowers per two randomly selected umbellets, average number of umbellets from two randomly selected umbellets, plant height (cm), umbel diameter (cm), and umbellet diameter (cm). Time sampled (morning, early afternoon, late afternoon), *D. radiella* presence (yes or no), flowering stage (refer to Chapter 2), study site location, date, and umbel position (primary or secondary) were treated as categorical variables. For the *D. radiella* data analysis, a linear mixed-effects model was analyzed where insect family count was treated as the predictor variable and the following three variables were treated as fixed coefficients: plants nested within elevation, *D. radiella* presence (yes or no), and umbel position (primary or secondary). For seed production data linear fixed-effects models were also used where total seed count and viable seed counts (untransformed) were treated as predictor variables. The following three coefficients were treated as fixed effects: *D. radiella* presence (yes or no), umbel position (primary or secondary), and plants nested within elevation.

### *Statistical Analysis*

I conducted data analysis using R Version 3.4.1 (R Development Core team, 2017). The 'Anova()' function was used to calculate *p-values* from the 'car' package and multiple linear regressions were conducted using the ‘lm’ function (Fox and Weisber, 2011). Estimated marginal means and Tukey adjusted pairwise comparisons were obtained with the ‘emmeans’ and ‘cld’ function from the ‘emmeans’ package with ±standard error (Lenth, 2018). A linear mixed model fit by REML t-tests using the Welch-Satterthwaite’s method from the ‘lme4’ package (Bates *et al.* 2015) was also utilized for *D. radiella* data analysis. Traditional backwards model selection in the ‘MuMIn’ package (Barton, 2018) was used to determine the best fit models for insect family count and viable seed counts in the multiple linear regressions. Viable seed counts treated as the response variable were log transformed, but data reported in figures is on the original scale.

## Results

### *Insect Visitors and Pollinators*

#### *i. Flowering Stage*

We identified 44 different insect families and approximately 46 species visiting *H. maximum* throughout 2017 and 2018 (Figure 6). Insect family count varied significantly among sites (F=15.804, df=13 and 216, $p<0.0001$), where lower elevation sites (Cebolla Creek and Cherokee Park Rd.) had the greatest insect family counts (1.90±SE 0.43, F=15.8, df=13 and 216, $p< 0.0001$) and (2.30±SE 0.31, F=15.8, df=13 and 216, $p<0.0001$), there was not a significant difference for insect family count at high elevation sites (-0.059±SE 0.84 to 0.368±SE 0.35, F=15.804, df=13 and 216, $p>0.05$). Secondary umbel position led to a significant decrease in insect family count (-0.696±SE 0.25, F=7.86, df=13 and 216, $p<0.01$).

The floral area or umbel diameter (cm) (0.0483±SE 0.027, F=3.273, df=13 and 216, p=0.07184) and the average flowers per umbellet (0.0322± SE 0.019, F=3.167, df=13 and 216, p=0.07653) were marginally insignificant in increasing insect family counts (Figure 14).

However, insect family count itself was not significantly different between sites (F=0.0017, df=13 and 216, p=0.9676) and averaged approximately 2 insect families per umbel within the 3-4 min time interval (F=20.42, df= 13 and 216 and 216, $R^2$=0.5513, $p<0.0001$). Joe Chamber's campsite had the least family insect visits per umbel at approximately (0.8608± SE 0.28) insect families when data was split by date, plant, umbel, flowering stage, and time sampled. Whereas Cherokee Park Rd. and Cebolla Creek had the most insect families (3.156±SE 0.18) and (2.760± SE 0.37) which were not significantly different from each other (F=15.804, df=13 and 216, $p>0.05$). Insect family counts were pooled to plant and umbel in (Figure 11) and represent greater diversity than splitting data above.

Flowering stage was a significant predictor for insect family count, from flowering ranks (2, 3, and 4): mature staminate male flowers with pollen, old staminate/ immature pistillate flowers in the transition between male and female stages, and mature pistillate female flowers with available nectar in the stylopodium) (Figure 12). Flowering ranks were not significantly different from each other ($p>0.05$) in respect to insect family count, rank 2 (male) flowers attributed to (1.402±SE 0.28, F=20.42, df=13 and 216, $p<0.0001$) insect families per timed interval, rank 3 (males with dehisced anthers) (1.262±SE 0.37,F=20.42, df=13 and 216, $p<0.0005$), rank 4 (female) flowers (1.406±SE 0.34, F=20.42, df=13 and 216, $p<0.0001$) insect families per visit (Figures 8 and 9). Flower ranks (1, 5, and 6) were representative of flowers emerging prior to pollen production or during fruit maturation, these stages were typically ignored for insect observations, and were attributed to the least insect counts.

### *ii. Elevation effects on insect diversity*

When elevation was treated as a continuous variable within the multiple linear regression model above insect family count was found to be decrease across elevation (m) (F=59.73, df=8 and 221, $R^2=0.5169$, $p<0.0001$), for every 1000m increase in elevation family insect count decreased by (2.81±SE 0.36). In addition to elevation, secondary umbels also decreased insect family counts (-1.15±SE 0.21, F=30.32, df=8 and 221, $p<0.0001$) in comparison to primary umbels, while average flowers per umbellet increased insect family counts (0.035±SE 0.018, F=3.949, df= 8 and 221, $p<0.05$). Similar to the data presented above, flowering stages (2, 3, and 4) led to significant increases in family insect count or insect diversity (1.331±SE 0.25 to 1.401±SE 0.31) (F=29.56, df=8 and 221, $p<0.0001$), however flowering stages were again not significantly different from each other. As such there were no significant differences in floral sex, male versus female stage flowers regarding insect family count, meaning pollen and floral rewards may be equally sought out by insects.

### *iii. D. radiella presence*

Across all 2017 and 2018 study sites approximately 15% of plants that had evidence of *D. radiella* in *H. maximum* stems or pedicels (48 infested, 272 not infested). Of the 48 plants, *D. radiella* counts were recorded in 34 plants where the average number of *D. radiella* per plant was approximately 7 larvae (6.941). Plants with *D. radiella*, had on average 5.286 or approximately (5) *D. radiella* entry holes per plant. The percentage of stems occupied with late instar, pupal, emerging adults, or parasitized *D. radiella* was 78%. Across elevation insect family count was not affected by *D. radiella* presence (and herbivory) (F=1.934, df=8 and 221, $p=0.166$) when insect family counts were split between plants, dates, umbels, flowering stages, and time sampled (Figure 10). However, when insect family count was pooled across plant and umbel in the linear fixed-effects model *D. radiella* presence significantly decreased insect family counts (-1.316±SE 0.54, F=5.878, df=1 and 117, $p<0.05$) (Figure 11). Where insect family count was significant across plants nested in elevation (2.933± SE 0.25, df=192, $p<0.001$). Secondary umbel position also decreased insect family counts (-0.8142±SE 0.26, F=9.877, df=1 and 118, *$p<0.005$*). There was a significant difference between plants with and without *D. radiella* for insect family count (*$p<0.05$*). Further umbel position (*$p<0.005$*) was also significantly different between primary and secondary umbels. Additionally, when umbel diameter (cm) is treated as a predictor variable in a multiple linear regression testing the coefficients: *D. radiella* presence (yes or no), plant height (cm), umbel position (primary or secondary), and elevation (m) it was found that *D. radiella* presence decreases floral area significantly (-4.68±SE 0.71, F=43.4093, df=4 and 331, *$p<0.0001$*), which may partially explain how *D. radiella* decreases insect family counts. Due to incomplete insect counts in the field in 2017, this data was not included for further analysis.

Total seed production and viable seed production was significantly impacted by *D. radiella* presence (Figure 15). Where plants with *D. radiella* produced more total seeds (167.03±SE 61.78, F=7.309, df=1 and 117, *$p<0.01$*) than plants without *D. radiella*. Additionally, plants with *D. radiella* also increased viable seed production by (107.15± SE 42.87, F=6.248, df=1 and 117, *$p<0.05$*) in comparison to *D. radiella* free plants (Figure 16). Secondary umbels produced significantly fewer total seeds (-240.66± SE 24.31, F=98.01, df=1 and 117, *$p<0.0001$*) and viable seeds (-135.65± SE 18.2, F=55.51, df=1 and 117, *$p<0.0001$*) in comparison to primary umbels.

#### *iv. Single Observation*

From a single observation of each of the six 2018 study sites, insect family count was significantly different across elevation (F=7.70, df=9 and 247, *$p<0.05$*). However, secondary umbel position decreased insect family count by (-0.805±SE 0.18, F=18.94, df=9 and 247, *$p<0.0001$*). Flowering stage (1, 2, 3, and 4) increased insect family count from (1.07± SE 0.28 to 3.12±SE 0.27, F=60.93, df=9 and 247, *$p<0.0001$*) and *D. radiella* presence increased insect family counts from single sampling dates (0.589±SE 0.22, F=7.42, df=9 and 247, $p<0.05$). Insect counts with flowering stages are depicted in all insect visit observations and a single observation in (Figure 12).

## Conclusion

Flowering stage and floral phenology matters when attracting insect visitors to *H. maximum*. Male stage flowers with pollen did not attract more insect families than female stage flowers with nectar, opposing results found in the congener *H. sphondylium* (Sheppard,1991). Unlike (Schiestl *et al.* 2004), my results may also indicate that fluctuations of floral rewards between male and female-stage flowers is less important in high elevation *H. maximum* plants. In *H. maximum* attracting different functional types of insects may not necessarily increase plant fitness. Although, plants at higher elevations may out-cross more frequently with dipteran species as compared to coleopteran species at lower elevations (Figure 13). Reduced insect diversity at higher elevations was neither attributed to *D. radiella* attack nor delays in flowering phenology in my study.

Our results showed that fewer insect families visited plants at higher elevation. Even though, flowering phenology did not lag exclusively at higher elevations in our study, low elevation sites were consistent in developing prior to high elevation sites. In relation to work that describes insect visitation matching plant phenologies under warming conditions (Memmott *et al.* 2004, Moreira *et al.* 2018), elevation as a proxy for climate change was not a clear driver for insect diversity in my study. Plant-insect community structure may have been further impacted by a combination of direct climate effects and indirect biotic insect interactions as discussed by (Gilman *et al.* 2010).

Elevation did not significantly affect plant seed production nor did *D. radiella* presence affect insect diversity between study sites. However, in plants with *D. radiella,* insect family counts decreased in comparison to un-attacked plants in the same study sites. Floral area and the average flowers per umbellet are suggested to benefit plants by increasing insect visits. In contrast, results show that *D. radiella* decreases umbel diameter (cm), thus potentially decreasing floral attraction, though it is equally possible that pollinators avoid damaged umbels (Zangerl and Berenbaum, 2009). Future work is needed to determine if *H. maximum* plants across increasing elevation gradients are producing different concentrations of chemical defenses in response to less *D. radiella* herbivory, similar to work conducted by (Anstett *et al.* 2018). Although, McClure Pass did have *D. radiella* infestation, rates of attack were 60% lower than in Cebolla Creek and Spring Creek, which may be attributed to large plant population at McClure Pass.

Secondary umbels were found to have less insect family visits than primary umbels, but there were no associated seed production losses with less insect visitors. In 2018 the proportion of viable seeds to total seeds was 56% in primary umbels as compared to 55% of secondary umbel seeds, indicating that slightly less than half of all seeds produced by *H. maximum* will be aborted. Further primary umbels produced 240 more total seeds, and 136 more viable seeds than the marked secondary umbels. However, because plants on average produced three secondary umbels plant fitness may be more equally distributed between umbels and not represent clear abortion patterns (Sheppard 1991). As *D. radiella* larvae typically attack primary umbels, our results showed that plants may over-compensate for lost fitness potential by producing more seeds and/or heavier seeds in un-attacked secondary umbels, similar to results found in *P. sativa* by (Hendrix and Trapp 1989). In instances where *D. radiella* consume flowers and fruit in both primary and secondary umbels it would seem unlikely that un-attacked secondary umbels will be able to compensate for extensive fitness losses.

Alternatively, preliminary greenhouse germination studies conducted in 2018 provide evidence that *H. maximum* may be self-compatible to some degree, where bagged umbels set fruit and seed following the exclusion of insect pollinators (Smith *unpublished data*). Studies reviewing *H. sphondylium* may suggest that congener *H. maximum* is amphimictic where inter-breeding occurs freely, and self-fertilization produces fertile offspring (Sheppard 1991), but further investigation is needed to suggest how out-crossing affects seed-set and fertility. As such differences in seed production needs to be investigated further to decipher if plants with fewer insect visitors may be more likely to self-fertilize, or if the amount of pollen transferred by pollinators significantly effects *H. maximum* seed production.

## Future Directions

Often, interesting questions can be proposed by simple observation. Elevation gradients should be further investigated to determine future impacts of global climate change on plant-pollinator communities and the relationships of plant-insect assemblages. Though, long-term observation on systems spanning elevational gradients is difficult, observation can be paired with experimentation to create stronger ecological models that map complex ecological interactions. For example, in the *H. maximum* system, further investigation is needed to look at underling mechanisms controlling and/or partitioning multitrophic plant-herbivore-pollinator relationships.

Further investigation of climatic conditions, seasonal temperatures, and precipitation rates would be beneficial in following *H. maximum* plant phenology up elevation gradients. With long-term studies compiling several years of insect data would help distinguish if patterns of insect orders, families, and species are truly attributed to floral timing and available floral resources in *H. maximum*. Functional insect type or the roles insect associates have while utilizing *H. maximum* would be a fascinating study. Distinguishing relationships of insect trophic levels while investigating *H. maximum* pollinators for pollen loads would increase our knowledge of *H. maximum* reproductive strategies utilized in variable climatic conditions.

Additionally, we can look at whether insect assemblages and multitrophic interactions interacting with *H. maximum* change in Colorado. I suggest investigation of *H. maximum* seed defensive chemistry and floral volatile cues produced by plants with and without *D. radiella* presence from plants in different elevations. It is possible that chemotype variation exists between plants in spatially separated populations, or alternatively the latitudinal herbivory-defense hypothesis might explain differences in defensive chemistry with less herbivore pressure and cooler temperatures as elevation increases. Regardless, the *H. maximum* system provides further outlets not only under the scope of plant-directed insect interactions, but several insect-directed trophic level functions have still yet to be determined.

# FIGURES

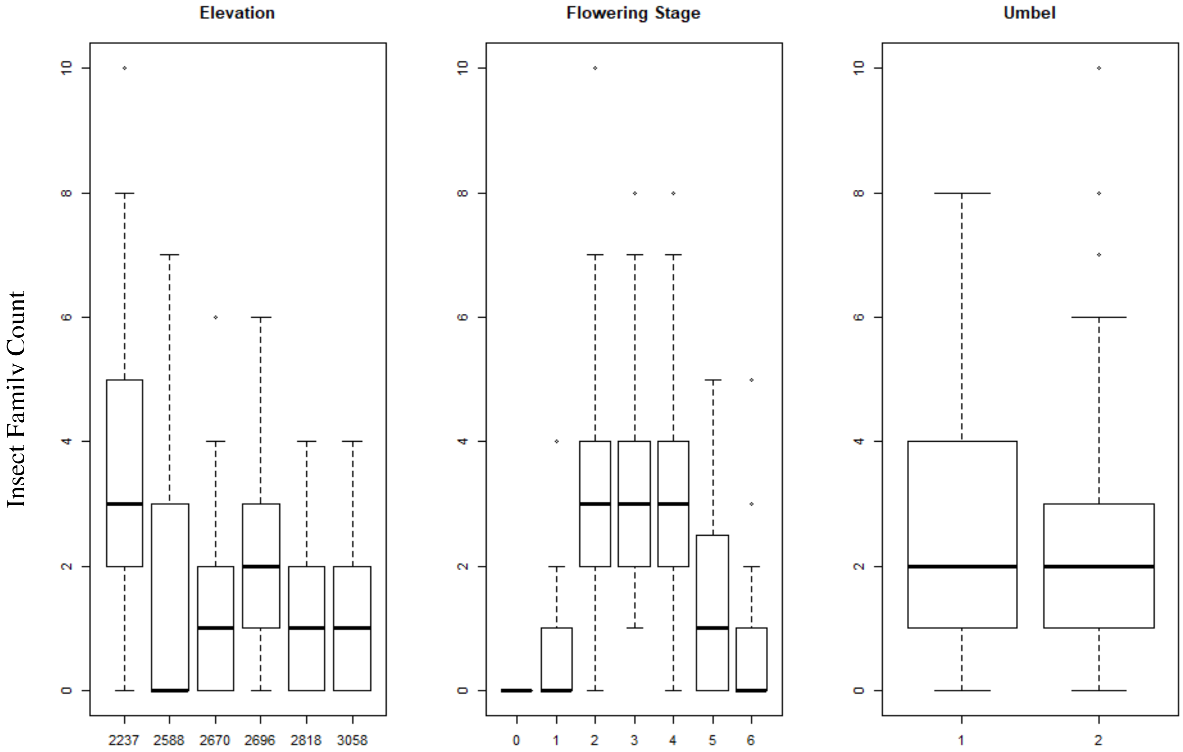

FIG. 10. The count of distinct insect families identified on *H. maximum* flowers across elevation, by flowering stage, or between primary and secondary umbels. Left-Insects are split by umbel, date visited, and time sampled such that overall insect family counts are reduced versus pooled values in Figure 9.

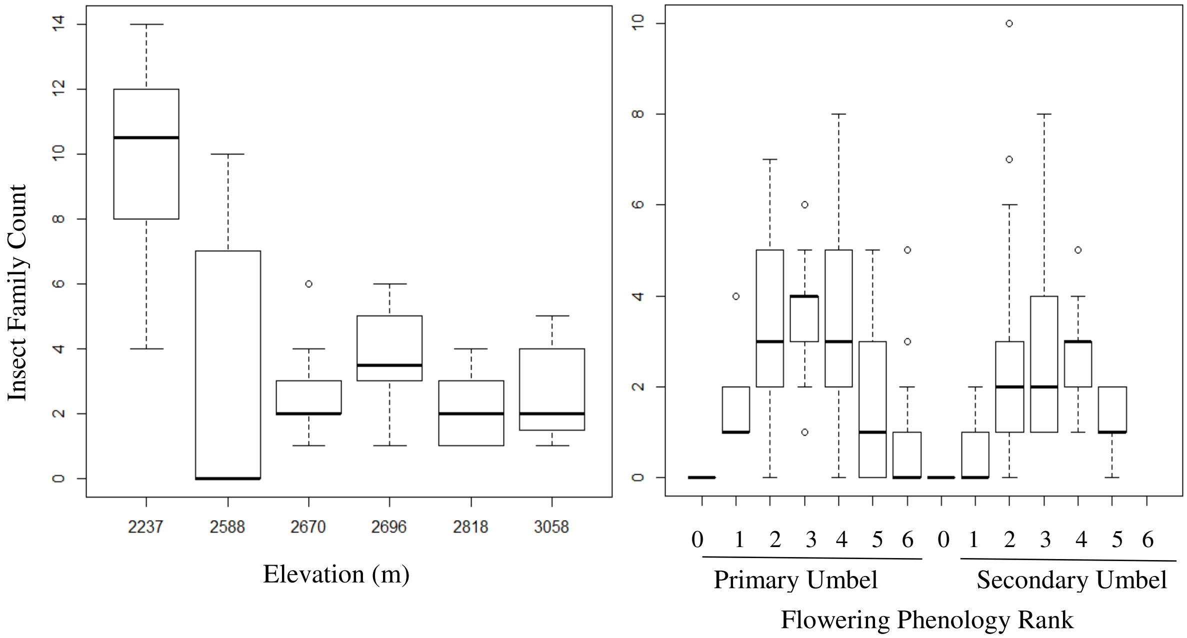

FIG. 11. Left-The insect families identified on *H. maximum* across elevation (insect counts pooled to whole plants). Right-Insect family count associated with flowering phenology in both primary and secondary umbels.

FIG. 12. Top-All study site visit data for insect counts and flowering stage, per insect Order. Bottom-Single observation of insect visitation across the six study sites.

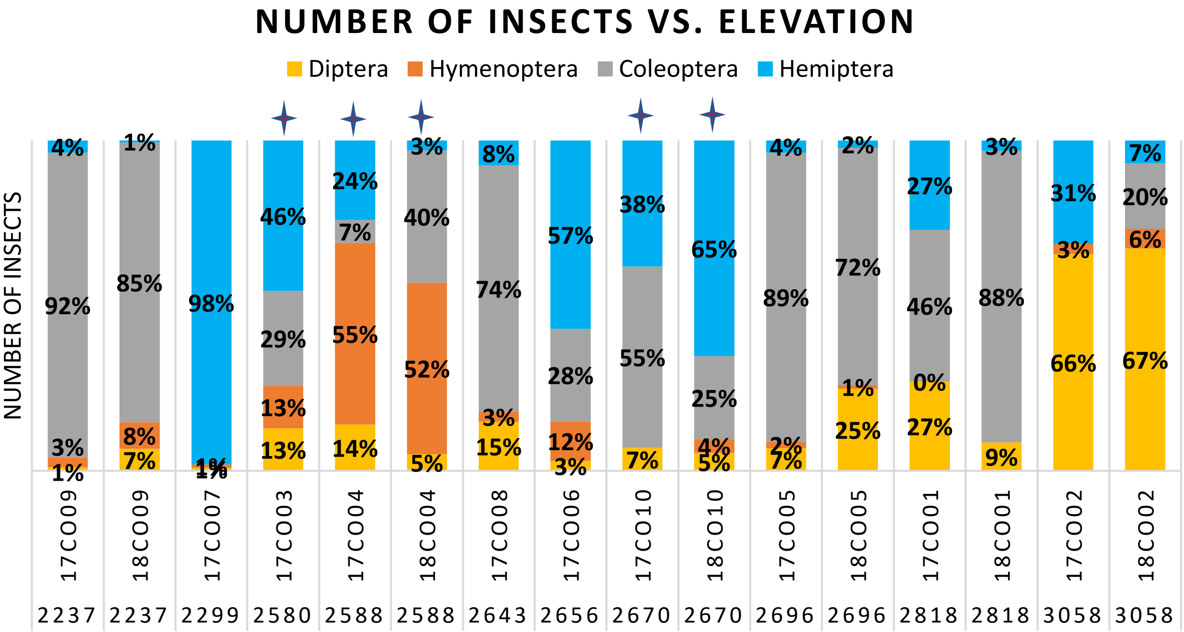

FIG. 13. Insect count percentages from all insect observations accumulated per site. Each bar represents insect order as a proportion of 100%, such that proportion of insect order is visualized by insect order per study site and year sampled. Asterisks represent sites with *D. radiella* presence.

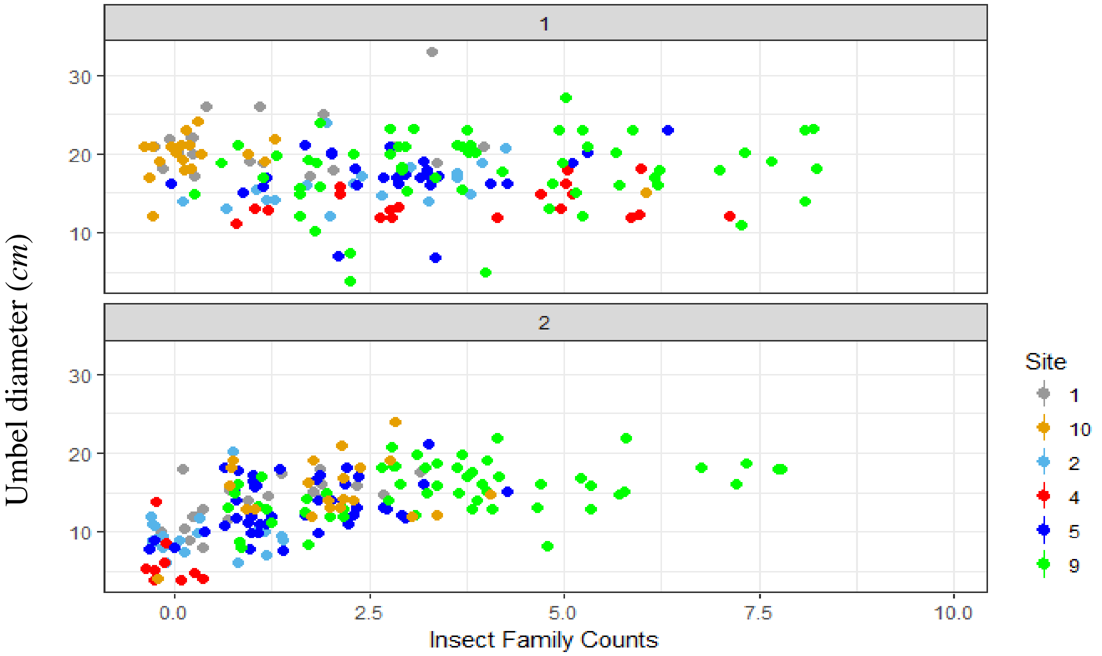

FIG. 14. The count of distinct insect families identified on *H. maximum* flowers as attributed to umbel diameter (*cm*). The top panel, box (1) represents insect counts and umbel diameters within primary umbels, while box (2) reflects the marked secondary umbel's insect family counts and umbel diameters. In the key Site 1(Joe Chamber's Campsite, 2818m), Site 10 (McClure Pass, 2670m), Site 2 (Crooked Creek Pass, 3058m), Site 4 (Cebolla Creek, 2588m), Site 5 (Shambhala/69E, 2696m), and Site 9 (Cherokee Park Rd., 2237m).

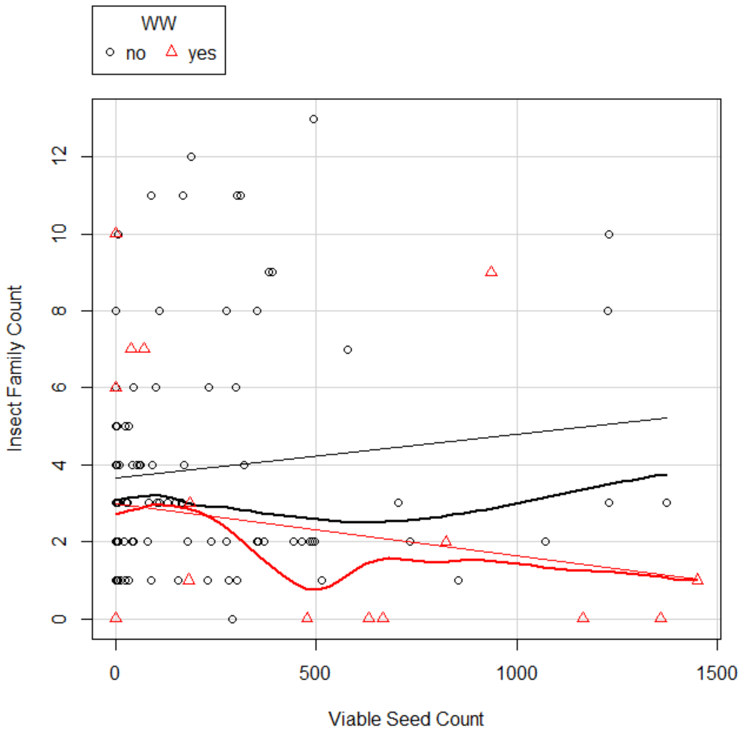

FIG. 15. *Heracleum maximum* viable seed count (seed production) increases slightly as more insect families visit flowers. Parsnip webworm (WW) presence (yes) and herbivory damage decreases the number of insect families that visit *H. maximum* which lowers viable seed production. Straight lines represent line of best fit linear regression lines.

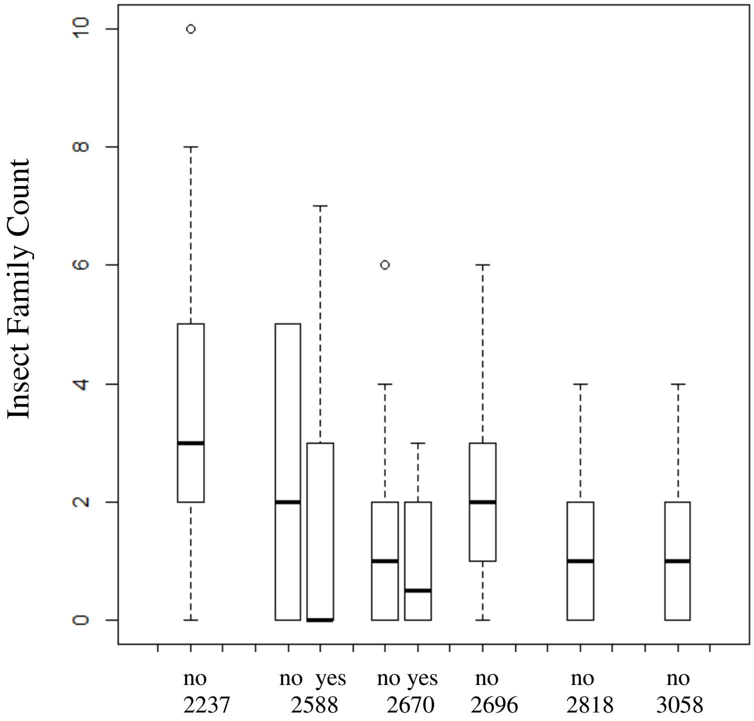

FIG. 16. The presence (yes) or absence (no) of *D. radiella* and the impact of herbivory on insect family count across elevation (m). Elevation(m) corresponding to boxplots are placed below.

# TABLES

TABLE 6. Insects identified by Dr. Boris Kondratieff on flowering *H. maximum* umbels. Common insects identified by sight in 2018 (*). Unk= unknown

| **Order** | (**Family**) *Genus species* |
|---|---|
| Coleoptera | *(**Scraptiidae**) *Anaspis rufa* (Say)<br>*(**Orsodacnidae**) *Orsodacne atra* (Ahrens)<br>*(**Cleridae**) *Trichodes ornatus*<br>*(**Mordellidae**) *Mordella spp.*<br>*(**Dermestidae**) *Anthrenus spp.*<br>*(**Scarabaeidae**): *Trichiotinus assimilis*, *Dichelonyx spp.*<br>*(**Cerambycidae**): *Acmaeops proteus proteus* (Kirby), *Cosmalia chrysocoma* (Kirby), *Stenocorus trivittatus* (Say), *Typocerus spp.*<br>(**Coccenellidae**): *Coccinella transveroguttata richardsoni*, *Coccinella septempunctata* (L.), *Hippodamia convergens* (Guérin-Méneville)<br>(**Elateridae**) Unk |
| Diptera | *(**Muscidae**) *Coenosia spp.*<br>*(**Syrphidae**) *Sphaerophoria spp.*<br>*(**Tephritidae**) *Rhagoletis basiola* (Sacken)<br>*(**Empididae**) Unk<br>*(**Agromyzidae**) Unk<br>*(**Phoridae**) *Phora spp.*<br>*(**Tachinidae**): *Adejeania vexatrix (*Sacken), *Gonia spp.*<br>(**Pallopteridae**) *Palloptera arcuata* (Fabricius)<br>(**Conopidae**) *Zodin spp.*<br>(**Sphaeroceridae**) Unk<br>(**Stratiomyidae**) *Stratiomys spp.*<br>(**Bibionidae**) *Dilophus spp.*<br>(**Psilidae**) Unk |
| Hemiptera | *(**Miridae**): *Arhyssus spp.*, *Coquillettia spp.*, *Lygus kalmii* (Stål)<br>(**Aphididae**) Unk<br>(**Anthocoridae**) *Anthocorus spp.* |
| Hymenoptera | *(**Ichneumonidae**) *Spiloptera vidinum melander* (Towns)<br>*(**Tenthredinidae**) *Tenthredo spp.*<br>*(**Vespidae**) *Vespula spp.*<br>*(**Gasteruptiidae**) Unk<br>*(**Formicidae**): *Myrmelachista spp., Formica spp.*<br>*(**Sphecidae**): *Crossocerus spp., Solierella spp.*<br>*(**Apidae**): *Apis melifera* (L.), *Bombus nevadensis*, *Bombus spp.*<br>(**Figitidae**) Unk<br>(**Braconidae**) Unk<br>(**Torymidae**) Unk<br>(**Chyrsididae**) Unk<br>(**Encrytidae**) *Copidosoma sosares* (Walker) |
| Lepidoptera | *(**Lycaenidae**) *Callophyrus gryneus* (Hübner)<br>*(**Noctuidae**) *Papaipema harrisii*<br>*(**Nymphalidae**): *Speyeria aphrodite*, *Aglais spp.*<br>*(**Depressariidae**): *Depressaria radiella* (Goeze), *Depressaria discipunctella*, *Agonopterix spp.*<br>(**Erebidae**) *Ctenchua venosa*<br>(**Geometridae**) Unk |
| Neuroptera | (**Raphidiidae**) *Agulla spp.* |

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